CUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

PhD THESIS

Gluten-free Fresh Pasta: Influence of Pregelatinized Starch, Inulin, and Probiotic Usage on Extrusion and Cooking Properties

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Department of Food Engineering

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PhD THESIS APPROVAL

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ABSTRACT

This study aims to define the best fresh gluten-free pasta (GFP) formulation, based on corn starch partially replaced (10, 20, and 30%) with pregelatinized starch (PGS), by hybrid multi-criteria decision making including AHP and TOPSIS, respectively, to enrich with inulin with two different degrees of polymerization (DP≥10 and DP≥23) and concentrations (4 and 8%), and then *Bacillus coagulans* GBI-30, 6086 as a probiotic source, respectively, and evaluate the extrusion attributes, cooking properties with different time intervals (0, 1, 5, 10, and 15 min), sensory characterization, and the cell viability of probiotics with/without inulin as a prebiotic source. The criteria weights of extrusion yield, cooking loss, yellowness index, hardness, and taste were 0.34, 0.22, 0.05, 0.11, and 0.29, respectively, as defined by the AHP method. According to TOPSIS results, the control sample based on durum wheat semolina was the first-ranked pasta, followed by 20% PGS-incorporated GFP, which was the best alternative to the control group. In general, the moisture, water absorption capacity, swelling index, cooking loss, volume increase, turbidity, and amylose leaching were increased, whereas color (L*, a*, b*, yellowness index, and color score) and texture profile attributes were decreased as the cooking time prolonged in all fresh GFP with/without inulin or probiotic source. The probiotic inclusion into fresh GFP with/without inulin generally negatively influenced the end-product quality. However, the viability of probiotics was above approximately 85% and 67% in inulin-fortified GFP with different DP and concentrations at 5 min and 10 min of cooking, respectively. Therefore, except for probiotic-enriched fresh GFP without inulin cooked for 10 min, the results were still above the acceptable threshold to show a potential positive impact on host health, which corresponds to at least 10^6 CFU/g of live strains.

Keywords: Synbiotic, *Bacillus coagulans*, Degree of polymerization, Cooking quality, Functional food.

ÇUKUROVA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ

DOKTORA TEZİ

Glutensiz Taze Makarna: Prejelatinize Nişasta, İnülin ve Probiyotik Kullanımının Ekstrüzyon ve Pişirme Özellikleri Üzerindeki Etkisi

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ÖZ

Bu çalışmada, mısır nişastasının prejelatinize nişasta (PJN) ile kısmen (%10, 20 ve 30) ikame edilerek ve sırasıyla AHP ile TOPSIS'den oluşan hibrit çok kriterli karar verme yöntemi uygulanarak, en iyi taze glutensiz makarna (TGM) formülasyonunun belirlenmesi, ve sırasıyla iki farklı polimerizasyon derecesinde (DP≥10 ve DP≥23) ve konsantrasyonda (%4 ve 8) inülin ve probiyotik kaynağı olarak *Bacillus coagulans* GBI-30, 6086 ile zenginleştirilip, ekstrüzyon niteliklerinin, farklı sürelerdeki (0, 1, 5, 10 ve 15 dk) pişirme özelliklerinin, duyusal karakterizasyonunun ve probiyotik hücre canlılığının değerlendirilmesi amaçlanmaktadır. AHP yöntemiyle tanımlanan, ekstrüzyon verimi, pişirme kaybı, sarılık indeksi, sertlik ve tat kriterlerinin ağırlıklandırmaları sırasıyla 0.34, 0.22, 0.05, 0.11 ve 0.29 olarak belirlenmiştir. TOPSIS sonuçlarına göre, durum buğdayı irmiği esaslı kontrol örneği en iyi makarna olarak birinci sırada yer alırken, bunu kontrol grubuna en iyi alternatif olarak %20 PJN içeren TGM takip etmiştir. Genel olarak nem, su absorpsiyon kapasitesi, şişme indeksi, pişirme kaybı, hacim artışı, bulanıklık ve amiloz sızması, inülin veya probiyotik kaynağı içeren/içermeyen tüm TGM'lerde, pişirme süresi uzadıkça artarken, renk (L*, a*, b*, sarılık indeksi ve renk skoru) ve tekstür profil özellikleri ise azalış göstermiştir. İnülin içeren/içermeyen TGM'ye probiyotik ilavesi, genel olarak nihai ürün kalitesini olumsuz yönde etkilemiştir. Ancak, 5 dk ve 10 dk pişirilen prebiyotik kaynağı olarak farklı DP ve konsantrasyonda inülin içeren TGM'lerin probiyotik hücre canlılığı, sırasıyla yaklaşık %85 ve %67'nin üzerinde belirlenmiştir. Bu nedenle, 10 dk pişirilmiş probiyotik ile zenginleştirilmiş inülin içermeyen TGM hariç tüm örnekler, canlı suşların konak sağlığı üzerinde potansiyel olumlu bir etki göstermesi için gerekli, en az 10^6 CFU/g'a karşılık gelen, kabul edilebilir eşiğin üzerindedir.

Anahtar Kelimeler: Sinbiyotik, *Bacillus coagulans*, Polimerizasyon derecesi, Pişirme kalitesi, Fonksiyonel gıda.

GENİŞLETİLMİŞ ÖZET

Çölyak hastalığına sahip kişilerin, buğdayda gliadin, çavdarda sekalin ve arpada hordein gibi prolamin proteinlerini içeren tahıl ve türevlerini diyetlerinden çıkarmaları ve ömür boyu bu glutensiz diyet uygulamasına devam etmeleri gerekmektedir. Ancak glutensiz gıda ürünleri, yapılarında gluten proteini içermediği için, nihai ürün genellikle kalite kayıpları ile karakterize edilmektedir. Bu nedenle glutensiz gıda formülasyonlarında, başta farklı bitkisel ve/veya hayvansal proteinler, hidrokolloidler ve emülgatörler olmak üzere farklı gıda bileşenleri ve/veya katkı maddeleri kullanılarak gluten yapısı ikame edilmektedir. Glutensiz makarna üretiminde ise bunun yanı sıra genellikle ekstrüzyon pişirme prosesi veya ham madde kaynağı olarak prejelatinize nişasta kaynaklarının kullanımı ön plana çıkmaktadır.

Öte yandan, glutensiz gıdalar genellikle mısır ve pirinç kökenli ham maddelerden elde edilmekte olup (Khairuddin ve Lasekan, 2021), protein, diyet lif, vitamin ve mineraller ile fitokimyasallar gibi birçok makro ve mikro bileşenlerden yoksun olduğundan dolayı besin değeri açısından yetersizdir (Scherf, 2019). Bu nedenle, çoğunlukla daha yüksek glisemik indeks değerlerine sahip olup, uzun vadede çölyak hastalığına sahip kişilerde metabolik sendrom riskini arttırmaktadır (Melini ve Melini, 2019). Bu bağlamda, glutensiz gıdaların besin ögelerince zenginleştirilmesi (Khairuddin ve Lasekan, 2021) ve insan sağlığı üzerinde faydalı olduğu bilinen probiyotik, prebiyotik ve sinbiyotikler gibi fonksiyonel gıda bileşenlerinin tahıl bazlı yeni gıda ürünlerinin geliştirilmesinde kullanılması ve günlük diyete dahil edilmesi oldukça önemlidir (Longoria-García ve ark., 2018). Ancak, sinbiyotik tahıl esaslı gıda üretimindeki temel problem, pişirme sırasında probiyotik bakterilerin ısıya duyarlılığıdır. Pişirme sırasında probiyotik bakterileri korumak için, nihai ürün probiyotik içeren yenilebilir bir filmle kaplanmakta, probiyotik mikroorganizma enkapsüle edilmekte (Majzoobi ve ark., 2019) veya yüksek sıcaklıklarda canlılığını koruyabilen *Bacillus coagulans* gibi spor oluşturan mikroorganizmalar gıda formülasyonuna dahil edilmektedir (Bader ve ark., 2012).

Başarılı bir fonksiyonel gıda ürünü geliştirilmesinde, fonksiyonel bileşenlerin işlevselliğini sürdürmesinin yanı sıra, nihai ürünün kabul edilebilir teknolojik kalite özelliklerine ve duyusal niteliklere de sahip olması gerekmektedir (Longoria-García ve ark., 2018). Ancak, bildiğimiz kadarıyla, literatürde *B.coagulans ve* farklı polimerizasyon derecesine sahip inülinin kombinasyon halinde kullanımının, glutensiz makarna üretiminde ekstrüzyon ve pişirme nitelikleri üzerindeki etkisini değerlendiren herhangi bir çalışma bulunmamaktadır. Bu nedenle, bu çalışmanın temel amacı, prejelatinize nişasta (PJN) ile kısmi olarak ikame edilen (%10, 20 ve 30) mısır nişastası esaslı taze glutensiz makarna (TGM) üretiminde, ekstrüzyon prosesi kapsamında, enerji ($G_{karıştırma}$, $G_{ekstrüzyon}$, spesifik mekanik enerji), verimlilik (ekstrüzyon süresi, ekstrüzyon verimi, ekstrüzyon akış hızı), ve boyutsal özellikler (genleşme oranı, kesitsel genleşme indeksi, birimsel yoğunluk, spesifik uzunluk) ile farklı sürelerde (0, 1, 5, 10 ve 15 dk) pişirme niteliklerini (nem, su absorpsiyon kapasitesi, şişme indeksi, pişirme kaybı, hacim artışı, pişirme suyunda bulanıklık ve amiloz sızması, renk, tekstür, optimum pişirme süresi, DSC, XRD, SEM ve duyusal analiz) değerlendirmek; ekstrüzyon (ekstrüzyon verimi), teknolojik (pişirme kaybı, renk, tekstür) ve duyusal (tat) özellikleri dikkate alarak sırasıyla analitik hiyerarşik süreç (AHP) ve ideal çözüme benzerliğe göre sıralama tercihi tekniği (TOPSIS) olmak üzere hibrit çok kriterli karar verme yöntemleri uygulayarak belirlenen en iyi TGM formülasyonuna, çalışmanın ilerleyen aşamalarında sırasıyla inülin ve probiyotik ilavesi gerçekleştirerek fonksiyonelleştirmektir.

AHP metoduna göre, ekstrüzyon verimi, pişirme kaybı, sarılık indeksi, sertlik ve tat özelliklerine ait kriter ağırlıkları sırasıyla 0.34, 0.22, 0.05, 0.11 ve 0.29 olarak belirlenmiştir. Bu sonuçlar, ekonomik kavramlarla ilişkili olan üretim verimliliği ile tüketici isteklerinin sürekliliğin sağlanmasında duyusal niteliklerin, glutensiz makarna seçiminde temel itici güçler olduğunu ortaya koymaktadır. İlgili analizin tutarlılık oranı 0.05 olup, bu değer 0.1'den küçük olduğundan, TOPSIS metodunda kullanımı için güvenilir olarak kabul edilmiştir. TOPSIS sonuçlarına göre ise, durum buğday irmiği esaslı kontrol makarnası, pozitif ideal çözüme en yakın ve negatif ideal çözüme en uzak mesafede olmak üzere en iyi makarna olarak belirlenmiş olup, bunu 20% PJN içeren TGM takip etmiştir. Bu nedenle, çalışmanın 2. ve 3. aşamalarında, mısır nişastasının %20 PJN ile ikame edildiği TGM formülasyonuna sırasıyla iki farklı polimerizasyon derecesinde (PD≥10 ve PD≥23) ve iki farklı konsantrasyonda (%4 ve 8) inülin ve ardından probiyotik (*Bacillus coagulans* GBI-30, 6086) ilavesi gerçekleştirilerek, fonksiyonel ve potansiyel sinbiyotik TGM üretilmiş, ekstrüzyon ve zamana bağlı (0, 1, 5, 10 ve 15 dk) pişirme özellikleri incelenmiştir.

Genel olarak, inülin ve/veya *Bacillus coagulans* içeren/içermeyen tüm TGM örneklerine ait $G_{ekstrüzyon}$ değerleri, $G_{karıştırma}$ değerlerinin yaklaşık üç katı olarak tespit edilmiştir TGM örneklerinde, enerji ile ilgili ekstrüzyon özellikleri ($G_{karıştırma}$, $G_{ekstrüzyon}$, spesifik mekanik enerji), PJN ikame oranı arttıkça artmış ve sırasıyla inülin ve probiyotik ilavesiyle kademeli olarak artış göstermesine rağmen, kontrol makarnasında elde edilen değerlere ulaşılamamıştır. Bu durum, kontrol örneğinin gluten içeriği ve besleme nem içeriğindeki azalmanın, daha fazla kayma gerilime neden olarak, yüksek akış viskozitesi ve makarna hamuru ile ekstrüder helezyonu iç çeperi arasında oluşan sürtünme kuvveti nedeniyle, daha yüksek enerji transferi ile ilişkilendirilmişir. Dolayısıyla, kontrol makarnası üretiminde ekstrüzyon prosesinin daha yüksek genel işletme maliyetlerine sahip olabileceği şeklinde yorumlanmıştır. Farklı oranlarda PJN içeren TGM üretiminde, ekstrüzyon verimi (EV) değerleri yaklaşık %64-86 aralığında değişkenlik göstermiştir. Mısır nişastasının %20 PJN ile kısmen ikame edildiği TGM formülasyonuna inülin ilavesi, toplam besleme materyalindeki artıştan dolayı, ekstrüzyon süresini uzatıp, ekstrüzyon akış hızını azaltırken, verimliliği ise olumlu yönde etkilemiştir. Bu formülasyonlara probiyotik ilavesinin ise, ekstrüzyon süresi uzamasına rağmen ekstrüder iç çeperi yüzeyindeki aşırı yapışkanlık nedeniyle EV değerlerini olumsuz yönde etkilediği kaydedilmiştir. Mısır nişastasının PJN ile ikame edilme oranı arttıkça, TGM'nin radyal genleşme oranında önemli bir düzeyde artış, birimsel yoğunluk ve spesifik uzunluk değerlerinde ise azalış gözlenmiştir. Soğuk ekstrüzyon koşulları altında gerçekleşen bu çalışmada, genleşmeyi etkileyen başlıca parametrelerden birinin viskozite olduğu ve bu durumun azalan nem içeriğinden kaynaklanabileceği öngörülmüştür. Yüksek jelatinizasyon derecesi, daha yüksek radyal genleşme ve artan spesifik mekanik enerji ile ilişkili olduğundan, benzer yönde bir ilişki, PJN ikame oranı arttıkça TGM örneklerinde de belirlenmiştir.

İnulin içeren tüm TGM örneklerinde, pişirme işleminin ilk 5 dk süresi boyunca 20-PJN esaslı TGM'ye kıyasla daha düşük nem içeriği, su absorpsiyon kapacitesi (SAK), şişme indeksi (Şİ) ve hacim artışı (HA) değerleri belirlenirken, uzayan pişirme sürelerinde (10-15 dk) özellikle %4 oranında kısa zincirli inülin (GR, PD≥10) içeren TGM'de, daha yüksek değerler kaydedilmiş ve bu nedenle pişirme ortamında bulanıklık ve amiloz sızması ile sertlik ve çiğnenebilirlik gibi tekstür özellikler ise genellikle azalma eğilimi göstermiştir. Pişirmenin ilk aşamalarında (0-5 dk) gerçekleşen bu durum, inülinin oldukça hidrofilik bir karaktere sahip olması ve hidrojen bağları yoluyla daha fazla miktarda su molekülüyle etkileşime girebilmesine rağmen, su için diğer hidrofilik moleküllerle rekabet halinde olması, nişasta granüllerini diyet lif yapısında enkapsüle edip etrafında fiziksel bir bariyer oluşturarak, su absorpsiyon ve/veya bağlama kapasitesi ile şişme kapasitesinde bir kısıtlamaya neden olması ile açıklanmaktadır. Bu eşit olmayan su dağılımı, PJN ve inulin arasında su bağlama hızı açısından farklılık ile sonuçlanmakta ve nihai ürün kalitesini tüm pişirme işlemi süresince etkilemektedir. Ayrıca, inülin daha düşük konsantrasyonlarda kullanıldığında, hidrojen bağları aracılığıyla daha iyi su bağlama kabiliyetine sahipken, yüksek oranlarda kullanıldığında ise makromoleküler biyopolimerlerin sürekliliğinin bozulması nedeniyle, su bağlama kapasitesinde bir azalışa yol açmıştır. Pişirme işlemi boyunca diyet lifin suda çözünür yapısı nedeniyle inülinle zenginleştirilmiş TGM'de, 20-PJN esaslı TGM örneğine kıyasla daha yüksek pişirme kaybı (PK) değerleri elde edilmiş ve bu değerler daha yüksek konsantrasyonda inülin kullanıldığında istatistiksel olarak anlamlı düzeyde artmıştır ($p<0.05$). TGM'ye inülin ilavesi sonucu optmum pişirme süresinde (OPS) bir düşüş belirlenmiş ve probiyotik eklenmesiyle bu süre kademeli olarak kısalmıştır. Bu durum, hem inülin hem *B. coagulans* içeren TGM'nin, daha yüksek PK değerlerine sahip olması, merkezi noktaya suyun difüzyon hızının artması sonucu yumuşamayı hızlandırması ve bu nedenle pişirme stresi dayanıklılığının daha düşük olduğu şeklinde yorumlanmıştır.

TGM'ye probiyotik ilavesi, nem, SAK, Şİ, ve HA'da düşüş; PK, ve pişirme ortamında bulanıklık ve amiloz sızmasında ise artışa neden olarak, nihai ürün kalitesini genellikle olumsuz yönde etkilemiştir. Ancak, 5 dk ve 10 dk pişirme işlemine tabi tutulan, farklı polimerziasyon derecesi ve konsantrasyonlarında prebiyotik kaynağı olarak inulin içeren TGM'de, sırasıyla yaklaşık %85 ve %67'nin üzerinde probiyotik hücre canlılığı belirlenmiştir. Bu bağlamda, 10 dk pişirilen inülin içermeyen probiotik ile zenginleştirilmiş TGM (P0) hariç, elde edilen sonuçlar konakçı sağlık üzerinde olumlu bir etki göstermek için gerekli olan en az 10^6 CFU/g canlı suşa karşılık gelen kabul edilebilir eşiğin üzerinde olarak belirlenmiştir.

İnülin ve/veya probiyotik kaynağı içeren/içermeyen tüm TGM’lerde pişirme süresi uzadıkça nem, SAK, Şİ, PK ve HA ile pişirme ortamındaki bulanıklık ve amiloz sızması değerleri genellikle artarken, renk değerleri (L*, a*, b*, sarılık indeksi ve renk skoru) ile tekstür profil özellikleri ise azalış göstermiştir. Mısır nişastasının kısmen (%10, 20 ve 30) PJN ile ikame edildiği, inülin ve/veya probiyotik kaynaklarınca zenginleştirildiği TGM'lerin SAK değerleri pişirme işleminin sonunda (15 dk) yaklaşık 2-3 kat artış göstermiştir. Ancak yüksek kaliteli makarna elde etmek için pişmiş makarnanın ağırlık artışının en az %100 olması gerektiğinden, bu kriter genellikle 5 dk süresince pişirilmeye tabi tutulmuş inulin ve/veya probiyotik içeren/içermeyen TGM örneklerinde karşılanmıştır. İnulin ve/veya *B. coagulans* içeren/içermeyen PJN ile kısmi olarak ikame edilmiş TGM’ye ait SI değerleri, pişirme işlemi sonunda yaklaşık 1-2 kat artış göstermiştir. Öte yandan, PK değerleri %10'dan fazla olan makarnalar genellikle tüketiciler tarafından tercih edilmemekte ve yüksek kaliteli bir makarna için en yüksek PK sınır değeri %6 olarak kabul edilmektedir. Bu nedenle, yüksek oranlarda PJN (%30) ile ikame edilen, yüksek konsantrasyonda inülin (%8) içeren ve probiyotik kaynağı ile zengineştirilen 5 dk pişirilmiş TGM örnekleri, bu gereksinimi karşılayamamaktadır. Genellikle yüksek kaliteli makarnalarda HA değerinin %200-300 aralığında olması gerektiğinden, en yüksek PJN oranı (%30) ile ikame edilen ve yüksek konsantrasyonda (8%) uzun zincirli inülin (PD≥23) içeren TGM örnekleri hariç, pişirme süresi sonunda inülin içeren/içermeyen tüm TGM örneklerinin bu gereksinimleri karşılayabildiği, ancak probiyotik içeren hiçbir TGM’nin ise bu gerekliliği pişirme işlemi boyunca karşılayamadığı tespit edilmiştir. Sonuç olarak TGM’ye yüksek konsantrasyon ve polimerizasyon derecesine sahip inülin ve/veya probiyotik ilavesinin, uzun pişirme sürelerinde yalnızca teknolojik değil duyusal özellikler ve besin nitelikleri üzerinde de olumsuz bir etkiye sahip olabileceği öngörülmüştür.

Diferansiyel taramalı kalorimetre (DSC) analizi sonuçlarına göre, durum buğdayı esaslı kontrol grubu ve inulin içeren/içermeyen TGM örneklerinde, nişasta jelatinizasyonu, protein denatürasyonu ve amiloz-lipit kompleksi oluşumu başta olmak üzere farklı reaksiyonları temsil eden endotermik bir pik elde edilmiştir. En yüksek entalpi değeri (ΔH), durum buğdayı irmiği esaslı kontrol grubunda gözlenmiş olup, bu durum güçlü gluten ağı içerisinde gömülü nişasta granülleri ile ilişkilendirilmiştir. Artan PJN ikame oranı ve inulin konsantrasyonu ile TGM örneklerinin ΔH değerlerinde ise azalma kaydedilmiştir. X-ışını kırınımı (XRD) analizi sonuçlarına göre, inulin içeren/içermeyen pişmemiş TGM örnekleri, A-tipi kristal forma sahip nişasta yapısı ile karakterize edilmekte olup, en yüksek bağıl kırınım şiddeti $2\theta=17^{o}$ ve 18^{o} piklerde tespit edilmiştir. Öte yandan inulin içeren/içermeyen pişmiş TGM örneklerinin ise en yüksek bağıl kırınım şiddeti $2\theta=17^{o}$ ve 20^{o} piklerde gözlenmiş olup sırasıyla B-tipi ve V-tipi kristal formda nişasta yapısına sahip olduğu belirlenmiştir. Taramalı elektron mikroskobu (SEM) ile 5 dk pişirilmiş makarna örneklerinde gerçekleştirilen mikroyapı incelemesine göre, durum buğdayı irmiği esaslı kontrol grubundaki gluten ağı eliptik şekle sahip nişasta granüllerini hapsederek kompleks bir yapıya sahip olup, daha düşük PK, turbidite ve amiloz sızması ile yüksek OPS ve ΔH değerlerine sahip olması ile uyumludur. Yüksek konsantrasyonda (8%) inülinle zenginleştirilmiş TGM örneklerinde ise suyun ileri düzeyde penetrasyonuna izin veren belirgin ve geniş gözeneklere sahip bir morfolojik yapı gözlenmiş olup probiyotik ilavesi ile bu etki daha da artarak yüksek PK değerleri ile uyumlu bir şekilde ileri düzeyde pürüzsüz bir yüzey elde edilmiştir.

Duyusal analiz sonuçlarına göre, farklı PJN seviyelerine sahip inülin içeren/içermeyen TGM örnekleri 15 dk'lık pişirme süresi boyunca panelistler tarafından al-dente'den aşırı pişmiş'e kadar geniş bir aralıkta tanımlanmıştır. Duyusal değerlendirme açısından inülin içeren/içermeyen "al-dente" pişirme seviyesine sahip TGM elde etme süresi 1 dk olarak belirlenmiş olup, bu değer, teknolojik olarak belirlenen OPS'den oldukça düşüktür. Bu durumun, yaygın olarak kullanılan OPS belirleme yönteminin buğday irmiği/unu esaslı makarnalar için geliştirilmiş olması sebebiyle, glutensiz makarna örneklerine uygun olmadığı ve/veya panelist tercihlerinin "al-dente" makarna hissi ile uyuşmamasından kaynaklandığı şeklinde yorumlanmıştır. Genel olarak en az tercih edilen pişirme süresi 15 dk iken, daha yüksek PJN ikame seviyelerine sahip (%30) ve yüksek konsantrasyonda (%8) uzun zincirli inülin (PD≥23) içeren TGM hariç olmak üzere, en çok tercih edilen pişirme süresi 5 dk olarak belirlenmiştir. Ayrıca, pişirme süresi tercih sıralaması, sırasıyla pişirmenin ileri aşamalarında (10-15 dk) ve neredeyse tüm pişirme süresince (1 dk hariç) PJN ve inülin oranındaki artıştan olumsuz yönde etkilenmiştir. Bu nedenle, belirtilen duyusal özellikler (yapısal bütünlük, renk, tekstür, tat, genel kabul edilebilirlik), inülin ve/veya probiyotik içeren/içermeyen tüm TGM örneklerinde 5 dk pişirmeye tabi tutulan örneklerde gerçekleştirilmiştir. Buna göre, TGM formülasyonunda mısır nişastasının PJN ile ikame edilme oranı ve inülin ilave edilme düzeyi arttıkça istatistiksel olarak anlamlı düzeyde olmamasına ragmen duyusal niteliklere ait değerlerin genel olarak azaldığı belirlenmiştir ($p>0.05$). Ayrıca, inülin içeren/içermeyen TGM'ye probiyotik ilavesinin teknolojik olarak belirlenen pişmiş nihai ürün üzerindeki olumsuz etkisi, tüm organoleptik özellikler için daha düşük puanlara sahip olması sebebiyle panelistler tarafından da doğrulanmıştır.

Sonuç olarak, sınırlı pişirme sürelerinde (5dk) teknolojik ve duyusal nitelikleri kabul edilebilir ve konakçı sağlık üzerinde olumlu etki görülmesi için yeterli düzeyde canlı probiyotik suş içeren potansiyel sinbiyotik TGM üretimi gerçekleştirimiştir. Ancak bundan sonraki çalışmalar, farklı kurutma sıcaklığı ve sürelerine tabi tutulan fonksiyonel glutensiz makarna örneklerinin, kurutma ve zamana bağlı pişirme özelliklerinin belirlenmesi ve kinetik olarak karakterize edilmesi, diyet lif başta olmak üzere besinsel niteliklerinin incelenmesi, nişasta sindiriminin *in vitro* kinetiğine dayalı, nişasta sindirim fraksiyonları (yavaş ve hızlı sindirilebilir) ile glisemik indeks değerlerinin belirlenmesi ve canlı suş sayısının gastrointestinal sistemde *in vivo* olarak da değerlendirilmesi üzerine odaklanmalıdır.

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SYMBOLS AND ABBREVIATIONS

°C	: Centigrade degree
%	: Percentage
AACC	:Cereals & Grains Association (formerly AACC International, American Association of Cereal Chemists)
abs	: Absorbance
ANOVA	: Analysis of variance
CFU	: Colony-forming unit
cm	: Centimeter
DP	: Degree of polymerization
EFSA	: European Food Safety Authority
FAO	: Food and Agriculture Organization
FDA	: Food and Drug Administration
g	: Gram
GRAS	: Generally Recognized as Safe
h	: Hour
ISAPP	: International Scientific Association for Probiotics and Prebiotics
log	: Logarithmic
mg	: Milligram
min	: Minute
mm	: Millimeter
NaCl	: Sodium chloride
sec	: Second
TPA	: Texture profile analysis
UV-vis	: Ultraviolet-visible

1. INTRODUCTION

In recent years, the close relationship between food and health has been noticed by consumers, including those who have to follow a strict dietary plan, such as a gluten-free diet. For this reason, food scientists and the food industry focus on "functional foods" that not only provide more nutritional value but also health benefits (Longoria-García et al., 2018). In this regard, prebiotics, probiotics, and synbiotics are one of the important pillars as functional food components in gluten-free food products.

Probiotics are defined as live microorganisms that are beneficial to the health of the host when taken in adequate amounts. According to the "International Scientific Society for Probiotics and Prebiotics" (ISAPP), it is defined as a foodstuff should contains at least 10^6 CFU/g of defined live strains that are expected to have potential positive effects on host health (Bultosa, 2016). However, bakery products are generally exposed to high temperatures while baking and/or cooking, and most of the probiotic microorganisms can not preserve their microbial viability (Longoria-García et al., 2018). Therefore, it has also been reported that spore-forming microorganisms such as *Bacillus coagulans* have improved viability and stability compared to other probiotic bacteria strains. Therefore, the development of functional bakery products by using this microorganism, thanks to its ability to withstand high-temperature processes such as cooking and boiling, has come into prominence (Bader et al., 2012).

Prebiotics are defined as compounds that can be fermented and thus result in specific changes in the composition and/or activity of the gastrointestinal microbiota and are thus beneficial for the health of the host (Kandil et al., 2017). Inulin-type fructans have prebiotic properties when fermented by probiotic microorganisms in the human intestine (Kaya et al., 2019). Therefore, it has been reported that they have many positive effects on health, such as increasing the bioavailability of some minerals, regulating the gastrointestinal system by affecting bowel movements and transit time, strengthening the immune system, reducing the risks of metabolic syndrome and cardiovascular diseases by affecting blood sugar regulation and lipid metabolism (Yabancı, 2010). Due to these positive effects on health, they are considered as functional foods (Kaya et al., 2019). The degree of polymerization (DP) of inulin varies between 3-60 (Özyurt and Ötleş, 2014). Short-chain inulin has better water-holding capacity, while long-chain inulin has higher viscosity values and gel-forming ability even when used at lower concentrations. For this reason, formulations of food products should be designed, depending on the DP of inulin, taking into account the nutritional value, textural properties, stabilization, emulsification, or gel formation needs of the final product (Ozturkoglu-Budak et al., 2019).

On the other hand, synbiotics are defined as a combination in which probiotics and prebiotics are applied together (Bultosa, 2016). According to the ISAPP report published in 2020, the concept of synbiotics has been updated as "a mixture containing living microorganisms and substrate(s) that are selectively used by host microorganisms that confer a health benefit on the host". In this context, "host" microorganisms may include external microorganisms, such as probiotics, that are both resident or colonized in the host and form a component of the host microbiota even if transiently present (Swanson et al., 2020).

It is known that the consumption of probiotics, prebiotics, and synbiotics is closely related to being beneficial for human health. Therefore, the development of functional cereal-based food products and incorporation into the daily diet are very important in terms of protecting and/or improving human health (Longoria-García et al., 2018). However, the main challenge in synbiotic bakery production is related to the heat sensitivity of probiotic bacteria during baking. In order to protect the probiotic bacteria during baking, the final product could be covered with an edible film, the probiotics could be microencapsulated and then added to the food structure (Majzoobi et al., 2019), or spore-forming microorganisms could be used. In order to develop a successful functional bakery product, it must not only have a prebiotic, probiotic, or synbiotic effect but also have acceptable sensory and physical quality properties (Longoria-García et al., 2018). In this context, it is reported that probiotics and prebiotics also could contribute to sensory properties such as taste and aroma, as well as technological properties such as specific volume and spreading rate in bakery products (Longoria-García et al., 2018).

On the other hand, modified starch is a form of starch in which its functional properties are improved by changing its physical and chemical properties. In this way, foods could be modified in terms of water retention and/or binding, thickening, emulsion stabilization, stickiness, gelling, and stabilization (Karaoğlu et al., 1998). Starches can be modified by cross-linking, acid treatment, oxidation, and esterification as chemical modifications; hydrothermal treatments and extrusion-cooking, or enzymatic and genetic methods. Among them, the starch aqueous suspension is heated to a temperature above the gelatinization temperature, then dried and ground for the production of pregelatinized starches. The most important feature of pregelatinized starches is their swelling ability in cold water. Therefore, they are commonly used, especially in instant foods, cake mixes, frozen foods, and some dessert formulations (Karaoğlu et al., 1998). To mimic the gluten network, the generation of a "scaffold" of retrograded starch is a way to enhance rigidity, decrease stickiness, and also prevent loss of soluble compounds in gluten-free pasta/noodle production. To enable a greater quantity of retrograded starch in these types of products, inducing starch disorganization should occur via heat treatments under specific moisture conditions, followed by a cooling process, during which part of the starch, particularly amylose, could form a three-dimensional network by strongly linking short chains of starch granules through junction zones. This could be carried out by technological process via subsequent heating and cooling stages, as applied in rice noodles production, extrusion at higher temperatures, using pregelatinized materials with a conventional production method similarly used in durum wheat semolina pasta, and/or incorporating food additives individually, or combining them with other approaches (Lucisano et al., 2012).

By the time of formulating a functional food, sustaining the ingredients' functionality together with the technological and sensorial attributes of end-products should be taken into consideration (Bavaro et al., 2024) in a holistic perspective. However, the number of studies in which prebiotic and probiotic sources are used together in gluten-free pasta/noodle production and evaluate the end-product quality is very limited in the literature. Moreover, as far as we know, there is no study in the literature about the production of gluten-free pasta, including both inulin with different DP and *Bacillus coagulans* as a prebiotic and probiotic source, respectively. Therefore, in the first chapter of this study, fresh gluten-free pasta based on native corn starch, partially replaced with pregelatinized tapioca starch at three different levels (10, 20, and 30%) as an independent variable, was produced, the extrusion and cooking properties at different cooking time intervals (0, 1, 5, 10, and 15 min) were evaluated. After that, a hybrid multi-criteria decision making (HMCDM) were applied regarding analytical hierarchical process (AHP) and technique for order preference by similarity to ideal solution (TOPSIS) methods, respectively, according to extrusion (extrusion yield), technological (cooking loss, color, texture), and sensorial (taste) attributes for the selection of the best gluten-free pasta formulation for the following chapter of the present study. In the second chapter of the study, inulin with different degrees of polymerization (DP≥10 and DP≥23) was incorporated at two different concentrations (4 and 8%) into the most suitable gluten-free pasta formulation, which was determined in the first chapter of the study with HMCDM, and their extrusion and cooking properties at different time intervals were examined. In the third chapter of the study, *B. coagulans* GBI-30, 6086, with a final concentration of 10^9 CFU/g, was blended with inulin-enriched GFP formulations, and their extrusion and cooking properties at different time intervals, together with the cell viability of the probiotic, were assessed.

2. PRELIMINARY WORK

Food hypersensitivity (FH) is an abnormal reaction that occurs in the body after consuming foods, which is mainly categorized as food allergy and food intolerance (Kadioğlu, 2017). While food allergy is an abnormal reaction that develops rapidly after the consumption of food and/or food additives triggered by the immune system, food intolerance arises from a disorder associated with the absorption and/or digestion of any food in the body (Akoğlu and Oruç, 2018). Generally, foods containing lactose, such as milk and dairy products, eggs, fish, and shellfish, among animal-origin foods; soy, peanuts, and nuts, and some grains that contain gluten-related proteins, especially wheat, as plant-based origin, are considered potential FH sources (Karakiliç et al., 2014). Among them, gluten-related disorders (GRD) include a wide range of intestinal and extra-intestinal symptoms (Hadjivassiliou et al., 2015). The GRD is primarily categorized into three major classes composed of autoimmune responses, IgE-mediated allergies, and non-autoimmune non-allergic reactions, which are essentially composed of celiac disease, wheat allergy, and non-celiac gluten sensitivity (NCGS), respectively (Lied, 2015), as illustrated in Figure 2.1 (Yazici et al., 2024).

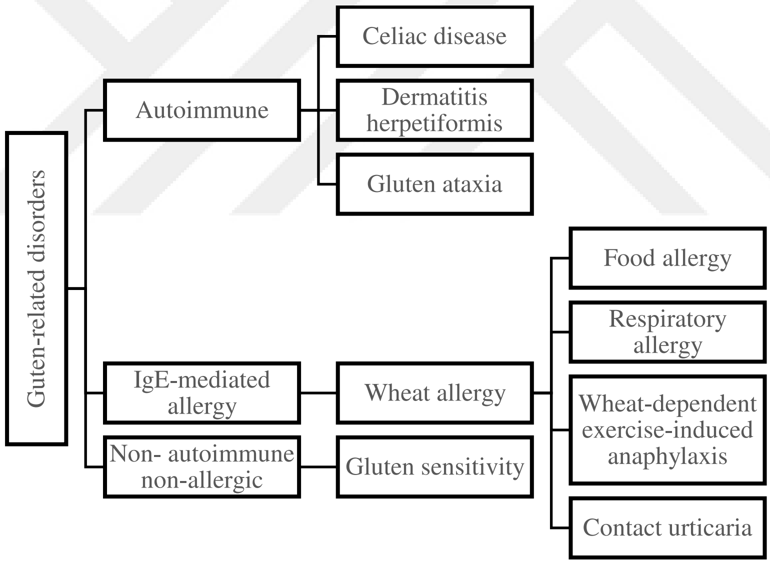

Figure 2.1. The main classification of wheat/gluten-related hypersensitivities

Celiac disease (CD), put it differently, coeliac disease, is a chronic autoimmune enteropathy influencing the small intestine (Scherf, 2019). Globally, CD influences nearly 0.5–1% of the population (Mustalahti et al., 2010; Gujral et al., 2012). The major symptoms of CD are micronutrient deficiencies, iron-deficiency anemia, weight loss, and diarrhea as a result of enteropathy and morphological changes based on atrophy and flattening, leading to a decrease in mucosal surface area and impairing nutrient absorption (Brouns et al., 2019). The potential contributing/related reasons for CD, apart from advancements in diagnostic methods that could underlie the rising prevalence of diagnosed- and self-reported GRD, include ancient grains, vital gluten, lectins, α-amylase/trypsin inhibitors, and irritable bowel syndrome (IBS). Among these, the hypothesis that is based on decreased immunogenicity of ancient grains, potentially owing to the absence of the D-genome, maintains an issue of ongoing debate. On the other side, growing exposure to gluten, especially through the widespread incorporation of vital gluten in several food products, and also the symptomatic overlap between CD and IBS, further complicate the clinical landscape of CD (Yazici et al., 2024).

Individuals with FH must adhere to a specialized diet (de Sousa Franckilin et al., 2023). In this regard, the only current treatment is strict compliance with a gluten-free diet (GFD) for individuals with CD (Sabença et al., 2021). The CD is triggered by consuming gliadin, which is a storage protein found in wheat (*Triticum aestivum*), and other prolamin proteins like secalin in rye and hordein in barley (Malalgoda and Simsek, 2017; Rodrigo and Hernández-Lahoz, 2018). The consumption of any species/variety of these grains is not strictly deemed appropriate for patients who suffer from CD (Wieser et al., 2020). Therefore, the GFD is composed of the complete absence of wheat, rye, barley, and derivatives or processed foods, including all these prolamin-containing cereals (Marciniak et al., 2021; Sharma et al., 2020). From the standpoint of nutritional value, GFD is mainly lacking essential compounds like proteins, dietary fiber, micronutrients such as vitamins and minerals, and phytochemicals (Scherf, 2019) because gluten-free (GF) foods are typically based on flour and starches originating from rice and maize/corn (Khairuddin and Lasekan, 2021). In addition to this, they are more prone to gelatinization and rapid digestion via α-amylase and thus have a role in having a higher glycemic index value, which leads to a rise in the risk of metabolic syndromes among individuals with CD in the long term (Melini and Melini, 2019). This could also be potentially owing to the lower amounts of resistant starch and dietary fiber in those GF raw materials (Khairuddin and Lasekan, 2021). As a consequence, the obligation of prolonged adherence to a GFD could culminate in nutritional imbalances (Zhu et al., 2023) and potential health problems in CD individuals. On the other side, GF products commonly remain incapable in terms of not only nutritional but also technological and sensorial attributes in contrast to conventional counterparts (Khairuddin and Lasekan, 2021). Because GF flours/starches are absent of both the strength and elasticity properties of gluten protein, even though they are physically-, chemically-, or biotechnologically modified (Zhu et al., 2023). Therefore, this led to GF bakery products with poor gas retention, low volume, pale color, and crumbly texture (Khairuddin and Lasekan, 2021). To sum up, the development of the new GF foods should be achieved by both fortification of the formulations to better support those on a GFD (Khairuddin and Lasekan, 2021) nutritionally and end-product quality.

2.1. Gluten-free Pasta

The replacement of the gluten network in the manufacturing of GF foods is a technological challenge because gluten is the major protein that is in charge of structural integrity. As a result of this, it is essential to the addition of components that substitute the viscoelastic attributes of gluten in GF formulations (Mariotti et al., 2011). Gluten network replacement in GF food products could be fulfilled by utilizing components that have the capability of water swelling. Together with the incorporation of these additives, which could modify the rheological features, suitable technological manufacturing processes should also be adopted for GF formulations with high quality (Mastromatteo et al., 2012).

In GF pasta/noodle development, performing technologies that promote starch structuring are crucial because they are typically based on GF ingredients that have low protein content, like rice and maize (De Arcangelis et al., 2020). It was proposed that the generation of a "scaffold" of retrograded starch may be a promising alternative to the gluten network of GF pasta/noodle. To mimic gluten's viscoelasticity, strategies are focusing on starch retrogradation for generating a rigid matrix to enhance texture, decrease stickiness, and reduce cooking loss of GF pasta/noodles. To obtain an adequate starch retrogradation level, starch is initially exposed to disorganization via heat treatment under controlled moisture conditions, and then a cooling process, in which particularly amylose enables making a 3D network through strong interactions of short starch chains by junction zones. Those structural modifications could be achieved during the GF pasta/noodle making process or fulfilled by the utilization of pregelatinized starches or starch-rich flours as raw materials in terms of ingredients (Mariotti et al., 2011).

Up to now, the manufacturing of GF pasta has been suggested primarily through two main approaches as using pretreated flour/starch by hydrothermal treatments (annealing, heat-moisture treatment, parboiling, and pregelatinization), and extrusion-cooking. While pretreated flours, where starch is disorganized by precooking, make them available for the traditional extrusion process, extrusion-cooking exposes raw starch to steaming and extrusion at temperatures above 100°C in a short time while enhancing in-situ gelatinization within the extruder (Marti and Pagani, 2013). Hydrothermal treatments have the ability to improve starch crystallinity, granule firmness, and the associations between polymer chains (Cham and Suwannaporn, 2010; Marti and Pagani, 2013; Padalino et al., 2016). As a result of this, these kinds of modifications suppress granule swelling, retard starch gelatinization, and enhance starch paste stability, which contributes to better cooking characteristics and developed textural attributes, especially in rice starch- or flour-based pasta and noodles (Cham and Suwannaporn, 2010; Marti and Pagani, 2013; Padalino et al., 2016).

Specifically, annealing is carried out at a low temperature (≈50–60°C) under high moisture conditions (>40%), whereas heat-moisture treatment is conducted at higher temperatures (≈100–120°C) with low moisture conditions (Marti and Pagani, 2013; Padalino et al., 2016). In this context, while heat-moisture treatment is more ideal for partially- or totally-dried pasta/noodles that need more improved gel strength, annealing is more proper for soft-textured, fresh GF pasta/noodles (Marti and Pagani, 2013). Therefore, the major difference between heat-moisture treatment and annealing is conducted at limited and intermediate or excess water, respectively. Secondly, while heat-moisture treatment broadens the endothermic transition, annealing reduces the temperature range of melting. The thermal stability of starch granules increases with both treatments (Zweifel et al., 2000) and should be implemented at temperatures below the gelatinization temperature (T_{gel}), but above the glass transition temperature (T_g) (Cham and Suwannaporn, 2010).

Parboiling is another hydrothermal method, is typically implemented on rice, including in the order of soaking, steaming, and drying steps (Marti et al., 2010; Taddei et al., 2021). It is promising in GF pasta/noodle development and thus, as patented by Grugni et al. in 2009 (Marti and Pagani, 2013). The parboiling process alters rice kernels' physicochemical, sensory, and nutritional characteristics by gelatinizing starch, generating lipid-amylose complex, limiting the leaching of amylose to cooking water, and micronutrient distribution. These changes decrease stickiness and improve kernel hardness through cooking (Marti and Pagani, 2013). Additionally, parboiling increases the content of resistant starch, thereby contributing to health benefits, as well (Taddei et al., 2021). Although there are some studies investigating hydrothermal-treated GF pasta/noodle development, including heat-moisture-treated or annealed rice (Hormdok and Noomhorm, 2007; Cham and Suwannaporn, 2010), cassava (Menon et al., 2016), and sorghum (Cervini et al., 2021) as raw materials, they still remain underexplored.

On the other side, the pregelatinized starch, which is also known as "instant starch" (Obadi et al., 2020), is a physically modified starch (Li et al., 2020) and is more frequently utilized owing to its lower cost (Padalino et al., 2016). It is commonly used to modify rehydration features, texture (Tang, 2019), rheological properties (Chillo et al., 2007), develop viscosity and processing attributes (Jeong and Lee, 2025), improve pasting characteristics, solubility and swelling in water with cold- or ambient temperature (Niyomwong et al., 2025) for enhancing cooking quality of pasta/noodle by a starch network formation (Chillo et al., 2007). It is also accepted as an alternative to hydrocolloids (Bello-Perez et al., 2022; Ding et al., 2024) or gluten mimicking in GF noodle/pasta production (Ding et al., 2024) owing to its swelling ability quickly in cold water and shows improved viscosity and textural properties (Li et al., 2020). It is generally produced by different methods, such as drum drying, twin screw extrusion drying (Li et al., 2020; Niyomwong et al., 2025; Jeong and Lee, 2025), spray drying (Niyomwong et al., 2025), hot-air drying, and freeze drying (Jeong and Lee, 2025), in which starch is exposed to a gelatinization process and then drying that alters the functional properties of native starch (Niyomwong et al., 2025). The alteration in the organization of starch granules by the application of physical and thermal treatment has been commonly accepted as a natural compound with high food safety. The goal of these treatments is to enhance the functional attributes of starch with no destroying its granular structure, which in turn removes any legislative limits as to its proportions in foods (Dib et al., 2018). Therefore, native starch, which is physically treated, such as gelatinization, is in GRAS status, and they are more commonly utilized than chemically-modified because of having some regulatory limitations (Scarton and Clerici, 2022). Nevertheless, adjustments to determine the precise water amount are needed, which generally corresponds to about 40%, which is higher than conventional pasta based on semolina (≈30%) (Marti and Pagani, 2013), owing to whose higher water-absorption capacity (Scarton and Clerici, 2022). On the other hand, higher proportions of pregelatinized starch may make the cold extrusion process almost impossible, and thus water may be incorporated into the flour/starch mixture in concentrations of nearly 60% to enable cold extrusion with obtain an extrudant with proper texture characteristics (Santacruz et al., 2012). In a study, GF noodles based on rice flour without pregelatinized flour were disintegrated during cooking, and quality-related analysis could not be conducted, owing to a lack of binding strength necessary for forming a cohesive noodle sheet, although they comprised hydroxymethyl cellulose as a hydrocolloid source. Therefore, this could be interpreted as pregelatinized flour/starch was essential in the case of the absence of a binding agent for sustaining dough elasticity (Jeong and Lee, 2025) in GF pasta/noodle.

Extrusion cooking, which is characterized as a high-temperature short-time process (Wang et al., 1999; Wang et al., 2012; Dhas et al., 2021), is widely implemented in the manufacturing of ready-to-eat/cook products in the food industry (Dhas et al., 2021). The other advantages of extrusion cooking that make it popular in the food industry are being efficient in both mechanical and thermal energy integration, which remains low costs of manufacturing while obtaining significant influences such as starch gelatinization, protein denaturation, enzyme inactivation, destruction of natural toxins, and microbial reduction in end-food products (Wolf et al., 2010). The critical issues in the development of GF pasta/noodles are disruption of starch granules, starch gelatinization, and protein denaturation during extrusion (Detchewa et al., 2022). Therefore, extrusion cooking is also a promising method for modifying starch structures to replace the gluten network in GF pasta/noodle production (Sajid Mushtaq et al., 2023). For this purpose, the GF flour is firstly exposed to heat treatment in an extruder with a heated wall and/or steam injection, after that, extruded, shaped, and lastly dried, respectively (Marti and Pagani, 2013). Due to the facilities in order of starch gelatinization and retrogradation, with both heating and cooling cycles, it improves the resistance of the starch network against cooking stress (Sajid Mushtaq et al., 2023). Moreover, extrusion cooking improves the association between amylose and amylopectin, resulting in products with greater firmness and decreased cooking loss values (Gao et al., 2018).

2.2. Prebiotic

Initially, the "prebiotic" term was described as "non-digestible" food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health" in 1995. Afterward, the definition was altered to "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers health benefits". This term was updated by ISAPP as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (Carlson et al., 2018). The prebiotics are isolated from plant sources, obtained by microbial production, or enzymatic synthesis, and breakdown of polysaccharides (Sharma et al., 2023).

The criteria must be met by eligible prebiotics are composed of able to reach the colon thereby being non-digestible during upper gastrointestinal tract (GIT) (Zeng et al., 2023) by resisting stomach acids, bile salts, and hydrolyzing enzymes (Sharma et al., 2023), easily fermentable by colonic microorganisms, stimulating selectively growth/activity gut bacteria (Ceylan and Atasoy, 2023), and make available to show proven health benefits to the host (Zeng et al., 2023). The "well-established" prebiotics are generally carbohydrates with a lower molecular weight and short chains. This enables more easier penetration during the colon and thus has a role in being a substrate for the endogenous colonic bacterium population (Rashidinejad et al., 2022). In typically, prebiotics are categorized as dietary fibers, whereas not all whole dietary fibers are classified as prebiotics (Sharma et al., 2023). The most commonly known, which dominate the market and are defined as "well documented" prebiotics according to the number of regulatory status and commercial applications, are oligosaccharides, particularly fructans and galactans, including fructooligosaccharides (FOS) and galactooligosaccharides (GOS), respectively (Cardoso et al., 2021). They are indigestible carbohydrates by humans, which have GRAS status (Sharma et al., 2023). On the other side, while lactosucrose, chitooligosaccharides, isomaltooligosaccharides, and xylooligosaccharides are categorized as "emerging prebiotics", epilactose, raffinose, and neoagaro-oligosaccharides are in the "under development" prebiotic status (Cardoso et al., 2021). In other respects, the natural gums (i.e., xanthan gum, guar gum, locust bean gum, tragacanth gum, arabic gum, karaya gum) are commonly utilized as stabilizers, foaming agents, but also edible coating and films material for the administration and encapsulation of probiotics have the potential for being a substrate for the host's microorganisms (Modrackova et al., 2019).

The health benefits of consumption of prebiotics are following as generation of some beneficial metabolits (i.e. short chain fatty acids) by enhancing activity and/or growth of beneficial gut bacteria, pathogen inhibition, modulation of immune system, promoted bowel function such as protecting from colorectal cancer, promote absorption of mineral compounds, metabolic impacts such as levels of insulin or cholesterol, controlling obesity and enhance satiety (Zeng et al, 2023). Therefore, there is a big demand from consumers for functional foods for retaining health that could be fulfilled by the incorporation of prebiotics as a healthy alternative to industrialized food products. The inclusion of prebiotics to food formulations brings along several advantages, such as balanced and better nutritional value, higher shelf-life, and promoting sensorial attributes (Aquino et al., 2017). Apart from these, prebiotics also play a role in baked goods, commonly as a sugar and/or fat substitute (Singla and Chakkaravarthi, 2017).

2.2.1. Inulin

Fructans are defined as oligomers or polymers of fructose, comprised mainly of fructosyl-fructose linkages. Due to being a heterogeneous group, they could be classified according to several parameters, such as source (plant, fungi, and bacteria), composition of the chain, degree of polymerization (DP), structure (linear, branched, and cyclic), and linkage conformation (β-(2-1) and β-(2-6). However, fructans are generally categorized into 3 main groups, termed as levan-type, inulin-type, and graminan-type, namely mixed-type vis-à-vis majorly fructosyl-fructose links. Among them, inulin-type fructans (ITF) are the most widely studied. They are principally considered as linear molecules, including predominantly β-(2-1) fructosyl-fructose links, and typically have a terminating glucose molecule (Mudannayake et al., 2022). ITF is a group of non-digestible carbohydrates, including inulin and oligofructose (Jackson et al., 2023). Therefore, inulin is involved in the dietary fiber class (Ahmed and Rashid, 2019). It was first discovered from *Inula helenium* root (Elecampane roots) in the early 1800s by a German scientist, Valentin Rose (Bhanja et al., 2022). He explored that it was a water-soluble polysaccharide that could be extractable from plant resources (Teferra, 2021). Later, this storage carbohydrate was named by Thomson as "inulin" in 1817 (Bhanja et al., 2022). Afterwards, another German scientist, Julius Sachs, as a plant physiologist, defined the inulin's spherocrystals precipitated by ethanol under the microscope. Later, those spherocrystals were separated from several tubers, such as *Helianthus tuberosus* and *Dahlia pinnata* (Ahmed and Rashid, 2019).

Inulin is a main storage carbohydrate in roots, tubers, and bulb parts of nearly 45,000 plant species that belong to mostly mono and dicotyledonous families such as Amaryllidaceae, Compositae, Gramineae, and Liliaceae (Ahmed and Rashid, 2019). Nearly 3,000 vegetables are also a natural inulin source, such as wheat (*Triticum aestivum*), leek (*Allium ampeloprasum*), onion (*Allium cepa*), garlic (*Allium sativum*), asparagus (*Asparagus falcatus*), apart from chicory roots (*Cichorium intybus* L.), Jerusalem artichoke (*Helianthus tuberosus* L.), and globe artichoke (Mudannayake et al., 2022). While banana, wheat, and onion have short-chain ITF regarding maximum DP<10, Jerusalem artichokes show medium-chain ITF, chicory root, and globe artichokes are characterized by long-chain ITF (Jackson et al., 2023). The novel and alternative plant-based inulin sources are given as dahlia (*Dahlia pinnata* L.), yacon (*Smallanthus sonchifolius*), blue agave (*Agave tequilana*), and dandelion (*Taraxacum officinale*) (Mudannayake et al., 2022). Inulin is generally obtained at the industrial level, which belongs to the Asteraceae family (Rubel et al., 2021). The commercial inulin and FOS on a large scale are majorly produced from chicory roots in Europe, followed by Jerusalem artichoke in the USA and some Asia Pacific countries (Rubel et al., 2021). The content of inulin based on fresh weight in these plants was found to correspond to 11-20% and 10-22%, respectively (Rubel et al., 2021). In other respects, some bacterial and fungal species, such as *Streptococcus mutans* and *Aspergillus sydowii*, respectively, are also utilized in inulin production. However, while plant-sourced inulin is a linear molecule with a low branching degree (1-2%), bacterial inulin exhibits very high DP and branching degrees (>15%) (Mudannayake et al., 2022).

The degree of polymerization (DP) of fructans ranges between 2-60, and the average DP value is 10–12 units (Ahmed and Rashid, 2019). Short-chain inulin exhibits a DP lower than 10, and long-chain inulin possesses a DP higher than 23 (Du et al., 2023). In this regard, the lower DP values less than 10 units or below are specified for oligofructose/FOS (Ahmed and Rashid, 2019). The DP is influenced by several factors, such as plant species, harvesting time, storing time, growing season, climatic conditions (Ahmed and Rashid, 2019), plants' physiological age, extraction methods, and process conditions (Mudannayake et al., 2022). The DP, together with its branching pattern, identifies both physicochemical and functional attributes of inulin, such as gelation attributes and gel integrity, melting point, and glass transition temperature. In this regard, DP-based fractionation eventuates in end-products with desired features (Ahmed and Rashid, 2019). The solubility of inulin is linked to its DP in a similar vein to other polymers (Teferra, 2021). Therefore, the inulin solubility is majorly dependent on its chain length (Bhanja et al., 2022), and there is an adverse relationship between them. In this regard, the solubility of inulin reduces with DP increase and thereby the length of the chain (Teferra, 2021; Bhanja et al., 2022). The short-chain ITFs (DP<10) and oligofructose are highly soluble (≈80%) at ambient temperature and in aqueous solution (Jackson et al., 2023). Moreover, inulin with higher solubility in comparison with native-counterparts was reported when enzymatically synthesized from sucrose. In addition, a positive relation was stated between the solubility of inulin with temperature (Teferra, 2021). Nevertheless, the solubility level of long-chain ITFs could not be attained by short-chain oligofructose, although it increased with temperature rise (Jackson et al., 2023).

The solubility and sweetness of short-chain inulin, such as oligofructose, with low DP (DP<10), are higher in contrast to native counterparts and long-chain inulin fraction. There is a comparable sweetness degree of low DP inulin with fructose (Teferra, 2021). Due to developed sweetness (≈30-35% of sucrose) and also low calorie values (≈1-2 kcal), short-chain inulin is generally utilized for partially or completely sugar replacement in foods (Ahmed and Rashid, 2019). The short-chain inulin also has a role in enhanced mouthfeel (Teferra, 2021) and satiety. Therefore, inulin is accepted as a good sucrose replacer in low-sugar, low-calorie, or diabetic diets. To achieve a balanced sweetness profile, inulin could also be combined with different sweeteners that have high intensities (i.e., acesulfame K, aspartame, or stevia) (Mudannayake et al., 2022).

On the other hand, the solubility of long-chain inulin fractions is lower, but they show relatively viscous behaviour (Ahmed and Rashid, 2019). Thus, they could form a gel-like structure when in contact with water (Bhanja et al., 2022), namely as "particulate gels" which are produced by the random aggregation of polymer chains (Mudannayake et al., 2022). However, a low DP is related to the inability to gels formation (Ahmed and Rashid, 2019). Therefore, inulin concentration should be at least 15-20% for gel formation (Mudannayake et al., 2022). Because the gel formation does not occur in inulin solutions at low concentrations (≤5%), owing to having inadequate particles for aggregation (Mudannayake et al., 2022). The gelation becomes evident when inulin concentration exceeds 30%, majorly because of the presence of dispersed, non-continuous particles. The gelation accelerates and gel formation is increased as the rise in inulin concentrations (40-45%), resulting in a gel with creamy consistency exhibiting as a fat mimic under these conditions. Although gel shows more firmer/rigid character at inulin concentrations up to 50%, it retains its fat-like properties (Ahmed and Rashid, 2019). The gel formation of inulin is achieved based on shear- and thermally-induced processes. In this context, these are materialized when shearing in high-concentration (≥25%) aqueous suspensions and heating at ≤85°C, then cooling (≤20°C) in aqueous solutions, respectively (Mudannayake et al., 2022). However, the gelation does not take place, and viscosity is considerably decreased when inulin chains are completely hydrated, during which the heating temperature is increased more than 80℃ due to being more prone to solubility of inulin (Ahmed and Rashid, 2019; Mudannayake et al., 2022). Their capability of generating the microcrystals while shearing in water or milk interacts and gives a fat-like sensation and creamy texture. Therefore, they could be utilized as a fat replacer for designing foods with low-fat content (Ahmed and Rashid, 2019). Complete fat replacement could successfully be achieved in foods with this inulin-based gel, which gives a smooth fatty mouthfeel, spreadable texture, and also a glossy appearance (Mudannayake et al., 2022). However, the gelling/thickening behaviour of inulin is different from that of hydrocolloids owing to its relatively lower molecular weight and limited water-binding capacity (Mudannayake et al., 2022). Although hydrocolloids are often commonly used in partial and/or complete sugar and/or fat-replaced food products to modify rheological properties, compromise textural quality, and thereby satisfaction of consumers, these additives serve limited nutritional value. On the other side, ITF could contribute not only to gel formation and viscosity development but also exhibit a valuable dietary fiber source, thus offering both functional and nutritional advantages (Jackson et al., 2023). Therefore, to modify viscosity and enhance rheological attributes of several food systems, inulin could be combined with hydrocolloids (i.e., alginate, β-glucan, carrageenan, guar gum, pectin, etc.) as a texture modifier agent (Mudannayake et al., 2022).

Inulin could be fermented in the colon due to its ability to selectively improve the proliferation of beneficial intestinal microbiota, resulting in advanced bowel function. The fermentation process causes an increase in the microbial populations, which considerably go toward fecal biomass by lowering pH with majorly short-chain fatty acids (SCFAs) production, as well (Ahmed and Rashid, 2019). Therefore, inulin fructans are well recognized as prebiotics (Mudannayake et al., 2022) and thus are widely utilized in functional food formulations to improve beneficial microorganism growth. They could also promote resilience of the host, prevent growth of harmful bacteria, improve intestinal peristalsis, and hinder diarrhea and constipation (Du et al., 2023) by rising stool rate/load with a bulking impact similar to other soluble dietary fibers (Shoaib et al., 2016). Several experimental studies based on animal and human have revealed that inulin could also demonstrate hepatoprotective, hypoglycemic, anti-tumor, and immunomodulatory activities along with beneficial gastrointestinal activity (Du et al., 2023).

In all European countries, inulin is legally classified as a non-additive but as a food or food ingredient (Ahmed and Rashid, 2019). In the USA, it is authorized for the inclusion of chicory inulin and oligofructose into the formulation of food products when officially accepted as a natural food ingredient with a generally recognized as GRAS status (Ahmed and Rashid, 2019; Rubel et al., 2021). They could be utilized in foods/drinks as an ingredient with no specific restraint, and labelled as dietary fiber (Ahmed and Rashid, 2019). In this context, the value of recommended daily intake for inulin and FOS as dietary fiber sources is in the range from 1 to 15 g/day. On the other side, it is declared that the daily intake tolerability of inulin was better than FOS, and thus interpreted that chain length also has a role in tolerance between fructan-type dietary fibres (Rubel et al., 2021). Inulin could not be hydrolyzed in the human gastrointestinal system and thus could not be exposed to digestion and/or absorption in the stomach and small intestine; it does not affect blood glucose levels. In this context, the glycemic index (GI) of commercially available native and purified inulin sources is 14 and 5, respectively, which are dramatically lower in contrast to the glucose GI, which corresponds to 100 (Mudannayake et al., 2022). In this respect, absorption of bile salts from the small intestine has a positive influence on lipid metabolism thanks to the efficiency of inulin and FOS in the glycemic response stabilization (Ahmed and Rashid, 2019). Therefore, they could be ideal and promising food for those individuals who suffer from diabetes because of not breaking down or absorption of inulin through digestion in the oral cavity and small intestine (Du et al., 2023).

2.2.2. Inulin-incorporated Pasta

Inulin is used in various food products as a fat or sugar substitute, majorly owing to its functional properties like the ability to enhance viscosity and texture. Additionally, it is a dietary fiber and a prebiotic source. These characteristics make inulin a valuable and promising ingredient in a wide range of applications, particularly dairy-based foods (yogurt, cheese, and ice cream, etc.), fruit juices, processed meats, bakery goods (such as bread, cakes, and breakfast cereals) (Jackson et al., 2023), and pasta, as well.

Over the past twenty years, the incorporation of inulin into pasta and noodle formulations has been studied at the 0.5-20% ratios in various studies, and the impact of extracted or commercial inulin, with varying DP, on the technological and nutritional properties of pasta were investigated, as shown in Appendix 1 and Appendix 2, respectively (Yazici et al., 2023a). Nevertheless, research exploring the application of inulin in gluten-free pasta still remains relatively scarce (Mastromatteo et al., 2012; Nechaev et al., 2021).

Generally, the moisture content increased as the inulin addition rise, which tends to be more pronounced at higher addition levels. This effect is ascribed to the inherent water-binding capacity of inulin (Saraç, 2021). The optimum cooking time (OCT) of inulin-enriched pasta/noodle has been determined to range between about 5.0-14.0 min (Aravind et al., 2012; Foschia et al., 2015a; Padalino et al., 2017; Gasparre and Rosell, 2019; Saraç, 2021; Zarroug et al., 2022; Difonzo et al., 2022), which is generally longer than that of control without inulin sources (Gasparre and Rosell, 2019; Foschia et al., 2015a; Difonzo et al., 2022). Conversely, a reduction in OCT upon inulin addition has also been detected in some studies (Padalino et al., 2017; Saraç, 2021; Zarroug et al., 2022). This discrepancy could be explained by the gluten network disruption that enables faster water penetration into starch granules. Variations in OCT are likely influenced by the type of inulin utilized, whose physicochemical attributes, and the specific processing parameters employed through pasta production (Difonzo et al., 2022).

Regarding water absorption, most studies reported an increase in pasta formulations including inulin in comparison to control with no inulin (Foschia et al., 2015a; Gasparre and Rosell, 2019; Saraç, 2021), although some exceptions exist (Brennan et al., 2004; Bustos et al., 2011). Swelling index (SI) values for inulin-enriched pasta were approximately ranged from 1.5 to 2.6 g/g (Brennan et al., 2004; Brennan and Tudorica, 2007; Manno et al., 2009; Aravind et al., 2012; Foschia et al., 2015a; Gasparre and Rosell, 2019; Zarroug et al., 2022; Difonzo et al., 2022; Padalino et al., 2017). A decrease in SI compared to control pasta could be linked to the generation of a dietary fiber matrix that encapsulates starch granules, thus restricting water access and competing with starch and protein for hydration (Bustos et al., 2011; Zarroug et al., 2022; Difonzo et al., 2022). In contrast, some studies have reported enhanced swelling properties in inulin-fortified pasta, which could be dedicated to the better water absorption and retention abilities of dietary fiber within the protein–starch–polysaccharide matrix (Foschia et al., 2015a; Gasparre and Rosell, 2019).

Cooking loss (CL), a critical indicator of pasta quality and mostly linked with the protein–starch matrix integrity, ideally should be below 7–8% (Saraç, 2021). Nevertheless, higher CL values were noted in some inulin-enriched pasta/noodle studies (Brennan, 2004; Foschia et al., 2015a; Gasparre and Rosell, 2019; Peressini et al., 2020; Zarroug et al., 2022). The integration of inulin has also been found to reduce pasta hardness/firmness in many studies (Brennan et al., 2004; Brennan and Tudorica, 2007; Bustos, et al., 2011; Filipović et al., 2014; Foschia et al., 2015a; Filipović et al., 2015a; Filipović et al., 2015b; Padalino et al., 2017; Ivkov et al., 2018; Peressini et al.,, 2020), likely because of the concurrent rise in SI and moisture content (Foschia et al., 2015a). On the contrary, other studies have reported increased firmness, which has been dedicated to the higher water absorption of dietary fiber in contrast to starch, causing a firmer textural structure (Difonzo et al., 2022; Singthong and Thongkaew, 2020).

In regard to sensory characteristics, the addition of inulin has been shown to have an adverse influence on the taste of cooked pasta when compared to control formulations (Saraç, 2021; Difonzo et al., 2022). Nevertheless, other sensory attributes, including the appearance of raw pasta (Saraç, 2021), elasticity, color (Padalino et al., 2017), bulkiness and adhesiveness of cooked pasta (Difonzo, 2022), break resistance, and overall quality of inulin-added pasta, did not show statistically significant differences from control samples (p>0.05) (Padalino et al., 2017), according to findings summarized in Appendix 1 (Yazici et al., 2023a).

From a nutritional perspective, the protein content in inulin-fortified pasta samples was mostly decreased (Brennan and Tudorica, 2007; Manno et al., 2009; Filipović et al., 2014; Filipović et al., 2015a; Filipović et al., 2015b; Padalino et al., 2017; Ivkov et al., 2018; Difonzo et al., 2022), as summarized in Appendix 2 (Yazici et al., 2023a). This reduction is majorly referred to as the dilution impact, sourced from reduced dietary fiber content in the formulation (Difonzo, 2022). As anticipated, dietary fiber levels in pasta rise proportionally with the quantity of inulin incorporated, typically ranging between nearly 3.5%-16% (Saraç, 2021; Padalino et al., 2017; Difonzo, 2022; Filipović et al., 2015a; Filipović et al., 2015b). Consequently, those formulations had the potential to meet labeling requirements for "high fiber content" or "source of fiber" claims in commercial food products (Difonzo et al., 2022). On the other side, the influence of inulin on starch digestibility was summarized in Table 2.1.

Table 2.1. The main influences of utilizing inulin in pasta/noodles on the starch digestibility

Major findings	**References**
Starch hydrolysis index↓, pGI↓	Difonzo et al., 2022
Starch digestibility↓ (more prominent in high DP inulin)	Padalino et al., 2017
pGI↔ (except durum wheat cultivar)	Garbetta et al., 2020
Reducing sugar release↓	Foschia et al., 2015b
Reducing sugar release↓ Pasta digestion decreased with increasing inulin addition pGI↔ (GI% decrease from control was 15% in inulin-added pasta at 10% concentration)	Brennan and Tudorică, 2004
Higher reducing sugar release at 20 min than β-glucan extract, psyllium Similar reducing sugar release with control at 60 min and 120 min IAUC↑	Peressini et al., 2020

IUAC: incremental area under the curve, pGI: predicted glycemic index, ↓ indicates increment is statistically different, ↑ indicates decrease is statistically different, ↔ indicates increment or decrease is not statistically different

The elevated fiber content introduced through inulin incorporation has been linked with reduced starch hydrolysis and less predicted glycemic index (pGI) values in pasta (Difonzo et al., 2022). However, no significant difference was found in the pGI of inulin-enriched pasta, regardless of the DP, when produced from 4 different durum wheat cultivars in comparison (except the cultivars of Timilia) with the control group (Garbetta et al., 2020). Similarly, in another investigation, while the pGI of pasta containing 2.5–10% inulin did not significantly differ from control, the percentage reduction in glycemic index increased from 2.3% to 15% as inulin concentration increased (Brennan et al., 2004). In addition, a greater reduction in starch digestibility was detected in pasta formulations with high-DP inulin compared to those with low-DP (Padalino et al., 2017). In another study evaluating the sugar release during digestion, pasta enriched with inulin exhibited the highest glucose release at 20 min, in contrast to samples containing other dietary fibers such as commercial β-glucan concentrates or psyllium. Nevertheless, the glucose release levels in inulin-enriched pasta were comparable to those of the control group by 60 min and 120 min of digestion (Peressini et al., 2020).

From a human health perspective, inulin-fortified pasta demonstrated promising health benefits in clinical contexts. In a study involving patients with Type 2 diabetes and chronic kidney disease (n=14), consumption of inulin-enriched protein-based pasta eventuated in significantly lower *in vivo* postprandial glucose levels at 90 min and 120 min following a meal. The glycemic index of the pasta was about 70%, suggesting whose potential utility in the dietary management of these conditions (Tubili et al., 2016). In another clinical trial with obese individuals (n=30) adhering to a calorie-restricted diet that included pasta enriched with 2% chicory inulin, no significant differences were defined in total cholesterol, HDL-cholesterol, triglyceride levels, or fasting blood glucose ($p>0.05$). However, notable decreases were reported in insulin levels, total body weight, and HbA1c following a glucose load, referring to beneficial metabolic impacts (Ivkov et al., 2018). Conversely, in the context of gastrointestinal digestion, inulin did not significantly enhance protein hydrolysis in multigrain noodles, unlike several other soluble dietary fibers such as arabinogalactan, arabinoxylan, β-glucan, FOS, GOS, and xylooligosaccharides ($p>0.05$). These alternative fibers enhanced protein digestibility through simulated digestion processes, particularly relevant for school-aged children (Gao et al., 2023).

2.3. Probiotic

Etymologically, the “probiotic” term originates from Greek words composed of “pro” and “biotic”, referring to “favor” and “life”, respectively (Sharma et al., 2023). They are generally defined as “a mono- or mixed live microorganism culture(s) which influence the host beneficially by enhancing the attributes of indigenous microflora” (Pramanik et al., 2023). According to the FAO and WHO guidelines in 2002, probiotics were described as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Majeed et al., 2016). They are also identified as “live biotherapeutics” by the FDA, which expresses that living microorganisms like bacteria and yeast with an intended locally or regionally therapeutic activity based on therapy and/or prevention of diseases in humans (Sharma et al., 2023).

The conventionally utilized major genera of probiotics are composed of *Lactobacillus* and *Bifidobacterium*, which are also present in the human GIT (Panghal et al., 2018), have GRAS status (Panghal et al., 2018), and a “Qualified Presumption of Safety” (QPS) status given by EFSA (Haranahalli Nataraj et al., 2024). To develop functional probiotic-included food, several other microorganisms, such as *Bacillus, Enterococcus, Streptococcus*, *Escherichia coli* strain Nissle 1917 (Majeed et al., 2016), *Propionibacterium, Pediococcus, Leuconostoc* (Panghal et al., 2018), and also some yeasts, such as *Saccharomyces boulardii* and *Saccharomyces cerevisiae,* have probiotic potential (Min et al., 2019). In this regard, they could be categorized as non-pathogenic, including *Lactobacillus, Bifidobacterium, Lactococcus, and Saccharomyces*, pathogens (i.e., *Bacillus cereus*), and opportunistic pathogens (other LABs and *Enterococcus*) (Panghal et al., 2018). On the other side, the new probiotic candidates from unconventional genera called “next-generation” probiotics include *Parabacteroides goldsteinii, Faecalibacterium prausnitzii, Clostridium beijerinckii, Bacteroides fragilis,* and *Akkermansia muciniphila* (Haranahalli Nataraj et al., 2024). The major factors for probiotic selection are based on safety criteria such as toxicity, pathogenicity, metabolic activity, infectivity, and intrinsic properties (i.e., antibiotic resistance). To introduce new probiotics in food products, the genera and strains should be prevalent intestinal microflora of humans, and the rate of risk-benefit together with safety should be carefully taken into consideration and evaluated as per CODEX Alimentarius (Panghal et al., 2018).

A microorganism must provide the following criteria to be classified as probiotic: resistive stomach acidity, bile salts, toxicity, bacteriocins, and toxins generated by endogenous bacteria, isolated from GIT healthy individulas originated from human or animal, capability of adherence to cells and mucins of intestine and colonize, generate anti-microbial components (i.e. bacteriosins) against to pathogens, being safe in therapeutic and both culinary settings (Sharma et al., 2023), and being non-pathogenic to host (Pramanik et al., 2023). They should be allowed a commonly regarded safety status and chosen according to their technological utility and functionality, apart from safety (Sharma et al., 2023), and also be available for production on a large scale (Sharma et al., 2023). The metabolic activity and viability of probiotics must be maintained from the manufacturing of food products to ingestion by consumers during their passage through the GIT (Soares et al., 2023). However, owing to the poor durability of *Lactobacillus* and *Bifidobacterium* against stomach acid and high temperatures, they become inactive through production processes, storing, and other harsh processing conditions of food production, resulting in a loss of viable probiotic bacteria. To improve probiotics’ survival, some methods, namely cold-chain management and microencapsulations, are available, which are an additional expenditure (Poshadri et al., 2022). On the other side, to overcome those restrictions, the implementation of spore-forming probiotics belonging to the *Bacillus*, *Brevibacillus*, *Sporolactobacillus*, and *Clostridium* genus could be a promising alternative, owing to be resistance to low pH, high temperature, high pressure, and also having the ability to germinate rapidly, and good stability (Soares et al., 2023).

When a food product is labeled with probiotics, it has to deliver live microorganisms in a required amount and exert health-beneficial effects on the host (Chaturvedi and Chakraborty, 2021). However, it is still debatable and has not reached a consensus (Ceylan and Atasoy, 2023) regarding the optimum concentration of probiotics for declaration as a functional probiotic product (Panghal et al., 2018). According to the ISAPP, it is stated that a food product should contain at least 10^6 CFU/g of defined live strains that are expected to have potential positive effects on host health (Bultosa, 2016). On the other side, it has been reported that the generally probiotic concentration should be 10^6 CFU/mL in the small intestine and 10^8 CFU/g in the colon to achieve a clinical effect (Minelli and Benini, 2008). In other respect, Canada's public health agency and Italian Health Ministry have suggested a daily intake of 10^9 CFU per serving size (Soares et al., 2023), and it is recommended by the Food Safety and Standards Authority of India that the viable amount of incorporated probiotic in food product should be more than 10^8 CFU in daily serving (Poshadri et al., 2022). Therefore, to demonstrate therapeutic influences, it is commonly admitted that the daily amount of live bacteria should be a minimum of 10^6–10^9 CFU/g (Ceylan and Atasoy, 2023).

The health benefits depend on the probiotic strain (Ceylan and Atasoy, 2023), which possesses its effects directly via the interactions of the host with ingested live microorganisms or indirectly as a consequence of the intake of microbial metabolites synthesized through fermentation (Rasika et al., 2021). Probiotics exert several health benefits, including hindering infections of the GIT, decreasing the levels of serum cholesterol, improving the immune system, vitamin synthesis (i.e., B complex vitamins), stimulating mineral absorption (i.e., Ca) (Rasika et al., 2021), enhancing lactose metabolism, and anti-carcinogenic, anti-microbial, anti-mutagenic, and anti-diarrheal properties (Vijaya Kumar et al., 2015). The potential mechanisms behind the health benefits are as follows: antimicrobial compound production, promoting immunity, rivalry for nutrients with pathogens, and extinction of toxin receptors (Ceylan and Atasoy, 2023). Furthermore, the probiotic incorporation into a GFD has been proposed as a new therapy to decrease symptoms of CD. Although the exact mechanism behind this phenomenon has not been completely discovered yet, gliadin toxicity could be suppressed by probiotic strains by the enzymatic hydrolysis of gliadin. In this regard, particularly using a combination of *Lactobacillus* and *Bifidobacterium* strains at sufficient concentration in diets could be a promising approach in CD by aiding in changes in gut microbiota, decreasing immune activation, gliadin detoxification, and alleviating the symptoms (Norouzbeigi et al., 2020).

The foods, including probiotics, are mainly composed of fermented dairy foods and non-dairy fermented products (Rashidinejad et al., 2022). Food is a good agent for carrying probiotics, particularly in dairy-based foods (i.e., milk, yoghurt, cheese, and ice cream) because it presents an optimum physical environmental condition, such as pH and temperature, with a sufficient substrate regarding chemical composition. In addition, those corresponding foods buffer probiotics while passing through the GIT. Hence, they could regulate colonization and enable bioactive components that improve the probiotics' functionality (Urrutia-Baca et al., 2024). Incorporation of probiotics in non-dairy food products is widely applied in juices, nutrition bars, and soy-based products (Pramanik et al., 2023). Even though several studies are showing no significant impact on overall acceptability with the addition of probiotic culture to food formulation, these products have yet to come to the market (Panghal et al., 2018). Because some sensorial alterations (i.e., aroma and taste) could be shown in non-dairy food products when incorporating probiotics into the formulation. The main reason behind this could arise from the generation of different metabolic compounds like lactic acid and other metabolites in living cells by different species through fermentation and over-storing duration (Panghal et al., 2018). In other respects, differences of storage temperature in probiotic (≈5°C) and non-dairy foods (ambient temperature) are another challenge regarding remaining an appropriate common temperature. On the other side, the interaction of food additives (coloring agents, sweeteners, artificial flavors) with the viability of probiotic strains should also be taken into consideration (Kumar et al., 2022).

2.3.1. *Bacillus coagulans*

The most broadly studied species of the *Bacillus* genus comprises *B. clausii*, *B. coagulans*, *B. cereus, B. licheniformis,* and *B. subtilis* (Adibpour et al., 2019). Among them, *B. coagulans* was initially isolated in 1915 by Hammer from spoiled canned milk. Firstly, they were mislabeled as *Lactobacillus sporogenes* owing to their ability to produce lactic acid (Lee et al., 2019), but following studies revealed that they need to be classified in the *Bacillus* genus (Cao et al., 2020). *B. coagulans* is a spore-forming, facultative anaerobic, catalase-positive, thermophilic bacterium whose optimum growth temperature is in the range of 35–50°C (Cao et al., 2020), but it could grow above 50°C. They are gram-positive bacterium (Zhao et al., 2023) and are tolerant to severe environmental conditions including such as bile, lactic acid, and salt (Zhao et al., 2023). It was declared that they are in GRAS status by the FDA and reported safe as EFSA and in the QPS list (Konuray ve Erginkaya, 2018). The probiotic activity and having role in decreasing chronic gastrointestinal symptoms and increasing resistance to infections have been supported by several clinical trials (Li et al., 2023a). Recently, it was also elucidated that absorption and the efficacy of dietary proteins were enhanced when consumed with *B. coagulans* GBI-30, 6086 (Adibpour et al., 2019).

Traditionally, *Lactobacillus* and *Bifidobacteria* exhibit surpassing probiotic activities, but their survivability is usually very poor (≈1-15%) (Cao et al., 2020). Due to *Lactobacillus* and *Bifidobacterium* being very delicate to temperature and harsh gastric conditions like stomach pH and bile salts, they are influenced throughout processes when manufacturing, storing, and transporting foods (Adibpour et al., 2019). In the first place, encapsulation or edible coating methods were achieved successfully for protecting probiotic bacteria in foods (Majeed et al., 2016) and maintained viability while producing, storing, and under severe conditions (acidic and including bile salts) (Majeed et al., 2016). Therefore, the viability and stability of probiotics could be promoted with encapsulation (Pramanik et al., 2023). To encapsulate probiotics, starch and its derivatives (i.e.resistant starch), are the most widely preferred materials, bringing along some benefits like thermal stabilization, extended shelf-life, and process-induced controlled release (Fuentes-Zaragoza et al., 2011). The most commonly used encapsulation techniques are fluidized bed drying, spray drying, and freeze-drying. However, they have some restrictions because of releasing most of the cells into the product through processing or storing phases and within the upper GIT parts (Rashidinejad et al., 2022). Particularly, hydrophilic, lipid-based encapsulants release probiotic cells when encountered with water and high temperature, respectively, or digested via lipase (Rashidinejad et al., 2022). Furthermore, these techniques are need of an extra cost for functional food production and thus are a main obstacle to an economically viable production process (Majeed et al., 2016). In addition to this, multi-stages for preparation could be a hurdle for application on an industrial scale (Poshadri et al., 2022). For this reason, spore-forming probiotic microorganisms caught the researchers' attention (Cao et al., 2020). The pros of utilizing the spore-forming probiotic microorganisms in contrast to traditional probiotics are comprising of ability to tolerate acidic enviroment (low pH) and accomplish passing from GIT, better resistant to thermal lethal influence, and also radiation or toxic chemicals in comparison with vegetative cells, conserve at ambient temperature in dried forms with no adverse impact on spores' survival, and inherent generation of macro- and micro- compounds such as proteins, enzymes, vitamins, anti-microbials, and pigments (i.e. carotenoids) (Adibpour et al., 2019). Those aforementioned advantages based on better survival and stability make probiotics with spore-forming abilities an ideal candidate for developing functional food (Cao et al., 2020), and ideally, functional choices for food formulations have a health-boosting effect (Adibpour et al., 2019), particularly in cereal-based foods, which are exposed to heat-treated processes like boiling or baking (Konuray ve Erginkaya, 2018). In this context, recently, many *Bacillus* species were declared as novel probiotics (Adibpour et al., 2019), and *B. coagulans* is one of the best choices for functional cereal-based food development (Cao et al., 2020) without the requirement of microencapsulation.

2.3.2. *Bacillus coagulans*-incorporated Pasta

Foods are better tools in comparison with supplements regarding human consumption of probiotics. The reason behind this fact could be explained with probiotics could be preserved via foods as they flow within the GIT, which is associated with whose buffering attributes and the presence of nutrients that could be utilized via the microorganisms. The survivability and multiplication of probiotics are influenced by chemical and physical features of the food matrix, as well. Apart from making available vital nutrients to remain metabolic activities and viability of probiotics, foods that include prebiotic components could positively impact in the affirmative both functionality and viability of beneficial microorganisms. Although the incorporation of spore-forming probiotics within food formulation is a recent advancement, they shouldn't germinate within the food matrices because they tend to spoil the foods under convenient conditions. In this context, food matrices should be selected based on probiotic strain or species (Soares et al., 2023).

Regarding the effect of *B. coagulans* on the technological properties of pasta/noodle, in a study, semolina pasta was enriched with *B. coagulans* GBI-30 and stored at ambient temperature for 6 months. The OCT accounts for 7 min in both *B. coagulans*-added and control pasta made from semolina, and thus, OCT was not influenced by either probiotic inclusion in pasta formulation and also storage. However, the water absorption and CL values were lower in pasta with probiotics than in semolina pasta, even though those corresponding values were increased over storage time, owing to gluten weakening. There was no statistically significant difference in lightness (L*) values between probiotic-enriched and control pasta. According to textural analysis results, the hardness was reduced with probiotic addition. However, probiotic addition to pasta formulation had no negative impact on overall acceptability scores in terms of sensory evaluation. To sum up, it was concluded that the pasta including *B. coagulans* maintained comparable technological and sensorial qualities in contrast to control pasta based on semolina that supported the potential of probiotics for the production of functional foods without compromising consumer satisfaction (Konuray and Erginkaya, 2020). In another study, the *Bacillus coagulans*-fortified pasta, based on a blend of composite flour (wheat, semolina, corn, pumpkin seed), was developed. The OCT, water absorption, volume increase, and CL values account for approximately 10 min, 52%, 145%, and 5%, respectively. In addition, lightness values were increased as the cooking process was applied to uncooked probiotic-pasta samples, which was attributed to the gelatinization process. According to sensorial analysis results, while appearance, stickiness, odor, and overall acceptability with not significantly differ from commercially available control pasta ($p>0.05$), tenderness, sensation of starch, flavor, and color were significantly higher in the control group than in *B. coagulans*-enriched pasta ($p<0.05$) (Piyadasa and Peera, 2019).

When the count/viability of *B. coagulans* exposed to cooking or storing processes of pasta/noodle was assessed, in a study, the initial *B. coagulans* count corresponded to 8.62 log CFU/g in the flour mixture for probiotic pasta production. A slight decrease was detected in viable counts after extrusion and drying processes, accounting for 8.49 log CFU/g and 8.30 log CFU/g, owing to mechanical and thermal stress, respectively. However, a proceeded reduction was observed in probiotic load during cooking, equal to 7.20 log CFU/g and 6.08 log CFU/g for 5 min and 11 min of cooking, respectively. In this regard, the minimum effective threshold for probiotic count (6 log CFU/g) was achieved even at the end of the longest boiling period. Moreover, the viability was maintained after 6 months of storage at ambient temperature. In this context, it was stated that *B. coagulans* could resist the producing, cooking, and storing, thereby maintaining adequate viability to categorize the end-product as probiotic upon shelf life (Konuray and Erginkaya, 2020). In another study, the concentration of *B.coagulans* MTCC 5856 was decreased to 6.78 log CFU/g from 7.17 log CFU/g, and thus the survival rate was 94.56% after the cooking process in noodles. These results confirmed that *B. coagulans* endured and retained its functionality with significant probiotic counts of more than 6 log CFU/g after high-temperature thermal food processing (Majeed et al., 2021). In another study, the *B. coagulans*-included pasta consistently sustained a viable count of probiotics during a 1-month of storing period at nearly 30°C. Therefore, it was over the threshold of the recommended daily intake, accounting for $1\ x\ 10^9$ CFU/serving at the end of the storing ($1.50\ x10^9$ CFU/g). However, the initial viability of uncooked pasta samples was reduced progressively in cooked samples from 1.46×10^9 CFU/g to 1.27×10^9 CFU/g at the same storage period, owing to exposure to thermal stress throughout cooking (Piyadasa and Peera, 2019). Since 4 months of storing, the modified atmosphere packaged (MAP) probiotic-included pasta samples presented probiotic genera such as *Bacillus*, *Lactobacillus*, and *Bifidobacterium*, whose average amplicon sequence variant abundances account for 2.55%, 6.12%, and 17.5%, respectively, supported by metagenomic tests. In addition to this, while efficient microbiological inhibition was observed together with inhibiting spoilage organisms' proliferation in probiotic-enriched MAP pasta, the unrestrained microbial loads, including LAB and total aerobic mesophilic bacteria, were increased in the control group packaged with the conventional protocol throughout storage. It was concluded that the contribution of bioprotective cultures was related to maintaining viable probiotic count and also promoting microbiological safety during prolonged shelf-life (Marzano et al., 2022).

2.4. Synbiotic

"Synbiotic" term is comprised of "sny and "biotic" stands for "together" and "life" in Greek, respectively. Initially, this term was intorduced as "a prebiotics and probiotics mixture salutarily influence the host by promoting the implantation and survival of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited amount of health-promoting bacteria, thereby enhancing welfare of host" in 1995 by Gibson and Roberfroid (Parhi et al., 2024). However ISAPP revised the definition in 2020 as "a mixture of live microorganisms and substrate(s) selectively utilized by host microorganisms that confer a health benefit on the host", and classified into two subsets as "complementary synbiotics" and "synergistic synbiotics" (Swanson et al., 2020). In the former, independently chosen to improve probiotics for a targeted biological activity and indigenous beneficial microbiota (Terpou et al., 2019), and thus demonstrate benefits on health with no need for any codependent function. In the latter, the substrate, not required to be prebiotic, is metabolized by a selectively co-administered live microbial population, which does not need to be probiotic (Chan and Liu, 2022). Hence, prebiotics are selected to promote specifically the chosen probiotic growth. Therefore, the prebiotic is involved in being selectively fermented by the probiotic strain, irrespective of the beneficial influence on the other bacterial population (Terpou et al., 2019). However, the health benefits have to be indicated in a human host in both cases (Chan and Liu, 2022).

Out-of-balance gut microbiota adversely influence the metabolism, intestinal health, and the gut-brain axis in irregular physiology and cell function. Thereafter, these alterations cause the development of some inflammatory, metabolic, cardiovascular, neurological, psychological, and chronic disorders and/or diseases in the host. However, those corresponding diseases/disorders could potentially be treated by the re-establishment of gut microbiota and its maintenance. In this context, previous clinical research proposed that the synbiotics have a potential influence to treat many disorders and/or diseases sourced from dysbiosis or abnormal gastrointestinal flora via conjoining the therapeutic potential of probiotics and prebiotics (Yadav et al., 2024). The therapeutic effectiveness of synbiotics was recorded as anti-microbial, anti-carcinogenic, anti-allergenic, anti-diarrheal effects, reduction in serum fats and blood sugars, regulation of the immune system, and also has a role in the treatment of cardiovascular diseases, prohibiting of osteoporosis, and immune system regulation (Yadav et al., 2024). Nevertheless, the exact mechanism of synbiotics for preventing or treating human diseases was still not elucidated in detail in previous research. However, the potential mechanisms may comprising of intestinal microbiota modulation, develop survival of the beneficial microorganism population in the GIT, reducing of pH make for more favorable to intestinal environment, growth inhibition or elimination of pathogenic microorganisms, improved generation of functional components (i.e. lactic acid, SCFAs and metabolites, mucin) as a result of prebiotics fermentation, enhanced functions of gut barrier and the integrity of the intestinal membrane, developed immune-modulation system in host, and also decrease infection, inflammation and restrict bacterial translocation (Yadav et al., 2024).

The global market for synbiotic products was worth approximately 919 million U.S. dollars in 2024 and is anticipated to be valued at nearly 1.27 billion by 2030 U.S. dollars by 2033, with sales increasing at a compound annual growth rate (CAGR) of 5.53% throughout the forecast period (Research and Markets, 2025). While synbiotic dairy-based and juicy products were well explored, non-dairy synbiotic food products were less investigated on a commercial scale, even though several research studies were conducted (Chaturvedi and Chakraborty, 2021). The major reason behind this sourced from less viability of probiotics within the capsule (vesicile) condition because of fewer protection ratio of encapsulation. However, co-encapsulation, put in other words, co-delivery, co-entrapment, of probiotics and prebiotics is a promising technique regarding both viability and stability of living microorganisms in the development of functional synbiotic food products. The most widely utilized co-encapsulation techniques are composed of freeze-drying, spray chilling, extrusion, coacervation, emulsification, electro-hydrodynamic atomization, and gelation as a low-temperature process, and also spray-drying as a high-temperature process. The crucial parameters include bacteria strain/species and environmental conditions, wall material type and safety, carrier size and stability, mass transfer, and mechanism of release in the gut, which affect entrapment/encapsulation techniques. On the other side, the viable probiotic bacteria must be delivered to the gut with no adverse impact on their physiological attributes with those encapsulation methods (Rashidinejad et al., 2022).

2.4.1. Synbiotic Pasta

Even though several researches have been focused on synbiotic bread production, there are only a numerus clausus studies that have explored synbiotic cereal-based goods (i.e., cakes, crackers, cookies, pasta/noodles, waffles, and breakfast cereals), as reviewed previously (Yazici et al., 2023b). The most frequently incorporated prebiotic sources in synbiotic combinations for direct usage are inulin and β-glucan. So far, the generally utilized probiotics are *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Limosilactobacillus fermentum*, *Levilactobacillus brevis*, and *Lacticaseibacillus rhamnosus* from the *Lactobacillaceae* family in synbiotic combinations for cereal-based end-products. To enhance the survivability and stability of probiotics in synbiotic combinations while processing/handling, storing, and transporting, the most commonly used wall materials in prebiotic coatings are composed of chitosan, Hi-maize (high-amylose maize starch), and various gums like acacia gum, carboxymethylcellulose, guar gum, gum arabic, κ-carrageenan, methylcellulose, and pectin. On the other side, lately, it has been reported that the *Saccharomyces boulardii* in cakes and the *Bacillus coagulans* in pasta/noodle have a potential in synbiotic combinations, as well, in combination with prebiotic compounds, including FOS, β-glucan, or barley flour (Yazici et al., 2023b). Nevertheless, there is a limited study regarding the application of both probiotic and prebiotic sources into GF pasta/noodle formulation, as shown in Table 2.2.

Table 2.2. Potential prebiotic and probiotic sources in synbiotic pasta/noodles

Product	**Probiotic source(s)**	**Prebiotic and potential source(s)**	**References**
Pasta	*L. paracasei* IMPC2.1 *E. coli* ATCC35401	Inulin[a]	Bavaro et al., 2024
Gluten-free pasta	*B. coagulans* Unique IS2 spores	Combination of pseudocereal flours and psyllium husk[a]	Achinna and Desphande, 2025
Gluten-free vermicelli	*B. coagulans* IS2 spores	Combination of pseudocereal flours and psyllium husk[a]	Poshadri and Desphande, 2024
Pasta	*B. coagulans* GBI-30, 6086	Barley flour[a]	Fares et al., 2015
Whole-grain pasta	*B. coagulans*	β-glucan[a]	Angelino et al., 2019
Pasta	*L. plantarum*, *L.acidophilus*, *L. Fermentum*	β-glucan[a]	Arena et al., 2014
Noodle	*L. plantarum* (MTCC 5422)	Fructooligosaccharide[b]	Rajam et al., 2015

a: direct usage; b: coating material

Moreover, there is no study in the literature based on inulin with different DP applications to formulations with probiotics incorporated into GF pasta, as far as we know. In this regard, more comprehensive evaluations of the nutritional, technological, and sensory properties of cereal-based products, together with assessing probiotic viability, are essential to fully understand and realize their synbiotic potential (Yazici et al., 2023c). The effect of *B.coagulans* addition together with prebiotic source on technological quality attributes of pasta/noodle was summarized in Table 2.3.

Table 2.3. Major influences of both prebiotic- and probiotic- incorporation on pasta/noodles technological properties

Major findings			Reference
↑	↓	↔	
WA, Color: L*	OCT, SI, Color: a*, b*, Sensory: color, odor, firmness, bulkiness, adhesiveness, taste, overall quality	Sensory: elasticity, fibrous	Bavaro et al, 2024
aw, moisture, hydration, CL, Color: a*, Texture: cohesiveness	OCT, cooked weight, WA, Color: L*, b*, Texture: firmness, chewiness, adhesiveness, springiness	–	Achinna and Deshpande, 2025
CL, Color(dry, cooked): a*, Texture: cohesiveness	OCT, cooked weight, WA, Color(dry, cooked):L*, b*, Texture: firmness, chewiness, springiness, adhesiveness	*aw*	Poshadri and Desphande, 2024
β-glucan	Yellow pigment, sensory, Nutrition: protein, phenolic acid (free, bound),	OCT, cooked weight, Texture: firmness	Fares et al., 2015
Moisture(fresh), solid loss(fresh), Color(fresh): a*	OCT(fresh), Color(fresh): L*, b*, color index(fresh)	Sensory(fresh): cream color, firmness, chewiness, starch aroma, cooked cereal aroma, overall quality	Rajam et al., 2015

aw: water activity, CL: cooking loss, OCT: optimum cooking time, WA: water absorption, SI: Swelling index ↓ indicates increment is statistically different, ↑ indicates decrease is statistically different, ↔ indicates increment or decrease is not statistically different.

The OCT of GF synbiotic pasta, formulated with pseudocereal flours, psyllium husk, and fortified with *B. coagulans,* exhibited a shorter time (nearly 5-6 min) than the control, corresponding to approximately 8 min. This was referring to a faster softening because of the lack of a gluten network. Moreover, the hydration capacity of synbiotic pasta was approximately doubled in contrast to control pasta, due to the hydrophilic attributes of the pseudocereal flours and additional fiber. Nevertheless, the water absorption through cooking was lower in the synbiotic pasta in comparison with the control, probably owing to reduced starch content and the presence of dietary fiber that modulates water uptake. As anticipated in GF pasta, CL was slightly higher in the synbiotic pasta relative to the control, indicating an increase in soluble solid leaching. In terms of texture, the synbiotic pasta had lower firmness, chewiness, and adhesiveness but greater cohesiveness values. The synbiotic pasta had acceptable and comparable values regarding sensory features, supporting its viability as a nutritious and functional synbiotic food (Achinna and Deshpande, 2025).

The GF synbiotic vermicelli-type pasta was developed by using a composite blend of psyllium husk and pseudocereal flour supplemented with *Bacillus coagulans* IS2. The OCT value of the synbiotic pasta was significantly lower and nearly double in the control pasta due to the gluten absence and the porous nature of pseudocereal structures. On the other side, the cooked weight values were lower in synbiotic pasta when compared with the control, which was attributed to a denser protein matrix restricting the water uptake. In a similar vein, water absorption was decreased in the synbiotic pasta relative to the control, referring to the higher contents of protein and fiber that prevent the swelling of starch. Although the CL values were slightly higher in the synbiotic pasta than in the control pasta, they were below the acceptable CL threshold level (10%), reflecting good structural integrity despite the lack of gluten. According to textural analysis, the synbiotic pasta was softer, indicating lower firmness values in comparison with the control group. In addition, chewiness and springiness values were also decreased, but exhibited greater cohesiveness values were observed in contrast to the control pasta without probiotics. Sensorial analysis revealed that although control pasta had higher values, synbiotic pasta had mean scores of overall acceptability exceeding 6, reflecting moderate to high liking, and thus still well accepted by the panellist (Poshadri and Desphande, 2024).

The comparable values of firmness and weight increase with control were defined in cooked synbiotic pasta, which involves probiotics and barley flour. The yellow pigment content, which is more prominent in the control, decreased by cooking in probiotic- and barley flour-added pasta samples that majorly include β-carotene and lutein to a lesser extent. According to a nutritional point of view, no significant differences were found in available carbohydrate and fat content of barley flour-based pasta, including *Bacillus coagulans,* than the control sample without either prebiotic or probiotic sources ($p>0.05$). The protein content of both raw and cooked probiotic-added pasta made with the combination of durum wheat-barley flour was lower than that of the control group made with durum flour. The total dietary fiber content of pasta with the addition of barley flour and *B. coagulans* was positively influenced by the cooking process. It was ascribed with both starch gelatinization and retrogradation processes, which alter part of it into resistant starch. Although there was no significant differences were obtained between glycemic index values of probiotic-enriched pasta including barley flour and control pasta, higher postprandial blood glucose levels were observed in the control sample (Fares et al., 2015).

The moisture content of potential synbiotic raw and cooked pasta was more than 2% and 11% higher than that of the control group, respectively. While the moisture content of encapsulated probiotic-added dried pasta at low temperature (LT-28°C) was higher than that of dried at high temperature (HT-55°C), related to a lower dehydration rate, comparable values were obtained after cooking. The shorter cooking time was needed for raw pasta, including encapsulated *Lactobacillus plantarum,* when compared to control pasta with no probiotic. This was explained by the fact that decreasing gluten levels with the incorporation of probiotic microcapsules led to quicker starch gelatinization, and thus lower cooking time. The OCT of pasta, including encapsulated *Lactobacillus plantarum,* was increased in dried pasta, and more pronounced in dried pasta at low temperatıre. The solid loss of both raw and dried pasta at low and high temperatures was higher than that of the control pasta. It was referred to as a discontinuous protein matrix due to the distribution of probiotic microcapsules into the gluten network. The solid loss of raw pasta with encapsulated probiotic was more prominent than dried ones, independently of drying temperature. The color index was reduced by the incorporation of probiotic microcapsules. According to the results of textural attributes of cooked noodles, the shear strength and adhesiveness were not significantly influenced by the addition of probiotic microcapsules in comparison to the control. No significant differences were determined in sensorial properties such as sweetness, firmness, and chewiness between probiotics, including raw noodle, with respect to control ($p>0.05$). Moreover, the acceptable raw and dried noodles containing probiotic microcapsules were acquired, according to having more than 7.5 overall quality score on a 15 cm scale of quantitative descriptive analysis (Rajam et al., 2015).

A single-blind, parallel, randomized, placebo study revealed that for 1 serve/day consumption of synbiotic whole-grain pasta including *B. coagulans* and β-glucans for 12 weeks by volunteers (n=41) who were healthy, overweight, or obese, the plasma LDL/HDL cholesterol ratio and high-sensitivity C-reactive protein were decreased. Moreover, slight changes in the gut microbiota composition were determined, regarding a decrease in *Bifidobacteria* in obese individuals, an increase in *Faecalibacterium prausnitzii* in hyperglycemic subjects after 12 weeks of consumption of synbiotic whole-grain pasta (Angelino et al., 2019).

3. MATERIAL AND METHOD

3.1. Material

The native corn starch and pregelatinized tapioca starch (Sinetex PE 20, Sino Thai Starch Co., Ltd., Thailand) used as the raw materials in the gluten-free pasta formulations were obtained from Sunar Group (Adana, Türkiye) and Sinerji Gida (Istanbul, Türkiye), respectively. The food additives used in gluten-free pasta formulations, such as xanthan gum (XN) (Ziboxan® LD, Deosen Biochemical (Ordos) Ltd.) and emulsifier (Palsgaard® SA 6610), were acquired from Azelis-Tunçkaya Chemical Substances (Istanbul, Türkiye). The durum wheat semolina, used in the control group in the first chapter of this study, was provided by Bagislar Flour Company (Adana, Türkiye). The other ingredients, which are present in both gluten-free and gluten-containing counterparts, as whole egg powder obtained by spray-drying sourced from *Gallus gallus* var. *domesticus*, were procured from Kor Agro Organic Food (Izmir, Türkiye), and iodized salt was bought from the local market. Two commercial inulins from chicory root with a low degree of polymerisation (DP) (Orafti® GR, DP≥10, Beneo GmbH, Germany) and high-DP (Orafti® HPX, DP≥23, Beneo GmbH, Germany) as the prebiotic source were supplied by Artisan Gida (Istanbul, Türkiye). The *Bacillus coagulans* GBI-30, 6086 (GanedenBC30®, Ganeden Biotech Inc., Maryfield Heights, OH) as a probiotic source with freeze-dried form was provided by Kerry Group (Istanbul, Türkiye).

3.2. Methods

3.2.1. Experimental Design

This study aims to produce and evaluate the extrusion and cooking properties of functional fresh gluten-free pasta (GFP) with prebiotic and probiotic sources, consisting of the three main chapters, as given below. To achieve this, the corresponding raw materials, additives, and other ingredients with their weight proportions according to the baker's ratio were shown in Table 3.1

i) 1st chapter: Determination of the extrusion and cooking properties with diferent intervals (0, 1, 5, 10, and 15 min) of GFP where native corn starch was partially substituted with pregelatinized starch (PGS) at 3 different concentrations (10, 20, and 30%) which were encoded as 10-PGS, 20-PGS, and 30-PGS, respectively, comparement with control group (C) made from durum wheat semolina (Table 3.1). Afterwards, hybrid multi-criteria decision making (HMCDM) was applied at the defined cooking time in order of analytical hierarchical process (AHP) and technique for order preference by similarity to ideal solution (TOPSIS) regarding extrusion (extrusion yield), technological (cooking loss, color, texture), and sensorial (taste) attributes to define the most appropriate GFP formulation for the following chapters of the present study.

ii) 2nd chapter: Incorporation of the short-chain of inulin with low degree of polymerisation (GR, DP≥10) and long-chain of inulin with high degree of polymerisation (HPX, DP≥23) as a prebiotic source, at two different ratios (4 and 8%) into the most appropriate GFP, which was defined in the 1st phase, were encoded as 4GR, 8GR, 4HPX, 8HPX, respectively, and evaluation of their extrusion and cooking properties.

iii) 3rd chapter: Addition of *B. coagulans* GBI-30, 6086 with a final concentration of 10^9 CFU/g as a probiotic source into inulin-enriched GFP formulation, which were encoded as P-4GR, P-8GR, P-4HPX, P-8HPX, and assessment of their extrusion and cooking properties against probiotic-fortified GFP without inulin (P0).

In this regard, the fresh GFP formula, including 15% whole egg powder, 2% xanthan gum, 1.5% salt, 0.5% emulsifier, and 60% water based on the weight of native corn starch, was replaced with 10, 20, and 30-PGS, as shown in Table 3.1. The whole dry ingredients and water ratio remained the same in the 2nd and 3rd chapters of the study, and enriched with inulin (4 and 8%) and probiotics with a final concentration of 10^9 CFU/g, respectively.

Table 3.1. The percentage weight fraction of ingredients used in the preparation of fresh pasta (%)*

Ingredients (%)	C	10-PGS	20-PGS	30-PGS
Durum wheat semolina	100	_	_	_
Native corn starch	_	90	80	70
Pregelatinized tapioca starch	_	10	20	30
Whole egg powder	15	15	15	15
Xanthan gum	_	2	2	2
Emulsifier	_	0.5	0.5	0.5
Salt	1.5	1.5	1.5	1.5
Water	40	60	60	60

* Weight proportions were given based on the baker's ratio

3.2.2. Fresh Pasta Production and Cooking Process

Both fresh durum wheat semolina-based pasta and GFP with/without prebiotic and probiotic sources were produced by using a benchtop pasta maker machine (HR2333/12, Viva collection, Philips Electronics Ltd., Eindhoven, Netherlands), providing a cold extrusion single-barrel system equipped with a spaghetti shaping disc (Appendix 3). The whole dry ingredients were premixed manually, and then transferred to the mixing chamber of the pasta machine. Later, tap water was poured gradually within the first 10 sec after the lid of the mixing chamber was attached. Then, the mixing process was automatically conducted and maintained at 3 min (1 cycle) in all pasta formulations, followed by an automated extrusion process. The spaghetti strains obtained in the first 10 sec were discarded, and then they were cutted as they exited from the extruder die whose time was defined according to the formulation, that is, 10, 20, and 30 sec for 10-PGS, 20-PGS together with durum with semolina pasta (C), and 30-PGS, respectively. Thereafter, fresh spaghetti strains were cut into small strips with a length of 5 cm by using a blade and wrapped with a plastic film to avoid drying until use for the cooking process (Appendix 3).

The weighted fresh pasta samples were transferred to a beaker filled with distilled boiled water without salt incorporation according to the AACCI method 66–05.01 guidelines, keeping the same rate of cooking water to pasta sample (25g /300 mL) (AACC, 1999). To prevent evaporation loss, maintain consistent temperature, and sustain the initial water volume, the beaker was covered with a watch glass (Appendix 3). The cooking process was materialized at about 100°C by using a magnetic stirrer with a heating plate (C-MAG HS-7, IKA-Werke GmbH and Co. KG, Germany), and the temperature was kept at this level for the entire cooking period, which lasted up to 15 min, and stirring was kept constant (mot 1) via using a magnetic stir bar. Finally, the cooked pasta was removed from the beaker, soaked in cold distilled water (4±1°C) to stop the cooking process, and then drained to remove excess water (Appendix 3). The values obtained at 10 sec at the beginning of cooking were labelled as 0 min. Cooking properties of pasta samples were generally measured at different time intervals (0, 1, 5, 10, and 15 min) to evaluate undercooking, optimum cooking, and overcooking conditions. The pasta production and cooking trial was done in duplicate for each pasta formulation.

3.2.3. Extrusion Parameters

Specific mechanical energy

The power values throughout the mixing (G_{mixing}) and extrusion processes ($G_{extrusion}$) were recorded individually by a powermeter (Uni-Trend Technology Co., Ltd, UT230B-EU, China) during the fresh pasta production, then averaged after subtracting the power consumed by the extruder in the process without pasta dough. The specific mechanical energy (SME) was obtained as given in Eq. 3.1 by proportioning the consumed mechanical energy and extrusion flow rate (de la Peña et. al, 2014; Sandhu et. al., 2015).

$$SME\ \left(\frac{J}{g}\right) = \frac{ME}{EFR} \tag{3.1}$$

ME= Mechanical energy (J/s)

EFR= Extrusion flow rate (g/s)

Residence time

The residence time (RT) is based on recording the total duration of the feed mixture passing from the extruder inlet to the die via a stopwatch (Attenborough et al., 2023). In this study, RT (min) was determined by excluding the standard mixing time (3 min) in all pasta formulations**.**

Extrusion yield

Extrusion yield (EY), which is one of the indicators of process efficiency, was calculated by dividing the total fresh pasta mass (Radwag AS 220.R2, Radom, Poland) obtained at the end of the extrusion process by the total weight of fresh pasta dough components (section 3.2.1, Table 3.1) in accordance with Eq. 3.2 (Zheng, 2018).

$$EY\ (\%) = \frac{m_{total}}{m_{dough}} * 100 \tag{3.2}$$

m_{total}: Total pasta weight (g)
m_{dough}: Pasta dough weight (g)

Extrusion flow rate

Extrusion flow rate (EFR) is necessary to obtain a uniform textural structure and a stable flow rate (Shukla et al., 2023). As a result of preliminary trials, the different intervals (10, 20, and 30 sec) were determined based on the total weight of fresh pasta samples and formulations (section 3.2.2), and the following equation (Eq. 3.3) was used for the calculation of average EFR as the extruded pasta mass per unit time (Shukla et al., 2023).

$$EFR\ \left(\frac{g}{min}\right) = \frac{m_m}{t} \tag{3.3}$$

m_m: Pasta weight obtained during the measurement period (g)

t: Extrusion cycle duration (min)

3.2.4. Physical Properties

Expansion ratio

The expansion ratio (ER), in other words radial expansion index, was obtained by measuring the average central diameter of the fresh pasta samples obtained during the extrusion with a digital caliper (AEK-Tech) and dividing the by the extruder die diameter, as given in Eq. 3.4 (Giménez et. al., 2013).

$$ER = \frac{R_p}{R_d} \tag{3.4}$$

ER: Expansion ratio

$R_{p=}$ Pasta diameter (mm)

$R_{d=}$ Spaghetti extruder die diameter (mm)

Cross-sectional expansion index

The cross-sectional expansion index (SEI) was calculated as given in Eq. 3.5, based on the average ER of the fresh pasta samples obtained during the extrusion (Alvarez-Martinez et. al., 1988).

$$SEI = ER^2 = \frac{{R_p}^2}{{R_d}^2} \tag{3.5}$$

SEI: Cross-sectional expansion index

R_p: Pasta diameter (mm)

R_d: Spaghetti extruder die diameter (mm)

Unit density

Fresh pasta samples obtained using the spaghetti extruder die were accepted as cylindrical (Mercier et al., 2011), and the average unit volume (V) values were calculated as given in Eq. 3.6 (Makdoud and Rosentrater, 2017; Paux and Rosentrater, 2018).

$$V\ (cm^3) = \pi \frac{{R_p}^2}{4} L \tag{3.6}$$

V: Unit volume of pasta (cm^3)

R_p: Pasta diameter (mm)

L: Pasta strand length (cm)

The average unit density (UD) value of fresh pasta samples was calculated according to Eq. 3.7 by dividing the pasta weight by the unit volume values obtained as given in Eq. 3.6 (Rosentrater et al., 2005; Makdoud and Rosentrater, 2017; Paux and Rosentrater, 2018).

$$\rho\left(\frac{g}{cm^3}\right)=\frac{m_p}{V} \quad (3.7)$$

ρ= Unit density of fresh pasta (g/cm^3)

$m_{p=}$ Unit weight of fresh pasta (g)

V= Unit volume of fresh pasta (cm^3)

Specific length

The average specific length (SL) values were calculated as given in Eq. 3.8, based on the proportion of the lengths of fresh pasta strain to their unit weight values.

$$SL\left(\frac{cm}{g}\right)=\frac{L}{m_p} \quad (3.8)$$

L: Pasta strand length (cm)

m_p: Unit weight of fresh pasta (g)

3.2.5. Cooking Properties

Moisture

According to AACC, Method 44-01.01, the moisture content (%) was calculated based on the weight difference resulting from the drying of cooked fresh pasta for different times (0, 1, 5, 10, and 15 min) in the drying oven (Binder FD53, Binder GmbH, Tuttlingen, Germany) at 105ºC until it reached its fixed weight (AACC, 1999).

Water absorption capacity

Fresh pasta samples cooked at different times (0, 1, 5, 10, and 15 min) based on AACC Method 66-50.01 were soaked with distilled water (≈4°C), then weighed (Radwag AS 220.R2, Radom, Poland) at room temperature, and the weight gain was calculated for water absorption capacity (WAC) measurement (Eq. 3.9) (Milde et al., 2020).

$$\text{WAC (\%)}=\frac{\text{Cooked pasta weight - Uncooked pasta weight}}{\text{Uncooked pasta weight}}*100 \quad (3.9)$$

Swelling index

The swelling index (SI) was calculated according to Eq. 3.10 as given below, by drying (Binder FD53, Binder GmbH, Tuttlingen, Germany) the fresh pasta samples cooked at different times (0, 1, 5, 10, and 15 min) at 105 ºC until constant weighing weight, based on AACC Method 66-50.01 (Rachman et al., 2019).

$$\text{SI (g/g)}=\frac{\text{Cooked pasta weight - Dried cooked pasta weight}}{\text{Dried cooked pasta weight}} \quad (3.10)$$

Cooking loss

The cooking loss (CL) was calculated according to Eq. 3.11 as given below (Raungrusmee et al., 2020) by drying at 105ºC (Binder FD53, Binder GmbH, Tuttlingen, Germany) of the samples

(5 mL) taken from the cooking water of fresh pasta samples cooked for different times (0, 1, 5, 10, and 15 min) based on AACC Method 66-50.01.

$$\text{CL (\%)}=\frac{\text{Residue weight after drying (g)}}{\text{Uncooked pasta weight (g)}}*100 \quad (3.11)$$

Volume increase

Fresh pasta samples cooked at different times (0, 1, 5, 10, and 15 min) based on AACC Method 66-50.01 were placed in a measuring cylinder containing vegetable oil (15 mL), the increase in oil level and sample volume were recorded and the volume increase (VI) was calculated (Eq. 3.12) (Levent, 2017).

$$\text{VI (\%)}=\frac{\text{Cooked pasta volume - Uncooked pasta volume}}{\text{Uncooked pasta volume}}*100 \quad (3.12)$$

Turbidity

The absorbance values of the cooking water of fresh pasta samples cooked at different times (0, 1, 5, 10, and 15 min) based on AACC Method 66-50.01 were recorded at 675 nm using a UV-Vis spectrophotometer (UV Mini-1240, Shimadzu, Japan) for turbidity measurement (Kim et al., 2014) after vortexed (IKA MS2 Mini Shaker, Wilmington, NC, USA).

Amylose leaching

Amylose leaching value was measured using a UV-Vis spectrophotometer (UV Mini-1240, Shimadzu, Japan) at 650 nm after vortexed (IKA MS2 Mini Shaker, Wilmington, NC, USA) of 1 mL cooking water taken from fresh pasta samples cooked for different times (0, 1, 5, 10, and 15 min) based on AACC Method 66-50.01, treated with 1 mL of iodine solution (2.0 g of KI plus 0.2 g of I_2 made up to 100 ml), and completed with 25 mL of distilled water (Susanna and Prabhasankar, 2013).

Color

Raw and pasta samples cooked for different times (1, 5, 10, and 15 min) based on AACC Method 66-50.01 were placed in petri dishes, and L*, a*, b* were recorded using a colorimeter (Hunter colorimeter Model D-25 optical Sensor, Hunter Associates Laboratory Inc., Reston, VA., USA). The chroma (C*), hue (h°) (Pessanha et al., 2023), yellowness index (YI) (Shah et al., 2019), color score (Jalgaonkar and Jha, 2016), and color change ($\Delta E^*_{cooking}$) (Lin et al., 2017) values based on the difference between the average color values (L_0^*, a_0^*, b_0^*) of raw pasta samples was calculated according to the equations as given below, Eq. 3.13, Eq. 3.14, Eq. 3.15, Eq. 3.16, Eq. 3.17, respectively.

$$C^*=\sqrt{(a^*)^2+(b^*)^2}, \quad (3.13)$$

$$h°=\arctan\frac{b^*}{a^*} \quad (3.14)$$

$$YI=\frac{142.86*b^*}{L^*} \quad (3.15)$$

$$\text{Color score}=\frac{L^*+(2b^*)}{20} \quad (3.16)$$

$$\Delta E^*_{cooking}=\sqrt{\left(L^*-L_0^*\right)^2+\left(a^*-a_0^*\right)^2+\left(b^*-b_0^*\right)^2}, \quad (3.17)$$

Texture

Based on slightly modified AACC Method 66-50.01 (AACC, 1999), texture profile analysis (TPA) was performed in cooked fresh pasta using a texture analyzer (TA.XT Plus, Stable Micro Systems, Godalming, UK) with an integrated P/36R probe and 5 kg load cell. The trigger force and pre-test speed, test speed, and post-test speed values were 10 g and 2 mm/s, respectively. In this regard, 5 number of cooked spaghetti strips (≈5 cm-long) were placed in parallel rows side by side on the plate, and double compression with 75% strain was applied, then the TPA parameters (hardness, chewiness, cohesiveness, springiness, and resilience) were determined from force-time curves (Kim et al., 2017).

Optimum cooking time

The optimum cooking time (OCT) of fresh pasta samples was determined using the modified AACC method 66-50.01 (AACC, 1999). Accordingly, a fresh pasta sample was taken each minute during 15 min, and after being immersed in cold distilled water (≈4°C), equal pressure was applied between two microscope slides (25.4*x*76.2 mm in size and 1-1.2 mm in thickness). After compression, the OCT (min) was determined when the white axis in the center disappears.

Differential scanning calorimetry

The thermal properties of raw fresh pasta samples were detected according to slightly modified Sutheeves et al. (2020) by differential scanning calorimetry (DSC 3^+ with FRS 5 + sensor, Mettler Toledo Inc., Columbus, OH, USA). An empty pan was utilized as a reference and calibrated with indium. The raw fresh pasta samples were placed and weighed in aluminum pans after being exposed to grinding and sieving. Then, they were hermetically sealed and inserted into the DSC. The samples were heated from 20°C to 130°C at a rate of 10°C/min, and then the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy change (ΔH) were determined from calorimeter curves.

X-ray diffraction pattern

The relative crystallinity of freeze-dried (FD8512, Ilshin Biobase, Kyunggi-do/Yangju-si/ Gyeonggi-do, Korea), grinded (KM75, Krups, Solingen, Germany), and sieved raw and 5 min-cooked fresh pasta samples were characterized according to the method of Yang et al. (2019) with minor modifications by using an X-ray diffractometer (Empyrean, PANalytical, Almelo, Netherlands), operated at 40 kV and 40 mA equipped with Cu Kα radiation (λ = 1.54 Å). The X-ray diffraction patterns were obtained in the region of the diffraction angle (2θ) from 3° to 40° at ambient temperature with a step of 0.05 and a scanning rate of 2°/min.

Microsturcture

To define the microstructure, first of all, 5 min of cooked fresh pasta samples were frozen at -80°C for 48h, then freeze-dried (FD8512, Ilshin Biobase, Kyunggi-do/Yangju-si/ Gyeonggi-do, Korea) lasted 24 h below 0.1 mPa, and milled by a grinder (KM75, Krups, Solingen, Germany). Afterwards, the corresponding samples were coated with gold–palladium to make them conductive. After sputtering with gold up to 15 nm (Quorum, Q150R ES, UK), specimen fields were screened under a high-vacuum Quanta 650 Field Emission scanning electron microscope (SEM) at 20 kV, and micrographs were obtained at a magnification of 3000 or 5000 times.

Viability of the probiotic cell

To determine the *B. coagulans* count in 5 min and 10 min cooked probiotic-added fresh GF pasta samples, 10 g of sample was aseptically weighed into a sterile stomacher bag (Interscience, Bag Light-400, France) including 90 mL of sterile diluent (0.85% NaCl), and then homogenized for 2 min using Bag Mixer (Interscience, Model 400P, St Nom, France). After that, samples were 10-fold serially diluted, aliquots of the decimal dilutions spread onto Glucose Yeast Extract agar (HiMedia, Mumbai, India), incubated for 48-72 h at 37°C to allow the growth, and then the mean viable typical probiotic count was enumerated and expressed as log CFU/g, based on the modified method of Fares et al. (2015).

To evaluate the enumaration accuracy and assess the potential of automated counting of bacterial colonies via Image J software program (ImageJ 1.47v, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) in contrast to plating method, petri images were captured by a digital camera, and then converted to 8 bits (gray level) from the original RGB image by the Color Space Converter plugin by using the ImageJ program. Afterwards, segmentation was carried out according to Otsu’s thresholding method, and the total number of probiotic colony cells was identified by the corresponding software.

The viability of *B. coagulans* in 5 min and 10 min cooked GFP samples were measured with respect to initial counts before cooking (Eq. 3.18), where N is the log cell number of viable bacteria per log CFU/g after cooking at the corresponding times, and N_0 is the log cell number of bacteria per log CFU/g before cooking, which was adapted from Rajam et al. (2015).

$$\text{Viability (\%)}=\frac{N}{N_0}*100 \tag{3.18}$$

Sensory analysis

In the first and second stages of sensory evaluation, the cooking status and cooking time preferences were determined, respectively, by using the cooking degree description (Table 3.2) in fresh pasta samples cooked at different times (1, 5, 10, and 15 min) in salt-incorporated boiled tap water accounting for 12.7 g/L (Bianchi et al., 2019), according to the “International Pasta Organisation.” After that, the sensorial attributes, such as structural integrity, color, texture, taste, and overall acceptability of fresh pasta samples cooked at the determined cooking time, were assessed by a total of 11 panelists from the Department of Food Engineering, Cukurova University, based on a 9-point hedonic scale in which 1 refers to "dislike very much" and 9 refers to "like very much" in the 3rd stage of sensory evaluation.

Table 3.2. Cooking degree levels and descriptions for sensory analysis

Cooking degree level	Cooking degree description	Cooking degree level	Cooking degree description
1	Raw	**6**	Medium-cooked
2	Uncooked	**7**	Cooked
3	Under-cooked	**8**	Well-cooked
4	Slightly-cooked	**9**	Over-cooked
5	Al-dente	**10**	Disintegrated

3.2.6. Hybrid multi-criteria decision-making

The AHP method was carried out for the determination of the relative importance of each criteria that influence the pasta quality by weighting a pairwise comparison matrix. In this context, a hierarchical structure was constituted (Figure 3.1) after identifying the aim, which is the best pasta formulation regarding different criteria, such as extrusion yield, cooking loss, color (yellowness index), texture (hardness), and taste. After the AHP method was applied, the technique for order of preference by similarity to ideal solution (TOPSIS) method was conducted to obtain the final ranking of pasta formulations (Yazici et al., 2023d).

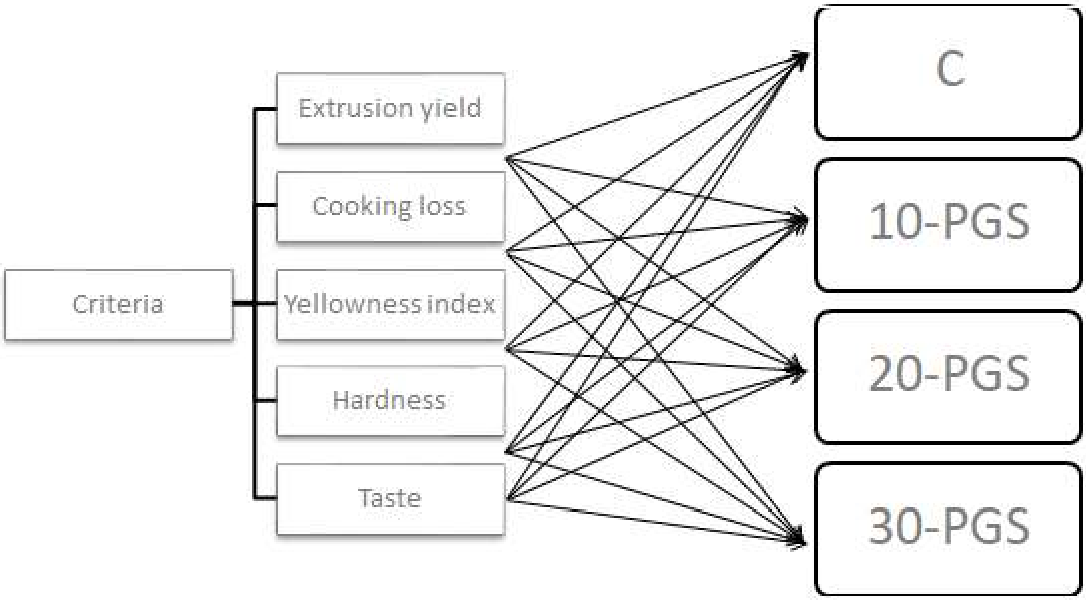

Figure 3.1. The structure of the decision hierarchy for selection by the AHP method

Analytic hierarchy process (AHP)

i) Pair-wise comparison matrix establishment

The pair-wise comparison matrix (*n x n*) was formed as given below, regarding the numerical rating between the criteria in terms of their relative importance as given in Table 3.3.

$$X_{ij} = \begin{bmatrix} 1 & x_{12} \dots & x_{1n} \\ 1/x_{12} & 1 \dots & x_{2n} \\ \vdots & \vdots & \vdots \\ 1/x_{1n} & 1/x_{2n} \dots & 1 \end{bmatrix}$$

ii) Comparison matrix normalization

As expressed in Eq. 3.19, a normalized comparison matrix was acquired from the division of each element of the column by the sum of the criteria per column.

$$n_{ij}=\frac{x_{ij}}{\sum_{i=1}^{n} x_{ij}} \quad j= 1, 2, \ldots, n \tag{3.19}$$

Table 3.3. Pairwise comparison rating scale of relative importance for the AHP method

Definition	Importance intensity
Equally importance	1
Moderately importance	3
Strongly importance	5
Very strongly importance	7
Extremely importance	9
Intermediate values between two adjoining judgments	2,4,6,8
Reciprocals for inverse comparison	

iii) Criteria weights calculations

The weights of criteria (W_i) were attained from the eigenvector of the comparison matrix based on the values that indicated the significance of particular process and quality attributes, obtained with the calculation of the average of the sum of all the criteria values in each row of the normalized comparison matrix (Eq. 3.20).

$$W_i=\frac{\sum_{j=1}^{n} n_{ij}}{n} \quad i= 1, 2, \ldots, n \tag{3.20}$$

iv) Consistency index and consistency ratio calculations and evaluation

The consistency ratio (CR), which should not be higher than 0.1, was calculated (Eq. 3.21) based on the values of the consistency index (CI) (Eq. 3.22), where λ_{max} is the highest eigenvalue of the matrix, and the random index (RI), as given in Table 3.4.

$$CR=CI/RI \tag{3.21}$$

$$CI=(\lambda_{max}-n)/(n-1) \tag{3.22}$$

Table 3.4. Random consistency index values

n	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
RI	0	0	0.58	0.90	1.12	1.24	1.32	1.41	1.45	1.51

Technique for order of preference by similarity to ideal solution (TOPSIS)

i) Decision-matrix establishment

The results of extrusion parameter (extrusion yield), technological properties (cooking loss, yellowness index, and hardness), and sensorial attribute (taste) of all pasta samples were used in the TOPSIS methodology for establishing the decision matrix, where A_{ij} is the decision matrix, *m,* and *n* are the decision points, and evaluation indicators, respectively.

$$A_{ij} = \begin{bmatrix} a_{11} & a_{12} \cdots & a_{1n} \\ a_{21} & a_{22} \cdots & a_{2n} \\ \vdots & \vdots & \vdots \\ a_{m1} & a_{m2} \cdots & a_{mn} \end{bmatrix}$$

ii) Decision matrix normalization

The normalized values were figured out (Eq. 3.23), and then the normalized decision matrix (r_{ij}) was generated, where r_{ij} is the normalized value and a_{ij} is the real value of the criteria, as given below.

$$r_{ij}=\frac{a_{ij}}{\sqrt{\sum_{i=1}^{m} a_{ij}^2}} \quad i = 1, 2,\ldots, m; j=1, 2,\ldots, n \tag{3.23}$$

$$r_{ij} = \begin{bmatrix} n_{11} & n_{12} \cdots & n_{1n} \\ n_{21} & n_{22} \cdots & n_{2n} \\ \vdots & \vdots & \vdots \\ n_{m1} & n_{m2} \cdots & n_{mn} \end{bmatrix}$$

iii) Weighted normalized decision-matrix establishment

The weighted normalized values were defined (Eq. 3.24), and then the weighted normalized decision matrix was established, where V_{ij} is the weighted normalized value, and W_{ij} is the weight of each criterion attained by the AHP method, as defined previously.

$$V_{ij}=W_{ij} * r_{ij} \tag{3.24}$$

iv) The positive (A^+) and negative ideal solution (A^-) determination

The positive (A^+) and negative (A^-) ideal solutions were determined using Eq. 3.25 and Eq. 3.26, respectively, where J is the benefit criteria (extrusion yield, color, texture, and taste) for maximization, and J' is the non-benefit criteria as cooking loss.

$$A^+ = \left\{\{max\, V_{ij} \middle| j \in \right\} J\}, \{min\, V_{ij}\; j \in J'\} \tag{3.25}$$

$$A^- = \left\{\{min\, V_{ij} \middle| j \in \right\} J\}, \{max\, V_{ij}\; j \in J'\} \tag{3.26}$$

v) Distance from the positive and negative ideal solutions determination

The distance from the positive (S_i^+) and negative ideal solutions (S_i^-) was identified based on Eq. 3.27 and Eq. 3.28, respectively.

$$S_i^+=\sqrt{\sum_{j=1}^{n} (V_{ij}-V_j^+)^2} \tag{3.27}$$

$$S_i^-=\sqrt{\sum_{j=1}^{n} (V_{ij}-V_j^-)^2} \tag{3.28}$$

vi) Relative closeness to the ideal solution measurement and final ranking of alternatives

The ranking of the pasta alternatives was determined based on performance index (P_i) values (3.29), which should be between 0 to 1 values, and higher values reflect that the option is closer to the optimal level.

$$P_i = S_i^- / (S_i^- + S_i^+) \quad (3.29)$$

3.2.7. Statistical analysis

All statistical analyses were performed using SPSS 20 statistical package (SPSS Inc., Chicago, IL, USA). Continuous variables were summarized as mean and standard deviation. The statistical level of significance for all tests was considered to be 0.05. For comparison of continuous variables between two groups, the Student's t-test or Mann-Whitney U test was used, depending on whether the statistical hypotheses were fulfilled or not, respectively. For comparison of more than two groups, One-way ANOVA or Kruskal-Wallis test was utilized, depending on whether the statistical hypotheses were provided or not, respectively. For the normally distributed data, Tukey, Games & Howell tests were applied for multiple comparisons of groups, regarding the homogeneity of variances. For non-normally distributed data, the Bonferroni-adjusted Mann-Whitney U test was conducted for multiple comparisons of groups. To evaluate the change in the measurements obtained in the time interval, the Repeated Measurements Analysis was applied. To evaluate the correlations between measurements, the Pearson Correlation Coefficient or Spearman Rank Correlation Coefficient was implemented, depending on whether the statistical hypotheses were achieved or not, respectively.

4. RESULTS AND DISCUSSIONS

4.1. Effect of Pregelatinized Starch on Extrusion Parameters of Gluten-free Pasta

4.1.1. Energy-related Properties

The controlled variables, such as product temperature, die pressure, torque, and specific mechanical energy (SME), are important indicators for the extrusion process (Fayose and Huan, 2015). The values of torque and mass output could vary from extrusion condition, even if running at full capacity with the feed rate. Because the mechanical action is directly associated with die pressure and SME throughout the extrusion system (Merayo et al., 2011). SME is defined as the amount of consumed energy per unit mass of extrudate in the extrusion system (de la Peña et al., 2014). It measures the competing influence change in viscosity because of alteration in the screw speed (pseudoplastic behavior) consequent on torque change that is needed to convey dough throughout the extruder (Rosentrater et al., 2005). Therefore, it is correlated with both physicochemical alteration in the end-product, which refers to the molecular breakdown during extrusion (Jia et al., 2023). It also influences the extrudates' mechanical and expansion attributes (Ryu and Ng, 2001). In this regard, the recorded energy values during mixing (G_{mixing}) and extrusion process ($G_{extrusion}$), and also SME values for the production of fresh semolina and GFP with different rates of pregelatinized starch (PGS) were given in Table 4.1.

Table 4.1. Energy-related extrusion values of semolina- and gluten-free pasta with pregelatinized starch

Sample	G_{mixing} (W)	$G_{extrusion}$ (W)	SME (J/g)
C	29.84±1.76^{a}	90.14±1.25^{a}	93.70±1.12^{a}
10-PGS	20.80±0.50^{c}	61.63±0.81^{d}	54.28±4.39^{c}
20-PGS	21.81±0.52^{c}	71.72±0.76^{c}	75.49±2.16^{b}
30-PGS	25.60±1.20^{b}	78.46±0.79^{b}	92.06±2.44^{a}

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$).

According to the achieved results, G_{mixing}, $G_{extrusion,}$ and SME values of all gluten-free pasta (GFP) samples ranged from 20.80-25.60 W, 61.63-78.46 W, and 54.28-92.06 J/g, respectively (Table 4.1). In this context, the $G_{extrusion}$ values of all pasta samples were almost tripled in regards to being those of the G_{mixing} values. In addition, the $G_{mixing,}$ $G_{extrusion,}$ and SME values were increased regarding the increase in the concentration of PGS ($p<0.05$) (Table 4.1). In a study, it was revealed that pregelatinization of red lentil flour for GFP production enhanced dough consistency, which in turn is expected to offer a high resistance during the extrusion process, indicating a higher SME (Bresciani et al., 2023). This phenomenon could also be related to the reduction in raw moisture content accepted equal to the feed material of the corresponding samples, as demonstrated in the following section 4.2.1 (Table 4.4). Therefore, a negative correlation was detected between SME and raw moisture content of pasta in the 1st chapter of this study ($r=-0.93$). Because the high moisture could cause a lubricant effect, ending in less shearing stress, thereby less energy transfer

(Giménez et al., 2013; Bouasla et al., 2017a). Moreover, rising in hydration level as moisture content increases is also related to a reduction in dough viscosity, and less friction between the interior barrel surface with pasta dough, and thus less required energy for extrusion (Manthey et al., 2004). A similar behaviour was reported, that as the moisture content increased in gluten-free spaghetti based on corn flour obtained from both soft and hard endosperm, SME values declined. It was attributed to increases in the friction level due to the decline in the moisture of the feed and the temperature of the extrusion process (Merayo et al., 2011). On the other side, it was stated that some hydrocolloid-containing doughs, such as xanthan gum (XN) and guar gum need for a high extrusion force and increase in extrusion torque for extruded snack production when the constant hydration was provided (Gasparre and Rosell, 2019), in GF products which are lacking the unique strength and elasticity properties of gluten proteins (Zhu et al., 2023). Moreover, it was also declared that the cereal-based food products demonstrate shear-thinning behavior under shear, whereas the cassava-based ones display shear-thickening with viscosity increase under increased extrusion time (Fayose and Huan, 2015). However, the G_{mixing}, $G_{extrusion,}$ and SME values of the control group based on durum wheat semolina were significantly higher in comparison with GFP counterparts ($p<0.05$) (Table 4.1), which could be attributed to its strength of gluten proteins. As a result of this, it could be interpreted that higher overall operational costs during the extrusion process (Sinaki and Koksel, 2024) were associated with semolina-based control pasta due to its higher SME values in contrast to GFP.

4.1.2. Efficiency-related Properties

Due to the extruder is not totally adiabatic, and also knowing that energy consumption could be materialized by both internal system and external sources, it is not expected that an extruder could work with 100% efficiency (Fayose and Huan, 2015). The throughput ratio of feed material is generally measured (Rosentrater et al., 2005) for defining extrusion yield (EY), thereby giving an expression for production rate (Anand, 2020). On the other side, extrusion flow rate (EFR) is defined as the ratio of extrudate weight to a particular periodic time from the die exit (Anand, 2020). Therefore, the EY, EFR, together with the residence time (RT) of all control and GFP samples, were shown in Table 4.2.

Table 4.2. Extrusion efficiency values of semolina- and gluten-free pasta with pregelatinized starch

Sample	RT (min)	EY (%)	EFR (g/min)
C	4.81±0.31^{b}	76.47±1.56^{b}	86.57±3.67^{a}
10-PGS	5.61±0.47^{b}	63.91±3.27^{c}	78.89±1.25^{b}
20-PGS	6.92±0.09^{a}	74.23±.2.00^{b}	66.43±2.21^{c}
30-PGS	7.84±0.54^{a}	86.34±2.34^{a}	47.15±2.94^{d}

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$).

In our study, EY as an indicator of process efficiency was varied from approximately 64% to 86% in GFP samples, as presented in Table 4.2. The GFP, including 30-PGS, exhibited the significantly highest EY ($p<0.05$), which could be expounded to the higher PGS amount in the corresponding formulation, which also resulted in a prolonged RT value. Therefore, a decrease in EY was observed in the case of the reduction in PGS ratio ($p<0.05$) (Table 4.2). This could be dedicated to the softening effects because of the higher water amount, and thus higher hydration levels as the PGS level decreased (section 4.2.1, Table 4.4), which possibly resulted in slippage of the dough at the surface of the barrel (de la Peña et al., 2014). It was also stated that elevated feed rate affects the fill degree and RT, in which the starch network degradation alters the rheological properties, and thus gives rise to the elastic impact and effect of the bulk density and expansion ratio of the end product (Sajid Mushtaq et al., 2023), the latter was discussed in section 4.1.3 (Table 4.3). However, the efficiency of durum wheat semolina pasta as a control group was higher than that of GFP samples, except for 30-PGS. It was stated that the process efficiency of cereal-based products is higher than that of cassava-based ones. This was announced as being difficult to process this kind of viscous material, typical for them throughout a dry-type extruder (Fayose and Huan, 2015).

The EFR values of GFP samples were between nearly 47 and 79 g/min, as demonstrated in Table 4.2. Based on these results, the EFR values of GFP were decreasing as the PGS amount increased, and those of GFP were lower than the control group (C). It could be associated with the hypothesised that the adverse relation between RT and screw speed of the extruder. In this regard, the control group made with durum wheat semolina had the lowest RT (Table 4.2), which stands for possibly having the highest screw speed. Moreover, it was declared that there is a positive correlation between screw speed and mass flow rate (Rosentrater et al., 2005). In this regard, the reduction in the EFR based on the increase in the level of PGS could be explained by incremental RT with a negative correlation between them ($r=-0.94$) in the 1st chapter of this study.

4.1.3. Dimensional Properties

The higher temperature, shear strain, and stress through the extrusion process could have an influence on the complex interplay between the chemical compounds, and thus change the eventual internal cellular structures that happen during the water evaporation output of the extruder die. Therefore, this brings about the expansion in the end-product from the die, which could be measured for the expansion ratio (ER) (Rosentrater et al., 2005). The ER, together with the bulk density, which have a strong negative relationship between them, play an important role in extruded products' acceptability (Sajid Mushtaq et al., 2023). The ER, in other words, radial expansion, is an expansion indicator by starch gelatinization, which is majorly subject to the starch composition of the feed material (Rosentrater et al., 2005). The ER generally depends on feed moisture content and extrusion temperature. Higher moisture level alters the molecular structure of amylopectin during the extrusion process and decrease the elasticity of starch-based feed materials via the plasticizing impact. As a result of this, while density is increased, the expansion ratio of extrudates is decreased (Sajid Mushtaq et al., 2023) by softening of molecular structures

(Rosentrater et al., 2005). On the other side, the cross-sectional expansion index (SEI) is described as the proportion of the diameter of the product to the extruder die (Anand, 2020), and also indicates radial expansion of extrudates similar to the ER (Varsha and Mohan, 2016). In our study, the cold-extrusion process, which is defined as a push-through compressive forming process at ambient temperature, was applied to all pasta samples under the same conditions. Due to this, the effect of such factors as temperature, the nozzle, and atmospheric pressure on dimensional properties could be negligible in the present study. The dimensional properties of semolina pasta as a control group and GFP with pregelatinized starch at different percentages (10, 20, and 30%) were given in Table 4.3.

Table 4.3. Dimensional properties of raw semolina- and gluten-free pasta with pregelatinized starch

Sample	**ER**	**SEI**	**UD (g/cm^3)**	**SL (cm/g)**
C	1.41±0.03^{bc}	1.81±0.09^{c}	1.28±0.03^{bc}	23.35±0.57^{a}
10-PGS	1.32±0.05^{c}	1.74±0.13^{c}	1.46±0.09^{a}	20.00±0.28^{b}
20-PGS	1.48±0.07^{b}	2.20±0.21^{b}	1.37±0.04^{ab}	17.81±0.42^{c}
30-PGS	1.77±0.02^{a}	3.14±0.07^{a}	1.13±0.07^{c}	14.23±0.40^{d}

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$).

The ER and SEI values of GFP were in the range of 1.32-1.77 and 1.74-3.14, respectively, as summarized in Table 4.3. It was reported by several researches that the gelatinized starch has a significant role in expansion (Anand, 2020). The greater geletainization degree is related to higher expansion (Giménez et al., 2013), which is in agreement with our results. In this regard, there was a significant increment in expansion pattern regarding both ER and SEI values of GFP, including higher PGS levels (20 and 30%) compared with that of the control pasta with durum wheat semolina ($p<0.05$) (Table 4.3). Besides, it was indicated that an increase in SME values resulted in increased SEI values for whole corn meal and wheat flour. It was also implied that the greater the expanded products' need for higher SME inputs (Ryu and Ng, 2001). In this context, a similar positive relationship between SME and SEI was also observed in our results, as the PGS level increased (section 4.1.1, Table 4.1). In addition, it was indicated that the volumetric expansion, which quantifies the multiplied effect of both longitudinal and radial expansion of extrudates after it has totally come outside the extruders' die, could be adversely affected by high feed moisture levels through extrusion (Varsha and Mohan, 2016). The viscosity might have dominated the expansion, and the moisture could be one of the major parameters influencing the expansion (Giménez et al., 2013), under the circumstances of cold-extrusion in this study. Therefore, an increase in the PGS concentration in GFP and thus reduced raw moisture content, which is accepted as similar to feed material (section 4.2.1, Table 4.4), likely increased the diameter of GFP samples, which in turn both corresponding ER and SEI values, as well.

The unit density (UD) measures the extrudate's weight per unit volume, which is another quantitative parameter of internal structure. However, the UD values are partially lower than the corresponding true density values because of potentially having entrained pores in extrudates (Rosentrater et al., 2005). The UD values of fresh GFP samples were between the range of 1.13-1.46 g/cm^3, as presented in Table 4.3. As for those results, the increase in the replacement ratio of native corn starch with PGS in GFP resulted in a decrease in UD ($p<0.05$). In a study, it was stated that the values of UD reduced as the volumetric expansion increased in the extrudates based on soybean meal-added corn masa by-product (Rosentrater et al., 2005). Therefore, a negative correlation between UD and ER was found out in the1st chapter of this study (r=-0.88). This pattern could be explained by the fact that more expansion in either radial or longitudinal direction implies a lesser density, which refers to a greater degree of starch gelatinization (Minweyelet et al., 2021; Awol et al., 2024), also aligns with their radial expansion-related values (ER and SEI).

The specific length (SL) is described as the length per unit weight for extrudates (Anand, 2020). It is valued as an index of the linear expansion per unit mass (Awol et al., 2024), which is useful to define product quality regarding its longitudinal expansion. The SL values of all pasta samples were given in Table 4.3. It is accepted that the higher SL values are ambitioned for the greater quality of the end-product (Varsha and Mohan, 2016). Based on the results, the highest SL values corresponding to 23.35 cm/g were found in the control group made from durum wheat semolina had the best quality in contrast to GFP. On the other hand, the SL values of fresh GFP samples were between the range of 14.23-20.00 cm/g (Table 4.3), and a significant decrease was detected with the rise in the replacement ratio of PGS with native corn starch ($p<0.05$). This is in agreement with the idea that the reduction in SL depends upon the decrease in screw speed of corn grits (Minweyelet et al., 2021), as explained previously (section 4.1.2). A negative correlation was identified between ER and SL in 1st chapter of this study (r=-0.79). Moreover, our SL results were in accordance with the phenomena based on the increase in SME values, bringing out the reduction in being values of SL (Ryu and Ng, 2001). In addition, a positive relation between SL with feed moisture (Ryu and Ng, 2001; Anand, 2020) and screw speed (Minweyelet et al., 2021), but a negative association with expansion (Ryu and Ng, 2001), was also consistent with our results regarding GFP, except control sample (C). This exception could arise from a lower initial water level (40%) in the formulation of durum wheat semolina-based control pasta section 3.2.1 (Table 3.1). When compared to the expansion pattern of durum wheat semolina and the GFP based on the combination of native corn starch and PGS, while the semolina was expanded in the axial direction, the gluten-free mixture was generally highly expanded in the radial direction. These results demonstrated that the expansion direction was different between durum wheat semolina pasta and gluten-free counterparts under these tested conditions**.**

4.2. Effect of Pregelatinized Starch on Cooking Properties of Gluten-free Pasta

Pasta is a complex food consisting of several compounds that are kept together by many interaction types, thereby generating an extensive microstructure variety, which could specify the end-product quality. Designating the pasta compounds is needed for finding out their nutritional, physicochemical, technological, and sensory attributes. Moreover, cooking pasta is also a determinant step that could alter pasta from high quality to poor quality in the case that the wrong cooking process is utilized (Lorenzo et al., 2018). The water is a good plasticizer for protein and starch, thereby making a strong depressing influence on glass transition (Lorenzo et al., 2018). When the water temperature reaches about 100°C through the cooking process, the pasta hydration eventuates via a process controlled by diffusion (Lorenzo et al., 2018). The starch granules undergo water absorption and swelling through the pasta cooking process. Two endothermic transitions composed of starch gelatinization followed by dissociation of amylose-lipid complexes are materialized as the cooking temperature increases (Steglich, 2013). The temperature-moisture conditions trigger starch gelatinization, which is the main alteration in its fraction by cooking. In the beginning, penetration of the water into native starches' amorphous growth occurs pursued as heating is maintained with hydration of the intercrystalline amorphous phase. Consequently, more water binds to starch as a result of an increase in the water-binding capacity of starch granules. The developed viscoelasticity and solubilization of starch are subordinate to the starch gelatinization process. In addition, water uptake into the matrix gives rise to a marked softening. This phenomenon makes a porous complex structure, including filamentous networks and swollen granules, which forms a honeycomb-like structure within the pasta's internal structure while partially and/or totally cooking regarding their microstructural change (Lorenzo et al., 2018). The hydration features do not just depend on gelatinized and damaged starch during cooking, but also on the resistance offered via the structures generated during the extrusion process (Giménez et al., 2013).

The penetration of water is materialized from the fissures existing on the surface of pasta to the protein matrix. As a result of this, the development of a smooth surface, protein hydration, volume expansion, and formation of additional fractures by produced tension take place (Lorenzo et al., 2018). In addition, the polymerization of gluten with starch gelatinization co-occurs in pasta cooking (Steglich, 2013). The water absorption on the part of the protein matrix produces a mobile diffusion between the surface and center through gelatinization (Lorenzo et al., 2018). This refers to the competition regarding not only water absorption but also penetration into the pasta. It is put forward that the large voids emerge round about swollen starch granules and attenuate in the intermediate part, and then fusing resulted in a discontinued and unsufficient protein network. The disintegration of starch granules and thus amylose leaching is observed to depend on the swelling, resulting in over-cooking loss and an increase in stickiness as a textural attribute. This phenomenon is related to the protein network, which could hinder the dissolution and leakage of starch granules.

On the other hand, it is declared that there is a two-stage transformation characterized by different diffusion coefficients that takes place in pasta cooking. In this context, while the starch gelatinization, which is based on a hydration-induced process in the outer layer, the heat-driven crystallite melting of come about in the pasta centre (Steglich, 2013).

As a consequence of the influence of heat and water uptake, such as starch gelatinization, protein swelling, rise in pasta's volume and weight, many reactions are generated through pasta cooking (Larrosa et al., 2016). In this regard, to assess the perception of pasta quality, examination of cooking behaviour is a critical step (Fradinho et al., 2019). In addition, the cooking time is the factor that defines the duration pasta requires to be in contact with boiling water for cooking and consuming (Milde et al., 2020). Therefore, in this study, the moisture, water absorption capacity, swelling index, cooking loss, volume increase, turbidity, and amylose leaching properties were investigated through 15 min of cooking in pasta samples with different time intervals (0, 1, 5, 10, and 15 min). The potential variations in this study, with literature about cooking behaviour, would be attributed to different ingredients utilized in the GFP formulations, the particular starch network formed in each sample through the pasta-making step, and different protein sources, rather than gluten, have ability to cooparate with starch for generating the final structure capable to preserve soluble compunds through cooking (Lucisano al., 2012).

4.2.1. Moisture

The moisture content of raw and cooked fresh pasta samples during 15 min of cooking with 5 different time intervals (0, 1, 5, 10, and 15min) was given in Table 4.4.

Table 4.4. Moisture contents of raw and cooked semolina- and gluten-free pasta with pregelatinized starch (%)

Cooking time (min)	C	10-PGS	20-PGS	30-PGS
Raw	31.89±0.18^{Fc}	37.06±0.20^{Fa}	36.57±0.41^{Ea}	35.02±0.41^{Db}
0	55.42±0.69^{Eb}	57.27±0.50^{Eb}	60.17±0.47^{Da}	50.89±1.40^{Cc}
1	63.09±0.84^{Db}	65.04±0.99^{Db}	68.37±0.42^{Ca}	60.97±0.80^{Bc}
5	69.48±0.58^{Cc}	73.42±0.53^{Cb}	75.81±0.48^{Ba}	64.77±0.75^{Bd}
10	75.13±0.77^{Bb}	79.29±0.13^{Ba}	78.87±0.55^{Aa}	73.64±0.43^{Ac}
15	78.15±0.41^{Ac}	83.18±1.14^{Aa}	80.92±0.38^{Ab}	75.74±0.81^{Ad}

Means followed by the different uppercase letters in the same column regarding cooking time were significantly different (p<0.05). Means followed by the different lowercase letters in the same row at the same cooking time were significantly different (p<0.05).

While cooking, including up to 5 min, the significantly highest moisture levels were determined in 20-PGS added GFP (p<0.05), but the greater values were observed in the 10-PGS formulated GFP at the latest phase of cooking (10 and 15 min). Both moisture contents of GFP admixtured with 10% and 20% PGS were higher during the whole cooking process as compared to those made from durum wheat semolina. This behavior could probably be attributed to the higher water-holding capacity of hydrocolloids, which were present as XN in the formulation GFP samples, and use a lower water amount (40%) in semolina pasta to obtain a better consistency (section 3.2.1, Table 3.l). Moreover, it could also be ascribed to a part of the hydrophobic attributes of the gluten network, which limit complete starch hydration (Menga et al., 2017). In addition, although moisture content of control pasta made from durum wheat semolina (C) were lower during cooking than those of GFP with 10- and 20-PGS, it may maintain limitied water absorption than GFP (except 30-PGS), but better outstand to further cooking without disruption because of its gluten content, that could be deduced from having the lowest CL values in contrast to GFP counterparts during whole cooking process (section 4.2.4, Table 4.7).

On the contrary, the lowest moisture value during all cooking steps was determined in the case of 30% replacement of native corn starch with PGS (Table 4.4). Probably, PGS has apparent higher amylose content in comparison to native counterparts. It is proposed that an excessive amount of amylose could hinder water absorption and swelling because of the maintenance of starch granule structure (Indrastuti et al., 2024), which could be escalated with the pregelatinization process. Therefore, this resulted in higher CL values (section 4.2.4, Table 4.7), which may refer that CL takes precedence over water absorption when 30-PGS is used in GFP formulations.

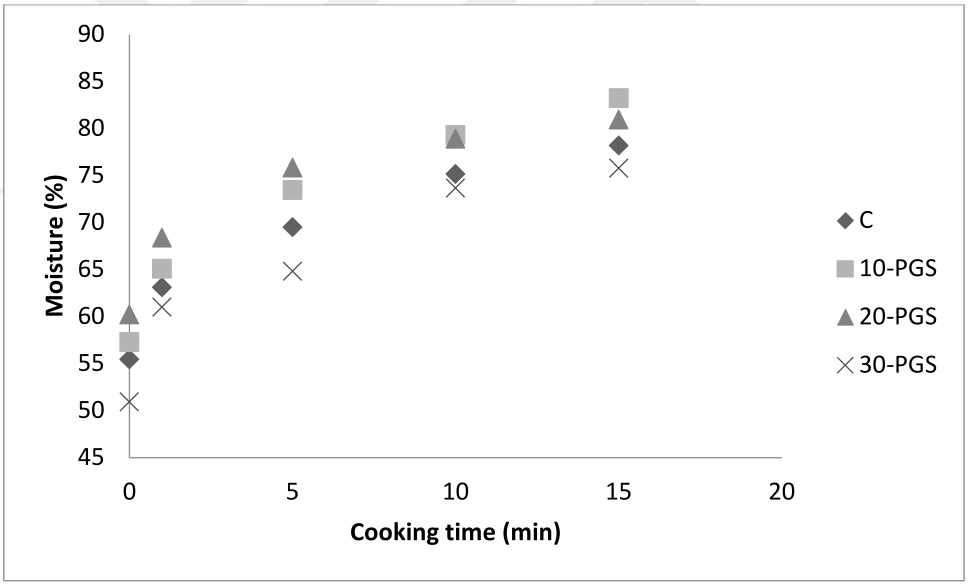

Figure 4.1. Moisture contents of semolina- and gluten-free pasta with pregelatinized starch during cooking

Additionally, an increase was observed in the moisture level during the cooking process in all fresh pasta samples, as shown in Fig. 4.1. According to the achieved results, the moisture values of fresh GFP samples were in the range between approximately 51-60% at the beginning of cooking (10 sec), reaching 76-83% at the end of the cooking (15 min) (Table 4.4). Similar trends were reported in previous studies (Sobota and Zarzycki, 2013; Sobota et al., 2013), although those values were lower than our results, likely because of differences in pasta compositions. The moisture content increases due to water absorption, depending upon the gradual rise in cooking duration, which would culminate in simultaneous textural changes (Sobota et al., 2013), which was explained in section 4.2.9.

4.2.2. Water Absorption Capacity

The water absorption capacity (WAC) is accepted as one of the most crucial features for pasta, as a degree of pasta hydration associated with directly affects the eating quality. Other terms, such as “cooking weight” or “water uptake” could also be utilized to stand for the WAC of pasta/noodle (Odabas and Cakmak, 2022). The WAC demonstrates the retrograded starch stability through the extrusion process. Because the extrusion alters the starch structure, leading to a rise in exposure of hydrophilic groups and starch gelatinization, which affects water absorption of the end-product (Sajid Mushtaq et al., 2023). Therefore, the availability of hydrophilic groups and the ability of gel forming in the macromolecules are in charge of the WAC (Udachan et al., 2022). The water absorption of pasta through cooking represents the swelling capability of starch and protein because of heating (Seetapan et al., 2019). Therefore, it includes a complex molecular modification, majorly hydration of proteins and starches (Larrosa et al., 2016). In this context, the source and/or composition and amount of protein have a role in hydration and absorption of water, which substantially affects the WAC (Bastos et al., 2016). The structural integrity of the network also takes part in water absorption intensity, namely, it may restrict water uptake with a well-cross-linked network structure (Lux et al., 2023). It is also connected with the level of damaged starch and the weakness of its granules (Bouasla et al., 2016), and there is a positive relation between them, which influences the preparation and texture of pasta dough (Seetapan et al., 2019). Moreover, it is also emphasized that the WAC could also be affected by other factors, such as cooking time and the form/shape of pasta (Sobota et al., 2013). It is declared that the weight should be doubled through cooking in the case of high-quality pasta (Fiorda et al., 2013b). In other words, it is desirable when the size of cooked pasta increases to twice the average (da Silva et al., 2016), put it differently, 100% change (Fiorda et al., 2013b). Additionally, it is also stated that higher water absorption is associated with the maintenance of prolonged satiety and faster satisfaction of hunger (Zarzycki et al., 2017; Thakur et al., 2022). On the contrary, the lower WAC is characterised by poor quality of cooked pasta because of its chewy texture (Bouasla et al., 2016). Therefore, the WAC of all fresh pasta samples during 15 min of cooking with 5 different time intervals (0, 1, 5, 10, and 15 min) was given in Table 4.5.

The hydration of gluten results in the formation of a stabilized protein network that has a high water retention capacity. Therefore, weak pasta structure brings about poor high water retention capacity through cooking, which is seen in the case of decreased and/or lack of gluten network in a pasta formulation (Bello-Perez et al., 2022). In this regard, significantly lower WAC values were recorded in GFP except 20-PGS within the first stages of cooking (0-5 min) in comparison with pasta made from durum wheat semolina (Table 4.5), which could be attributed to the gluten fraction of control pasta. However, lower hydration of control pasta in prolonged cooking durations (10-15 min) than that of 10- and 20-PGS (Table 4.5) could be explained by the formation of a strong gluten structure could also restrict water diffusion to starch molecules and swelling (Sozer and Kaya, 2008). Because it was declared that water absorption is associated with the integrity of the structural network, which could be limited with a well cross-linked network structure (Lubowa et al, 2021). Therefore, this could refer to its compactness, owing not only to the gluten protein network but also to the applied high pressure during extrusion in the pasta-making process (Achinna and Deshpande, 2025).

Table 4.5. Water absorption capacity of semolina- and gluten-free pasta with pregelatinized starch (%)

Sample	Cooking time (min)				
	0	1	5	10	15
C	62.10±1.62^{Ea}	88.74±2.80^{Dab}	124.96±2.63^{Cb}	143.11±2.72^{Bc}	162.72±2.43^{Ab}
10-PGS	53.10±1.76^{Eb}	80.66±0.95^{Dbc}	122.38±0.33^{Cb}	168.31±4.61^{Ba}	198.09±3.00^{Aa}
20-PGS	64.18±1.19^{Da}	97.21±4.31^{Ca}	132.02±0.54^{Ba}	154.76±2.38^{Ab}	168.93±2.12^{Ab}
30-PGS	49.45±2.42^{Eb}	73.69±1.06^{Dc}	110.90±1.36^{Cc}	129.40±3.51^{Bd}	150.56±3.14^{Ac}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$).

In other respects, cold extrusion could not allow for the totally gelatinization of starch granules. In this regard, the gelatinization and predominance of the weight increase would take place through pasta cooking (da Silva et al., 2016), as seen in the control group with durum wheat semolina in our study, which may limit the potential of having further high WAC levels. Moreover, the lower water level used in control pasta formulation (40%) than that of gluten-free counterparts (60%), as given in section 3.2.1 (Table 3.1), may also be the reason behind this. In contrast, in a study, wheat-flour-based noodles had a higher water absorption rate than gluten-free pasta partially replaced with pregelatinized rice flour (15%). This may potentially arise from exposing different cooking times to gluten-containing and gluten-free noodles, corresponding to 12 min and 4 min, respectively (Jeong and Lee, 2025). Similarly, significantly lower water absorption values were yielded in gluten-free noodles made from rice flour partially replaced with (5, 10, and 15%) pregelatinised high-amylose maize starch together with Ca^{2+}-induced setting of alginate, in comparison to control pasta prepared by rice flour ($p<0.05$). This was attributed to the extent of the generation of junction zones and starch retrogradation via $CaCl_2$ cross-linked sodium alginate (Lubowa et al., 2021).

However, in our study, the statistically highest WAC content was observed in GFP prepared with 20- and 10-PGS in the first (0-5 min) and last stages of cooking (10-15 min), respectively ($p<0.05$), that could be attributed to their formulation, including both hydrocolloid and pregelatinized starch (section 3.2.1, Table 3.1). Because a greater water absorption could be achieved in the presence of hydrocolloids by their high water-binding ability (Gasparre and Rosell, 2019), as a result of hydrophilic behavior (Widelska et al., 2019). As confirmed by a study that greater water absorption and swelling in XN-supplemented (3%) rice noodles were monitored (Yalcin and Basman, 2008). Because XN has the ability to enhance the capacity of water binding and thus swelling of starch molecules (Indrastuti et al., 2024). Additionally, an easy water penetration occurs with the capability of diffusing water in the protein-starch matrix because of having a more open structure of XN (Ertaş et al., 2023).

The higher WAC of GFP (except 30-PGS) could also be related with water absorption and binding ability/capacity of pregelatinized flour (Rafiq et al., 2018) by rising molecular mobility, destruction of granules, swelling, and also starch matrix expansion (Suo et al., 2022). Because the hydrogen linkages that hold the structure would weaken, thereby allowing the absorb water and swell when starch granules are heated in the presence of water (da Silva et al., 2016). It was also supported by another study that PGS could absorb a higher amount of water than that of the corresponding native starch forms (Santacruz et al., 2012). However, the WAC of 20-PGS, including GFP, was lower compared to the ones formulated with 10-PGS at the last phases of the cooking (10-15 min) (Table 4.5). The reason behind this could be explained by the destruction of the molecular order of starch, gradually breaking down the hydrogen bonds and irreversible loss of crystallinity due to absorption of high water quantity (Albuja-Vaca et al., 2020).

Moreover, the lowest values of the WAC were obtained for GFP produced from 30-PGS, although they include the same type and concentration of hydrocolloid, corresponding to 2% XN (section 3.2.1, Table 3.1). This could be announced with the idea that swelling power could be weaker, and absorbed water may not be held by the pasta strands if excessive levels of starch are already gelatinized (da Silva et al., 2016). Moreover, this may also be disclosed with the having the highest cooking loss values (section 4.2.4, Table 4.7). Because water absorption takes place from outside to inside of pasta, otherwise, the transfer of ingredients comes from pasta to the cooking water medium through cooking, namely, cooking loss occurs (Indrastuti et al., 2024). This behaviour is also consistent with the findings of Dib et al. (2018), who reported that the WAC values were increased up to 6% incorporation of pregelatinised rice flour, but reduced after the addition of the maximum level. In alignment with this, the WAC declined from almost 71% to 58% when the substitution level of rice flour with pregelatinized high-amylose maize starch was increased from 5% to 10% (Lubowa et al., 2021).

According to the results, the WAC of control made with durum wheat semolina and GFP samples was in the range of nearly 49-64% to 151-198% from the initially cooked to extended cooked, corresponding to 10 sec (0 min) and 15 min, respectively (Table 4.5). The WAC values were approximately 2-3 times increased at the end of the cooking, and thus the WAC values in all pasta were increased as cooking time progressed, as (Figure 4.2). Similar statements were also made by other researches (Dziki and Laskowski, 2005; Lucisano al., 2012; Sobota et al., 2013; Oduro-Obeng et al., 2021; Lux et al., 2023). Moreover, it is generally accepted that the minimum weight gain of cooked pasta characterized with high quality should be doubled, which is synonymous with a 100% increase (Fiorda et al., 2013b; Pessanha et al., 2023). Therefore, the 100% increase in achieved WAC values at 5 min of cooking could be considered satisfactory in all pasta samples. However, typically, the rapidly increasing period in initial water absorption is followed by a slower rate in later stages of the hydration curves. The kinetics of water uptake depend upon water diffusion via the matrix and the melting kinetics of the crystalline domains. Both phenomena are majorly in charge for the time at which disappearing the unpenetrated central core and the melting of all the starch crystals (Larrosa et al., 2015). Therefore, it is also mentioned that fully cooking could significantly reduce the weight increase because of higher cooking losses in those pasta samples (Dziki and Laskowski, 2005). In this regard, it could be the hypothesis that overcooking, such as prolonging cooking duration to 20 min, would be concluded with reduced WAC values in GFP samples with different levels of PGS, in our study.

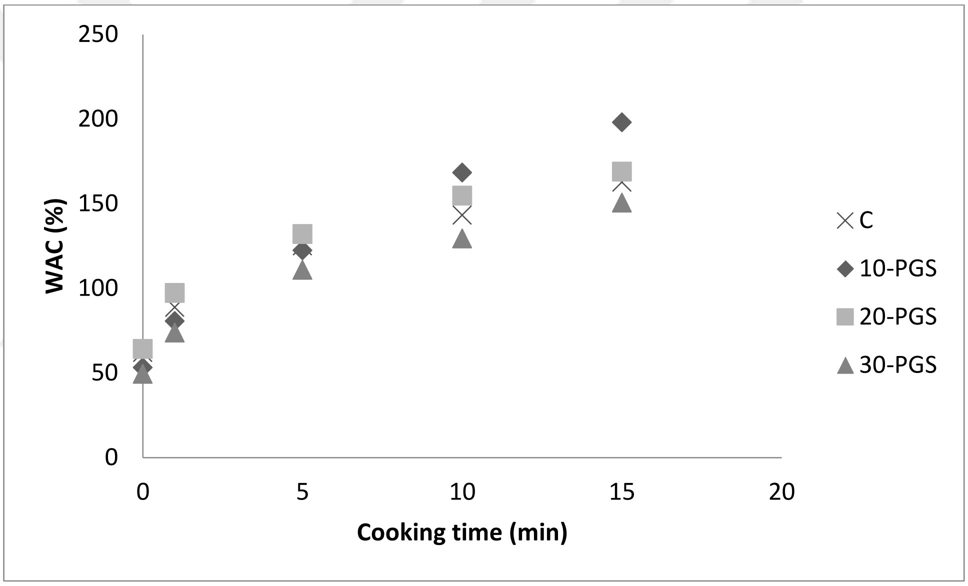

Figure 4.2. Water absorption capacity of semolina- and gluten-free pasta with pregelatinized starch during cooking

In the literature regarding WAC of pasta/noodle with PGS was in the range of approxiametely 48-59% in alkaline noodle made from sorghum flour partially replaced (30, 50 and 70%) with pregelatinized sorghum flour (Duemchok and Thongngam, 2011), 47-53% in noodle made from rice flour partially replaced with pregelatinized rice flour (15%) prepared by utilizing 3 different drying methods (hot air drying, freeze drying, and twin-screw extruder drying) (Jeong and Lee, 2025), 80-84% in semolina pasta including pregelatinized amaranth flour (50%) with different gelatinization degrees (50 and 100%) obtained by a single screw-extruder (Bello-Perez et al., 2022), 78-98% in noodle prepared from wheat flour partially substituted with pregelatinized corn starch (5, 10, and 15%) (Yousif et al., 2012), 78% in macaroni-shaped pasta based on pregelatinized red lentil flour (Bresciani et al., 2023), 58-71% in noodle based on rice flour partially replaced (5, 10, and 15%) with pregelatinized high-amylose maize starch in combined with sodium alginate set by Ca^{2+} (Lubowa et al., 2021), 73-76% in noodle prepared from pregelatinized white kidney bean starch obtained by drum drying at different temperatures (120, 130, and 140°C) for total substitution (100%) of wheat flour (Niyomwong et al., 2025), 108-121% in noodle based on the combination of wheat flour and native corn starch partially substituted with (10%) extruded and drum-dried corn starch, individually (Li et al., 2020), and 129-146% in noodle prepared by wheat flour incorporated with pregelatinized highland barley flour at 3 different ratios (30, 50 and 70%) (Wang et al., 2025a). The discrepancy between our results and the literature may arise from the differences in formulation, together with the type form of of pasta/noodle, cooking time, and the source and level of PGS, as well.

4.2.3. Swelling Index

The swelling index (SI) refers to water absorption via protein and starch through pasta cooking for utilization in hydration and gelatinization, respectively (Padalino et al., 2013; Fradinho et al., 2019; Faheid et al., 2022). Therefore, while water absorption is associated with yield after pasta cooking (Fradinho et al., 2019), SI is more characterized by the capability of water absorption and holding it within the starch-protein network (Veena et al., 2022). Therefore, the SI of all fresh pasta samples during 15 min of cooking with 5 different time intervals (0, 1, 5, 10, and 15 min) was given in Table 4.6.

Table 4.6. Swelling index values of semolina- and gluten-free pasta with pregelatinized starch (g/g)

Sample	**Cooking time (min)**				
	0	**1**	**5**	**10**	**15**
C	1.27±0.05^{Eb}	1.76±0.04^{Dc}	2.55±0.02^{Cc}	3.49±0.06^{Bc}	3.66±0.04^{Ac}
10-PGS	1.35±0.02^{Eb}	1.93±0.05^{Db}	2.83±0.03^{Cb}	3.95±0.05^{Ba}	4.58±0.02^{Aa}
20-PGS	1.70±0.17^{Ea}	2.42±0.05^{Da}	3.18±0.04^{Ca}	3.71±0.06^{Bb}	4.21±0.04^{Ab}
30-PGS	1.17±0.06^{Db}	1.69±0.04^{Cc}	2.45±0.08^{Bc}	3.11±0.12^{Ad}	3.57±0.09^{Ac}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different (p<0.05). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different (p<0.05).

The SI values were significantly higher throughout all cooking process, where 10% and 20% of native corn starch were replaced with PGS in GFP formulation compared with the control pasta made with durum wheat semolina (p<0.05). Marti et al. (2013) displayed a similar behaviour regarding the significantly higher account for tripled water swelling index of GFP made with gelatinized rice flour, in contrast to untreated rice flour. This could be interrelated with a weak matrix that is formed when the gluten from durum wheat semolina interacts with other proteins from whole egg powder, which reduces water retention ability (Bello-Perez et al., 2022). It is also reported that proteins could competitively decrease the needed water amount for starch swelling (Ungureanu-Iuga et al., 2020), and diffusion of water to starch granules could be prevented by generating a strong protein network as protein amount increases (Aydin, 2022), which may be the reasons for the lower SI values of the control group prepared with durum wheat semolina including gluten than that of GFP except for 30-PGS.

Moreover, utilizing hydrocolloids could also induce a lesser relation of soluble compounds by increasing bound water to proteins and starches (Gasparre and Rosell, 2019). This could be supported that the swelling ability of corn and mung starch granules was enhanced with hydrocolloids via preventing leakage of starch compounds through gelatinization (Levent, 2017). In that case, trapping the starch molecules through cooking and restricting extreme swelling and diffusion of amylose into cooking medium could be taken under control, particularly using XN in GFP formulations (Milde et al., 2020), and also a formed polymer network between protein chains (Milde et al., 2020), possibly prove the higher SI values in GFP (except for 30-PGS) than control pasta.

On the other hand, the lowest SI value during the whole cooking process was determined in fresh GFP with 30-PGS. The hydrogen linkages that keep the structure will weaken while heating starch granules in the presence of water, thereby enabling starch granules to absorb and swell. However, when the greater starch content is already gelatinized, the swelling ability weakens, and absorbed water could not be kept by the pasta/noodle strands (da Silva et al., 2016). Moreover, it was stated that lower water absorption indicates a lower increase in swelling volume (Aydin, 2022), which agrees with the findings in our WAC results of 30-PGS added GFP, in the present study (section 4.2.2, Table 4.5). Therefore, a strong positive correlation was determined between WAC and SI (r=+0.99) regarding the 1st chapter of this study.

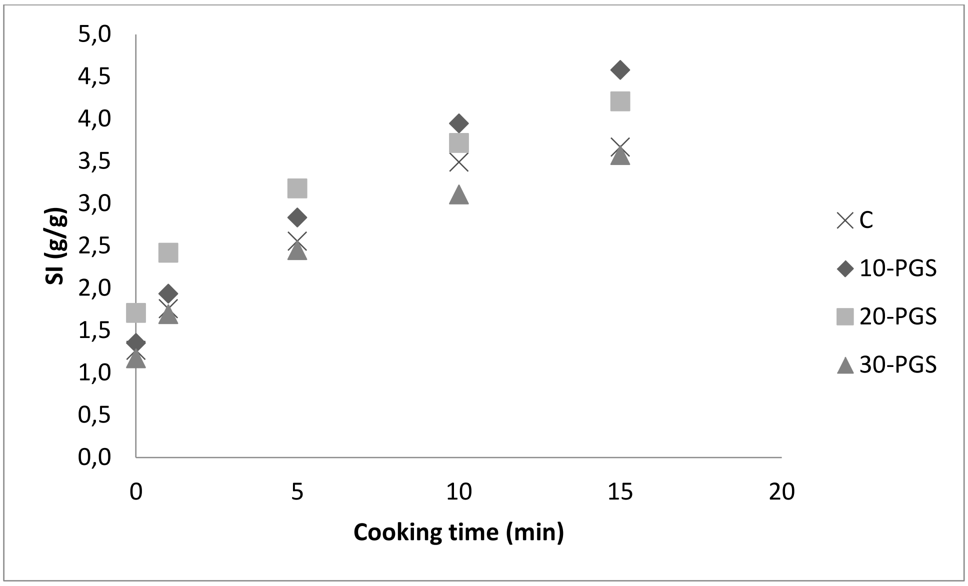

Figure 4.3. Swelling index of semolina- and gluten-free pasta with pregelatinized starch during cooking

According to the results, the SI of control made with durum wheat semolina and GFP samples was in the range of about 1.17-1.70 g/g to 3.57-4.58 g/g (Table 4.6). A similar tendency with WAC, regarding a 1-2 times increase for SI at the end of cooking (15 min), was observed as cooking time progressed (Figure 4.3). In a similar vein, an increase in SI values throughout cooking was recorded in fresh GFP based on amaranth flour (up to 1:10 water ratio), including 1% alginate (Lux et al., 2023).

4.2.4. Cooking Loss

Throughout the cooking process, pasta is subjected to boiling water, and particularly gelatinized swollen starch granules are lost from the surface to the cooking medium (Heo et al., 2014). Moreover, other soluble solid substances such as non-starch polysaccharides, proteins (Lorenzo et al., 2018), and minerals (Odabas and Cakmak, 2022) are also released from the pasta and pass through the cooking medium as the pasta cooking process progresses (Lucisano al., 2012). However, each kind of pasta should retain its shape and attributes, and thus should not disperse and deform through cooking (Aydin, 2022). This proceeds to different extents depending on the structural compactness (Lucisano al., 2012).

The cooking loss (CL) is defined as the quantity of soluble solids leached out to cooking water (Lorenzo et al., 2018). CL is also termed as “dry matter loss” (alternatively, i.e., loss of dry matter) or “solid loss” (alternatively, i.e., loss of solid), as well (Fradinho et al., 2019). It is commonly accepted as an indicative of the overall cooking performance of pasta, because it expresses a sign of resistance to disintegration through cooking (Sosa et al., 2019). Therefore, a desirable, high-quality pasta is characterised with the transmission of a lower quantity of solids/residue to cooking water (Fradinho et al., 2019). However, there are different acceptances about the limits of CL values, which are generally valid for durum wheat pasta. In this context, acceptable CL values for a good quality pasta should be as below and/or equal to 6% (Fradinho et al., 2019), 7-8% (Susanna and Prabhasankar, 2013; Odabas and Cakmak, 2022), 8% (Baah et al., 2022; Süfer, 2022), 10% (Llavata et al., 2019), 12% (Giuberti et al., 2015; Albuja-Vaca et al., 2020). The classification of pasta quality according to CL values could be materialised when pasta loses up to 6% solids could be considered as excellent-very good-quite good; 8% is regular-good quality, and 10% is poor-low quality-maximum acceptable level (Fiorda et al., 2013b; da Silva et al., 2016; de Oliveira et al., 2022). Otherwise, it could be encountered with a sticky surface, viscous/turbid cooking water as undesirable quality features (Kim et al., 2019), which decreased the nutritional values of the end product, as well (Odabas and Cakmak, 2022).

Maintaining physical integrity through cooking is associated with a protein-starch matrix (Fradinho et al., 2019). Typically, a strong and stable network of coagulated gluten proteins is formed, which entraps starch granules, thereby, lowering CL values connected with lower stickiness and better consistency achieved in semolina/wheat flour-based pasta/noodle (Gasparre and Rosell, 2019). However, the starch granules are inadequately entrapped in the matrix through cooking due to the absence of gluten proteins in GF pasta/noodles. Therefore, some soluble compounds from swollen starch molecules and small fragments easily pass into the cooking medium, resulting in higher CL values (Seetapan et al., 2019). In this context, the CL mainly occurs because of the solubility of gelatinised starch with loosely bound from the surface. This behaviour majorly depends upon the strength of the retrograded starch network surrounding the gelatinised starch and the quantity of gelatinised starch (Wang et al., 2012). It is stated that there are many other factors that negatively influence the CL of pasta, such as damaged starch and coarse particle size (Kim et al., 2019). On the other side, the moisture content could also affect CL regarding osmatic balance through pasta cooking (Pessanha et al., 2023).

The GFP, which is made by non-conventional ingredients, is frequently characterized by higher CL values as a result of the absence of gluten (Hager et al., 2012). Therefore, it is stated that GFP could exhibit 3-4 times higher CL (da Silva et al., 2016), reaching the excessive values that account for 20–25% (Giuberti et al., 2015). The starch molecule is accepted as the major structural network in GFP. The generation of retrograded starch with a compact structure around the gelatinized starch is generally achieved by extrusion cooking, which could protect the pasta integrity through hydration with hot water, resulting in fewer leaching of gelatinized starch out from the surface of pasta (Bouasla and Wojtowicz, 2021). However, complete starch gelatinization may not be achieved by cold extrusion (da Silva et al., 2016), and cooking pasta directly after cold extrusion leads to an increase in the gelatinization degree of the starch granules, permitting a major fraction leaching of amylose from the starch molecules (Santacruz et al., 2012). Therefore, the single-barrel system cold extrusion process, which was applied to all pasta samples in the present study, may cause higher CL values in all pasta samples, including control and GFP with different PGS ratios (10, 20, and 30%) during 15 min of cooking with 5 different time intervals (0, 1, 5, 10, and 15 min), as given in Table 4.7.

Table 4.7. Cooking loss values of semolina- and gluten-free pasta with pregelatinized starch (%)

Sample	**Cooking time (min)**				
	0	**1**	**5**	**10**	**15**
C	2.27 ± 0.03^{Dc}	3.43 ± 0.22^{Cb}	4.27 ± 0.18^{Cc}	6.53 ± 0.15^{Bd}	8.31 ± 0.48^{Ac}
10-PGS	2.91 ± 0.11^{Eab}	3.89 ± 0.26^{Dab}	5.68 ± 0.21^{Cb}	7.25 ± 0.07^{Bc}	9.02 ± 0.57^{Ac}
20-PGS	2.72 ± 0.07^{Eb}	3.65 ± 0.14^{Dab}	4.80 ± 0.16^{Cc}	8.25 ± 0.10^{Bb}	10.78 ± 0.24^{Ab}
30-PGS	3.11 ± 0.11^{Ea}	4.13 ± 0.17^{Da}	7.09 ± 0.27^{Ca}	11.83 ± 0.28^{Ba}	17.06 ± 0.53^{Aa}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$).

As expected, the lowest CL values throughout the whole cooking progression were determined in the control group based on durum wheat semolina (Table 4.7). It was emphasized by several studies that the CL is associated with protein content and composition, particularly gluten proteins. Because there was a positive relationship between pasta quality and glutenin:gliadin ratio. Particularly, a strong elastic structure is formed after hydration owing to the high amount of glutenins. On the other side, especially γ-45 subunits in the event of gliadin fractions also generate a strong gluten network, have a role in pasta having high cooking quality, including lower CL through cooking (Sobota et al., 2013). In this regard, the lowest dry matter loss of the control group could be dedicated to the encapsulation of starch and other compounds within the gluten network (Detchewa et al., 2016), that supported by microstructure images in the following section 4.2.13 (Figure 4.9a). Therefore, it could be stated that the pasta with the most resistant to disintegration while boiling (Foschia et al., 2017) is the control pasta made from durum wheat semolina.

However, it was demonstrated by Leandre (2020) that the pasta formulation based on egg protein, XN, and especially PGS, which homologise with our GFP formulation in this study (section 3.2.1, Table 3.1), may interfere with excessive CL through cooking. Therefore, one of the reasons behind the comparable CL values of control pasta with GFP (except for 30-PGS) could be the presence of PGS. The positive influence of pregelatinized flour on CL by enhancing the tolerance to cooking stress is referred to as acting as a binder, repolymerization into a network surrounding the starch granules through the extrusion process (Marti et al., 2013). The second possible explanation about the disintegration resistance and the decreased release of compounds through the cooking of 10- and 20-PGS-added GFP could be dedicated to hydrocolloid incorporation into formulations. Because they have the ability to keep more solids into the matrix by behaving as a barrier (Losano Richard et al., 2022) due to be in charge of the formation of a gel-like network through cooking (Alinovi et al., 2023), via hydrogen bonds and hydrophilic interactions as a result of the interplays between starch granules and hydrocolloids (González et al., 2022). In particular, the XN incorporation could aid in terms of trapping starch granules and thus a stronger three-dimensional network formation to avoid diffusion of amylose out (Indrastuti et al., 2024). Thus, the comparatively lower CL is owing to the hypothesis that the hydrocolloid XN forms a network around the starch granules, holding them in place while cooking. It can be assumed that XN encapsulated and integrated the polysaccharide network, strengthening the structural integrity of the pasta by the interaction between XN and starch, which hinders the starch dissolution of starch on the pasta surface during the cooking process, leading to a decrease in the CL of the pasta (Lux et al., 2023). The third potential factor regarding having a better cooking tolerance and maintaining the integrity (Sobota and Zarzycki, 2013) of GFP, those prepared with 10- and 20-PGS could also be disclosed with egg proteins, particularly albumen proteins. Even in the lack of gluten, they have the ability a reticule forming to ensure good cohesiveness and consistency by thermal denaturation via heating. Ovalbumin proteins could modify the rheological attributes of carbohydrates by neutralizing their surface charge, and thereby, repulsion between starch granules does not eventuate. Moreover, the alteration of the water absorption within the starch granules could be justified by a complex formation between amylose and an emulsifier sourced from egg proteins. Thus, facilitating the moulding process causes pasta to have a smoother surface, which reduces lixiviation of solids through cooking (Fiorda et al., 2013b; Ferreira et al., 2016). Nevertheless, the generated structure via ovo-lacto proteins is still not as strong as that of the traditional wheat flour pasta (Milde et al., 2020), which is probably one of the reasons that occurred in this study**,** making explicit the predominance of control pasta as compared to the GFP. However, the dry matter loss is also fulfilled because of the solubility of loosely bound starch gelatinization on the surface of pasta. Because CL depends upon both starch gelatinization degree and the power of the starch network surrounding the gelatinized starch (Lux et al., 2023). Moreover, probably the structural integrity of XN-starch network was not strong enough in owing to many interference areas originating from high PGS amount in 30-PGS, resulting in loosely-bound gelatinized starch leached into the cooking medium, as declared for alginate and amaranth flour (Lux et al., 2023). In this context, the GFP with the highest level of PGS (30%) had the highest CL values throughout the cooking step (Table 4.7).

According to the results, the CL of all pasta samples was in the range of about 2.27-3.11% to 8.31-17.06% (Table 4.7). The CL values of all pasta samples were increased as the cooking maintained, as shown in Figure 4.4. This trend is in accordance with previously published studies (Dziki and Laskowski, 2005; Sobota and Zarzycki, 2013; Sobota et al., 2013). This could be expounded by the gradual hydration of pasta within the deeper layer. Entrapped starch granules within the protein matrix swell and gelatinize, and then the structure of the protein undergoes fewer compactness. After that, more intensive penetration of water into pasta and leaching majorly dry matter of soluble compounds out (Sobota and Zarzycki, 2013).

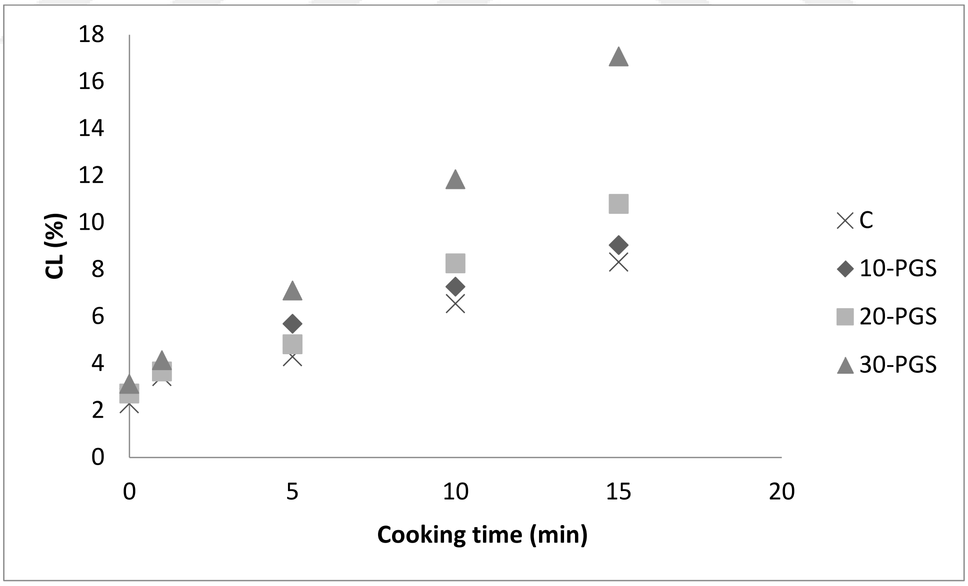

Figure 4.4. Cooking loss of semolina- and gluten-free pasta with pregelatinized starch during cooking

When accepting the 6% as the maximum limit for CL, control, and all GFP, excluding 30-PGS, could be considered high-quality pasta within 5 min of cooking duration. This is also aligned with a 100% increase in WAC, as previously mentioned in section 4.2.2. However, cooking time extension to 5 min also resulted in nearly doubled CL values in all samples. Therefore, the prolonged cooking time of more than 5 min may adversely affect both technological and likely nutritional values of GFP samples.

In the literature regarding CL of pasta/noodle comprising of PGS was determined as in the range between nearly 1-1.5% in rice noodle based partially substituted (5, 10, and 15%) with pregelatinized high-amylose maize starch together with Ca^{2+}-induced setting of alginate (Lubowa et al., 2021), 6% in macaroni-shape pasta with pregelatinised red lentil flours (Bresciani et al., 2023), 6-7% in noodle made from pregelatinized white kidney bean starch prepared by drum drying with different temperatures (120, 130, and 140°C) for totally replacement of wheat flour (Niyomwong et al., 2025), 6-8% noodle made from wheat flour partially replaced (4, 8, and 12%) with pregelatinized *Canna edulis* starch (Santacruz et al., 2012), 9% in semolina pasta partially replaced with (50%) pregelatinized amaranth flour with two different gelatinization degrees (50 and 100%) prepared by a single screw-extruder, 11-12% in gluten-free spaghetti based on amaranth flour partially substituted (5, 7, and 9%) with pregelatinized corn starch (Chillo et al., 2007), 9-10% in noodle made from wheat flour enriched with pregelatinized highland barley flour at different proportions (30, 50 and 70%) (Wang et al., 2025a), 11-12% in noodle prepared by the wheat flour and native corn starch combination which is partially substituted with (10%) pregelatinized corn starch obtained by different methods (extrusion drying and drum-drying) (Li et al., 2020), and 13.5-21% noodle made from in wheat flour parially substituted with pregelatinized corn starch (5, 10, and 15%) (Yousif et al., 2012). The discrepancy between our results and the literature may arise from the differences in formulation, together with the type form of of pasta/noodle, cooking time, and also the type and/or level of PGS.

4.2.5. Volume Increase

A high volume increase (VI) is desired through pasta cooking; otherwise, this refers to absorbing fewer water and thus resulting in a firmer texture thereafter cooking (Köten and Ünsal, 2022). The VI is generally ascribed to enhanced water absorption and also the swelling index of pasta (Ray et al., 2023). Therefore, the VI values of semolina-based control and GFP with different proportions of PGS during cooking time (1, 5, 10, and 15 min) were given in Table 4.8.

In this context, the highest VI within the first 5 min and 10-15 min of the cooking process belonged to the 20-PGS-included GFP, and 10-PGS-added GFP, respectively. An increase in the VI of GFP could be disclosed with the rise in their water uptake at the same time (Ertaş et al., 2023). Because both weight and volume properties were dedicated to WAC and the shape of the pasta (Pessanha et al., 2023). In this regard, the positive relationship between volume of pasta with water absorption (Aydin, 2022) was consistent with our corresponding results, as given in section 4.2.2 (Table 4.5). Therefore, a positive correlation between WAC and SI with VI was recorded regarding the 1st chapter of this study (r=+0.93 and r=+0.94, respectively). This was also supported by recent literature that one of the highest VI, which was comparable to control noodles based on wheat flour, was obtained when replacing 10% and 15% with pregelatinized corn starch (Yousif et al., 2012). In contrast, lower volume values were recorded in 4 min-cooked gluten-free noodles made from rice flour and pregelatinized rice flour (15%) than those of the control group based on wheat flour (Jeong and Lee, 2025).

Table 4.8. Volume increase values of semolina- and gluten-free pasta with pregelatinized starch (%)

Sample	Cooking time (min)			
	1	5	10	15
C	121.67±11.67^{Cab}	155.00±15.00^{BCa}	185.00±15.00^{ABa}	215.00±15.00^{Aab}
10-PGS	111.36±6.82^{Cb}	127.56±9.38^{Cb}	213.64±13.64^{Ba}	240.91±13.64^{Aa}
20-PGS	138.24±2.95^{Ca}	179.07±3.28^{Ba}	200.00±10.19^{ABa}	230.09±0.68^{Aa}
30-PGS	107.14±7.15^{Cb}	119.64±5.36^{Bb}	176.79±8.93^{Aa}	183.93±16.07^{Ab}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$).

In other respects, the higher VI values of 10- and 20-PGS-included GFP, in contrast to the control with durum wheat semolina, could be relevant with the presence of XN in the formulation. Because it could swell in cold water and is frequently utilized as a binder and volume enhancer, as stated for guar gum (Chauhan et al., 2017). Similar behavior has already been reported for GFP based on pregelatinized rice starch:corn starch (1:1), including XN, which had the highest VI compared with those values that comprise other additives such as transglutaminase and hydroxypropyl methylcellulose (Ertaş et al., 2023). On the other side, although higher VI values were observed in control pasta than GFP with 10-PGS within 5 min of cooking, there was a significant reduction in VI values of control pasta at the end of cooking (15 min) ($p<0.05$). This could be referred to as its higher protein content regarding gluten of the control group with respect to GFP samples. Since volume expansion in cereal-based extrudates is positively correlated with starch, whereas negatively correlated with protein contents. Thus, the incorporation of proteins into high starch flours decreases starch conversion, which in turn causes fewer expansion and has more resistance to water dispersion (Varsha and Mohan, 2016).

On the other side, the lowest VI values were observed in the GFP, including 30-PGS, compared with those of not only other GFP but also control pasta based on durum wheat semolina, which tends to show a similar trend with our results regarding lower WAC, as previously announced in section 4.2.2. Moreover, this phenomenon could be associated with having the highest CL values (section 4.2.4, Table 4.7), surpassing their water absorption and swelling, which could prevent volume expansion. In addition to this, a positive correlation between feed moisture and volume expansion (Rafiq et al., 2018) could be another reason for having the lowest VI values of GFP, including the highest PGS ratio (30-PGS), which had the lowest raw pasta moisture values among other GFP samples, as well (section 4.2.1, Table 4.4).

The VI values of control and GFP with different ratios of PGS were in the range from approximately 107-138% to 184-241% from the initial to the end of cooking, as given in Table 4.8. Therefore, the VI values of all pasta samples were increased during the cooking process, as presented in Figure 4.5. This was in line with our results that the water absorption and swelling of the starch granules upon cooking culminate increase in volume expansion and pressure on the protein network (Steglich, 2013). Current results were in agreement with Lux et al. (2023) mentioned that the pasta volume increased with cooking time duration, and also Suo et al. (2022), who declared that the values of thickness gain and length increased during cooking. It was also indicated that the VI of a good-quality pasta/noodle based on wheat flour should be between 200-300% (Pessanha et al., 2023). In particular, the volume expansion of all pasta samples was doubled at the end of the 15 min of cooking, except for 30-PGS (Table 4.8), likely owing to its highest CL values (section 4.2.4, Table 4.7). Regarding all GFP samples, except for 30% PGS, agreed with this requirement at 15 min of cooking, and the lowest level of PGS-added GFP (10-PGS) was the sample closest to providing these conditions, but required a prolonged cooking process.

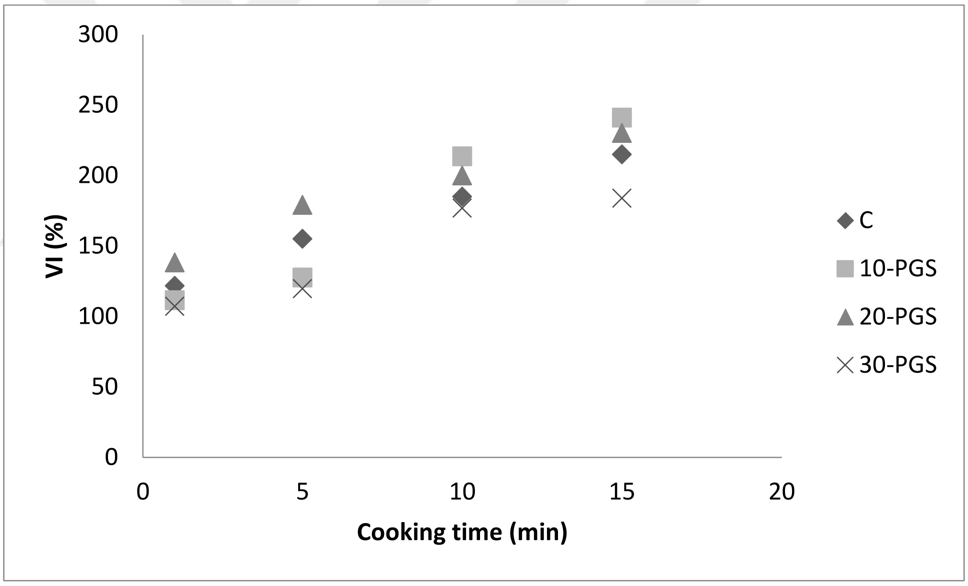

Figure 4.5. Volume increase of semolina- and gluten-free pasta with pregelatinized starch during cooking

4.2.6. Turbidity

The turbidity of the cooking water refers to the solid loss degree from the pasta strands, which is caused by leaching soluble solids out (Kim et al., 2014), resulting in viscous (Heo et al., 2013; Heo et al., 2014), cloudy cooking water (Jang et al., 2015), and a sticky surface of pasta (Heo et al., 2013; Heo et al., 2014). The positive link between turbidity and stickiness was also supported by other research (Geng et al., 2021; Bouasla and Wojtowicz, 2021), thereby is not favourably acknowledged by consumers (Kim et al., 2014). In this context, high light transmittance of cooking water, and thus low turbidity, is associated with a better pasta quality (Li et al., 2024). Therefore, the turbidity values in cooking water of fresh control pasta made from durum wheat semolina and GFP blended with 3 different PGS rates (10, 20, and 30%) during the whole cooking process with 5 different time intervals (0, 1, 5, 10, and 15 min) were screened in Table 4.9.

Table 4.9. Turbidity values in the cooking medium of semolina- and gluten-free pasta with pregelatinized starch (abs at 675 nm)

Sample	Cooking time (min)				
	0	1	5	10	15
C	0.05±0.01^{Ec}	0.16±0.01^{Db}	0.28±0.03^{Cb}	0.40±0.02^{Bc}	0.55±0.02^{Ac}
10-PGS	0.07±0.01^{Eb}	0.17±0.01^{Db}	0.31±0.03^{Cb}	0.47±0.04^{Bc}	0.63±0.01^{Ac}
20-PGS	0.06±0.01^{Ebc}	0.12±0.01^{Dc}	0.29±0.01^{Cb}	0.71±0.01^{Bb}	1.34±0.03^{Ab}
30-PGS	0.10±0.01^{Ea}	0.19±0.01^{Da}	0.45±0.09^{Ca}	1.20±0.07^{Ba}	1.67±0.05^{Aa}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$).

According to Table 4.9, while the lowest turbidity levels among all fresh pasta samples were determined in the control group based on durum wheat semolina, the highest values were observed in GFP made with 30-PGS over all cooking times. This attitude could correspond to their lowest and highest CL levels, respectively, as mentioned in section 4.2.4 (Table 4.7). It was revealed that CL may indicate loss of weight and/or volume during cooking, which could be measured by turbidity (Jeong and Lee, 2025). In a similar vein, the positive relationship between the CL, namely solid loss, and turbidity was also addressed in the literature with several studies (Heo et al., 2013; Jeon et al., 2014; Moon and Kweon, 2021; Park et al., 2022; Bak et al., 2023; Li et al., 2024), which is aligned with the results of the 1st chapter of this study (r=+0.97).

A stronger structure of gluten network represents fewer solids leaching out from pasta (Park et al., 2022), as seen in the control pasta with the lowest turbidity of cooking water in our work. In a study, a similar trend revealed that the turbidity of the wheat-based noodle was usually much lower in contrast to the rice noodle, culminating in less cooking water viscosity because of less CL (Kim et al., 2014). In a similar vein, the cooking medium of the control noodle made from wheat flour had significantly lower turbidity values when compared with the rice flour-based gluten-free noodle, including 15% pregelatinized rice flour (hot air-dried, freeze-dried, twin screw extrusion dried). This turbidity increase was associated with the gluten absence and thus a decline in the ability of the dough forming, resulting in leaching out and thus high turbidity (Jeong and Lee, 2025). However, the severe CL, which indicates excessive leaching out of starch and other soluble compounds, eventuates in the disintegration of pasta structure during the hot-water cooking process, which is undesirable to consumers (Kim et al., 2014). This behaviour seemed as is similar to the case of 30-PGS incorporated GFP, which resulted in the highest turbidity, in our study. Because of having insufficient strength while heating resulted in weakness of pasta' binding capacity (Jeon et al., 2014), and greater solubility sourced from high starch damage (Heo et al.,

2013) could be valid when increased PGS level and cooking time, as viewed in our study. In a recent literature the lowest water absorption, highest CL and turbidity in brown rice noodles with additional plant-based protein sources (rice protein, lentil protein) ended up with coarse texture and sticky surface, respectively (Geng et al., 2021), which had a similar characteristics with 30-PGS that during the whole cooking time, as exhibited in the section 4.2.2 (Table 4.5), section 4.2.4 (Table 4.7), respectively.

On the other side, the significantly lowest turbidity among GFP samples was possessed by the 20-PGS-added sample within the first 5 min of cooking ($p<0.05$). However, the comparable values with the control group based on durum wheat semolina, which has the lowest value among all the pasta samples, were obtained in GFP, including 10-PGS within 10-15 min of cooking ($p>0.05$)**.** Inclusion of XN in 10-PGS-formulated GFP could be one of the reasons for the lowered turbidity values to a comparable level to that seen with the control pasta made with semolina (Table 4.9). In a study, the turbidity of buckwheat noodles without hydrocolloids was 3-fold higher than compared of the wheat flour-based ones. Because it is pointed out that the hydrocolloids, in a dose-dependent manner, demonstrated a rise in water absorption and swelling index, which has a correlation with the viscoelastic behavior of pasta dough, whereas a significant reduction in CL and turbidity in noodles**.** Therefore, they also emphasized that the hydrocolloid concentration rather than the type of hydrocolloid had a crucial influence on CL and turbidity (Jang et al., 2015).

The cooking water turbidity values of all pasta samples were changed from 0.05-0.10 abs to 0.55-1.67 abs (at 675 nm) during cooking, as presented in Table 4.9. As reflected in Figure 4.6, the cooking water turbidity of all fresh pasta samples increased as the cooking continued, reaching approximately more than 10-fold values at the end of the cooking (15 min) against to the initial obtained in 10 sec (0 min). This could represent more induced breakdown of soluble solid materials, which is consistent with their corresponding CL values as previously described (section 4.2.4, Table 4.7). This agreed with the findings that the noodle soup’s turbidity was significantly rised with not only the psyllium husk amount but also cooking time (Bak et al., 2023). In another study, similar behaviour was displayed with this present study, where the water absorption, volume change, and also the turbidity of noodles were increased as cooking time increased (Bak et al., 2023), as similarly cited in our study, as well, in the section 4.2.2 (Figure 4.2), 4.2.5 (Figure 4.5), and 4.2.6 (Figure 4.6), respectively. Nevertheless, there was a common agreement that higher CL, which most probably accompanies increased turbidity arising from the weak strength of the protein network (Moon and Kweon, 2021), and lower connectivity degree in pasta structure (Park et al., 2022). This phenomenon suggested that GFP would likely have higher CL than gluten-containing counterparts, although including egg proteins comes from whole egg powder and XN for gluten mimic in its formulation. However, the differences between control pasta based on durum wheat semolina and GFP regarding turbidity were more apparent in the prolonged cooking times (10-15 min), particularly in higher PGS amounts (20- and 30-PGS). Therefore, it could be interpreted that, in the case of limited cooking times, an adequate amount of PGS in the presence of hydrocolloid and additional animal- or plant-based protein could have potential in GFP production regarding lower CL and turbidity when durum wheat semolina is accepted as a reference.

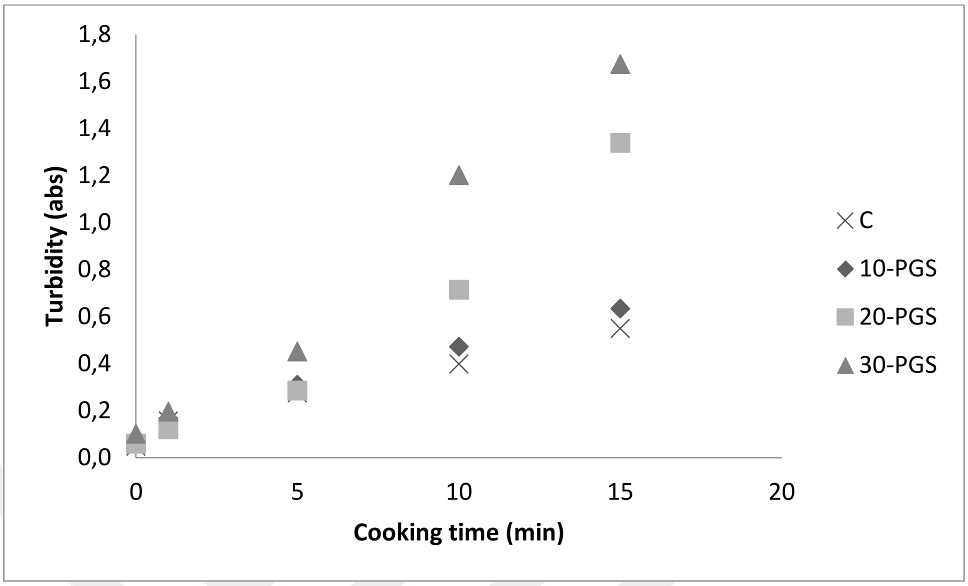

Figure 4.6. Turbidity values in the cooking medium of semolina- and gluten-free pasta with pregelatinized starch during cooking

4.2.7. Amylose Leaching

During cooking, proteins denature, almost complete starch gelatinization takes place, and the granule goes through the changes from the crystalline structure loss with no granule rupture to dextrinization and leaching. These changes in the starch are contingent upon many parameters, such as concentration, mechanical stress level through the cooking process, and treatment duration (Milde et al., 2020). The amylose is the linear chain of glucose molecules linked by α-1,4 D-glucose units, solubilized from gelatinized starch molecules (Susanna and Prabhasankar, 2013). Under the conditions based on heating and gelatinization of starch, a measure of molecules, especially amylose, leak from the starch granules, and amylopectin in small quantities dissociate within the heating process (Xu et al., 2021). The starch granules lose their integrity, and amylose could leach out, leading to excessive CL and a rise in stickiness, depending upon the starch swelling level (Steglich, 2013). The loosely-bounded gelatinized starch leaches out cooking water of pasta, and the iodine test could demonstrate, for a quick method, that starch in the cooking medium (Lux et al., 2023). Therefore, Table 4.10 summarized the amylose leaching (AL) values in cooking medium obtained for fresh control pasta made from durum wheat semolina and GFP samples with PGS at different levels (10, 20, and 30%) during the whole cooking duration (0, 1, 5, 10, and 15 min).

Table 4.10. Amylose leaching values in the cooking medium of semolina- and gluten-free pasta with pregelatinized starch (abs at 650 nm)

Sample	**Cooking time (min)**				
	0	**1**	**5**	**10**	**15**
C	0.07±0.01^{Eab}	0.19±0.02^{Dc}	0.49±0.01^{Cb}	0.89±0.04^{Bc}	1.20±0.15^{Ab}
10-PGS	0.08±0.01^{Ea}	0.27±0.03^{Db}	0.56±0.02^{Cb}	1.07±0.04^{Bbc}	1.34±0.01^{Ab}
20-PGS	0.06±0.01^{Eb}	0.23±0.01^{Dbc}	0.53±0.02^{Cb}	1.27±0.02^{Bb}	1.45±0.01^{Ab}
30-PGS	0.09±0.01^{Ea}	0.32±0.01^{Da}	0.68±0.06^{Ca}	1.70±0.23^{Ba}	2.16±0.21^{Aa}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$).

According to Table 4.10, the lowest amylose leaching values were generally recorded in control samples produced from durum wheat semolina, possibly originating from its strong gluten network, which demonstrates a similar pattern with turbidity (section 4.2.6, Table 4.9) and CL (section 4.2.4, Table 4.7). Therefore, a positive correlation was defined between AL and CL (r=+0.97) in 1st chapter of this study. However, the GFP admixtured with PGS in the presence of XN could form a more compact pasta structure, thereby lessening the leaching of amylose out when cooked, presumably inducing a less sticky texture among GFP samples, as similarly proposed previously (Kamsiati et al., 2019). This result was in line with a previous study that the incorporation of XN in the case of cassava starch brings about higher cohesiveness, and thereby less amylose leaching to the medium while cooking (Indrastuti et al., 2024). The hydrothermal treatment applied to rice flour for pregelatinization formed more highly regulated crystalline are in starch granules, leading to generate a rigid network. As a result of this, a stronger starch matrix could preserve the amyloceous compounds from increased leaching out. Consequently, it has resulted in lower CL, fewer release of amylose fractions to aqıuous phase and improved texture regarding an increase in firmness and reduction in stickiness (Dib et al., 2018). In another observation, it was revealed that the heat-moisture treatment implemented on sweet potato starches could possibly promote cross-linking within the starch molecules as strong internal bonds, which act like strong glue around the noodle's surface. Thus, this resulted in reducing and/or limiting its solubility, cooking loss, swelling ability, and amylose release into the aqueous medium (Tsakama et al., 2013). Therefore, comparable AL results of control with GFP (except for 30-PGS) could be sourced in the presence of a lower or moderate amount of PGS and hydrocolloid in their corresponding formulation. Moreover, emulsifiers could also have a role, which is also present in GFP formulation (section 3.2.1, Table 3.1), owing to forming a complex with amylose to limit starch leaching (Ji et al., 2025). However, structural integrity between the interaction of XN with high concentrations of PGS (30%), particularly in the late phase after 5 min of cooking, may not be strong enough, and thus loosely-bound gelatinised starch leaches into cooking water. The weak structural stability of this network could be sourced from many interference areas (Lux et al., 2023) because of greater PGS content. This could be associated with water migration behavior from more strongly/tightly bound sites, such as proteins, to more weakly bound sites, such as starch, which restricts water for starch granules (Sozer and Kaya, 2008). On the contrary, it was suggested that higher quantities of PGS could behave better during the cooking process owing to major amylose leaching and further retrogradation, stemming from the PGS, which could absorb higher amounts of water in contrast to native starch. However, although the retrograded amylose acquired during the cooling of pasta forms a network that prevents the starch components' dispersion from the pasta to the cooking medium during the boiling, the straight cooking of pasta/noodle after cold extrusion permits to rise in the gelatinization degree of the starch, causing in a major amylose leaching from the starch (Santacruz et al., 2012) may be one of the major reason behind this.

The amylose leaching values of control and GFP were altered from 0.06-0.09 abs to 1.20-2.16 abs (at 650 nm) during cooking, as presented in Table 4.10. As indicated in Figure 4.7, the amylose leaching was increased in both the control and all GFP samples with a prolonged boiling process. In this regard, there was a positive relation between the T and AL in the 1st chapter of this study (r=+0.67) because of being one of the reasons for turbidity in cooking water.

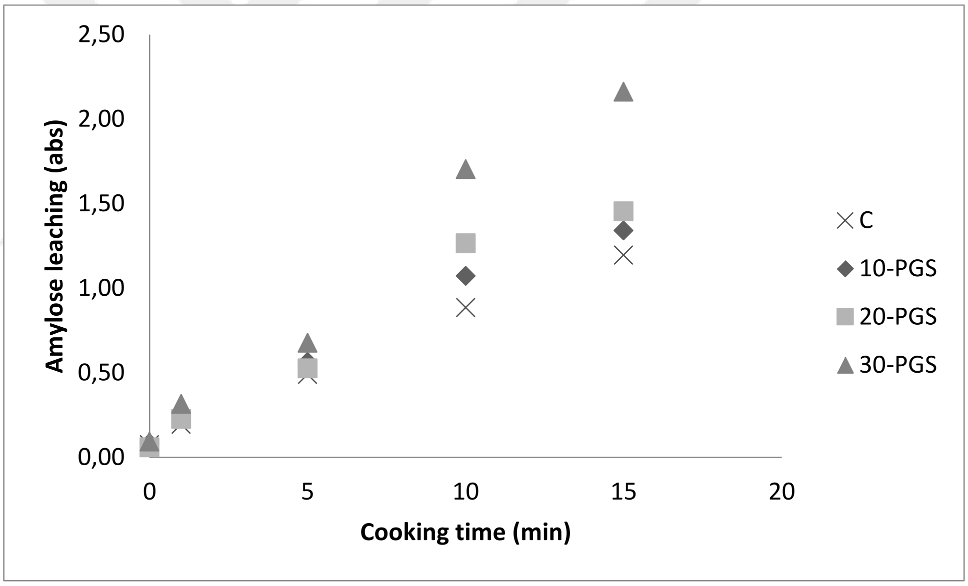

Figure 4.7. Amylose leaching values in the cooking medium of semolina- and gluten-free pasta with pregelatinized starch during cooking

4.2.8. Color

Color is one of the most substantial quality parameters that impacts the preference of consumers (Odabas and Cakmak, 2022). In a traditional wheat flour/semolina-based pasta, a golden and/or deep amber color is favored depending upon the quantity of yellow pigments (Lorenzo et al., 2018). The pasta color is influenced by several factors, such as formulations with regard to raw materials and ingredients, but also process conditions (Marti et al., 2014). A cooked pasta with a bright yellow color is generally attributed to formulations including egg yolk (Lorenzo et al., 2018). The yellowish color is also highly desirable in erişte (Turkish noodle) by means of egg content in the formulation (Odabas and Cakmak, 2022). On the other side, the carotenoid content present in the wheat grain and degradation via lipoxygenases through the process is the major determinant in the pasta color (Lorenzo et al., 2018). Apart from pigment degradation, non-enzymatic browning reactions, such as the Maillard reaction and caramelization, could take place during the pasta extrusion process, which directly influence both color and appearance of end-products (Sajid Mushtaq et al., 2023). In particular, the lightness of GF extrudates is significantly influenced by high extrusion temperature via an increase in the die temperature (Sajid Mushtaq et al., 2023). Moreover, the drying process in pasta-making also has a significant role in the color because of the outset of the Maillard reactions and the following generation of the colored components (Marengo et al., 2018). In addition, differences about hydration kinetics of kernel or flour (Marti et al., 2018), the strength of ionic linkages and pH (Ungureanu-Iuga et al., 2020) also have a role in pasta color**.**

The instrumental methods for color identification were developed to neglect the individual effect and ensure consistent statistical information because evaluating the color and appearance of pasta is subjective (Lorenzo et al., 2018). In this context, the L* value represents lightness, which is in the range of 0-100, indicating dark to light, respectively; a* value refers to the red to green color degree, and the b* value remarks the yellow to blue color degree. Therefore, particularly L* and b* values are particularly specific color features for pasta quality, which is directly associated with consumer acceptability (Lux et al., 2023). The chroma (C*) expresses the color intensity perceived by the products (Giri and Sakhale, 2022), and hue (h°) states the color location in a 3D diagram in which 90° indicates a color close to pure yellow (Pessanha et al., 2023). In other respects, the higher color scores, which are in the range between 1-10, are more pleasing (Lorenzo et al., 2018), and the yellowness index (YI) should maintain its stability throughout pasta cooking (Lux et al., 2023). On the other side, color differences (ΔE*) account for more than 2, are accepted as noticeable by consumers (Motta Romero et al., 2017; Llavata et al., 2019), but the limit degree of ΔE* about the incapability of detecting by the naked eye is accepted as 5 in another study (Weber et al., 2023).

Therefore, the color parameters regarding L*, a*, b*, C*, h°, and also derivatives, including YI, color score, $\Delta E^*_{cooking}$, of both raw and cooked (1-15 min) fresh control and GFP samples were given in Table 4.11 and Appendix 4. Both semolina-based control pasta and all GFP samples with 3 different ratios of PGS had positive b* values in the whole cooking process, which is accepted as a desirable pasta color (Caratini and Rosentrater, 2019). This could be interrelated with the presence of whole egg powder at the same concentration (15%) in all pasta samples (section 3.2.1, Table 3.1). The positive effect of egg albumen in rice-flour-based GFP (Marengo et al., 2018) and egg powder in rice-flour-based yellow alkaline noodle (Seetapan et al., 2019) on b* values was also stated in previous studies**.** However, among them, the control group based on semolina had the significantly highest b* and YI values in comparison with all GFP samples throughout the cooking process ($p<0.05$), although the relatively high lipoxigenase activity endogenously present in semolina had a role in bleaching yellow pigments via oxidation upon pasta processing (Caratini and Rosentrater, 2019). The greater yellowness of control pasta may arise from higher carotenoid content, including lutein and zeaxanthin of wheat, in contrast to corn starch, as well.

Table 4.11. Color properties of raw and cooked semolina- and gluten-free pasta with pregelatinized starch

Color parameter	Cooking time (min)	C	10-PGS	20-PGS	30-PGS
L*	Raw	61.80±1.53^{Cc}	79.96±0.60^{Aa}	76.55±1.20^{ABab}	73.67±0.99^{ABb}
	1	66.56±0.77^{Cc}	74.74±0.53^{Ba}	72.87±0.59^{Bb}	71.56±0.50^{Bb}
	5	69.68±1.21^{Ba}	73.48±1.79^{Ba}	71.72±0.52^{BCa}	70.31±1.14^{BCa}
	10	72.05±1.16^{Aa}	71.65±1.33^{Ca}	70.81±0.60^{Ca}	69.69±0.36^{BCa}
	15	74.94±0.27^{Aa}	70.71±0.16^{Cb}	69.58±0.57^{Cc}	68.87±0.53^{Cc}
a*	Raw	2.32±0.05^{Aa}	0.72±0.09^{Ab}	0.81±0.14^{Ab}	0.86±0.13^{Ab}
	1	-2.38±0.07^{Ba}	-2.99±0.13^{Bc}	-2.80±0.09^{Bbc}	-2.59±0.06^{Bab}
	5	-2.75±0.06^{Ca}	-3.39±0.03^{Cc}	-3.20±0.02^{Cb}	-3.11±0.04^{Cb}
	10	-3.48±0.05^{Da}	-3.85±0.03^{Dc}	-3.62±0.01^{Db}	-3.48±0.03^{Da}
	15	-3.79±0.08^{Eb}	-4.07±0.03^{Ec}	-3.73±0.01^{Eab}	-3.67±0.03^{Ea}
b*	Raw	33.25±0.84^{Aa}	31.20±0.34^{Aa}	26.68±0.53^{Ab}	24.84±0.39^{Ab}
	1	27.22±0.68^{ABa}	22.45±1.21^{Bb}	21.45±0.21^{Bb}	20.98±0.13^{Bb}
	5	23.09±0.65^{BCa}	19.06±0.19^{Bb}	17.53±0.42^{Cc}	16.44±0.28^{Cc}
	10	22.22±0.26^{Ca}	15.44±0.21^{Cb}	14.50±0.23^{Dc}	13.83±0.51^{Dc}
	15	19.80±0.35^{Da}	13.96±0.13^{Db}	13.53±0.27^{Db}	12.85±0.21^{Dc}
YI	Raw	76.83±1.48^{Aa}	55.74±1.03^{Ab}	49.85±1.35^{Ac}	48.18±0.10^{Ac}
	1	58.40±1.06^{Ba}	42.94±2.49^{ABb}	42.05±0.76^{Bb}	41.88±0.54^{Bb}
	5	47.38±1.24^{Ca}	37.10±1.29^{Bb}	34.91±0.59^{Cb}	33.45±1.50^{Cb}
	10	44.08±1.23^{Da}	30.79±0.98^{Cb}	29.26±0.21^{Dbc}	28.35±0.90^{CDc}
	15	37.75±0.54^{Da}	28.21±0.32^{Cb}	27.78±0.77^{Dbc}	26.65±0.63^{Dc}
Color score	Raw	6.41±0.23^{Abc}	7.12±0.01^{Aa}	6.50±0.06^{Ab}	6.17±0.09^{Ac}
	1	6.05±0.16^{ABa}	5.98±0.19^{Bab}	5.79±0.01^{Bab}	5.68±0.02^{Bb}
	5	5.79±0.01^{Ba}	5.58±0.07^{Bb}	5.34±0.07^{Cc}	5.16±0.07^{Cd}
	10	5.82±0.04^{Ba}	5.13±0.05^{Cb}	4.99±0.05^{Dc}	4.87±0.07^{Dc}
	15	5.73±0.05^{Ba}	4.93±0.01^{Db}	4.83±0.01^{Ec}	4.73±0.01^{Dd}
$\Delta E^*_{cooking}$	1	9.11±0.34^{Dab}	10.96±0.85^{Ca}	7.37±0.10^{Db}	4.96±0.01^{Cc}
	5	13.85±1.15^{Ca}	14.46±0.65^{Ca}	11.11±0.57^{Cb}	9.40±0.27^{Bb}
	10	16.16±0.90^{Bb}	18.44±0.43^{Ba}	14.18±0.45^{Bc}	11.81±0.54^{Ad}
	15	19.78±0.09^{Ab}	20.14±0.03^{Aa}	15.58±0.03^{Ac}	13.00±0.04^{Ad}

Means followed by the different uppercase letters in the same column regarding cooking time for each color parameter were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same row at the same cooking time for each color parameter were significantly different ($p<0.05$).

On the other side, the L* values of control pasta were higher than all GFP samples within the last phase of cooking (10 and 15 min), but lower within the first 5 min of cooking. Therefore, the most yellowish and brightest pasta, also supported by its highest YI, was recorded in the control pasta in the last two intervals of cooking (10 and 15 min), which are the qualities desired by consumers. The differences in the characteristics of pasta reflectance could originate from interaction with other molecules, culminating in structural changes (Oduro-Obeng et al., 2021). In a study, L* values of gluten-free noodles based on rice flour partially replaced (15%) with pregelatinized rice flour obtained by different methods (hot air-dried, freeze-dried, and twin screw extruder-dried) were lower than the control noodle made from wheat flour. This was linked with high-heat treatment application for the gelatinization process, probably reducing the natural whiteness of the rice flour (Jeong and Lee, 2025).

The increase in PGS incorporation level into GFP causes a slight reduction in lightness (L*) values, as well (Table 4.11). The decline in L* values with PGS introduction was also supported by the previous studies that pregelatinized quinoa flour-added (20-40%) gluten-free spaghetti (Caratini and Rosentrater, 2019), and partially pregelatinized chayotextile flour-replaced (10-30%) semolina-based pasta (González Victoriano et al., 2021). The darkness increase, namely decrease in L* in GFP including PGS at different proportions, was possibly because of the Maillard reaction, based on reducing sugars in the pregelatinization process, by heating of flour and water (Caratini and Rosentrater, 2019). This could also be explained by the idea of the positive relationship between lightness and water absorption (section 4.2.2, Table 4.5) (Indrastuti et al., 2024) and the negative relationship between CL and lightness (section 4.2.4, Table 4.7). It was proposed that the enhanced saturation and brightness, but reduced cooking loss and turbidity, upon cooking and/or steaming, led to the developed consumers' appeal (Li et al., 2024). Therefore, it could be interpreted that consumers' tendency towards color may be negatively influenced as the PGS level increases in GFP formulation. It was proved by sensorial analysis results (section 4.2.14 (Table 4.18), although the differences in color scores between the GFP samples were not significantly different ($p>0.05$). In accordance with our results, the rise in pregelatinized quinoa flour ratios from 20% up to 40% led to a reduction in L* values, accounting for nearly 43 to 40 in gluten-free spaghetti samples (Caratini and Rosentrater, 2019). In contrast, 10% cassava starch addition into taro flour-based dried noodle significantly increased lightness values ($p<0.01$), meaning brighter in contrast to cassava starch-free ones (0%) (Indrastuti et al., 2024).

On the other side, significantly lower b* values of GFP with different levels of PGS were defined in comparison with the control made from semolina ($p<0.05$), and this reduction was more prominent as the PGS level increased (Table 4.11). This was in line with the hypothesis that the higher yellowness could be observed in more corn flour/starch in GF cooked pasta samples (Sosa et al., 2019), aligning with that the corn starch ratio was decreased as the replacement proportion with PGS increased, in this study. In a similar vein, b* values were reduced from approximately 17 to 4 in the increase of pregelatinized chayotextile flour from 10% to 30% in semolina pasta, although there was an increment was recorded in 20% partially replaced pasta samples (b*:≈23) (González Victoriano et al., 2021). The differences in color values between the present study and those assessed by other authors could be sourced from the differences in both quantity and quality of pigments present in the raw materials utilized in the different pasta formulations (Fiorda et al., 2013a). Moreover, a slight decrease in YI and color score was also observed as the concentration of PGS raised in GFP in the present study (Table 4.11).

On the other hand, the L* values of the control group prepared with semolina were increased, whereas corresponding values of GFP samples, regardless of the PGS amount, were decreased as the cooking process continued (Table 4.11). This is consistent with prior findings demonstrating that cooking generally decreased in noodle lightness (Jeong and Lee, 2025). The decrease in the L* value with cooking time increase refers to a browning of the end-product (Sanguinetti et al., 2015) agrees with the findings previously reported by other authors studying gluten-free spaghetti based on rice flour admixed with bean flour at different amounts (80:20, 60:40) (Giuberti et al., 2015), gluten-free pasta made from proso millet (Motta Romero et al., 2017), and corn flour and starch-based GFP including grape peels and whey powders (Ungureanu-Iuga et al., 2020). This could also be attributed to the fact that the excessive release of molecules from whole egg powder in GFP formulations to the cooking medium may tend to enhance the Maillard reaction, eventuate in a non-enzymatic browning on the surface, which possibly masks the lightness source from gelatinization (Sanguinetti et al., 2015). The higher CL values, and thus the compounds, give rise to high turbidity and amylose leaching of GFP than which of control pasta may be caused by covering the pasta surface, which may mask the lightness.

The a* and b* values of both control and all GFP samples containing 3 different ratios of PGS were also decreased within the cooking duration (Table 4.11). The cooking process led to a reduction in both color parameters in agreement with the literature, such as fenugreek incorporated GFP based on tigernut-chickpea combination (Llavata et al., 2019), and gluten-free traditional Turkish noodle with *Macrolepiota procera* mushroom (Süfer et al., 2022). In particular, the reduction in b* values during the pasta cooking process, in contrast to corresponding raw values, because of the relation with pasta yellowness, was supported with several studies (Giuberti et al., 2015; Bouasla et al., 2017b; Ungureanu-Iuga et al., 2020; Süfer, 2022). This behaviour is explained as a consequence of thermal degradation and dissolution of pigments, resulting in leaching into the cooking medium with boiling water (Giuberti et al., 2015; Bouasla et al., 2017b). The major carotenoids present in both wheat semolina and corn starch are zeaxanthin and lutein, which show heat-labile character; a color degradation was expected once all of the pasta samples were cooked (Motta Romero et al., 2017). A similar decrement manner is also recorded in YI and color score values with cooking, in line with a* and b* color values. This could be disclosed with the predominant calculation based on the b* values. In addition to this, the CL increase and further protein denaturation could be the charge of the reduced YI (Lux et al., 2023), aligned with increasing CL values as cooking prolonged in all pasta samples, as previously given in section 4.2.4 (Table 4.7). However, although the YI decrease was observed in all pasta samples as cooking time prolonged, its intensity changed according to pasta composition (Lux et al., 2023). Similarly, YI was declined in gluten-free fresh amaranth-based pasta (amaranth flour: water 1:2, 1:4, and 1:6), including 1% alginate without excepting 15 min; and 1.5 alginate (amaranth flour: water 1:2, 1:4, 1:6, and 1:8) up to 10 min, in the same specific conditions (Lux et al., 2023). Regarding pasta colour stability upon cooking, $\Delta E_{cooking}$ was increased as the cooking process continued up to 15 min (Table 4.11), probably stemming from color pigment degradation by cooking (Fradinho et al., 2020). In terms of color stability during cooking, the ΔE* of all cooked pasta samples could be noticeable by regular human vision (Table 4.11),

4.2.9. Texture

Texture analysis is one of the main quality features that influence the eating quality of pasta/noodle (Sajid Mushtaq et al., 2023). It is utilized to assess the influence of ingredients and process methods on raw or cooked pasta/noodle, and ultimately the acceptance of consumers (Patiño-Rodríguez et al., 2018). In general, highly soft and sticky pasta is unacceptable, but firm and elastic pasta, namely “al-dente”, is generally accepted as desirable (Hager et al., 2012). Both starch gelatinization and protein coagulation, which occur while the pasta cooking process, give rise to structural changes and thus affect the texture of the end-product (Sozer and Kaya, 2008). The absorption and swelling of water by protein should occur more rapidly in comparison with starch granules while cooking a pasta with high quality (Sozer and Kaya, 2008). Therefore, to obtain a good cooking quality pasta with a firmer texture and less stickiness, the onset of the starch gelatinization should be materialized after the protein hydration (Sozer and Kaya, 2008; Susanna and Prabhasankar, 2013). The CL is one of the most determining factors in pasta texture. In case of higher CL, which reflects lower cooking tolerance and higher starch solubility, a turbid cooking medium can cause a sticky mouthfeel. In contrast, a chewy texture is achieved in the conditions of less cooking weight because of poor WAC (Wang et al., 2012). The hardness/firmness of the GFP is mainly associated with the individual hydrated starch granules and/or gelatinized starch granules embedded in the denatured external protein matrix (Ertaş et al., 2023). The implementation of the treatments, including heating and cooling, which cause gelatinization and retrogradation of the starch, respectively, forms a starch network able to endure cooking stress (Wang et al., 2012). Therefore, starch becomes a crucial structural element in the textural properties of the GFP due to its macromolecular structure (Mirhosseini et al., 2015). However, GFP is generally described with a lower hardness, chewiness, and springiness, and higher adhesiveness values (Faheid et al., 2022). To compete this, stabilization and emulsifying agents should be incorporated into GFP formulation to achieve better starch adherence in the structure with a firmer and less sticky structure (Ertaş et al., 2023). Therefore, the firmness of GFP is considerably influenced by raw materials and also food additives (Ertaş et al., 2023). As a result, textural quality regarding the meet of structural integrity, including firmness and elasticity with a certain degree of less stickiness, as texture parameters, becomes a more critical factor in GFP production (Milde et al., 2021).

The texture profile analysis (TPA) is a double compression test for the measurement of the mechanical and physical attributes of food samples by simulating a bite process. While hardness, resilience, and adhesiveness are obtained during the first bite, chewiness, cohesiveness, and springiness are attained during the 2nd compression cycle (Faheid et al., 2022). Among them, hardness is defined as the required force to give a deformation to pasta (Gasparre and Rosell, 2019; Faheid et al., 2022), which is associated with the structures' strength under compression (Faheid et al., 2022), and a good index for consistency of pasta (Gasparre and Rosell, 2019). Chewiness is described as needed energy for chewing till suitable to swallow pasta (Udachan and Sahoo, 2017; Gasparre and Rosell, 2019), and is associated with the protein matrix's elastic strength (Lorenzo et al., 2018; Pessanha et al., 2023). Cohesiveness measures the internal bond strength of pasta (Udachan and Sahoo, 2017; Bolarinwa and Oyesiji, 2021), thus it is indicative about the ability of the pasta to hold together throughout the cooking process (Sozer and Kaya, 2008; Lorenzo et al., 2018; Sosa et al., 2019), which depends on the competition between starch, proteins, and additives (i.e., hydrocolloids) for forming a continuous network in GFP (Lorenzo et al., 2018). Springiness defines elasticity by quantifying the extent of recovery of the pasta between the 1st and the 2nd cycle of compression (Faheid et al., 2022). Resilience is the capability for the regaining of the original shape of pasta after 1st compression (Lorenzo et al., 2018). Therefore, the TPA parameters (hardness, chewiness, cohesiveness, springiness, and resilience) of the control based on durum wheat semolina and GFP with different PGS ratios (10, 20, and 30%) during 15 min of cooking with intervals (0, 5, 10, and 15 min) were given in Table 4.12.

Although XN could mimic the elastic nature of gluten and improve the firmness of pasta (Palavecino et al., 2017; Milde et al., 2020), owing to binding the water-soluble starch (Ertaş et al., 2023), and thus the capability of forming a gel with high consistency in aqueous solutions (Palavecino et al., 2017), all textural attributes were higher throughout the cooking process in the control group based on semolina in comparison with GFP samples (Table 4.12). Protein structures are bound together by disulfide, hydrogen, and hydrophobic links to form a matrix that gives viscoelastic features to cooked pasta made by durum wheat semolina. The strength and continuity of the protein matrix are shaped based on these inter- and intra-molecular bonds (Tudorică et al., 2002; Foschia et al., 2015a). In this context, the firmness of durum wheat pasta is characterized by gluten proteins, which form a protein network with cross-linking mechanisms that enhance protein aggregation and the generation of further interchain disulfide bonds (Menga et al., 2017). Therefore, this could be interpreted as semolina pasta as the control group has a strong structure because of gluten proteins, enabling the hardness while still allowing a good water absorption capacity (Lubowa et al., 2021). Conversely, hardness values of gluten-free noodles made from rice flour partially substituted (15%) with pregelatinized rice flour prepared by different drying methods (hot air, freeze-drying, and twin-screw extrusion drying) exhibited higher hardness values than the wheat flour-based noodle. In another study, the gluten-free noodles consisting of extruded pregelatinized rice flour at high temperature with short time also demonstrated higher hardness and chewiness values against to the control group made from wheat flour (Jeong and Lee, 2025). On the other hand, the strength and continuity of protein matrix held by intra- and inter-molecular bonds (disulfide, hydrogen, and hydrophobic bonds), which bring viscoelastic features to cooked pasta (Foschia et al., 2015a), and release of exudates while starch granule gelatinization is observed during the cooking process, and thus, probably may cause an increase in cohesiveness on the surface of the cooked pasta (Tudorică et al., 2002; Foschia et al., 2015a). Similar to our results, higher cohesiveness values were established in traditional wheat pasta, contrary to corn- or quinoa-based GFP, which is linked with the gluten network's cohesive characteristic, and whose interactivity with the gelatinized starch granules. This was interpreted as holding the GFP structure after the OCT is more difficult (Lorenzo et al., 2018), which aligns with our results in section 4.2.10 (Figure 4.8).

Table 4.12. Texture properties of cooked semolina- and gluten-free pasta with pregelatinized starch

Texture parameter	Cooking time (min)	C	10-PGS	20-PGS	30-PGS
Hardness (N)	**0**	10.89±0.42^{Aa}	5.99±0.10^{Ac}	4.34±0.16^{Ad}	7.62±0.56^{Ab}
	5	9.42±0.15^{Ba}	5.16±0.50^{Ab}	3.49±0.05^{Bc}	4.65±0.24^{Bb}
	10	8.16±0.62^{Ca}	3.96±0.39^{Bb}	2.86±0.13^{Cc}	2.38±0.37^{Cc}
	15	7.48±0.04^{Ca}	3.62±0.05^{Bb}	2.42±0.02^{Dc}	1.78±0.06^{Cd}
Chewiness (N)	**0**	45.00±2.14^{Aa}	4.16±0.12^{Ab}	2.60±0.06^{Ab}	4.42±0.17^{Ab}
	5	34.02±0.46^{Ba}	2.75±0.31^{Bb}	1.54±0.13^{Bc}	1.93±0.32^{Bbc}
	10	27.14±1.33^{Ca}	1.82±0.18^{Cb}	0.97±0.12^{Bb}	0.73±0.05^{Cb}
	15	19.96±1.21^{Ca}	1.42±0.02^{Cb}	0.65±0.03^{Cb}	0.44±0.07^{Db}
Cohesiveness	**0**	0.79±0.01^{Aa}	0.69±0.01^{Ab}	0.66±0.01^{Ac}	0.65±0.02^{Ac}
	5	0.72±0.01^{Ba}	0.57±0.01^{Bb}	0.53±0.01^{Bc}	0.52±0.02^{Bc}
	10	0.70±0.02^{BCa}	0.51±0.01^{Cb}	0.47±0.03^{BCbc}	0.45±0.01^{Cc}
	15	0.66±0.01^{Ca}	0.48±0.02^{Db}	0.44±0.01^{Cc}	0.43±0.01^{Dc}
Springiness	**0**	5.19±0.17^{ABCa}	1.00±0.02^{Ab}	0.90±0.01^{Ab}	0.90±0.01^{Ab}
	5	4.98±0.06^{Ba}	0.94±0.01^{Bb}	0.83±0.05^{ABbc}	0.79±0.07^{ABCc}
	10	4.77±0.03^{Ca}	0.90±0.01^{Cb}	0.72±0.02^{Bc}	0.69±0.05^{Bc}
	15	4.05±0.21^{Da}	0.82±0.02^{Db}	0.60±0.03^{Cb}	0.58±0.06^{Cb}
Resilience	**0**	0.59±0.02^{Aa}	0.54±0.02^{Aab}	0.51±0.01^{Ab}	0.48±0.04^{Ab}
	5	0.55±0.02^{Aa}	0.46±0.01^{Bb}	0.42±0.03^{Abc}	0.38±0.02^{Ac}
	10	0.50±0.01^{Ba}	0.42±0.02^{Cb}	0.33±0.01^{Bc}	0.31±0.01^{Bc}
	15	0.43±0.04^{Ba}	0.38±0.03^{Da}	0.30±0.01^{Cb}	0.29±0.01^{Bb}

Means followed by the different uppercase letters in the same column regarding cooking time for each texture parameter were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same row at the same cooking time for each texture parameter were significantly different ($p<0.05$).

The hydrothermal treatments modify the starch organization and are in charge of new macromolecular structure by stimulating the variation in the physical and chemical attributes of starch (Dib et al., 2018). Starch granules are destroyed during hydrothermal treatments, causing an increase in the intergranular attraction, which strongly bonded the compounds in the pasta (Ding et al., 2024). The hydrothermal treatments partially dispose the swelling of granules by decelerating the starch gelatinization and improving the starch paste stability (Dib et al., 2018). Therefore, the PGS could increase the dough viscosity, strengthen the network generated by natural starch particles, and enhance the starch gel network formation, which causes a reduction in CL (Dib et al., 2018) pasta with firmer structure (Ding et al., 2024), more consistent (Dib et al., 2018), less sticky, and thus improved textural structure (Dib et al., 2018). In the case of 10-PGS, this may be attributed to this, and in the presence of egg albumin originating from whole egg powder, which introduces an emulsification role, helping in the protein network formation. This network probably led involvement of the starch, restricting the water access to the starch granules present in the flours when submitted to gelatinization during cooking, limiting swelling of the granules and as a consequence reducing the amylose solubility, give rise to a more resistant film the formation on the pasta surface, which in turn elevated the hardness (Fiorda et al., 2013a) among the other GFP samples (Table 4.12). Similarly, the raw GFP including 40% pre-gelatinized flour made from cassava starch and cassava bagasse (70:30) demonstrated increased firmness, which was referred to a dominant pregelatinized ingredient characterized by high retention of water (Fiorda et al., 2013a). In an analogue study, hardness and chewiness values were increased when 10% cassava starch, which was pregelatinized by steaming, and added into fermented taro flour-based noodle dough, including XN (Indrastuti et al., 2024). However, the lowest hardness and chewiness values were determined in 20-PGS GFP samples within from the beginning to 5 min of cooking (Table 4.12). The reason behind this could be explained by having the highest WAC and SI values in the corresponding period, as declared previously in section 4.2.2 (Table 4.5) and section 4.2.3 (Table 4.6), respectively. In addition, the cohesiveness, springiness, and resilience values were generally reduced as the replacement ratio of semolina with PGS was increased in GFP (Table 4.12). Therefore, the highest and lowest hardness, chewiness, cohesiveness, springiness, and resilience values, whereafter control pasta, were belonged to GFP with 10-PGS and 30-PGS, since 5 min of cooking, respectively (Table 4.12). This could be interpreted as excessive amounts of PGS could worsen the GFP structure, but it could enhance endurable when used up to a specific level, particularly at prolonged cooking. Because water has a greater tendency to more firmly bound of water to protein than starch granules, which results in an insufficient amount of water for starch granules (Sozer and Kaya, 2008). Therefore, this could be arsied from the fact that not totally gelatinized starch while suspension preparation, owing to the limited water amount. As a result of this, swelling of ungelatinized starch starts, and it destroys the gel structure partially, causing softening in pasta texture (Lux et al., 2023). It could be supported by the tendency of being vulnerable to highly swollen starch to rupture as observed in β-glucan-enriched gluten-free rice noodle (Heo et al., 2014), and excessive water absorption is counterproductive to textural quality as monitored in chickpea-pasta, including carboxymethyl cellulose, and thus led to a decrease in firmness values (Losano Richard et al., 2022). In a study, a significant decrease was recorded in hardness and chewiness values when the semolina was partially (50%) replaced with PGS extruded amaranth flour ($p<0.05$) (Bello-Perez et al., 2022). In another study, the springiness declined when an excessive amount of pregelatinized highland (hull-less) barley flour was used in noodle formulation, owing to gain the viscous-dominated gel structure character, which from elastic after hydrothermal treatment (Ding et al., 2024).

The adverse relationship between hardness/firmness and CL was supported by other researchers (Wang et al., 2012; Alinovi et al., 2023; Indrastuti et al., 2024) reflecting changes in the strcture because of the change in pasta formulation is in charge of for change in mass transfer and texture phenomena while cooking (Alinovi et al., 2023), which was consistent with our CL results particularly obtained in the latest phase of cooking (10-15 min), as given in the section 4.2.4 (Table 4.7), although there was some controversial positive links between firmness and CL, as well (Sobota et al., 2013). A negative link between hardness/firmness with turbidity (Park et al., 2022), chewiness with CL (Milde et al., 2020), and also amylose leaching (Palavecino et al., 2017; Milde et al., 2020; Alizadeh et al., 2024) was aligned with our corresponding results acquired in the latest phase of cooking (10-15 min), and demonstrated in the section 4.2.6 (Table 4.9), section 4.2.4 (Table 4.7), and section 4.2.7 (Table 4.10), respectively. On the other hand, a number of studies have revealed that there was also a negative associaton between hardness and water absorption/holding ability/capacity (Wang et al., 2012; Duta et al., 2019; Garcia-Valle et al., 2021; Oduro-Obeng et al., 2021; Odabas and Cakmak, 2022; Aydin, 2022**;** Weber et al., 2023) which consistent with our WAC results particularly obtained in the first phase of cooking (0-5 min) as situated in the section 4.2.2 (Table 4.5) which confirmed the weakening influence of highly absorbed water during cooking (Odabas and Cakmak, 2022). An inverse interrelation between hardness/firmness with moisture (Brennan and Tudorica, 2007) and SI (Brennan and Tudorica, 2007; Difonzo et al., 2022; Aydin, 2022) was followed up by our corresponding results achieved in the first phases of cooking (0-5 min), and detailed in section 4.2.3 (Table 4.6), respectively. Controversially the positive association between firmness with water absorption or weight increase was also declared (Lux et al., 2023; Sobota et al., 2013) and SI (Lux et al., 2023) are more eligible for the extended cooking time (10-15 min) in GFP samples, regarding as presented previously in the section 4.2.2 (Table 4.5) and section 4.2.3 (Table 4.6), respectively. Therefore, different phases of the cooking process could be dominated by different parameters, and this way, the influence on end-product quality could be changed according to their manner.

A gradual hydration in the deeper layers of pasta with further cooking durations, which causes changes in the pasta properties (Sobota et al., 2013). It was emphasised by several researchers that the pasta which originates from durum wheat grain as a raw material, is generally characterized with high protein content and a strong gluten network, allowing it to be still endurable better overcooking and the consistency changes in consequence of increased cooking times (Sobota et al., 2013), align with our hardness results of control pasta (Table 4.12). Although the pasta structure should retain non-sticky and firm after cooking (Pessanha et al., 2023), a reduction was generally recorded in all textural features of both control and GFP samples as the cooking progressed (Table 4.12). In a similar way, the hardness was reduced in spaghetti made by durum wheat semolina (Cimini et al., 2019), gluten-free lasagna dough with air yams (Weber et al., 2023), gluten-free traditional Turkish noodle, including *Macrolepiota procera* mushroom, in contrast to that of uncooked samples (Süfer, 2022), and gluten-free starch noodles made from sweet potato (Menon et al., 2016). In a similar vein, less firm spaghetti was acquired irrespective of wheat cultivar or flour type as cooking time progressed because of absorbing more water (Oduro-Obeng et al., 2021), and chewiness also declined in gluten-free fusilli based on raw whole millet (Pessanha et al., 2023) as a result of the continued cooking process. Hardness/firmness was displayed 2-fold loss when extending the OCT (Biernacka et al., 2019). In our study, the hardness values of 10- and 20-PGS GFP decreased by half at the end of cooking, whereas they further declined in GFP with the highest amount of PGS (30-PGS) (Table 4.12). On the contrary, the firmness of amaranth flour-based gluten-free pasta, including alginate (1.5%), was increased with progressive cooking, probably due to the dramatic loss of hydrogen bonds with heating, enabling the escape of water from pasta, resulting in a firm texture (Lux et al., 2023). In another controversial study, springiness and cohesiveness values in gluten-free lasagna dough, including air yams, were increased with cooking due to gelling attributes of starch (Weber et al., 2023). The resilience also showed an increase in durum wheat semolina spaghetti as the cooking time progressed, which is interrelated with the progressive coagulation and interactions of proteins that led to the generation of a reinforced and continuous network within which starch granules were trapped (Cimini et al., 2019).

In the literature regarding hardness/firmness values of pasta/noodle comprisng of PGS was between in likely 13-14 N in semolina pasta comprising of extruded pregelatinized amaranth flour (50%) with different gelatinization degrees (50 and 100%) (Bello-Perez et al., 2022), 710-716 N in pasta produced by pregelatinised red lentils (Bresciani et al., 2023), 79-82.5 N in noodles based on highland barley flour with 3 differet ratios of pregelatinized highland barley flour (5.0, 7.5, and 10%) (Ding et al., 2024), 73-82 N in pregelatinized sweet potato starch-incorporated (1, 2, 3 and 4%) highland barley noodle (Ding et al., 2024), 94-117 N in rice noodle partially substituted with pregelatinized rice flour (15%) by utilizing different drying methods (hot air drying, freeze drying, and twin-screw extruder drying) (Jeong and Lee, 2025), 43-51 g in noodle where totally replaced of wheat flour with drum dried starch at different temperatures (120, 130, and 140°C) pregelatinized white kidney bean (Niyomwong et al., 2025). The discrepancy between our results and the literature may arise from the differences in formulation, together with the type/form of pasta/noodle, cooking time, and also the source and level of PGS.

4.2.10. Optimum Cooking Time

The acceptability of pasta from consumers is based on its cooking behavior associated with a proper development of starch-protein and protein-protein matrix (Marengo et al., 2018). In this context, the overall cooking quality of pasta depends on several phenomenon taking place through cooking as denaturation of proteins (Milde et al., 2020), hydration and complete gelatinization of starch, and interactivity with non-starchy matrices (Giuberti et al., 2015), crystalline structure loss with no rupture of the granule to dextrinization and leaching (Milde et al., 2020). When some of the starch gelatinize and then become a part of the pasta structure, others are leaching into the cooking medium along with amylose chains, ending in an undesirable pasta with a sticky surface and turbid cooking water (Indrastuti et al., 2024). The phenomenon that occurs in starches depends on many factors, such as concentration, mechanical stress level through the cooking process, and treatment duration (Milde et al., 2020). In this regard, the cooking time is the parameter that specifies the contact duration of pasta with boiling water to gelatinize for cooking and consuming (Milde et al., 2020; Indrastuti et al., 2024). Optimum cooking time (OCT) is identified as the precise time for the disappearance of the white center core in the pasta strand (Lorenzo et al., 2018). It is determined to achieve resilient, firm, non-sticky pasta, namely "al-dente", where a starch fraction is still not gelatinized (Diantom et al., 2019). It is a crucial parameter in the culinary attributes (Córdoba-Cerón et al., 2022) in terms of nutritional and textural properties of pasta, but also has a role in the energy cost of the cooking process (Patiño-Rodríguez et al., 2019).The OCT is interconnected with the water diffusion ratio into pasta, which is highly associated with formulation/composition and process conditions of pasta, which both influence the matrix structure of the end-product (Lorenzo et al., 2018), and pasta shape (Milde et al., 2021). In this regard, the longer OCT is influenced by higher protein and amylose content, particle shape, and size (Aydin, 2022). In particular, a stronger matrix form is generated in higher ratios of proteins (Milde et al., 2021). Therefore, a higher value of OCT mentions requires a longer time period for pasta cooking (Phongthai et al., 2017). However, the prolongation of OCT resulted in extreme soluble solid loss into water (de Oliveira et al., 2022). Therefore, shorter cooking time with low CL values is characteristic of a good quality pasta. Because while the pasta with a hard and coarse texture is obtained when the cooking time is not sufficient, excessively soft and sticky pasta is achieved with a very long cooking time (Indrastuti et al., 2024). However, the OCT is generally short owing to the need for water to attain a pasta center through cooking is low when there is a lack of gluten network, as in the case of GFP. Because GFP has fewer an impact in contrast to the 3D gluten network present in wheat. Thus, the faster penetration ratio of water into the core structure of pasta occurs in GFP (Seetapan et al., 2019). On the other side, the majority of GFP samples had a less distinctive outer layer, which could possibly lead to a failure in determining OCT by compressing the samples between 2 glass plates according to the AACC Approved Method (Hager et al., 2012). The OCT of control pasta and GFP samples was given in Figure 4.8, and representative visuals for OCT determination, including raw and 4 different cooked time intervals (1, 5, 10, and 15 min), were given in Appendix 5 (a-d).

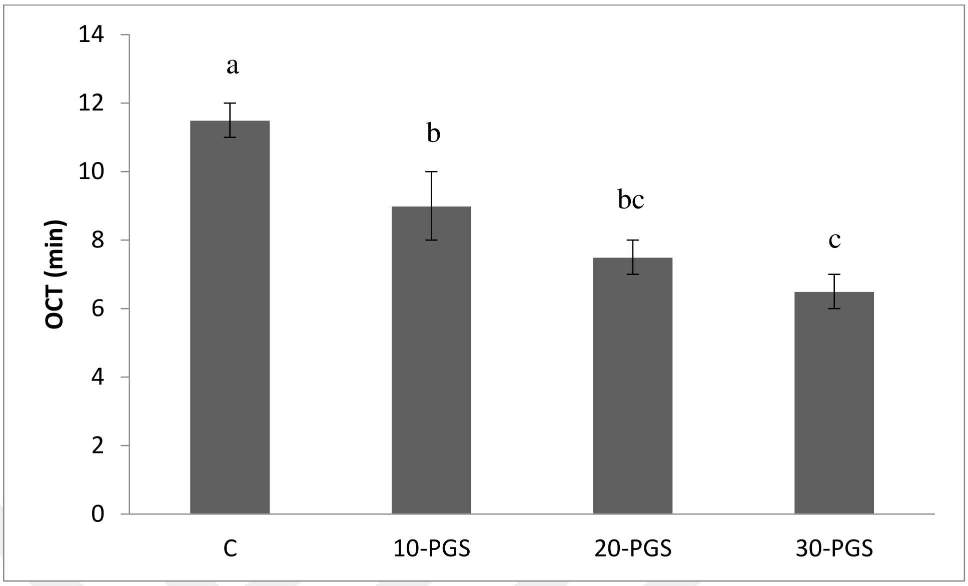

Figure 4.8. Optimum cooking time of semolina- and gluten-free pasta with pregelatinized starch

The significantly highest OCT belonged to the control group produced from durum wheat semolina, in contrast to all GFP samples, including different PGS ratios ($p<0.05$). This reflects that the time required for starch to fully gelatinize in the control pasta becomes longer. The higher OCT could also be ascribed to protein content increase, causing firmer textural structure of the end-product (Giri and Sakhale, 2022). Because longer OCT is materialized with protein increase by absorbing more water and letting fewer water available for gelatinization of starch (Rachman et al., 2019). This refers to a more homogenous and compact structure of the starch-protein matrix (Witek et al., 2020) as monitored in the control group, including gluten, supported by its microscopic images (section 4.2.13, Figure 4.9a). On the other side, in a study where enrichment of pasta with whole egg was facilitated, the cooking was achieved by rapid water penetration (Witek et al., 2020).

The pregelatinized starch has effectively absorbed and retained water, which in turn exhibited shorter cooking times (Lubowa et al., 2021). It was also declared that a shorter OCT is expected if the pregelatinized flour is utilized (Bento et al., 2021) or pregelatinization occurs in the extrusion process (Baah et al., 2022), in GFP production. This was consistent with our OCT values of GFP samples, which were between 6.5-9 min (Figure 4.8). This could be dedicated to the enhancement of both the water absorption and swelling ability of starch (Aydin, 2022), which led to faster starch hydration during cooking, ended up with lower OCT (Baah et al., 2022). Moreover, the decrease in OCT of gluten-free pasta/noodle could be explained by accelerated softening and loss of capability to retain mechanical attributes in the presence of water (Milde et al., 2020), resulting in poor pasta structure to allow faster transfer and penetration/diffusion ratio of water into the centeral zone of the pasta dough (Rachman et al., 2020). This could be interpreted as having a less compact structure, incapable of enduring the cooking stresses (Bresciani et al., 2021), although utilizing hydrocolloids in GFP samples have a potential to prolong OCT (Gasparre and Rosell, 2019) because of limitation of water (Gasparre and Rosell, 2019) by binding water with their several hydroxyl groups which cause a competition between the starch to acquire water (Indrastuti et al., 2024) and thereby postpone the starch gelatinization by rising in the gelatinization temperature onset (Gasparre and Rosell, 2019). Even though the hydrocolloids could support the network generation in GFP (Patiño-Rodríguez et al., 2019), and particularly XN is stable under hot conditions, which could put off the starch granules from swelling (Indrastuti et al., 2024), the comparable values with semolina pasta were not accomplished in our GFP samples with different PGS. As a result, in general, GFP needs fewer cooking times in contrast to durum wheat flour/semolina because of the absence of a compact gluten network (Ungureanu-Iuga et al., 2020), which expresses the generation of a weaker protein network (Garcia-Valle et al., 2021), as observed in our study (Figure 4.8). This is in accordance with previously published GFP studies, such as those made from amaranth (Islas-Rubio et al., 2014), banana-cassava flour (Rachman et al., 2019; Rachman et al., 2020), and faba bean (Rosa-Sibakov et al., 2016). A study based on the evaluation of commercial GFP from different brands in the market in comparison with gluten-containing counterparts as a control group revealed that the OCT of gluten-free pasta samples was in a wide range, corresponding to 4.49-12.87 min, which was also generally lower than the control (Hayıt et al., 2023). The higher cooking time values, rather than the results of our study, could be explained by the fact that those GFP samples were in the dried form in the market. In contrast, higher OCT in gluten-free formulations than those of traditional wheat flour/semolina pasta (Lorenzo et al., 2018), as also monitored in corn flour-based gluten-free spaghetti (Gasparre and Rosell, 2019), and spriuluna-enriched protein maize gluten-free pasta (Veena et al., 2022). This was attributed to the restriction of water diffusion towards the pasta center in the case of spirulina protein (Veena et al., 2022). In another study, gluten-free noodles where rice flour was partially substituted with (5, 10, and 15%) with pregelatinised high-amylose maize starch in combined with Ca^{2+}-induced setting of alginate, had statistically higher OCT, ranging between nearly 7-8 min than control pasta based on only rice flour without PGS (≈3 min) ($p<0.05$). This was explained by limiting water uptake because of the influence of network strengthening by PGS maize starch with 1% sodium alginate set by Ca^{2+} (Lubowa et al., 2021). On the other hand, the potentially slightly shorter OCT of the control group in our study, in contrast to commercial counterparts in the market, could be explained by being in the form of fresh pasta.

As given in Figure 4.8, the OCT was decreased with the increase in the PGS ratio in the GFP formulation. It could also be related to the positive correlation between water absorption and OCT (Giuberti et al., 2015; Duta et al., 2019), which expounded to more water diffusion and interaction with both protein and starch matrices because of increasing accessibility to the protein polar amino-acid groups, while cooking, in consequence of the protein denaturation, could promote the water affinity (Giuberti et al., 2015). However, the adverse relation between CL and OCT (Bello-Perez et al., 2022) of GFP pasta samples could be the major reason, instead of WAC, in our cases, particularly seen in its influence on prolonged cooking time subsequent to 5 min, in the present study. On the other hand, the positive association between OCT and firmness (Wang et al., 2012) was also consistent with our results recorded in the extended cooking times beyond 5 min, as previously reported (section 4.2.9, Table 4.12). However, there were also contradicting observations suggesting that a greater content of cassava starch or pregelatinized flour usage in gluten-free pasta/noodle makes the recorded OCT longer. This was clarified with different hypothesis based on acting as a binder of cassava starch (Bolarinwa and Oyesiji, 2021), the interactions of starch with protein and lipids take place during extrusion related with the influence of starch retrogradation while drying of pregelatinized flour preventing the cooking of pasta/noodle (de Moura et al., 2016), and this reflects the macroscopic result of the more ability of the exposed-hydrophilic groups for binding water molecules and generate a gel form (da Silva et al., 2016).

In the literature OCT of pasta/noodle consisting of pregelatinized starch was defined as 6 min in noodle based on wheat flour fortified with pregelatinized highland barley flour at different levels (30, 50 and 70%) (Wang et al., 2025a), 6.5 min in gluten-free spaghetti prepared from amaranthus flour and partially replaced (5, 7, and 9%) with pregelatinized corn starch (Chillo et al., 2007), 10-12 min in pasta partially (50%) substitued with pregelatinized amaranth flour (Bello-Perez et al., 2022), 7-8 min in rice noodle partially replaced (5, 10, and 15%) with pregelatinized high-amylose maize starch combination with 1% sodium alginate set by Ca^{2+} (Lubowa et al., 2021), 14.5 min in wheat flour-based noodle partially substituted (5, 10, and 15%) with pregelatinized corn starch (Yousif et al., 2012). The discrepancy between our results and the literature may have arisen from the differences in formulation, together with the type/form of pasta/noodle, and also the type and replacement level of PGS.

4.2.11. Differential Scanning Calorimetry

Under heating conditions with temperature change, while the protein molecules are denatured and polymerised into a more stable and uniform reticular structure, the starch compounds break into the paste (Zhao et al., 2024). The gelatinization process accounts for a progressive crystallinity loss of amylopectin chains in starch owing to water and thermal plasticization (Laurent et al., 2023). Therefore, a disruption in the ordered molecules occurs while the heating process towards excess water occurs throughout the starch granules' gelatinization process (Jia et al., 2023). As a result of this, both hydration and swelling of the amorphous regions in the starch granules are conducted by the gelatinization process, which includes double helices and crystalline regions' melting. Throughout amorphous regions, stress is caused on the polymer chain and crystalline region, and taken away from the surface of the starch crystallite, resulting in an increment in gelatinization temperatures (Dib et al., 2018). The primary structural alterations are protein insolubilization and starch gelatinization through cooking, which happen at almost similar temperatures and moisture degrees. According to the DSC outcome, almost all (≈99%) of the endotherm of starch gelatinization disappears in cooked pasta (Laurent et al., 2023). To detect temperature for characterizing starch gelatinization at different phases, differential scanning calorimetry (DSC) is a beneficial method. While the onset temperature (T_o) and endset temperature (T_c) correspond with the beginning and end of the gelatinization process, the peak temperature (T_p) indicates maximum heat flow related to the main phase transition (Dib et al., 2018). All of these melting temperatures (T_o, T_p, T_c) denote the heat stability of starch crystallites (Chen et al., 2024a). The T_p differences may originate from variation in amylose amount, amylose-lipid complex, amylopectin brain-chain length, rate of crystalline to amorphous in the granule structure (Tazrart et al., 2019), and larger particle size distribution (Lawal et al., 2021). On the other side, the enthalpy (ΔH) is measured by the peak area (Riva et al., 2000) reflects starch gelatinization degree within the starch- or flour-based system (Tudorică et al., 2002), and thus the melting degree of starch crystallites (Jia et al., 2023), also the quantity of the molecular order structures' loss (Seetapan et al., 2019) that is closely associated with single or double helical structure and crysal amounts (Chen et al., 2024a). It was announced that the gelatinization enthalpy had a positive association between starch with higher amylopectin proportion (Park and Baik, 2004), and longer-chain amylopectin is more associated with the need for higher energy for dissociation, culminating in ΔH increase (Tazrart et al., 2019). Therefore, the thermal parameters of raw control made from semolina and GFP samples with different PGS levels were measured by the DSC, and presented in Table 4.13.

Table 4.13. Thermal properties of semolina- and gluten-free pasta with pregelatinized starch

Sample	**T_o (°C)**	**T_p (°C)**	**T_c (°C)**	**ΔH (J/g)**
C	53.27±1.15[c]	69.13±0.76[b]	105.09±0.13[b]	10.83±1.13[a]
10-PGS	60.08±0.65[b]	70.29±0.92[b]	109.00±1.44[ab]	8.46±.1.08[a]
20-PGS	63.89±1.63[a]	73.79±0.11[a]	111.21±2.06[ab]	5.94±0.89[b]
30-PGS	66.09±1.37[a]	77.02±2.40[a]	112.36±4.05[a]	4.36±0.63[b]

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$).

All of the control pasta and GFP pasta samples generally displayed a single endotherm peak between nearly 53-112°C. The T_p of control pasta made with durum wheat semolina was located at about 69°C (Table 4.13), which aligned with the specific pattern in the DSC endotherm of the durum wheat semolina marked around 50-70°C, indicating native starch gelatinization in excess water (Laurent et al., 2023). In a similar vein, the T_p of raw wheat starch was measured as 69.61°C. However, lower values were also recorded in other studies, which could probably have arisen from the wheat starch origin (Tian et al., 2020). This could also be associated with gluten denaturation, although being slightly higher than the previously declared values (63-65°C) (Bello-

Perez et al., 2022), In another study, the first gelatinization peak of durum wheat starch were between 55-60°C with 13-14 mJ/mg ΔH, but a second endothermic transition peak were also present around 100°C (Vansteelandt and Delcour, 1999). However, the interpretation of DSC endotherms could be difficult due to endothermic phenomena are owed to different molecular events, which frequently overlap (Laurent et al., 2023). This could be attributed to the superimposition of peaks of starch gelatinization and protein denaturation because of occurring nearly temperatures (Kim et al., 2014). However, the mechanisms associated with the denaturation of gluten proteins during DSC heating are weakly endothermic compared to starch gelatinization, and thus may be neglected (Laurent et al., 2023). Moreover, the presence of possibly overlapped multiple endothermal peak located at higher T_c values may indicate formation of amylose-lipid complex by damaged amylose chains' double helix structure after cooking by heat and shear force (Seetapan et al., 2019) with lipid possibly derive from whole egg powder which had minimum 39% fat content as declared by the producer company, used as a one of the main raw material in both gluten-free and gluten-containing control pasta formulations (section 3.1).

On the other hand, higher T_o, $T_{p,}$ and T_c values were measured in GFP samples when compared with the control pasta based on semolina (Table 4.13). Considering that the T_o values are higher than 60°C in GFP, it may be hypothesized that cold extrusion would not gelatinize starch, but the cooking process would enhance starch gelatinization, being conducted in excess of boiling water (Bresciani et al., 2023). Higher gelatinization temperatures reflect that more temperature is required for commencing the gelatinization process (Dib et al., 2018), and also higher peak temperature implies higher cooperative unit or more perfect crystals, namely larger crystal size or longer chains in the crystal (Tazrart et al., 2019). Higher transition temperatures could be explained by the structural alterations of starch granules and the related interactions between amylose or amylose–lipid. The gelatinization process drives the hydration and swelling of amorphous regions, which comprises melting the double helices and crystalline regions. Stress is induced in both polymer chains and crystallite regions during amorphous regions' swelling, and thus crystalline regions are removed from the starch crystallite surface (Dib et al., 2018). Therefore, this could be linked with the starch gelatinization in excess water, which means external amylopectin chains lost their double helical crystalline structure (Tazrart et al., 2019). However, gelatinization attributes are not only subjected to source and chemical structure of starch but also environmental factors such as heat-moisture treatments, annealing, non-ionic compounds/electrolytes, and also hydrophilic hydrocolloids (Tazrart et al., 2019). In this regard, the increase in transition temperatures could be explained by higher and stronger water absorption of hydrocolloides as XN used in GFP formulations, and competition between starch and protein molecules for available water while heating, thus bringing about a reduction in starch binding ratio and retard gelatinization time (Zhao et al., 2024). As a result of this, it could be interpreted that a higher temperature is required for swelling and breaking crystallite areas of PGS-included GFP.

Moreover, an increment in all transition temperatures was determined when the PGS amount was increased in the GFP formulation (Table 4.13). Similarly, although the differences between them were not significant ($p>0.05$), a slight increase in transition temperatures was recorded as the pregelatinized starch ratio increased in raw noodles prepared from native wheat starch combination (Tang et al., 2019). In another study, the transition temperatures of the second peak, which is associated with starch gelatinization, were between 69-78°C in gelatinized amaranth flour obtained by a single screw-extrusion process used at 50% gelatinization degree in pasta production, and increased to73-81°C when pregelatinization level at 100% (Bello-Perez et al., 2022). On the other side, the difference between T_o and T_c is an indicator of gelatinization process length (Dib et al., 2018). Therefore, the shorter length of transition, namely gelatinization process, in 30-PGS-included GFP may also be indicative of its lowest OCT values, as given in section 4.2.10 (Figure 4.8) in comparison with other GFP samples. In alignment with our results, rice-field bean GFP, including hydrothermally treated pregelatinized rice flour, was characterized by a higher T_o, T_p, and T_c between around 51-172°C, and a lower length of transition, namely gelatinization process, in comparison with the control without PGS (Dib et al., 2018).

According to thermal flow patterns of GFP with different proportions of PGS for substitution of native corn starch, lower ΔH were recorded in contrast to pasta made from durum wheat semolina. The higher enthalpy of control pasta could be expounded by the phenomenon that the embedded starch in a strong gluten network enables it to be more difficult for gelatinization (Jia et al., 2023), as proved by its microstructure images (section 4.2.13, Figure 4.9a). On the other side, the lower ΔH refers to less energy being needed for melting (Marti et al., 2010), and thus breaking down the starch structures (Lawal et al., 2021) denotes crystalline structure loss of starch granules and being more gelatinized (Seetapan et al., 2019), as observed in all GFP samples with different levels of PGS compared to the control group. This was aligned with the researchers' declaration that higher T_o and T_p but lower enthalpies were obtained in heat-moisture treatment-applied starches (Yue et al., 1999).

On the other hand, a reduction in enthalpy was monitored when the replacement ratio of the native corn starch with PGS was increased in GFP samples (Table 4.13). In this context, it was interpreted as partial gelatinization, which was fulfilled upon using PGS in GFP formulations as a raw material, supported by the case that the lower heat stability of partially gelatinized some molecular fractions (Dib et al., 2018). Because less energy is required in the status of a higher degree of gelatinization for fully gelatinization, the crystal strength and crystalline regions within the starch particles diminish as a function of the starch gelatinization degree (Bresciani et al., 2023). This is consistent with the results of previous studies, which showed that the lowest gelatinization enthalpy belongs to the noodle made from the wheat starch with the highest PGS ratio because it has less native starch and lower crystallinity (Tang et al., 2019). Moreover, the lower ΔH is associated with double helices' disorganization (Dib et al., 2018; Jia et al., 2023), revealing both crystalline and non-crystalline character, decline in the melted and unbend double helices during gelatinization could be noticed (Dib et al., 2018). Therefore, a higher ratio and extent of the polymers with non-helical order may leach out and thus cause increased CL (Jia et al., 2023), consistent with our results, as increased CL values when the PGS amount increased (section 4.2.4, Table 4.7). In this regard, a negative correlation was defined in ΔH between T and AL in the 1st chapter of the present study (r=-0.90, r=-0.70, respectively). Additionally, a negative relationship between SME and ΔH was also declared (Jia et al., 2023), which demonstrates a similar tendency in our results as given in section 4.1.1 (Table 4.1).

4.2.12. X-ray Diffraction Pattern

The wide-angle X-ray diffractometry (XRD) is utilized for defining the crystallites that have a long-range ordered structure, formed from double helices (Tang et al., 2019), generally used for defining the structure of the starch-based foodstuff brought about by exposing granular starch to thermal or physical treatment (Jia et al., 2023). It is intrinsically related to the compound characteristics, such as amylose and amylopectin content or retrogradation of food products (Manno et al., 2009). To achieve the pasta with a high cooking quality, a specific cooking degree has to be fulfilled, which accounts for 50% of native crystallinity loss. However, while firmness would decrease, stickiness would increase when this corresponding value is went beyond (Merayo et al., 2011). The X-ray diffractograms of the control group and GFP samples, where corn starch was replaced with different rates of PGS (10, 20, and 30%), were shown in Table 4.14 and Appendix 6a-d.

Table 4.14. Relative intensities in X-ray diffraction patterns of semolina- and gluten-free pasta with pregelatinized starch (%)

Pos. [°2θ]	C		10-PGS		20-PGS		30-PGS	
	Raw	**5 min**	**Raw**	**5 min**	**Raw**	**5 min**	**Raw**	**5 min**
15	74.05	-	81.63	-	77.88	-	76.45	-
17	91.61	80.30	84.68	100.00	84.76	100.00	82.98	100.00
18	100.00	-	100.00	-	100.00	-	100.00	-
20	35.45	100.00	24.61	80.14	26.89	71.28	29.60	67.30
23	75.98	-	79.21	-	78.24	-	75.17	-

- not defined at the corresponding angles (2θ).

The diffraction peaks of raw control sample made from durum wheat semolina and GFP with different amount of PGS were shown at angles (2θ) of 15, 17, 18, and 23^{o} (Table 4.14), and thus presented a typical A-type crystalline pattern (Marti et al., 2010; Detchewa et al., 2016; Seetapan et al., 2019; Zweifel et al., 2000; Yan et al., 2023; Yue et al., 2023), which is generated by amylopectin crystalline structure (Jia et al., 2023). This orthorhombic crystalline structure type (A-type starch) is characteristic of the corn starch-based food products (Hernandez-Hernandez et al., 2023), which is aligned with being the main ingredient in our GFP formulations (section 3.2.1, Table 3.1). Moreover, it is also a typical crystalline structure for semolina wheat starch (Detchewa et al., 2016), which was used as a raw material in the control sample (section 3.2.1, Table 3.1). It was declared that tapioca starch belongs to the C-type polymorph group comprising both A- and B-type polymorphs. In addition, the diffraction lines for tapioca starch were also observed at 15, 17, 18, and 23^{o} angles ensured proof to support the crystallinity type for tapioca starch in the literature (root family). The peaks detected at these angles also corresponded for tapioca starch quite well for the description of amylopectin crystallites, as well (Rakhshi et al., 2024).

The main diffraction doublets with the highest relative intensities appeared at 2θ of 18°, followed by 17° in the raw control group and all GFP samples with different PGS levels (Appendix 6a-d). In addition to this, the endothermic peak in the DSC spectrum reflecting the onset of melting in A-type crystalline pattern with a temperature was nearly 53-66 °C as T_o values (section 4.2.11, Table 4.13) representing the A-type crystals melting of was aligned with our result of XRD, similar to what is defined as in Chinese gluten-free rice noodle exposed to dual high-temperature retrogradation process brings in viscoelasticity (Yan et al., 2023).

After cooking, probably the ordered structure of wheat starch and corn starch of the control and GFP samples, respectively, were destroyed and disordered, similarly as observed in potato starch after steaming (Li et al., 2023b), and native rice flour after extrusion cooking by heat and shear force which damaged amylose chains' double helix structure, and thus, enabled the amylose-lipid interaction (Seetapan et al., 2019), and double helices loss of amylopectin (Yang et al., 2019). Therefore, the typical A-type crystalline peaks were generally reduced or disappeared after 5 min cooking. The prominent diffraction peaks with the highest intensities were observed at 2θ~17^{o} and followed by 20^{o,} which represent B- and V-type crystal structure, respectively, in cooked control and GFP samples (Table 4.14 (Appendix 6a-d). While the gelatinized starch with relatively lower moisture contents (≈30-33%) have more tendency to form A-type retrograded crystals, (include 8 H_2O molecules/structural unit), higher moisture levels account for about 60-90% are favored for generating B-type crystals (include 36 H_2O molecules/structural unit) as the former was observed in rice noodle (Yan et al., 2023), that consistent with our results as given in the section 4.2.1 (Table 4.4) for raw and cooked pasta samples, respectively. On the other side, the peaks mainly detected at 2θ of 7, 13, and 20^{o} are typical for V-type crystal structure, indicating forming more ordered packed single helical complexes with amylose-lipid (Jia et al., 2023). As the PGS ratio was increased, the relative intensity values of peaks at 2θ of 20^{o} were decreased (Table 4.14). This narrowing effect suggests that less organized helices need a lower melting energy of helices could be supported by DSC analysis (Marti et al., 2010) with their lower ΔH values, as given in the section 4.2.11 (Table 4.13). On the other hand, V-type crystals melting generally need more higher temperature values (90–110°C) in the DSC spectrum (Yan et al., 2023), and thus our results of DSC (section 4.2.11 and Table 4.13) were consistent with our XRD results. The positive relationship between initial crystallinity and gelatinization enthalpy was also mentioned previously (Detchewa et al., 2016; Tang et al., 2019). Moreover, the 30-PGS, which had the lowest relative intensity situated at 20° supported by having the highest amylose leaching values throughout the cooking process, as mentioned in section 4.2.7 (Table 4.10). Additionally, a positive association between crystallinity and hardness/firmness was obtained by texture analysis (Merayo et al., 2011; Tang et al., 2019), which may be consistent with our results obtained at the last stages of cooking (10-15 min), where hardness reduced as the PGS level increased, as shown in section 4.2.9 (Table 4.12).

4.2.13. Microstructure

The microscopic images could help to examine the structural integrity, appearance, and texture of food products (Dib et al. 2018). To investigate the microstructure of GFP with scanning electron microscopy (SEM) is crucial with regard to determining the interactions and dimensional structure of compounds in the formulation, and also informing the physical structure of the end-product (Ertaş et al., 2023). The structure of cooked pasta alters continually from the surface to the core, while a relatively low uniform structure was generally observed in raw pasta; the greatest changes occur on the pasta surface that has been exposed to the influence of cooking for the longest period (Sozer and Kaya, 2008). The cooking process in pasta causes structural alterations, majorly both starch gelatinization and protein coagulation, which could be reflected in micrographs. In this regard, an apparent outer layer could be monitored. The granular starch is trapped by a strengthened and continuous network with protein denaturation and interaction in the interspaces between granules. Those interspaces are also obstructed by swelling and gelatinization of starch (Hager et al., 2012). In the internal structure of cooked pasta, the starch granules are destroyed and form starch aggregates with fragmented surfaces (Tian et al., 2020), and mostly starch granules are not apparent because of likely enveloping by protein network and forming a complex composite structure (Chauhan et al., 2017). This structural property is more explicit for cooked semolina-based pasta samples, that is, the charge of the characteristic "al dente" mouthfeel (Hager et al., 2012). Therefore, the cooked pasta made from durum wheat is mainly defined as a compact matrix with starch granules entrapped in a protein network (Lucisano al., 2012). Therefore, in this study, the morphology of 5 min-cooked fresh control pasta made from durum wheat semolina and GFP based on corn starch, replaced with 3 levels of PGS (10, 20, and 30%) at *x*3000 magnification, was observed by analyzing their cross-sectional microstructure under SEM and presented in Figure 4.9.

In this study, the control pasta made from semolina, the starch granules were encapsulated with gluten as a protective coat (Veena et al., 2022). In this context, some starch granules, have a oval-shaped (Che et al., 2024), which in turn elliptical starch granules (Ji et al., 2025), were embedded in the protein network structure, (Tian et al., 2020) which seem like a protective cover film on the surface of protein and starch (Che et al., 2024), as represented by a red circle in control pasta, suggesting well-developed protein network matrix owing to gluten in contrast to GFP samples (Figure 4.9a). However, the cracked and/or damaged starch granules, which are characteristic morphologically for heat-moisture-treated ones, were not clearly visible under those conditions (Ji et al., 2025). The microstructure of control pasta had a more compact and thick gelatinized starch framework (Veena et al., 2022). As a result of this, less decline in subsequent starch degradation and leaching out during cooking (Veena et al., 2022) was recorded (section 4.2.7, Table 4.10).

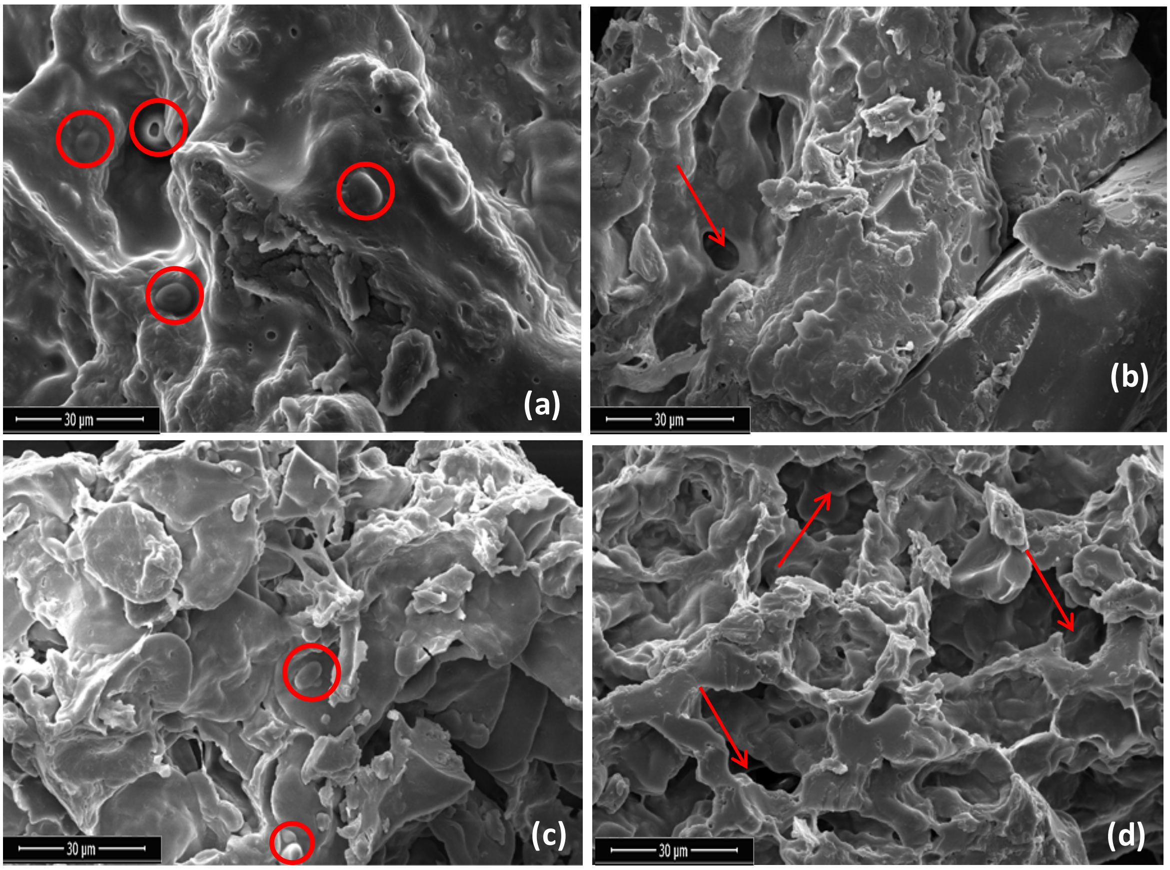

Figure 4.9. Scanning electron microscopy micrographs of cooked semolina- and gluten-free pasta with pregelatinized starch at a magnification level of *x*3000: (a) Control with semolina, (b) 10-PGS, (c) 20-PGS, (d) 30-PGS

Although the incorporation of XN enables to develop more uniform (Indrastuti et al., 2024) and compact internal structure with some visible starch molecules' agglomerates embedded in the fibrous protein-hydrocolloid matrix, and thus has a role in encapsulating starch granules within the gum network, having an influence on decreased leaching out while cooking (Widelska et al., 2019), the protein network of GFP was not similar to the gluten network (Susanna and Prabhasankar, 2013). Those protein matrices did not form, or a thin layer of protein matrix was formed in GFP samples, which led to the detachment of protein parts from the network and starch granules exposed outside (Veena et al., 2022). A coagulated diffuse protein matrix could keep the gelatinized starch in place while exposing the cooking process (Larrosa et al., 2015). In the case of 10-PGS, as given in Figure 4.9b, gelatinized starch had fewer encompassed starch granules, which is likely due to the present lower amount of PGS (Fiorda et al., 2013b). Although all microstructure images were captured for the pasta samples exposed to the same duration of cooking (5 min), GFP with 20-PGS demonstrated both intact and gelatinized starch granules (Figure 4.9c) more complete gelatinization process was observed, and thus was not completely able to distinct between the starch-gum matrix. Particularly, starch granules were agglomerated or wrapped by PGS and/or protein (Tang, 2019) or gum, as shown in 20-PGS. This more continuous phase formed by agglomerates of proteins and starches are surrounded by PGS which is in charge of better CL an less passing starch compounds into cooking medium (Dib et al., 2018) align with lower CL, turbidity, and amylose leaching values in contrast to other PGS-added GFP samples, as mentioned in section 4.2.4 (Table 4.7), section 4.2.6 (Table 4.9), and section 4.2.7 (Table 4.10), respectively. On the other hand starch granules, were agglomerated poorly and separate granules were more visible on the surface (Dib et al., 2018), where greater substitution level as seen in 30-PGS-included GFP that which had a more loose, corrugated and rough surface with big holes, as shown in red arrws (Figure 4.9d), which may allow excessive water access and penetration, which in turn higher CL, turbidity, and amylose leaching values in contrast to other PGS-added GFP samples, as mentioned in section 4.2.4 (Table 4.7), section 4.2.6 (Table 4.9), and section 4.2.7 (Table 4.10), respectively. This is consistent with the morphology of the fractured surfaces of the raw noodles, which have lower initial crystallinity with higher levels of PGS, have a more continuous and denser structure with more wrapped starch granules, but are composed of a few holes, as observed under SEM (Tang et al., 2019).

4.2.14. Sensory Evaluation

Sensorial analysis is one of the most comprehensive methods for the subjective estimation of consumer attitude and selection (Weber et al., 2023) by utilizing human senses to define organoleptic properties of the food products regarding their quality. In addition, sensorial evaluation ensures crucial reference information for comparison with the results of chemical and/or instrumental methods (Faheid et al., 2022), and is also beneficial for further detecting the relation between the final product and raw material (González Victoriano et al., 2021). Producing with improved quality of gluten-free pasta/noodles is especially challenging from the perspective of consumers due to their formulation influencing the technofunctional and sensory features of this kind of product (Alinovi et al., 2023).

The sensorial evaluation is conducted three major steps in this present study including firstly characterization of the cooking degree of pasta samples based on 4 different cooking time (1, 5, 10, and 15 min) (Table 4.15), secondly the preference rank of the cooking times of each samples, individually (Table 4.16 and Table 4.17), and lastly the assessment of sensorial attributes (structural integrity, color, texture, taste, and overall acceptability) at the defined cooking time in the second step of sensoy analysis (Table 4.18, Figure 4.10).

Table 4.15. Cooking degrees of semolina- and gluten-free pasta with pregelatinized starch determined by sensory analysis

Sample	Cooking time (min)			
	1	5	10	15
C	Under-cooked	Al-dente	Medium-cooked	Cooked
10-PGS	Al-dente	Medium-cooked	Cooked	Well-cooked
20-PGS	Al-dente	Cooked	Well-cooked	Well-cooked
30-PGS	Al-dente	Cooked	Well-cooked	Over-cooked

In the first step of the sensory analysis, GFP samples were defined in a wide range from "al-dente" to "over-cooked" through 15 min of cooking. In this regard, neither control based on semolina nor GFP pasta samples were described as "disintegrated", which indicates that all pasta samples, even including gluten or not, had the potential to endure 15 min of cooking. According to Table 4.15, all GFP with different ratios of PGS were characterized as "al-dente" in by panelists at 1 min of cooking, although the technologically identified OCT of GFP samples were substantially higher (section 4.2.10, Figure 4.8) which was conducted based on the define completely disappearance time of the white core particles of uncooked starch after compressing the pasta between two pieces of transparent glass (Detchewa et al., 2022). This could arise from that the OCT method retrieved from AACC 66-50.01 is developed mainly based on wheat flour/semolina pasta/noodle (AACC, 1999). Moreover, the OCT of pasta was determined with no salt in distilled water according to the AACC 66-50.01 method, whereas the sensorial analysis was conducted with tap water with salt addition, whose amount was decided according to the "International Pasta Organisation" as 12.7g/L (Bianchi et al., 2019). Therefore, the differences between the results based on technological and sensorial perspectives could arise from this implementation discrepancy. In addition, this could also be associated with the preference of the consumers who do not tend to consume "al-dente" pasta, as well.

Table 4.16. Cooking time preferences of semolina- and gluten-free pasta with pregelatinized starch by sensory analysis

Sample	Cooking time (min)			
	1	5	10	15
C	3.73 ± 0.47^{Aa}	2.27 ± 0.65^{Ba}	1.82 ± 0.35^{Ba}	2.18 ± 1.08^{Bb}
10-PGS	2.91 ± 0.94^{ABab}	1.91 ± 0.70^{Ca}	2.00 ± 0.33^{BCa}	3.18 ± 0.38^{Aab}
20-PGS	2.18 ± 0.33^{BCbc}	1.45 ± 0.69^{Ca}	2.64 ± 0.67^{Ba}	3.73 ± 0.65^{Aa}
30-PGS	1.55 ± 0.69^{Cc}	1.73 ± 0.90^{Ca}	2.82 ± 0.40^{Ba}	3.91 ± 0.30^{Aa}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$).

On the other hand, control pasta made from semolina was characterized as "under-cooked" in 1 min of cooking, followed by a lower cooking degree when compared to gluten-free counterparts at each cooking time. This manner could stem from having the lowest CL values of the control group in contrast to all GFP samples as previously described (section 4.2.4., Table 4.7), which was also consistent with having the highest OCT values (section 4.2.10, Figure 4.8). Moreover, the highest cooking degree, as “over-cooked”, was obtained in 30-PGS included GFP at 15 min of cooking, whose higher cooking degree tendency was continued in other cooking intervals, could also be associated with its highest CL values at the corresponding cooking times (section 4.2.4, Table 4.7).

Table 4.17. Preference ranking of cooking time in semolina- and gluten-free pasta with pregelatinized starch by sensory analysis

Rank	**Cooking time (min)**			
	1	**5**	**10**	**15**
1	30-PGS	20-PGS	C	C
2	20-PGS	30-PGS	10-PGS	10-PGS
3	10-PGS	10--PGS	20-PGS	20-PGS
4	C	C	30-PGS	30-PGS

The measured OCT measured according to the AACC Approved method 66-50.01 (section 4.2.10, Figure 4.8) was generally agreed with the first-ranked cooking time preference of panelists. According to the preference ranking test in the second step of sensory analysis, the least preferred pasta cooking time was 15 min, except for the control sample based on semolina (Table 4.16). While 1 min of cooking was the most would rather in the GFP with 30-PGS, it was the last choice for the control group that contained gluten (Table 4.17). Moreover, the preference rank was adversely influenced by the increase in PGS ratio at 10 and 15 min of cooking (Table 4.17). On the other side, the most chosen cooking time was defined as 5 min of cooking in all pasta samples except for 30-PGS (Table 4.16). This could be explained by the fact that the panelists did not generally favor pasta that was “al-dente” and also no more than “cooked”. In this context, the common cooking time was decided as 5 min in all pasta samples for the third step of sensorial evaluation. This also agrees with technological quality parameters regarding weight gain should be doubled (Pessanha et al., 2023) in high-quality pasta, as met in all GFP samples cooked at 5 min, as previously mentioned in section 4.2.2 (Table 4.5).

Table 4.18. Sensory properties of semolina- and gluten-free pasta with pregelatinized starch

Sample	**Structural integrity**	**Color**	**Texture**	**Taste**	**Overall acceptability**
C	7.36±1.21[a]	7.45±0.53[a]	7.73±0.45[a]	6.82±1.33[a]	7.00±0.43[a]
10-PGS	7.27±0.66[a]	7.18±0.72[a]	6.82±0.50[ab]	7.00±0.59[a]	7.27±0.90[a]
20-PGS	7.09±0.49[a]	7.00±0.60[a]	5.73±0.56[ab]	6.73±1.19[a]	7.18±1.25[a]
30-PGS	6.00±0.56[a]	6.82±1.17[a]	4.73±0.68[b]	5.55±0.53[a]	6.00±0.54[a]

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$).

In the third step of sensory analysis, the sensorial attributes regarding structural integrity, color, texture, taste, and overall acceptability in all fresh pasta samples cooked at 5 min were given in Table 4.18 and Figure 4.10. According to this, although there were no significant differences between control and GFP regarding the scores of structural integrity, color, taste, and overall acceptability ($p>0.05$), the lowest scores of all sensorial attributes were found in the GFP with the highest level of PGS (30%). Moreover, the color values of GFP samples were decreased as the increasing in PGS level increased ($p>0.05$), which was also in agreement with the values confirmed with the instrumental method regarding especially the yellowness index and color score obtained in 5 min of cooking (section 4.2.8., Table 4.11). In addition, there was a positive link between the scores of structural integrity and instrumentally defined cohesiveness values, which are reduced as the PGS level increased (section 4.2.9, Table 4.12). On the other hand, while GFP with 20-PGS had the lowest hardness values determined by TPA within 5 min of cooking, the lowest texture score belonged to GFP, where native corn starch replaced PGS at the highest ratio (30-PGS). This could be attributed to higher CL values of 30-PGS, and thus probably sticky structure may adversely influence panelists' perception.

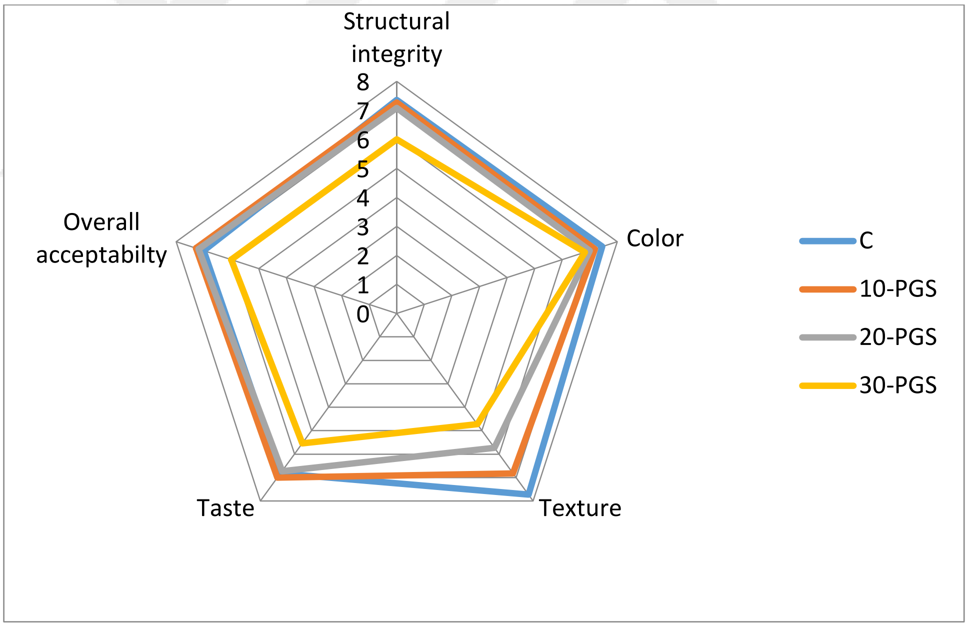

Figure 4.10. Sensory properties of a radar plot of semolina- and gluten-free pasta with pregelatinized starch

Although the control pasta made from semolina had the highest structural integrity and color scores, which is coherent with the values obtained via the instrumental method, as explained in section 4.2.9 (Table 4.12), and section 4.2.8 (Table 4.11), respectively, the highest texture score possibly negatively affects its overall acceptability. Moreover, more than 5 min of cooking was preferred by panelists, as previously revealed in the second step of sensory evaluation (Table 4.16), which in turn was likely evaluated as having a coarse texture. While the least amount of PGS addition (10%) was positively influenced by taste score, higher incorporation adversely affected GFP samples, in contrast to semolina pasta. This could arise from the limited concentration of PGS may mask dominant egg flavor, thereby having a positive impact on overall acceptability, as well. However, increased PGS amount in GFP negatively influenced the taste score, which could be interpreted as excessive PGS sourced from tapioca, may have characteristic unfamiliar taste. This supports the dominance and holistic impact of taste perception on sensory evaluation carried out by panelists. As a result, all sensory parameters declined as the replacement ratio of native corn starch with PGS increased in the GFP formulation ($p>0.05$).

4.2.15. Multi-criteria Decision Making

The value of the global market for gluten-free pasta was nearly accounted for $1 billion, and it is expected to reach $1.7 billion by 2027 (Statista, 2022). Nevertheless, studies on consumers' choice of particularly GF pasta are still very scarce (Xhakollari et al., 2025). In a study, online survey results were collected from 7296 gluten-free consumers across 13 European countries, and type of the most often procured gluten-free product among the 20 products (cake mixes, flatbread, crackers, condiments, etc.) was pasta for all the consumer groups (Dean et al., 2024). Moreover, while consumers were more tended to heavily criticize gluten-free bread, they were more satisfied with gluten-free pasta in general (Dean et al., 2024). However, it has been stated that worst taste, low nutritional value, together with high prices were the reasons behind the preference for gluten-free pasta among consumers who do not suffer from gluten-related disorders (Zerbini et al., 2024). Therefore, current GFP development was projected to maintain comparable technological and sensorial quality in contrast to conventional durum wheat pasta for enhancing the acceptability of consumers (Ismail et al., 2024). In this regard, the hybrid multi-criteria decision making (HMCDM) was conducted taking into consideration the benefits of producers/manufacturers, research and developers, and also consumers. In this regard, extrusion yield, technological properties (cooking loss, yellowness index, and hardness), and taste as a sensory evaluation parameter were selected based on showing regard for those three pillars, respectively. The last couple of criteria were determined regarding the most encountered problems and/or lack of properties, and consumers' expectations and demand for GFP in comparison with gluten-containing counterparts.

Therefore, exturion-related, technological, and sensory values of 5 min-cooked pasta samples were used for the TOPSIS method. In this perspective, first of all, the normalized weights of criteria, such as extrusion yield, cooking loss, yellowness index as a color attribute, hardness as a textural properties, and taste as sensorial features, were defined by the AHP technique (Table 4.19).

Table 4.19. Normalized pair-wise comparison matrix

Parameter	Extrusion yield	Cooking loss	Yellowness index	Hardness	Taste
Extrusion yield	0.343	0.367	0.300	0.353	0.313
Cooking loss	0.171	0.183	0.250	0.353	0.156
Yellowness index	0.057	0.037	0.050	0.029	0.063
Hardness	0.086	0.046	0.150	0.088	0.156
Taste	0.343	0.367	0.250	0.176	0.313

Then the criteria weights of extrusion yield, cooking loss, yellowness index, hardness, and taste were defined as 0.34, 0.22, 0.05, 0.11, and 0.29, respectively (Table 4.20). Thus, the extrusion yield was found to be the most important criterion, followed by taste, respectively (Figure 4.11).

Table 4.20. Weights of criteria and consistency ratio obtained by the AHP method

	Extrusion yield	Cooking loss	Yellowness index	Hardness	Taste	CR
W_i	0.34	0.22	0.05	0.11	0.29	0.046
Rank	1	3	5	4	2	

This manner enlightens that the efficiency of production and providing continuity with satisfying consumers' requests were determined as the main drivers of decision-making in GFP selection. In general, it was declared that taste remains one of the most crucial intrinsic properties for consumers to identify a decision of repurchase (Xhakollari et al., 2025).

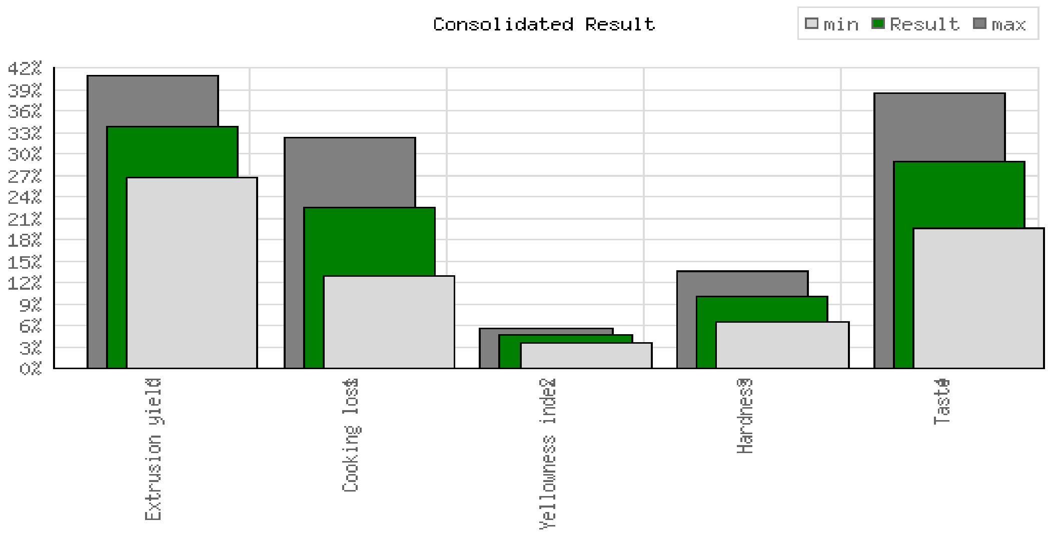

Figure 4.11. Consolidated results of the AHP method

In a study that focused on consumers' decisions and willingness to pay for GFP in Italy, consumers were relatively contented with the GFP, and the characteristics defined as "extremely important” in higher ratios were cooking, taste, and consistency (Xhakollari et al., 2025). In another study, taste was designated as “very important” criteria, corresponding to nearly 86%, followed by quality and price (Laine, 2016). The consistency ratio (CR) with regard to experts' viewpoints was found to be 0.05, which was less than 0.1, thereby accepted as consistent and reliable for further analysis using TOPSIS (Table 4.20).

Secondly, the alternatives were ranked as reported in Table 4.21, according to the order of these P_i values. Regarding Table 4.21, the control group based on semolina was the best pasta (P_i =0.908988) with the nearest distance to the positive ideal solution (S_i^+=0.022220) and also the farthest distance to the negative ideal solution (S_i^-=0.221925), according to TOPSIS results of HMCDM. On the contrary, GFP with 30-PGS had the last rank among all pasta samples, which was consistent with having the lowest overall acceptability score (section 4.2.14, Table 4.18) as determined in sensorial evaluation (section 4.2.14, Table 4.18). However, the 20-PGS incorporated GFP had the highest ranking among the GFP samples, which makes it the best alternative to semolina pasta. Therefore, 20-PGS was selected to be enriched with inulin and *B. coagulans*, respectively, for the following chapters.

Table 4.21. Final ranking of pasta obtained by the TOPSIS method

Sample	S_i^+	S_i^-	P_i	Ranking
C	0.022220	0.221925	0.908988	1
10-PGS	0.068124	0.215018	0.759399	3
20-PGS	0.059471	0.214182	0.782678	2
30-PGS	0.077370	0.196979	0.717986	4

P_i: performance index, S_i^+: distance from the positive ideal solutions, S_i^-: distance from the negative ideal solutions

4.3. Effect of Inulin Incorporation on Extrusion Parameters of Gluten-free Pasta

4.3.1. Energy-related Properties

The monitored energy-related values while mixing (G_{mixing}) and extrusion processes ($G_{extrusion}$), and also specific mechanical energy (SME) values of GFP, including inulin with different chain length and proportions (4 and 8%) in contrast to GFP without inulin (20-PGS), were summarized in Table 4.22.

Table 4.22. Energy-related extrusion values of gluten-free pasta with/without inulin

Sample	G_{mixing} (W)	$G_{extrusion}$ (W)	SME (J/g)
20-PGS	21.81±0.52^c	71.72±0.76^b	75.49±2.16^c
4GR	22.65±0.65^c	72.74±0.56^b	77.04±2.54^c
8GR	24.80±0.60^{ab}	76.55±1.33^a	81.93±0.69^{ab}
4HPX	23.40±1.20^{bc}	73.95±0.77^b	78.83±0.66^{bc}
8HPX	26.30±0.70^a	78.12±0.64^a	83.35±0.70^a

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$).

According to the obtained results, G_{mixing}, $G_{extrusion,}$ and SME values of all inulin-incorporated GFP samples were in the range between 22.65-26.30 W, 72.74-78.12 W, and 77.04-83.35 J/g, respectively (Table 4.22). In this regard, the $G_{extrusion}$ values of all inulin-added GFP samples were nearly more than tripled in contrast to being that of the G_{mixing} values. Moreover, the 4% inulin incorporation into GFP formulation resulted in a slight rise in the $G_{mixing,}$ $G_{extrusion,}$ and SME values in comparison with GFP without inulin (20-PGS) ($p>0.05$), but further increase was observed as the inulin amount doubled ($p<0.05$). Therefore, the higher SME values indicate that the overall operational cost (Sinaki and Koksel, 2024) of inulin-added GFP during the process could be higher than that of GFP based on 20-PGS with no inulin. This manner may be associated with the slight decrease in raw pasta moisture values accepted similar to the feed moisture of these corresponding samples (section 4.4.1, Table 4.25). This was explained with an increment in hydration degree, with the moisture amount rise is also linked with the decrease in dough viscosity (Manthey et al., 2004; de la Peña et al., 2014; Ryu and Ng, 2001; Varsha and Mohan, 2016). In this context, a negative relationship between the moisture content and different variables, such as the SME (Manthey et al., 2004; Ryu and Ng, 2001; Giménez et al., 2013; de la Peña et al., 2014; Bouasla et al., 2017a), torque (Fayose and Huan, 2015), and die pressure (de la Peña et al., 2014), which was mentioned previously, could be valid in our study. Because higher moisture gives rise to a lubricant influence, causing a fewer shearing stress, and thus less energy transfer (Giménez et al., 2013; Bouasla et al., 2017a). Therefore, a negative correlation between SME and raw moisture content was found in the 2nd chapter of this study ($r=-0.85$). It was also highlighted that the influence of moisture is commonly more significant on the friction level in comparison with temperature (Merayo et al., 2011). The lubricating impact within the extruder barrel also depends on several factors, such as the degree of the interactions between water and dietary fibres, and also other compounds like starch polymers, such as amylose and amylopectin (Sinaki and Koksel, 2024). Similarly, in the presence of insoluble fiber, the viscosity of the feed increased, owing to less affinity with starch (Varsha and Mohan, 2016). On the contrary, SME and pressure were decreased as a consequence of higher fiber and protein content originating from enrichment of corn flour-based spaghetti with broad bean flour, owing to a decline in friction level, which led to a decrease in flow viscosity (Giménez et al., 2013). In another study, the SME and pressure values of spaghetti made by corn- and broad bean flour mixture (70:30) through an extrusion-cooking process with different temperatures were reduced as the moisture content was increased. This behaviour was ascribed to the decline in the friction degree as a result of the higher protein and fibre levels contributed by broad bean flour, which could generate a discontinuity in the formed structure, giving rise to a reduction in flow viscosity (Giménez et al., 2013). Another controversial study revealed that the torque, SME, and pressure values of extrudates based on red lentil, including fibers (carrot powder, wheat bran, red lentil hulls), were lower than those of the extrudates with no additional fiber. This was interpreted as fewer energy input needs for fiber-fortified flours. The reason behind the decline in these variables of the extrusion process (torque, SME, and pressure) may be linked with a decline in the melt viscosity likely sourced from shear-thinning behaviour, which is incompatible with other components (i.e., starch) possessed by dietary fiber suspensions.

4.3.2. Efficiency-related Properties

In this present study, the residence time (RT), extrusion yield (EY), and extrusion flow rate (EFR) values as the parameters related to extrusion efficiency of GFP with/without inulin were shown in Table 4.23.

Table 4.23. Extrusion efficiency values of gluten-free pasta with/without inulin

Sample	RT (min)	EY (%)	EFR (g/min)
20-PGS	6.92±0.09^{b}	74.23±2.00^{c}	66.43±2.21^{a}
4GR	7.23±0.44^{b}	77.23±0.38^{bc}	63.72±1.34^{ab}
8GR	7.55±0.29^{b}	80.66±4.15^{b}	60.06±1.50^{bc}
4HPX	7.38±0.44^{b}	79.11±2.42^{bc}	62.16±1.20^{b}
8HPX	8.70±0.46^{a}	89.72±0.27^{a}	57.86±0.54^{c}

Means followed by the different lowercase letters in the same column were significantly different (p<0.05).

According to this, the EY was in the range from nearly 77% to 90% in GFP samples, including inulin with different DP and levels (4 and 8%) (Table 4.23). The inulin addition into GFP gave rise to an increase in EY, along with RT up to nearly 9 min, as well. Moreover, a further increment in both efficiency-related parameters was observed in the case where the inulin ratio was doubled (p<0.05). The inulin addition was conducted as enrichment within a constant hydration level, not the replacement of other ingredients present in the GFP formulation. Therefore, this could have arisen from the increase in the amount of the total feed material. The EFR values of GFP samples consisting of different chain lengths (short- and long) and concentrations (4 and 8%) were varied between nearly 58-64 g/min as seen in Table 4.23. Based on the given results, the EFR values of inulin-enriched GFP were decreasing as the inulin amount increased, and those values were significantly lower than those of GFP based on 20-PGS without inulin (p<0.05) (Table 4.23). This may be ascribed to the negative relationship between RT and extruders' screw speed. In this context, the GFP made with 20-PGS with no inulin had the lowest RT could refer to having the possibly highest screw speed, which caused the highest EFR. Furthermore, it was stated that there is a positive link between mass flow rate and screw speed (Rosentrater et al., 2005). Therefore, the decline in the EFR based on the increase in the inulin level was disclosed with the increase in the RT, and thereby possibly a decrease in the screw speed. In this context, a negative correlation between EFR and RT was monitored in the 2nd chapter of this study (r=-0.78).

4.3.3. Dimensional Properties

It was declared by several studies that although gelatinized starch has a crucial role in expansion via enabling the gas-holding ability to the extrudate melt, some ingredients, such as dietary fibers together with proteins, sugars, and fats, play as diluents or dispersed phase fillers which decrease the starchy matrix's stretchability (Anand, 2020). Therefore, the dimensional properties (ER, SEI, UD, and SL) of GFP with inulin against 20-PGS with no inulin were presented in Table 4.24.

Table 4.24. Dimensional properties of raw gluten-free pasta with/without inulin

Sample	ER	SEI	UD (g/cm^3)	SL (cm/g)
20-PGS	1.48±0.07^a	2.20±0.21^a	1.37±0.04^a	17.81±0.42^a
4GR	1.53±0.01^a	2.31±0.06^a	1.34±0.02^{ab}	16.87±0.20^{ab}
8GR	1.55±0.14^a	2.44±0.32^a	1.26±0.02^{bc}	16.21±0.09^{bc}
4HPX	1.54±0.04^a	2.36±0.11^a	1.32±0.04^{ab}	16.48±0.10^b
8HPX	1.58±0.02^a	2.50±0.07^a	1.18±0.05^c	15.20±0.91^c

Means followed by the different lowercase letters in the same column were significantly different (p<0.05).

The ER and SEI values of inulin-fortified GFP were over the range in 1.53-1.58 and 2.31-2.50, respectively, as shown in Table 4.24. In this context, there was a slight increment in the expansion trend regarding both ER and SEI values of GFP with inulin, and when its level was doubled compared with that of the GFP sample with no inulin (20-PGS) (p>0.05). This was aligned with the declaration that the inulin incorporation into extruded cereal products enables several functional properties, such as greater expansion (Lobato et al., 2011). It was also related to the phenomenon that greater expanded extrudate requires more SME inputs (Ryu and Ng, 2001), and thus a similar positive association between SEI and SME may also be monitored in the present study (section 4.3.1, Table 4.22). Moreover, it was mentioned that the expansion of extrudates commonly takes place at low moisture but high temperature of extrusion conditions (Minweyelet et al., 2021). The volumetric expansion could be negatively influenced by higher moisture levels of feeds during extrusion (Varsha and Mohan, 2016). In a similar vein, a negative relationship between moisture content and radial expansion index was also stated. This manner was elucidated by reducing the melt viscosity by acting as a lubricant inside the extruder barrel of moisture (Sinaki and Koksel, 2024). In this regard, an increase in the inulin amount and thus a decline in raw pasta moisture content accepted similar to that of feed material (section 4.4.1, Table 4.25), could increase the diameter of GFP samples, and thus a slight increase of both corresponding ER and SEI values (p>0.05). Conversely, expansion was decreased in the presence of insoluble dietary fiber in feed viscosity increase, a reduction in elastic attributes, and less affinity between starch (Varsha and Mohan, 2016). In another controversial study, oat bran and soy flour had a deleterious influence on radial expansion, causing more compact texture and less expanded puffed extrudates due to their fiber content, which ruptures external surface and air cell walls in extrudates, and thus hinders the air bubbles' full expansion (Lobato et al., 2011).

The UD values of inulin-enriched GFP samples were in the range between 1.18-1.34 g/cm^3, as given in Table 4.24. According to the results, the incorporation and increase in the inulin ratio of the GFP formulation gave rise to a decline in the UD. This was in line with the negative association of UD with volumetric expansion (Rosentrater et al., 2005). Therefore, a negative correlation between UD and ER was found out in the 2nd chapter of this study (r=-0.67).

The SL values of all inulin-added GFP samples were summarized in Table 4.24. Based on the results, the SL values of GFP samples, including inulin, were over the range of 15.20-16.87 cm/g (Table 4.24), and a reduction was determined in the increase in inulin incorporation ratio into GFP formulation based on 20-PGS ($p<0.05$). The results were in line with the phenomenon regarding the increase in SME values resulting in the decline in being values of SL (Ryu and Ng, 2001), similarly seen in the 2nd chapter of this study (r=-0.89). In addition to this, a positive relationship between the SL with feed moisture (Ryu and Ng, 2001; Anand, 2020) and also screw speed (Minweyelet et al., 2021), whereas an adverse link with expansion (Ryu and Ng, 2001) was agreed with out results, as well (Table 4.24). Therefore, a positive correlation was observed between SL and raw moisture levels in the 2nd chapter of this study (r=+0.86). Moreover, a negative correlation was identified between ER and SL in the 2nd chapter of this study (r=-0.63). In contrast, the reduction of diameter while length enlargement of extrudates with increasing content of dietary fiber in the feed was also reported by researchers, which was explained with increases in air cell wall thickness and interference with the formation of air bubbles (Varsha and Mohan, 2016).

4.4. Effect of Inulin Incorporation on Cooking Properties of Gluten-free Pasta

4.4.1. Moisture

The moisture amount of all fresh raw and cooked GFP samples based on 20-PGS with/without inulin, during 15 min cooking with 5 different time intervals (0, 1, 5, 10, and 15 min), was given in Table 4.25.

When cooked within the first 5-min duration, the highest moisture contents were determined in 20-PGS added GFP with no inulin, and thus, inulin incorporation reduced the moisture values (Table 4.25). Similarly, the incorporation of inulin (Frutafit-HD; DP: 8-13) in a spaghetti formulation increased the dry matter (Brennan et al., 2004), and thus inferred that the moisture content was decreased, similar to the result acquired in the first stages of cooking duration (0-5 min) in our study. However, the greater moisture values were observed in the 4% inulin added GFP regardless of chain length at the latest phase of cooking (10 and 15 min). This could be attributed to differences in the speed of water uptake of PGS and inulin. Similarly, in a study, significantly higher moisture content was observed in pasta where semolina was replaced with both short-chain (Raftiline GR, DP=10) and long-chain inulin (Raftiline HPX, DP=23) at 15% when comparing with the control group without inulin ($p<0.05$). The reason behind this was ascribed to the dietary fiber attributes of inulin, which would result in higher SI but lesser firmness (Foschia et al., 2015a).

Table 4.25. Moisture contents of raw and cooked gluten-free pasta with/without inulin (%)

Cooking time (min)	20-PGS	4GR	8GR	4HPX	8HPX
Raw	36.57 ± 0.41^{Ea}	36.16 ± 0.25^{Fab}	34.00 ± 0.03^{Fc}	35.57 ± 0.19^{Fb}	34.61 ± 0.26^{Fc}
0	60.17 ± 0.47^{Da}	58.62 ± 0.56^{Ea}	52.87 ± 1.23^{Eb}	57.16 ± 1.27^{Ea}	50.95 ± 1.32^{Eb}
1	68.37 ± 0.42^{Ca}	65.56 ± 1.04^{Db}	61.18 ± 0.13^{Dc}	63.90 ± 0.96^{Db}	60.12 ± 0.60^{Dc}
5	75.81 ± 0.48^{Ba}	74.03 ± 0.78^{Cab}	71.44 ± 0.47^{Cc}	72.09 ± 1.64^{Cbc}	70.78 ± 0.79^{Cc}
10	78.87 ± 0.55^{Ab}	80.44 ± 0.21^{Ba}	77.87 ± 0.15^{Bc}	79.01 ± 0.53^{Bb}	74.11 ± 0.15^{Bd}
15	80.92 ± 0.38^{Aab}	81.57 ± 0.24^{Aa}	79.72 ± 0.36^{Ac}	80.47 ± 0.46^{Abc}	76.08 ± 0.34^{Ad}

Means followed by the different uppercase letters in the same column regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same row at the same cooking time were significantly different ($p<0.05$).

Generally, a decline was defined in moisture content as the inulin level increased to 8% ($p<0.05$) (Table 4.25), which is agreed with the study revealed that the moisture amount of bread was decreased as the inulin incorporation degree increased ($p<0.05$) align with the findings of water absorption of the bread dough determined by farinograph (Majzoobi et al., 2019). Similarly, a gradual increase in dry matter, thereby deducing a decrease in moisture content, was observed in spaghetti as the inulin concentration increased (2.5, 5, 7.5, and 10%) (Brennan et al., 2004). In the same way, the dry matter of 7 min of cooked pasta increased from nearly 34% to 38% when the inulin level doubled from 7.5% to 15% (Tudorică et al., 2002), and thus, figured out that the adverse influence was observed in moisture content, which aligns with our study regarding the tendency and the values obtained. On the other hand, some non-starch polysaccharides (NSPs), such as inulin (Frutafit-HD; DP: 8-13), pea fiber, bamboo fiber, or locust bean gum, did not make a significant difference in dry matter and thus moisture levels in comparison with pasta without NSPs ($p>0.01$) (Brennan and Tudorica, 2007). Accordingly, the moisture content (6-6.5%) of inulin-fortified (5, 10, and 15%) dried durum wheat pasta did not vary significantly between the control pasta with no inulin fibre fraction (Manno et al., 2009). These corresponding lower values and differences from our study could originate from being in a dried form and gluten-containing counterparts.

In terms of the viewpoint of the inulin chain-length, the moisture of GFP with low DP (GR, DP≥10) was significantly higher than that of including long-chain inulin (HPX, DP ≥ 23) ($p<0.05$), as demonstrated in Table 4.25. Corresponding with our results that the moisture content was significantly higher in low-DP-inulin-fortified durum wheat semolina pasta than that of having a higher chain length used at the same level ($p<0.05$) (Foschia et al., 2015a), On the other side, in a study, moisture content was not significantly influenced by the DP of inulin introduced to durum wheat pasta ($p>0.05$) (Peressini et al., 2020).

Additionally, a gradual increase was detected upon the moisture content throughout cooking in all GFP samples with inulin, as shown in Figure 4.12. According to the obtained results, the moisture levels of all fresh GFP samples, including inulin, were in the range between approximately 51-59% at the beginning of cooking (10 sec), reaching 76-82% at the end of the cooking (15 min) (Table 4.25). Similar trends were reported in the first chapter of our study regarding the effect of PGS (section 4.2.1), and also previous research of Sobota and Zarzycki (2013) and Sobota et al. (2013), even though the corresponding values were lower in contrast to our results of the present study, probably owing to differences in pasta formulations. Similar vein, in another study, all cooked GFP samples fortified with different ratios of (20, 30, and 40%) chestnut flour increased after 2.5 min of cooking in contrast to raw ones (Alinovi et al., 2023), and those corresponding values were lower than our results could be arise from not only the differences in the formulation but also in primarily in cooking time duration.

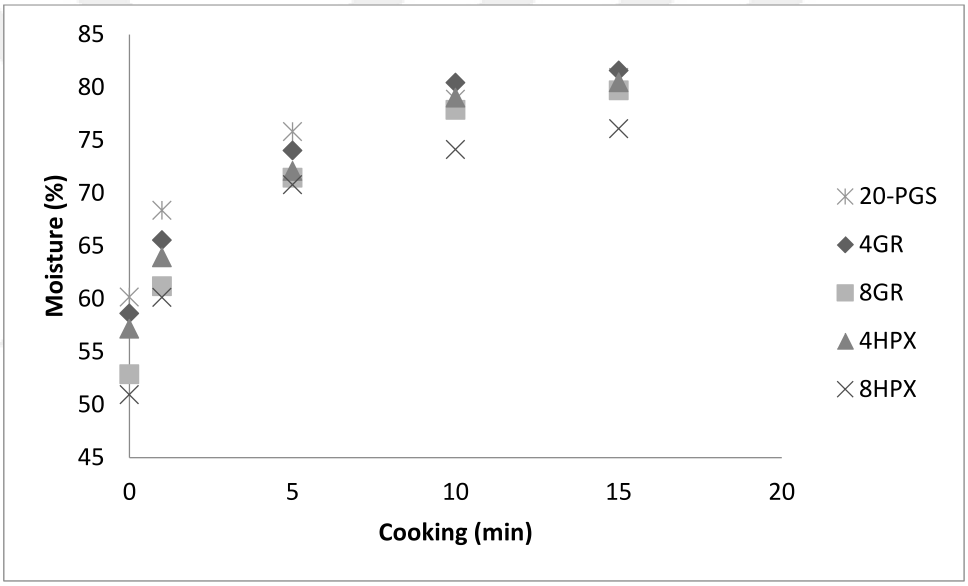

Figure 4.12. Moisture contents of gluten-free pasta with/without inulin during cooking

In the literature regarding moisture content of inulin-enriched pasta/noodle were between nearly 68% (deduced from 32% dry matter content) in 7 min-cooked inulin-added (2.5, 5, 7.5, and 10%) fresh durum wheat pasta as a soluble non-starch polysaccharides (Brennan and Tudorica, 2007), approximately 65-68% (deduced from 32-35% dry matter content) that in 7 min-cooked inulin-fortified (2.5, 5, 7.5, and 10%) spaghetti made from commercial durum semolina flour (Brennan et al., 2004), 66-67% in spaghetti based on durum wheat semolina partially substituted (15%) with inulin has different DP used as a soluble dietary fiber source (Peressini et al., 2020), 62-66 g/100g (inferred from 34-38 g/100g dry matter content) in 7 min-cooked pasta produced with durum wheat semolina partially replaced with inulin at diferent levels (7.5, 10, 12.5, and 15%) (Tudorică et al., 2002). The discrepancy between our results and the literature may arise from not only differences in formulation, together with the type of pasta/noodle, but also differences in the source, chain length/DP, and concentration of inulin.

4.4.2. Water Absorption Capacity

One of the critical characteristics of GFP is the capability of water absorption, which depends on the composition of feed material and process conditions that lead to differentiation in both macro- and microstructures (Poshadri and Deshpande, 2024; Achinna and Deshpande, 2025). In this context, pasta hydration is likely to be associated with protein denaturation degree along with the hydrophilic macromolecules (i.e., starch and dietary fiber) (Achinna and Deshpande, 2025). The differences of WAC among different dietary fibers could also referable to their differences in particle size and structures (Foschia et al., 2015a; Saraç, 2021). Therefore, the WAC of inulin-enriched GFP pasta samples with different DP and concentrations (4 and 8%) compared to control GFP made without inulin (20-PGS) throughout the 15-min cooking process with 5 different time intervals (0, 1, 5, 10, and 15 min) were shown in Table 4.26.

Table 4.26. Water absorption capacity of gluten-free pasta with/without inulin (%)

Sample	Cooking time (min)				
	0	1	5	10	15
20-PGS	64.18±1.19^{Da}	97.21±4.31^{Ca}	132.02±0.54^{Ba}	154.76±2.38^{Abc}	168.93±2.12^{Ab}
4GR	60.29±1.29^{Eb}	92.43±0.69^{Dab}	129.70±0.57^{Cb}	165.21±1.67^{Ba}	182.54±1.02^{Aa}
8GR	54.99±1.99^{Ec}	81.78±2.29^{Dc}	116.34±0.53^{Cd}	150.87±2.14^{Bcd}	165.75±1.03^{Ab}
4HPX	56.88±1.61^{Ebc}	86.82±1.51^{Dbc}	124.56±0.62^{Cc}	160.50±2.01^{Bab}	178.83±2.03^{Aa}
8HPX	49.84±0.38^{Ed}	78.77±0.73^{Dc}	114.81±1.24^{Cd}	143.35±2.38^{Bd}	154.40±1.74^{Ac}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$).

The WAC values of all fresh inulin-added GFP samples were lower in comparison with the control group with no fiber inclusion (20-PGS) within the first 5 min of cooking ($p<0.05$), as given in Table 4.26. Inulin is a polysaccharide that has a highly hydrophilic character and can interact with greater amounts of water molecules via hydrogen linkages through hydroxyl groups (−OH). Nevertheless, the competition with other hydrophilic molecules, such as protein, non-starch polysaccharides, and damaged starch, could prevent their hydration in the dough by forming a barrier around them, resulting in less water absorption (Majzoobi et al., 2019). This was also supported by the idea that a barrier could be formed around starch by inulin, surpassing water swelling. The restriction of water absorption increase could be collectively comprised of this and formed a dense structure of starch granules via heat-moisture-treated raw material (Ji et al., 2025). It was proven by the farinograph outputs that the water absorption (≈53-55%) of fresh noodle dough where 5% proportion of wheat flour was substituted with short-chain and long-chain inulin, was lower in contrast to the control without inulin (≈60%). This was explained with a high quantity of hydroxyl groups, which have the ability to form hydrogen bonds with water that make for free water more inconvenient to migrate, thus obstructing the water-binding capacity of starch, which in turn decreases water absorption (Chen et al., 2023). The diminished water absorption was also determined in pasta enriched with different fibers apart from inulin, such as resistant starches (RS-type II and RS-type IV) or oat bran, compared to control pasta with no fiber (Bustos et al., 2011). In a similar vein, the water absorption reduction was associated with the competition between fiber-rich ingredients with water, which led to a weaker dough network, as observed in GFP, including grape peel (Ungureanu-Iuga et al., 2020). In another study, even though the greater values in mass increase were obtained in higher amounts of the defatted flaxseed flour (DFF) as a soluble fiber, which coud absorb 16–30 times its weight in water, the water absorption and thereby mass increase was decreased when DFF admixtured with rice flour for GFP production, because of the characteristics and potential interactions of DFF with other compounds (de Moura et al., 2016). Correspondence with a study revealed that inulin-enriched pasta (2.5-7.5%) produced by the straight dough method yielded a significant reduction in water absorption determined via farinograph, and this decrement was increased as the inulin addition level increased ($p<0.05$) (Majzoobi et al., 2019). This could be explained by the phenomenon based on the components that have the ability to absorb water could influence weight gain and volume increase, and among these non-conventional components, some of them, such as dietary fibers and proteins, could decrease gelatinized starch amount, and thus water absorption, weight, and volume of pasta (da Silva et al., 2016; Pessanha et al., 2023).

On the other side, the greater WAC values were determined in the GFP, including 4% of inulin irrespective of DP at the last stages of cooking (10 and 15 min), in contrast to control GFP based on 20-PGS with no fiber addition, in the present study (Table 4.26). The WAC increase with inulin incorporation to gluten-free noodles in the presence of XN was associated with the –OH group in inulin fiber responsible for H-bonding (Raungrusmee et al., 2020). Therefore, the WAC increase could be accredited to the high water absorption abilities of dietary fibers (Foschia et al., 2015a; Saraç, 2021). It was also indicated that protein matrix disruptions may enhance water absorption and subject starch granules to swelling and rupture (Foschia et al., 2015a), which may also have a role in all GFP samples with inulin, including whole egg powder. Moreover, the durability of PGS against overcooking may be enhanced with inulin addition, owing to competition with water, and thus delay the disruption.

However, the increment in inulin concentration from 4% to 8% resulted in a reduction of WAC values, as presented in Table 4.26. This behaviour could be interpreted as inulin could better bind water via hydrogen bonds at lower concentrations owing to continuity disruption at higher levels of inulin caused by incompatibility of macromolecular biopolymers, which in turn causes a reduction in water holding (Chen et al., 2024b). Similarly, the WAC durum wheat pasta was reduced as the inulin amount in the formulation raised (2.5, 5, 7.5, and 10%), although the differences were not significant between them ($p>0.05$). This was announced with the highly hydrophilic character of inulin, which absorbs the water preferentially, thereby inhibiting water absorption and swelling of starch that could result in the structural changes of the pasta (Brennan et al., 2004). However, the water absorption index of fresh functional pasta including inulin extracted from globe artichoke roots did not significantly influence by different inulin concentrations (5, 10, and 15%) (p>0.05) (Difonzo et al., 2022). In contrast, a slight increment was recorded in terms of water absorption when the inulin proportion increased from 2.5% to 5% and 7.5% in the noodle based on heat-moisture-treated wheat starch, although the differences were not significant (p>0.05) (Ji et al., 2025). Similarly, an increase in water absorption was observed in bread what flour-based pasta when inulin incorporation was increased from 2.5% to 5%, 7.5, and 10% (p>0.05) (Bustos et al., 2011).

When assessed from the point of inulin DP, in general, the GFP-enriched inulin with low DP (GR) had higher WAC than that of high DP (HPX) at the same incorporation degrees (Table 4.26). Align with that, higher WAC values were recorded in partial replacement (15%) of durum wheat semolina with short-chain inulin than that of including long-chain inulin (HPX) (Foschia et al., 2015a). In contrast, WAC was increased in durum wheat spaghetti inulin with high DP extracted from cardoon roots and further augmented with inulin level from 2% to 4%, whereas commercial inulin sourced from chicory has low DP, decreased WAC, and worsens as the inulin level raised (Padalino et al., 2017). In aonther study, while WAC was not affected when semolina was replaced by inulin with higher DP (Frutafit-HD, DP: 12-14) at different levels (2.5-20%), a significant decrease in water absorption was recorded as the substition ratio of inulin with lower DP (TIC PRETESTED LV-100, DP: 7-8) was increased (p<0.05). This diminishment was associated with decreased SI at 7.5% and 10% replacement levels disclosed with water competition of inulin with the starch through pasta formation (Aravind et al., 2012).

According to the achieved results, the WAC of GFP with inulin was in the range of nearly 50-60% to 154-183% from the beginning to the end of cooking (0-15 min) (Table 4.26). In this context, the WAC values were increased and nearly tripled at the end of the cooking in all GFP samples with/without inulin. Similar behaviour was stated in other studies, as well (Dziki and Laskowski, 2005; Lucisano al., 2012; Sobota et al., 2013; Oduro-Obeng et al., 2021; Lux et al., 2023). In general, the quick rise in water absorption at the initial stage was shown to continue at a slower rate at later periods in hydration curves (Larrosa et al., 2015). A similar behaviour was defined in this study, as shown in Figure 4.13, which is also aligned with the statement that totally cooking may diminish weight increase significantly owing to greater CL in pasta (Dziki and Laskowski, 2005). This could be interpreted as meaning that ongoing cooking up to 20 min probably would adversely influence the WAC of inulin-enriched GFP samples in the present study. Furthermore, it was commonly declared that the least weight gain of cooked pasta should be a 100% increase, which, namely, implies that it should double to obtain high-quality pasta (Fiorda et al., 2013b; Pessanha et al., 2023). In light of this information, the corresponding values were obtained for inulin-added GFP cooked at 5 min could be the most appropriate among the other cooking durations that were also proven as the most desirable by consumers in sensory evaluation, as shown in section 4.4.13 (Table 4.36).

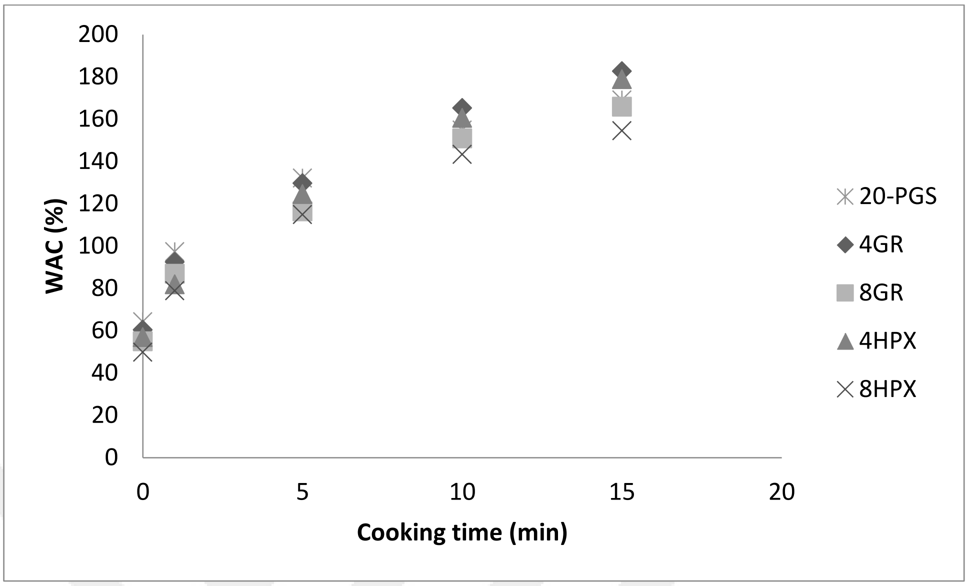

Figure 4.13. Water absorption capacity of gluten-free pasta with/without during cooking

In the literature regarding the WAC of inulin-enriched pasta/noodle were over the range of proximate 49% and 54% in fresh noodle based on 5% proportion wheat flour substituted with short-chain- and long-chain inulin, respectively (Chen et al., 2023), 74-83% in 7 min-cooked inulin-fortified (2.5, 5, 7.5, and 10%) spaghetti made from commercial durum semolina flour (Brennan et al., 2004), roughly 73% g/100g in fresh semolina pasta where inulin extracted from globe artichoke roots used at 3 concentration (5, 10, and 15%) (Difonzo et al., 2022), 105.5 g/100g in gluten-free noodle prepared by tiger nut flour including inulin as a hydrocolloid (Gasparre and Rosell, 2019), 96% in pasta enriched with 12% inulin from chicory (Bavaro et al., 2024), 128-149% in spaghetti produced with 4 wholemeal durum wheat flour and fortified with 4% inulin from cardoon roots with high-DP and chicory with low-DP (Garbetta et al., 2020), 134-136% and 141-144% in wholemeal durum wheat spaghetti comprise of commercial inulin (2 and 4%) with low DP produced from chicory and inulin extracted from cardoon roots with a high DP, respectively (Padalino et al., 2017), 133% and 126% in pasta made with wholemeal flours at 2 different substitution levels (2 and 4%) with low-chain chicory- and long-chain topiinambur-inulin, respectively (Cardullo et al., 2022), 138 g/100g and 123 g/100g for short-chain- and long-chain inulin-fortified (15%) functional durum wheat semolina pasta, respectively (Foschia et al., 2015a), 152-156 g/100g in pasta substituted with inulin for a portion of bread wheat flour (2.5, 5.0, 7.5 and 10.0%) (Bustos et al., 2011), 144-172% and 165-170% in durum wheat semolina spaghetti including inulin (5%) with DP of 7–8 and 12-14, respectively (Aravind et al., 2012), about 185% in Turkish noodle made from wheat flour partially replaced with inulin (4%) (Saraç, 2021). The discrepancy between our results and literature may arise from not only differences in formulation together with the type form of of pasta/noodle, but also differences in the source, chain length/DP, and concentration of inulin.

4.4.3. Swelling Index

The SI values of all fresh GFP pasta samples with/without inulin during the 15-min cooking process with 5 different time intervals (0, 1, 5, 10, and 15 min) was presented in Table 4.27.

Table 4.27. Swelling index values of gluten-free pasta with/without inulin (g/g)

Sample	Cooking time (min)				
	0	1	5	10	15
20-PGS	1.70±0.17[Ea]	2.42±0.05[Da]	3.18±0.04[Ca]	3.71±0.06[Bb]	4.21±0.04[Aab]
4GR	1.51±0.04[Eab]	1.97±0.05[Db]	3.02±0.05[Cb]	3.96±0.10[Ba]	4.31±0.05[Aa]
8GR	1.34±0.06[Ebc]	1.74±0.02[Dc]	2.58±0.03[Cd]	3.61±0.07[Bb]	3.88±0.04[Abc]
4HPX	1.41±0.05[Ebc]	1.96±0.04[Db]	2.83±0.03[Cc]	3.77±0.07[Bb]	4.25±0.31[Aab]
8HPX	1.25±0.03[Dc]	1.61±0.01[Cd]	2.46±0.09[Bd]	3.41±0.02[Ac]	3.66±0.14[Ac]

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$).

Inulin had a negative influence on the SI values of GFP regardless of its level and type within the first 5 min of cooking (Table 4.27). This reduction stemmed from the adsorption of inulin creates cross-linked regions on starch surfaces, suppressing granular swelling because of establishing a physical barrier (Ji et al., 2025). Therefore, one of the main reasons behind this manner could originate from a strong rivalry between inulin and other compounds, such as protein and starch, for water, as declared for durum wheat semolina (Zarroug et al., 2022). The restriction of water penetration to the starch granules, and thus swelling, is derived from starch granules encapsulation within the reticule of fiber whose hydroxyl groups compete for water absorption with protein and starch (Difonzo et al., 2022). This uneven water distribution in the pasta matrix causes starch granules to less swelling and eventually would results in increased CL owing to protein-starch network breakdown (Bastos et al., 2016). It was noted that this SI decrease, together with water absorption, is associated with the high hydrophilic character of inulin, which presumably absorbs water preferentially, causing starch swelling inhibition (Brennan et al., 2004). In a similar vein, a SI reduction was stated in the commercial inulin-incorporated (Raftilose® Synergy, 12% w/w) durum wheat semolina pasta in contrast to the control group without inulin (Bavaro et al., 2024). The decline in SI with inulin addition into pasta formulation was also observed in different studies such as durum wheat pasta when inulin (Frutafıt-HD) utilized at higher concentrations (5, 7.5, and 10%) (Brennan et al., 2004), durum wheat spaghetti fortified inulin with 7–8 DP (TIC PRETESTED LV-100) at higher amounts (7.5 and 10%) (Aravind et al., 2012), and semolina pasta including commercial inulin with DP≥23 (Zarroug et al., 2022). It was also stated that the highest SI values belong to XN-added pasta samples in contrast to those of involving other NSPs, such as inulin, guar gum, locust bean gum, β-glucan, pea fiber, and bamboo fiber (Brennan and Tudorica, 2007). In contrast, dietary fiber-enriched pasta, including short-chain inulin (Raftiline® GR, DP=10) and long-chain inulin (Raftiline® HPX, DP=23), had higher SI values than control spaghetti made from semolina (Foschia et al., 2015a).

On the other side, the higher SI values were determined in GFP, including 4% short- (GR) and long-chain inulin (HPX) in the extended cooking duration (10 and 15 min) in comparison with GFP without dietary fiber source (20-PGS). It could be interpreted that the unfavorable effect of PGS at prolonged cooking could be shortened with the addition of inulin at lower levels (4%). However, the concentration increase of inulin, irrespective of DP, adversely affected the SI values in inulin-enriched GFP samples (Table 4.27). This result was in agreement with the study, based on the SI values of durum wheat semolina spaghetti fortified with inulin (Frutafit-HD) was significantly reduced after 2.5% concentration of inulin ($p<0.05$) (Brennan et al., 2004). The SI reduction was also recorded when the level of commercial inulin (DP≥23) in durum wheat semolina pasta was increased from 5% to 10% (Zarroug et al., 2022). In a similar way, when semolina was replaced with lower DP inulin (TIC PRETESTED LV-100, DP: 7–8) at high concentrations (7.5 and 10%), a decrease in SI values was detected in durum wheat spaghetti (Aravind et al., 2012). Controversially, although lower SI values were found in pasta made from bread wheat flour with inulin (2.5-10%) than control pasta without any dietary fiber source, the corresponding values were increased with the augmentation in inulin amount (Bustos et al., 2011). Furthermore, in the case of increasing usage level of soluble or insoluble NSPs, the SI of fresh pasta samples was increased, which was dedicated to their water holding capacity (Brennan and Tudorica, 2007), and also retaining water in a well-improved network based on starch-protein-polysaccharide (Foschia et al., 2015a; Tudorică et al., 2002), influenced by dietary fiber types that have different hydration and swelling abilities/capacities (Difonzo et al., 2022).

When evaluated in terms of the inulin chain length, the SI values of pasta samples including inulin with lower DP were higher than those of higher DP (Foschia et al. 2015a, Aravind et al., 2012), which also agrees with our results, as monitored in Table 4.27. Conversely, significantly higher SI values were declared in spaghetti made by whole-meal durum wheat consisting of inulin extracted from cardoon roots with high DP than that of consisting of commercial inulin produced from chicory has low DP when utilized at the same levels (Padalino et al., 2017). In another controversial study, spaghetti produced with 4 wholemeal durum wheat flours (cv SenatoreCappelli, cv Margherito, cv Russello, cv Timilia) and fortified with 4% inulin from cardoon roots with high-DP had slightly higher SI (except for cv Timilia) than that of chicory with low-DP, although the average values were not found significantly different ($p>0.05$) (Garbetta et al., 2020).

According to the acquired results, the SI of the inulin-enriched GFP samples was in the range of about 1.25-1.51 g/g to 3.66-4.31 g/g (Table 4.27). A less increase in swelling volume is characterized by lower water absorption (Aydin, 2022), and thus the specified SI results in all GFP samples with/without inulin were consistent with the WAC trend, as previously mentioned (section 4.4.2, Table 4.26). Therefore, a strong positive correlation was discovered between WAC and SI in the 2nd chapter of this study (r=+0.99). This similar inclination with WAC was monitored as cooking duration proceeded, regarding more than a 2 times increase for SI values in inulin-included GFP samples at the end of cooking (15 min), in contrast to their initial SI values obtained cooking at 10 sec (0 min) (Figure 4.14). The rise in SI values during the cooking process was also defined in fresh amaranth flour-based GFP (up to 1:10 water ratio) with 1% alginate (Lux et al., 2023).

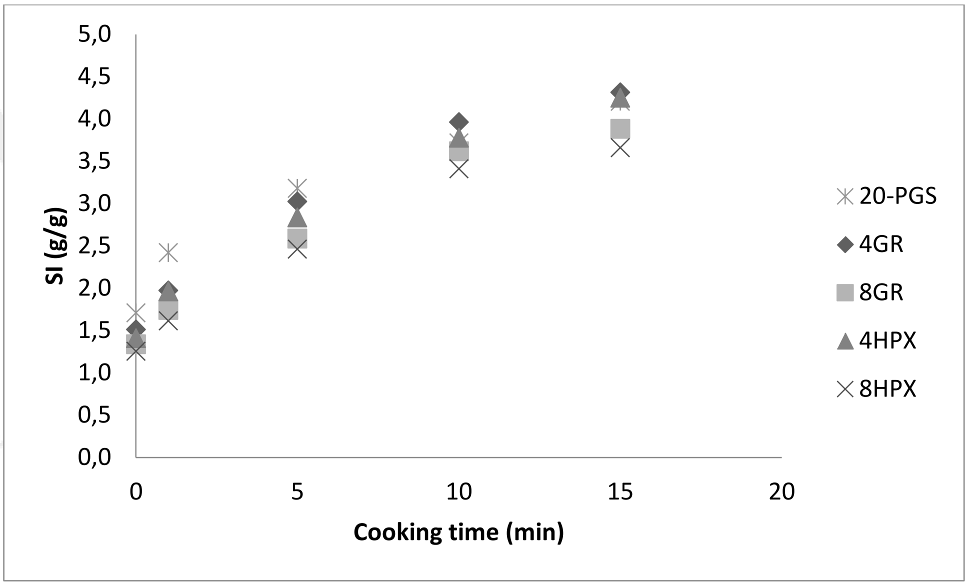

Figure 4.14. Swelling index of gluten-free pasta with/without inulin during cooking

In the literature regarding the SI of inulin-enriched pasta/noodle were between the range of approximately 2 g/g in durum wheat semolina spaghetti including inulin (5%) with DP of 7–8 or 12-14 (Aravind et al., 2012), 2.13 g water/g DM in inulin-added (2.5, 5, 7.5, and 10%) fresh durum wheat pasta as a soluble non-starch polysaccharide (Brennan and Tudorica, 2007), 1.84-2.15 g/g in 7 min-cooked inulin-fortified (2.5, 5, 7.5, and 10%) spaghetti made from commercial durum semolina flour (Brennan et al., 2004), 1.98-2.08 g/g in pasta made from bread wheat flour substituted with inulin at different portions (2.5, 5.0, 7.5 and 10.0%) (Bustos et al., 2011), 1.47-1.53 g/g in fresh semolina pasta where inulin extracted from globe artichoke roots used at 3 concentration (5, 10, and 15%) (Difonzo et al., 2022), 2.57 g water /g dry pasta and 2.35 g water/ g dry pasta for short-chain- and long-chain inulin-fortified (15%) functional durum wheat semolina pasta, respectively (Foschia et al., 2015a), 1.5 g/g in gluten-free noodle prepared by tiger nut flour including inulin as a hydrocolloid (Gasparre and Rosell, 2019), about 1.7 g/g and 1.8 g/g in wholemeal durum wheat spaghetti comprise of commercial inulin (2 and 4%) with low DP produced from chicory and inulin extracted from cardoon roots with a high DP, respectively (Padalino et al., 2017), 1.65-2.02 g of water/g of dry pasta in 7 min-cooked pasta produced with durum wheat semolina partially replacing with inulin at diferent levels (7.5, 10, 12.5, and 15%) (Tudorică et al., 2002), approximately 1.8 g/g in pasta enriched with 12% inulin from chicory (Bavaro et al., 2024), 2.19 g/g and 2.09 g/g in pasta made with wholemeal flours at 2 different substitution levels (2 and 4%) with low-chain chicory- and long-chain topiinambur-inulin, respectively (Cardullo et al., 2022), 1.58-1.88 g/g in spaghetti produced with 4 wholemeal durum wheat flour and fortified with 4% inulin from cardoon roots with high-DP and chicory with low-DP (Garbetta et al., 2020). The discrepancy between our results and literature may arise from not only differences in formulation together with the type form of of pasta/noodle, but also differences in the source, chain length/DP, and concentration of inulin.

4.4.4. Cooking Loss

One of the most influential pasta quality parameters by fiber addition into the pasta formulation is CL, owing to the fact that uniform cooking water diffusion is affected by the protein matrix integrity, hence protein content and dietary fiber type (Foschia et al., 2015a). It was reported by several studies that CL values generally yielded an increase with dietary fiber incorporation (Saraç, 2021). Therefore, the CL of 20-PGS-based fresh GFP enriched with inulin against 20-PGS during 15 min cooking with 5 different time intervals (0, 1, 5, 10, and 15 min) was given in Table 4.28.

Table 4.28. Cooking loss values of gluten-free pasta with/without inulin (%)

Sample	**Cooking time (min)**				
	0	**1**	**5**	**10**	**15**
20-PGS	2.72±0.07^{Ed}	3.65±0.14^{Dd}	4.80±0.16^{Ce}	8.25±0.10^{Bd}	10.78±0.24^{Ad}
4GR	3.14±0.16^{Ecd}	3.96±0.15^{Dcd}	5.58±0.24^{Cd}	8.74±0.16^{Bcd}	12.00±0.63^{Acd}
8GR	4.06±0.08^{Eb}	5.08±0.24^{Db}	7.32±0.40^{Cb}	11.36±0.36^{Bb}	15.86±0.68^{Ab}
4HPX	3.49±0.16^{Ec}	4.24±0.13^{Dc}	6.44±0.23^{Cc}	9.40±0.21^{Bc}	13.55±0.49^{Ac}
8HPX	4.57±0.26^{Ea}	5.60±0.14^{Da}	8.19±0.24^{Ca}	13.44±0.49^{Ba}	18.13±0.77^{Aa}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$).

The CL ratios showed generally a significant increase with inulin fortification in GFP samples during the whole cooking process in reference to the sample without inulin (20-PGS) ($p<0.05$), as shown in Table 4.28. In GFP, the major reason behind the CL is gelatinized starch leaching owing to a lack of gluten network, and higher dietary fiber content could give rise to leaching of more gelatinized starch (Ungureanu-Iuga et al., 2020). Therefore, the primary contributor of CL is accepted as partial inulin dissolution while pasta cooking (Ji et al., 2025) in this case. The potential driving force from this behaviour primarily originated from disruption of interactions between protein and starch, and also unequal water distribution in the pasta matrix because of being tendency to competitive hydration of fiber (Foschia et al., 2015a; Bastos et al., 2016; Padalino et al., 2017; Saraç, 2021; Baah et al., 2022) that restrains optimal hydration for protein while the formation of pasta dough (Padalino et al., 2017). Therefore, a negative correlation was defined between CL and water absorption/mass gain in the literature (Li et al., 2020; Bento et al., 2021). In the case of the presence of water-soluble compounds, the gluten network, which is in charge of retaining the solid materials when cooking, is also weakened (Foschia et al., 2015a). In this regard, a degree of inulin as a soluble dietary fiber leaches from the pasta during cooking, which makes a contribution to the CL (Aravind et al., 2012). These results also parallel the data obtained by studies that included inulin-blended pasta, which possessed higher CL values than those of not including inulin (Bustos et al., 2011; Aravind et al., 2012; Foschia et al., 2015a; Padalino et al., 2017; Peressini et al., 2020; Zarroug et al, 2022; Chen et al., 2023). A greater CL values in inulin-enriched pasta samples were monitored in comparison with pasta containing oat bran, RS type II and RS type IV at different usage levels (5, 7.5, and 10%) (Bustos et al., 2011), 15% oat bran replaced in semolina pasta (Foschia et al., 2015a), 4% apple fiber incorporated wheat flour-based Turkish noodle (Saraç, 2021), fresh noodle based on 5% proportion of wheat flour substituted with short-chain- and long-chain inulin (Chen et al., 2023), and comparable values were also observed when replacing semolina with 15% psyllium husk seed (Peressini et al., 2020). Conversely, the addition of dietary fiber, such as inulin, caused a reduction in CL values in comparison with control groups without inulin (Gasparre and Rosell, 2019; Saraç, 2021).

On the other side, CL values were increased irrespective of the length of the chain as the concentration of utilized inulin in GFP formulation was doubled from 4% to 8% within the same chain length of inulin (Table 4.28). These results were consistent with previous studies where inulin level was increased from 5% to10% for semolina replacement in pasta (commercially available inulin from chicory roots, average DP≥23) (Zarroug et al., 2022), 2.5% up to 10% with a significant increase in pasta made from bread wheat flour (Orafti HP) (Bustos et al., 2011), 2% to 4% in whole-meal durum wheat spaghetti which was more promenent with commercial inulin produced from chicory (low DP) than that of inulin extracted from cardoon roots (high DP) (Padalino et al., 2017), 5% up to 15% with significant increase in fresh pasta with inulin extracted from globe artichoke roots (Difonzo et al., 2022), and 4.98% to 5.30% in pasta made with wholemeal flours with low-chain chicory- and long-chain topiinambur-inulin when its level was doubled from 2% to 4% (Cardullo et al., 2022). However, while the CL was increased as the inulin (DP ≥ 23) level was raised from 2.5% to 5%, a decline was screened from 5% to 7.5 in noodles made from heat-moisture treatment applied wheat starch. This was explained by the formation of an inadequate continuous network enabling it to be dominated by inulin leaching in the case of lower inulin ratios, whereas more intermolecular interactions allow the generation of a gel-like, robust, and continuous network both within the pasta matrix and on granule surfaces at higher inulin proportions, which plays as a physical barrier that prevents amylose leaching and also influentially entraps smaller fractions of soluble inulin that otherwise could be lost. Therefore, it was stated that inulin can regulate granule stability and also water distributions when used at higher concentrations during cooking (Ji et al., 2025), which disagrees with our results.

Moreover, the polymerization degree of inulin also had an influence on CL values. In line with our results (Table 4.28), the spaghetti-shaped pasta based on durum wheat semolina incorporated inulin with short chain (GR), lower CL values were declared in comparison with long-chain inulin (HPX) (Peressini et al., 2020). In contrast, the short-chain inulin having lower DP usage in pasta production resulted in a greater CL value in prior research (Aravind et al., 2012; Foschia et al., 2015a; Padalino et al., 2017; Garbetta et al., 2020; Chen et al., 2023). As the inulin DP was increased, the CL reduction was ascribed to the competition between inulin and semolina compounds, such as protein and starch, while the formation of pasta dough for water (Padalino et al., 2017).

According to the results, the CL in GFP samples, including inulin with 2 different DP at 4% and 8% were in the range of about 3.14-4.57% to 12.00-18.13% (Table 4.28). Thus, there was an increasing trend in CL values of all GFP samples with inulin, as cooking duration sustained (Figure 4.15), which is consistent with previous research studies (Dziki and Laskowski, 2005; Sobota and Zarzycki, 2013; Sobota et al., 2013). This phenomenon stems from hydration occurring progressively, including the entrapping of starch granules in a protein matrix, followed by swelling and gelatinizing; consequently, a less compact protein structure results in soluble loss and leaching to the cooking medium (Sobota and Zarzycki, 2013). This protein matrix with discontinuous and weak structure could not only increase CL but also adhesiveness (Padalino et al., 2013).

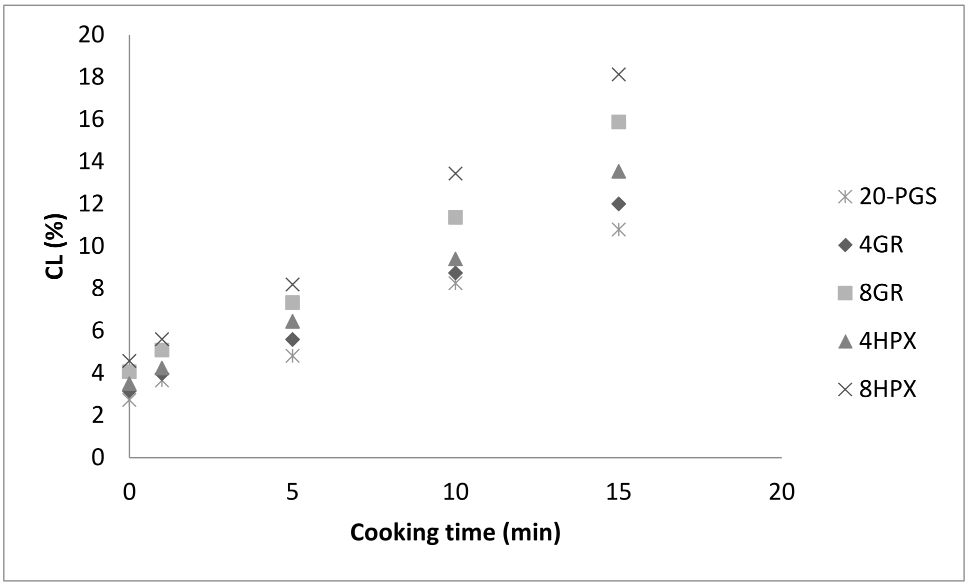

Figure 4.15. Cooking loss of gluten-free pasta with/without inulin during cooking

In the case of the maximum CL limit for pasta, which is accepted as 6%, control GFP without inulin (20-PGS), and 4% inulin-incorporated GFP at the 5-min of cooking could meet this requirement, which also compromises with the necessity of a 100% increase in WAC values for being high-quality pasta, as well (section 4.4.2, Table 4.26). At the end of the cooking, CL values of nearly all inulin-added GFP samples were more than doubled in comparison with those values acquired at 5 min of cooking. In this context, extended cooking had a negative impact not only on the technological but also likely nutritional properties of the inulin incorporated GFP formulations.

In the literature regarding the CL values of inulin-added pasta/noodle was account for 2.70-3.62% in fresh semolina pasta where inulin extracted from globe artichoke roots used at 3 diffferent concentration (5, 10, and 15%) (Difonzo et al., 2022), 3-5.5% according to NaCl and kansui included noodle based on wheat flour enriched with long-chain inulin (Yue et al., 2023), about 5% in fresh noodle based on 5% proportion wheat flour substituted with either of short-chain- or long-chain inulin (Chen et al., 2023), 6-9% and 6-10% in durum wheat semolina spaghetti including inulin (5%) with DP of 7–8 and 12-14, respectively (Aravind et al., 2012), ranged between 5.32-7.54% in spaghetti produced with 4 wholemeal durum wheat flour and fortified with 4% inulin from cardoon roots with high-DP and chicory with low-DP (Garbetta et al., 2020), 6.4-8% in pasta substituted a portion of bread wheat flour with inulin (2.5, 5.0, 7.5 and 10.0%) (Bustos et al., 2011), about 7-8% and 7% in wholemeal durum wheat spaghetti comprise of commercial inulin (2 and 4%) with low DP produced from chicory and inulin extracted from cardoon roots with a high DP, respectively (Padalino et al., 2017), 6.3% in Turkish noodle made from wheat flour partially replaced with inulin (4%) (Saraç, 2021), 6.72-8.06% in 7 min-cooked pasta produced with durum wheat semolina partially replacing with inulin at diferent levels (7.5, 10, 12.5, and 15%) (Tudorică et al., 2002), about 5% in pasta made with wholemeal flours at 2 different substitution levels (2 and 4%) with both low-chain chicory- and long-chain topiinambur-inulin (Cardullo et al., 2022), 9.7% in inulin-added (2.5, 5, 7.5, and 10%) fresh durum wheat pasta as a soluble non-starch polysaccharides (Brennan and Tudorica, 2007), about 8-9% in 7 min-cooked inulin-fortified (2.5, 5, 7.5, and 10%) spaghetti made from commercial durum semolina flour (Brennan et al., 2004), nearly 10% in spaghetti based on durum wheat semolina partially substituted (15%) with inulin has different DP used as a soluble dietary fiber source (Peressini et al., 2020), almost 11 g/100g and 9 g/100g for short-chain- and long-chain inulin-fortified (15%) functional durum wheat semolina pasta, respectively (Foschia et al., 2015a), 16.52 g/100g in gluten-free noodle prepared by tiger nut flour including inulin used as a hydrocolloid (Gasparre and Rosell, 2019). The discrepancy between our results and literature may arise from not only differences in formulation together with the type form of of pasta/noodle, but also differences in the source, chain length/DP, and concentration of inulin.

4.4.5. Volume Increase

The VI, which was obtained against the volume values measured in 10 sec (0 min), of inulin-added fresh GFP in 2 types, as low- and high-DP at 2 levels (4 and 8%) during cooking time (1, 5, 10, and 15 min), was given in Table 4.29.

Table 4.29. Volume increase values of gluten-free pasta with/without inulin (%)

Sample	Cooking time (min)			
	1	5	10	15
20-PGS	138.24±2.95^{Ca}	179.07±3.28^{Ba}	200.00±10.19^{ABb}	230.09±0.68^{Ab}
4GR	131.08±3.30^{Da}	164.58±2.09^{Cab}	234.44±4.49^{Ba}	277.78±12.83^{Aa}
8GR	109.09±5.25^{Cb}	145.45±5.25^{Bcd}	181.82±5.25^{ABbc}	218.18±5.25^{Abc}
4HPX	119.23±6.66^{Cab}	156.90±3.84^{Cbc}	205.77±3.33^{Bab}	234.62±6.66^{Ab}
8HPX	86.43±3.71^{Cc}	130.00±5.77^{Bd}	170.00±5.77^{Ac}	190.00±5.77^{Ac}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different (p<0.05). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different (p<0.05).

In the initial phase of cooking (1 and 5 min), lower VI values were found in inulin-blended GFP samples, irrespective of their type and concentration, in contrast to control GFP, which is non-inclusive of inulin (20-PGS). Both the weight and volume of pasta/noodles, and thus weight gain and volume increase, respectively, are associated with the components and their proportions, which have the ability to absorb water, but are also influenced by other compounds such as proteins, lipids, and also dietary fibers, which could lower the gelatinized starch amount while cooking and the end products' weight (Pessanha et al., 2023). The reason behind this manner could be explained by the different water absorption speed of dietary fiber and starch, hence having lower VI degrees (Saraç, 2021). In this regard, the positive correlation between volume of pasta with water absorption (Aydin, 2022) and swelling (Lux et al., 2023) was consistent with our results as given in the section 4.4.2 (Table 4.26) and section 4.4.3 (Table 4.27), corresponding to r=+0.96 and r=+0.93, respectively. On the contrary, the substitution of wheat flour with 4% dietary fiber, apart from inulin, such as carrot fiber and apple fiber, generally resulted in an increase in the VI values of noodle samples (153-195%), compared to samples without fiber (160%) in Turkish noodle samples (Saraç, 2021). In another study, the addition of psyllium husk to gluten-free noodle formulation made by soaked-and-dried soybean led to an increase in volume values when compared with the wheat noodle with no fiber addition (Bak et al., 2023).

On the other hand, GFP enriched with 4% inulin was positively influenced by the VI values at the last phases of the cooking (10 and 15 min) vis-à-vis the control GFP group not contain inulin (20-PGS) (Table 4.29). However, when the effect of the inulin concentration increase on VI values in GFP samples was assessed, a decline was observed regardless of its DP values, as the amount of inulin was doubled in the same type of inulin (p<0.05) (Table 4.29). This could be originated from that predominance of CL on water absorption, and swelling that may limit the expansion of volume that could be proven with highest CL values of 8% inulin-included GFP, as previously mentioned in section 4.4.4 (Table 4.28). Similar behaviour regarding a decline in VI was defined as the proportion of β-glucan, which is a soluble fiber, was doubled from 5% to 10% in durum semolina-based fusilli (p<0.05), although no significant differences were obtained at greater levels of β-glucan were used (15 and 20%) (p>0.05) (Krawęcka et al., 2020). In other respects, while no statistical difference was observed in VI values of semolina pasta supplemented with black rice bran, which is an insoluble fiber, when the ratios were between 5-15% ($p>0.05$), a decrement was recorded at higher incorporation rates (20-25%) ($p<0.05$). This was associated with the relatively greater contents of fractions as protein and fiber, compared to total starch proportions in the pasta (Sethi et al., 2020). Conversely, as the psyllium husk level was increased from 10% up to 40%, the volume of gluten-free noodles was generally significantly increased ($p<0.05$) (Bak et al., 2023).

The VI values of control GFP with no inulin (20-PGS) and GFP samples with inulin (short- and long-chain), used at 2 different ratios (4 and 8%), were in the range of approximately 86-131% to 190-278% from the top to the end of cooking, as given in Table 4.29. Therefore, an increment was observed in all fresh GFP samples with/without inulin sources throughout 15 min of cooking, as shown in Figure 4.16. This was also supported by the statement that the volume expansion is fulfilled as a result of starch granules' absorption and swelling of water during the cooking process (Steglich, 2013). Similarly, in a study, the addition of psyllium husk as a dietary fiber source made a gradual increase in volume values within different cooking time cycles (3, 6, and 9 min) in soaked-and-dried soybean-based gluten-free noodle compared with a control noodle based on wheat flour (Bak et al., 2023). It was noted that a high-quality pasta/noodle made from wheat flour should have VI values in the range of 200-300% (Pessanha et al., 2023). In view of this information, all GFP samples with inulin met this requirement at the end of cooking, except for 8HPX, as a GFP with a high level and DP of inulin (Table 4.29).

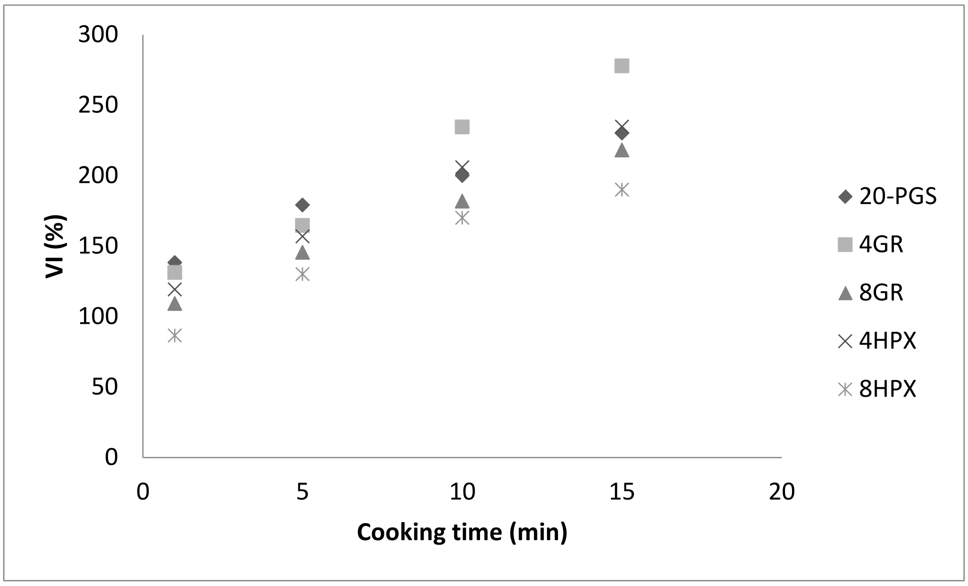

Figure 4.16. Volume increase of gluten-free pasta with/without inulin during cooking

4.4.6. Turbidity and Amylose Leaching

The turbidity (T) and amylose leaching (AL) values of fresh GFP included 2 types of inulin with low- and high-DP at 2 amounts (4 and 8%) during cooking time (0, 1, 5, 10, and 15 min) were given in Table 4.30.

Table 4.30. Turbidity and amylose leaching values in the cooking medium of gluten-free pasta with/without inulin (abs at 675 nm and 650 nm, respectively)

Sample	Cooking time (min)	T (abs)	AL (abs)
20-PGS	0	$0.06{\pm}0.01^{Eb}$	$0.06{\pm}0.01^{Ec}$
	1	$0.12{\pm}0.01^{Dc}$	$0.23{\pm}0.01^{Da}$
	5	$0.29{\pm}0.01^{Cc}$	$0.53{\pm}0.02^{Cc}$
	10	$0.71{\pm}0.02^{Bb}$	$1.27{\pm}0.02^{Bab}$
	15	$1.34{\pm}0.03^{Aab}$	$1.45{\pm}0.01^{Ac}$
4GR	0	$0.06{\pm}0.01^{Db}$	$0.08{\pm}0.01^{Eb}$
	1	$0.13{\pm}0.03^{Cbc}$	$0.25{\pm}0.02^{Da}$
	5	$0.29{\pm}0.01^{Bc}$	$0.54{\pm}0.03^{Cbc}$
	10	$0.64{\pm}0.01^{Ab}$	$1.03{\pm}0.06^{Bc}$
	15	$1.07{\pm}0.21^{Ab}$	$1.33{\pm}0.02^{Ad}$
8GR	0	$0.07{\pm}0.01^{Ea}$	$0.09{\pm}0.01^{Eb}$
	1	$0.16{\pm}0.01^{Dab}$	$0.27{\pm}0.01^{Da}$
	5	$0.43{\pm}0.06^{Cab}$	$0.67{\pm}0.03^{Ca}$
	10	$0.88{\pm}0.06^{Ba}$	$1.28{\pm}0.01^{Bab}$
	15	$1.32{\pm}0.11^{Aab}$	$1.62{\pm}0.08^{Ab}$
4HPX	0	$0.06{\pm}0.01^{Eab}$	$0.08{\pm}0.01^{Db}$
	1	$0.17{\pm}0.01^{Da}$	$0.26{\pm}0.02^{Ca}$
	5	$0.34{\pm}0.02^{Cbc}$	$0.63{\pm}0.05^{Bab}$
	10	$0.71{\pm}0.02^{Bb}$	$1.19{\pm}0.08^{Ab}$
	15	$1.16{\pm}0.12^{Aab}$	$1.39{\pm}0.01^{Acd}$
8HPX	0	$0.07{\pm}0.01^{Ea}$	$0.12{\pm}0.01^{Ea}$
	1	$0.18{\pm}0.01^{Da}$	$0.29{\pm}0.05^{Da}$
	5	$0.50{\pm}0.06^{Ca}$	$0.68{\pm}0.05^{Ca}$
	10	$0.92{\pm}0.07^{Ba}$	$1.39{\pm}0.05^{Ba}$
	15	$1.39{\pm}0.04^{Aa}$	$1.73{\pm}0.02^{Aa}$

Means followed by the different uppercase letters in the same column regarding cooking time for each parameter were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same row at the same cooking time for each parameter were significantly different ($p<0.05$).

Within the first 5-min cooking, all inulin-enriched GFP samples had higher T and AL values than the control group, consisting of 20-PGS without fiber (Table 4.30). In a similar vein, 20% replacement of brown rice flour with red lentil flour, whose coat is rich in crude fiber, significantly increased the turbidity values in rice noodles (Geng et al., 2021). Therefore, the turbidity increase with inulin incorporation into GFP was sourced from being soluble dietary fiber nature. This could be announced with the positive association between turbidity with CL, as previously stated with many studies in the literature (Heo et al., 2013; Jeon et al., 2014; Moon and Kweon, 2021; Park et al., 2022; Bak et al., 2023; Li et al., 2024) that emphasised that excessive CL may cause severe soluble components to be released during pasta cooking (Kim et al., 2014). Therefore, a strong positive correlation of CL with T and AL was supported in the 2nd chapter of this study (r=+0.97, r=+0.97, respectively). On the other hand, a decreasing influence in both T and AL values was recorded in 4% inulin-added-GFP samples, regardless of its type, in the extended cooking process (10 and 15 min) vis-à-vis 20-PGS without inulin (Table 4.30). Similarly, while cooking in a short time (3 min) made a slight increase in turbidity of gluten-free noodle based on soaked-and-dried soybean, lower turbidity values were monitored as cooking time prolonged (6 and 9 min) in comparison with the control noodle (Bak et al., 2023). Moreover, there was a positive relation between the T and AL in the 2nd chapter of this study (r=+0.76) because of being one of the reasons for turbidity in cooking water.

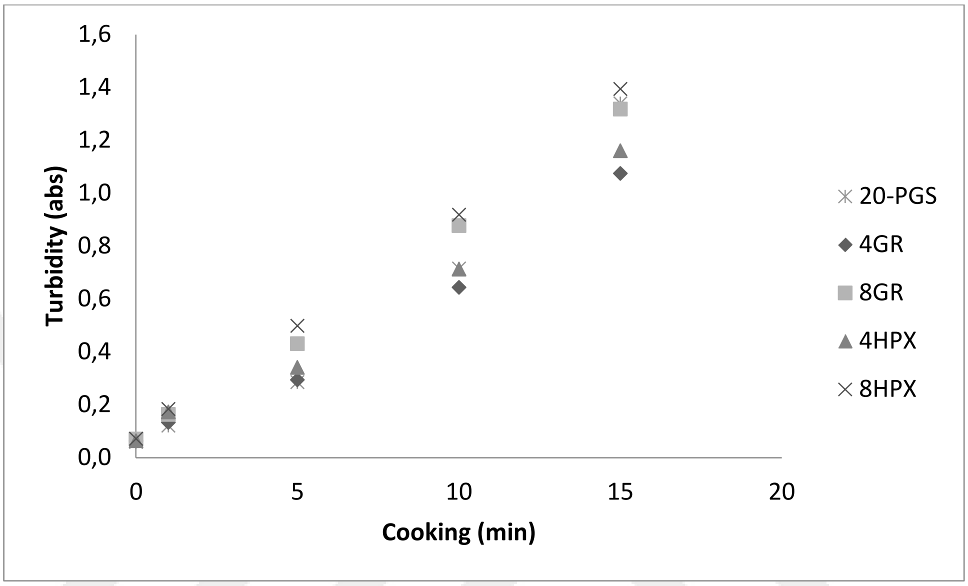

Figure 4.17. Turbidity in the cooking medium of gluten-free pasta with/without inulin during cooking

In terms of inulin amount, there was an increase in both T and AL values throughout the cooking process, as presented in Figure 4.17 and Figure 4.18, respectively. Contrary to this, psyllium husk incorporation level had a decreasing influence on turbidity in gluten-free noodles, as the replacement level with soybean flour increased from 10% up to 40% (Bak et al., 2023). In another controversial study, a gradual reduction corresponding to nearly from 18% to 12% in leached amylose ratio was determined when the inulin addition was fulfilled from 2.5% to10% in noodle formulation. This behaviour was expounded with the adsorption of inulin on starch granule surfaces and thus inconvenience amylose leaching from starch granules. Furthermore, amylose content may be influenced by the amylopectin crystal structures' melting degree while starch granules' swelling in an endothermic process, and thus, inulin incorporation could effectively sustain the order of starch crystal structure, an order that complicates escaping amylose from the crystal structure (Xu et al., 2021). It was also mentioned that higher concentrations of inulin (7.5%), as a threshold level, could form more continous, robust, gel-like network as a result of extensive intermolecular interactions within pasta/noodle matrix and granule surfaces, as well that not only efficiently trap of soluble smaller fractions of inulin, if not may be lost, but also behave like a physical barrier which prevent AL (Ji et al., 2025).

The T and AL values of GFP samples with different amounts and types of inulin were over the range of 0.06-1.39 abs and 0.08-1.73 abs, respectively (Table 4.30). As presented in Figure 4.17 and Figure 4.18, the T and AL of cooking water in all inulin-fortified GFP samples, irrespective of their polymerization degree, were increased as the cooking process was maintained, which is aligned with their corresponding CL values throughout the boiling process, as previously described (section 4.4.4, Table 4.28). There was an agreement that the results of a study referring to the turbidity of psyllium husk-incorporated with different rates of gluten-free noodle soup were significantly increased with prolonged cooking time up to 9 min (Bak et al., 2023). However, there is a limited study in the literature regarding evaluating the influence of the incorporation of inulin and other dietary fiber sources on the T and AL values of GFP. Therefore, the values and effects found in the literature may originate from the differences in types, contents, and concentrations of ingredients utilized and the different production methods and/or processes with different flowcharts followed**.**

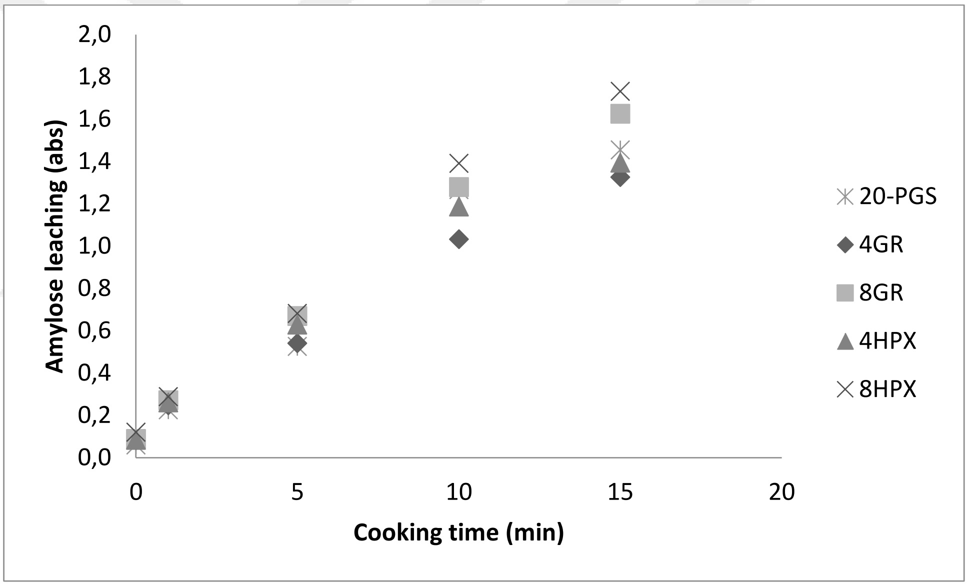

Figure 4.18. Amylose leaching in the cooking medium of gluten-free pasta with/without inulin during cooking

4.4.7. Color

The color parameters regarding L*, a*, b*, C*, h°, and also their derivatives comprise of the yellowness index (YI), color score, $\Delta E^*_{cooking}$ of both raw and cooked (1-15 min) fresh control GFP (20-PPGS with no inulin) and GFP samples including inulin with different chain length (short- and long-chain) utilized at 2 different rates (4 and 8%) were summarized in Table 4.31, and Appendix 4.

Table 4.31. Color values of raw and cooked gluten-free pasta with/without inulin

Color	Time (min)	20-PGS	4GR	8GR	4HPX	8HPX
L*	**Raw**	76.55±1.20^{ABb}	78.12±0.48^{Aab}	78.93±0.42^{Aab}	80.29±0.84^{Aa}	80.97±1.62^{Aa}
	1	72.87±0.59^{Bc}	76.28±0.64^{Bb}	76.43±0.13^{Bb}	77.35±0.46^{ABab}	78.05±0.35^{ABa}
	5	71.72±0.52^{BCc}	74.95±0.34^{Cb}	75.32±0.41^{Cb}	76.46±0.42^{Ba}	77.46±0.39^{Ba}
	10	70.81±0.61^{Cc}	74.17±0.35^{Db}	74.60±0.57^{Cb}	75.16±0.03^{Cb}	76.67±0.22^{Ca}
	15	69.58±0.57^{Cc}	73.22±0.32^{Db}	73.56±0.29^{Db}	74.19±0.40^{Dab}	75.05±0.13^{Da}
a*	**Raw**	0.81±0.15^{Ac}	1.45±0.13^{Aa}	1.24±0.01^{Aab}	1.12±0.08^{Ab}	1.04±0.13^{Abc}
	1	-2.80±0.09^{Ba}	-2.87±0.11^{Ba}	-3.10±0.03^{Bb}	-3.18±0.06^{Bbc}	-3.33±0.07^{Bc}
	5	-3.20±0.02^{Ca}	-3.31±0.04^{Ca}	-3.46±0.08^{Cb}	-3.52±0.02^{Cbc}	-3.57±0.01^{Cc}
	10	-3.62±0.01^{Db}	-3.51±0.07^{Da}	-3.64±0.03^{CDb}	-3.58±0.01^{Dab}	-3.65±0.03^{Cb}
	15	-3.73±0.01^{Ebc}	-3.62±0.04^{Ea}	-3.75±0.02^{Dbc}	-3.71±0.04^{Eb}	-3.80±0.03^{Dc}
b*	**Raw**	26.68±0.53^{Ab}	27.61±0.54^{Aab}	28.87±1.07^{Aa}	27.90±0.40^{Aab}	28.33±0.25^{Aab}
	1	21.45±0.22^{Bb}	21.88±0.23^{Bb}	22.06±0.23^{Bb}	23.28±0.36^{Ba}	23.87±0.30^{Ba}
	5	17.53±0.42^{Cc}	17.87±0.43^{Cbc}	18.35±0.19^{Cabc}	18.57±0.16^{Cab}	18.88±0.31^{Ca}
	10	14.50±0.23^{Dd}	14.88±0.17^{Dcd}	15.37±0.10^{Dc}	15.93±0.16^{Db}	17.00±0.31^{Da}
	15	13.53±0.27^{Dc}	13.98±0.32^{Ebc}	14.32±0.59^{Dbc}	14.80±0.13^{Eab}	15.50±0.16^{Ea}
YI	**Raw**	49.85±1.35^{Aa}	50.49±1.30^{Aa}	52.26±1.27^{Aa}	49.65±1.22^{Aa}	49.99±0.57^{Aa}
	1	42.05±0.76^{Bbc}	40.98±0.09^{Bc}	41.22±0.49^{Bc}	42.99±0.41^{Bab}	43.69±0.35^{Ba}
	5	34.91±0.59^{Ca}	34.06±0.67^{Ca}	34.81±0.55^{Ca}	34.69±0.48^{Ca}	34.82±0.40^{Ca}
	10	29.26±0.21^{Dcd}	28.66±0.18^{Dd}	29.44±0.04^{Dc}	30.28±0.29^{Db}	31.66±0.48^{Da}
	15	27.78±0.77^{Dab}	27.28±0.74^{Eb}	27.82±1.26^{Dab}	28.50±0.10^{Eab}	29.50±0.35^{Ea}
Color score	**Raw**	6.50±0.06^{Ac}	6.67±0.03^{Abc}	6.83±0.09^{Aab}	6.80±0.01^{Aab}	6.88±0.11^{Aa}
	1	5.79±0.01^{Bc}	6.00±0.06^{Bb}	6.03±0.02^{Bb}	6.20±0.06^{Ba}	6.29±0.05^{Ba}
	5	5.34±0.07^{Cd}	5.53±0.06^{Cc}	5.60±0.01^{Cbc}	5.68±0.01^{Cab}	5.76±0.05^{Ca}
	10	4.99±0.05^{Dd}	5.20±0.04^{Dc}	5.27±0.04^{Dbc}	5.35± 0.02^{Db}	5.53±0.04^{Da}
	15	4.83±0.01^{Ed}	5.06±0.02^{Ec}	5.11± 0.04^{Dc}	5.19±0.03^{Eb}	5.30±0.01^{Ea}
$\Delta E^*_{cooking}$	**1**	7.37±0.10^{Db}	7.43±0.28^{Db}	8.45±0.16^{Ca}	6.97±0.40^{Db}	6.89±0.39^{Db}
	5	11.11±0.57^{Cab}	11.30±0.46^{Cab}	12.08±0.08^{Ba}	11.11±0.01^{Cab}	11.08±0.39^{Cb}
	10	14.18±0.45^{Bb}	14.22±0.23^{Bab}	15.00±0.25^{Aa}	13.84±0.15^{Bb}	12.99±0.33^{Bc}
	15	15.58±0.03^{Ab}	15.35±0.18^{Ab}	16.29±0.43^{Aa}	15.23±0.26^{Ab}	14.94±0.08^{Ab}

Means followed by the different uppercase letters in the same column regarding cooking time for each color parameter were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same row at the same cooking time for each color parameter were significantly different ($p<0.05$).

According to the obtained results, the inclusion of inulin in the GFP formulation with semolina replacement led to a generally significant increase in L* values ($p<0.05$) (Table 4.31). This increase was possibly occured due to the natural white color of the commercial inulin (Difonzo et al., 2022). Similar results were recorded where inulin (Orafti HP) was added at 2.5-10% to bread wheat flour-based pasta, in contrast to the control without any dietary fiber sources. This was associated with the whiter color of inulin and also resistant starches in comparison with flour, which in turn, brightness values changed with fiber replacement (Bustos et al., 2011). In a similar vein, brightness was increased in wheat flour-based fresh noodles with the incorporation of modified (phosphorylated) long-chain inulin up to 6%, but then a decline was also observed in those corresponding values in higher replacement ratios (8 and 10%) (Che et al., 2024). This was also explained with the phosphorylated long-chain inulin probably acted like an emulsifier, enabling more sufficient interactions of starch–protein-water, concluding with a more uniform internal structure. This uniform tissue allows an enhanced light reflection when the pasta/noodles are illuminated with light, which increases the brightness (L*) of the end-product (Che et al., 2024). In another study, L* values were higher in long-chain inulin blended spelt pasta in contrast to control without additive, and a progressive significant increase was also determined as the replacement level with inulin was doubled from 10% to 20% ($p<0.05$) (Filipović et al., 2015a). The lightness was also increased significantly in spaghetti made from durum wheat semi-whole-meal flour, including 12% (w/w) inulin ($p<0.001$) (Bavaro et al., 2024). While some dietary fibers sourced from apples, carrots, and peas made the pasta have a darker color (lower L*), inulin yielded a brighter color in Turkish noodles made from whole wheat flour, among other non-starch polysaccharides, and also higher in comparison with the control with no fiber (Saraç, 2021). In a similar vein, partial replacement (15%) of durum wheat semolina with both short-chain inulin (Raftiline GR) and long-chain inulin (Raftiline HPX) resulted in an increase of L* values in fresh cooked pasta samples, whereas psyllium, oat bran, and glucagel led to a reduction (Foschia et al., 2015a). In a study, it was stated that inulin-included cooked noodles made from non-conventional flour (tiger nut) were brighter, namely have higher L* values (Gasparre and Rosell, 2019), although an obverse pattern was defined in corresponding to their raw counterparts (Foschia et al., 2015a; Gasparre and Rosell, 2019). Correspondingly, higher L* values were monitored where partial semolina substitution was conducted with inulin than barley balance and psyllium, which were also lower in contrast to no fiber-added control samples (Peressini et al., 2020). Therefore, it was highlighted that dietary fiber inclusion in pasta making generally darkened the end-products (Foschia et al., 2015a; Saraç, 2021) except for inulin.

On the other side, the GFP with high-DP inulin (HPX) had higher L* values throughout cooking than that of low-DP inulin (GR) (Table 4.31). Similarly, significantly lower L* values were identified when short-chain inulin (Raftiline GR, DP=10) was used ($p<0.05$), but no significant difference was determined in long-chain inulin (Raftiline HPX, DP=23) added uncooked spaghetti samples ($p>0.05$), in the case of partially replacement of durum wheat semolina (15%) (Peressini et al., 2020). In another study, there were no significant differences in L* values between short-chain and long-chain inulin blended raw and cooked semolina pasta ($p>0.05$) (Foschia et al., 2015a). On the contrary, a decline in L* values, lightness, was recorded as a function of the increasing proportion of commercially available inulin from chicory roots (DP≥23) (Zarroug et al., 2022), Jerusalem artichoke powder (Singthong and Thongkaew, 2020), and phosphorylated long-chain inulin (Fibruline XL, DP≥23) (Che et al., 2024) in the formulation of corresponding pasta/noodle samples.

On the other hand, the b* values of raw and cooked fresh GFP samples were also increased when 20-PGS GFP was fortified with inulin with 2 different chain lengths and ratios (Table 4.31). A similar pattern was stated in both raw and cooked whole wheat flour-based Turkish noodles substituted with 4% inulin (Saraç, 2021), semolina pasta partially replaced (5 and 10%) with commercially available inulin from chicory roots (Zarroug et al., 2022), long-chain inulin-fortified (10 and 20%) spelt pasta (Filipović et al., 2015a), and cooked gluten-free noodles based on tiger nut flour, including 0.5% inulin (Gasparre and Rosell, 2019). In contrast, a reduction in both red index and yellow index, which refer to a* and b* color values, respectively, was seen in 12% (w/w) inulin-fortified durum wheat pasta samples compared to the control group with no inulin ($p < 0.001$) (Bavaro et al., 2024). Controversial tendency was also recorded in semolina spaghetti enriched with both short- and long-chain inulin at 15% (w/w) (Peressini et al., 2020), pasta made from bread wheat flour comprising of different concentrations of inulin (2.5-10%) (Bustos et al., 2011), and this reduction was generally associated owing to a dilution influence by the substitiution of semolina/wheat flour (Peressini et al., 2020). The b* increment was slightly prominent when the addition amount of inulin was 8%, on the conditions of the same chain length, in the present study ($p>0.05$) (Table 4.31). In agreement with data reported in the literature, the b* values, which in turn affect yellowness, of glass noodles were increased when the proportion of Jerusalem artichoke (*Helianthus tuberosus)* powder was increased (Singthong and Thongkaew, 2020). This could originate from their lower volume increase values in comparison with lower amounts of inulin-added (4%) GFP samples (section 4.4.5, Table 4.29). It was elucidated with as previously mentioned for β-glucan, that a decline in volume increase when the amount of utilized fiber increased could contribute a greater levels of carotenoids, thereby increasing b* values in the cooked pasta (Krawęcka et al., 2020). Conversely, b* values were reduced in raw and cooked pasta samples as the substitution for durum wheat semolina with high DP inulin extracted from globe artichoke roots was increased (5, 10, and 15%) (Difonzo et al., 2022).

In other respects, a reduction in L*, a*, and b* values in all inulin-added GFP samples was recorded throughout the 15-min of cooking process (Table 4.31). The partial hydrolysis of inulin during boiling into its two constitutive monomers (glucose and fructose) can enhance surface darkening through Maillard reactions (Majzoobi et al., 2019). Therefore, the primary cause behind this could be the nature of inulin-type fructans, which are a mixture of reducing and non-reducing oligosaccharides, making them susceptible to participating Maillard reaction (Jackson et al., 2023). Parallel with this, L*, a*, and b* values of fresh tagliatelle made from durum wheat semolina comprising inulin (5, 10, and 15%) extracted from globe artichoke roots were also decreased as a result of the cooking process (Difonzo et al., 2022). The reduction of b* values yielded with the cooking process of inulin-incorporated GFP could originate from pigments predominantly present in carotenoids (zeaxanthin and lutein), which exhibit heat-labile behaviour (Motta Romero et al., 2017), and dissolution/degradation, by thermal treatment, concluding with leaching out to the boiling water (Giuberti et al., 2015; Bouasla et al., 2017b). Conversely, an increase in L* (lightness) was found in consequence of the cooking process of inulin-incorporated pasta/noodle (Gasparre and Rosell, 2019; Saraç, 2021). In the present study, a gradual decline in YI and color score, with different intensities according to formulation, which are based on a* and b* values, was also determined throughout the cooking process in inulin-fortified GFP. This may be attributed to the maintained increase in CL values of those corresponding pasta samples, as previously presented in section 4.4.4 (Table 4.28). The ΔE* values between raw and cooked pasta, which are indicative of color stability during cooking, mainly sourced from degradation of color pigments (Fradinho et al., 2020), were increased with prolonging the cooking process, as expected, and in turn GFP samples with inulin at all cooking times could be noticeable by regular human vision (Table 4.31).

4.4.8. Texture

The hardness/firmness differences in cooked pasta are majorly based on the gluten, but some compounds, such as protein, dietary fiber, resistance starch, and amylose, are also determinants in the texture properties of GFP (de Oliveira et al., 2022). It was also emphasized that not only the dietary fiber type but also its proportions included in pasta formulation may influence the textural characteristics (Bustos et al., 2011). Inulin causes a competition for water with protein and starch during pasta hydration. Therefore, this may cause an unncompleted gluten hydration, and thus may lead to a weak gluten network formation in semolina-based pasta, which is explained by physicochemical characteristics of inulin, possible protein network discontinuity, and disruptive effect on starch-protein binding while cooking (Padalino et al., 2017), and could interfere with the structure and textural properties of GFP, as well (de Oliveira et al., 2022). Therefore, the hardness, chewiness, cohesiveness, springiness, and resilience values as the TPA parameters of the inulin-added fresh GFP with low- and high-DP at 2 levels (4 and 8%) against 20-PGS during the whole cooking process with 4 time intervals (0, 5, 10, and 15 min) were demonstrated in Table 4.32.

Firmer and chewier GFP with inulin was more pronounced at the beginning of the cooking (0-5 min) when comparing with the GFP sample without inulin (20-PGS) ($p<0.05$) (Table 4.32). A firmer pasta may be obtained because reducing starch granules' swelling could be explained by forming a physically cross-linked network by fibers around the starch granules, which entraps them during cooking (Peressini et al., 2020). This negative correlation between hardness/firmness and SI (Brennan and Tudorica, 2007), aligned with our results in the 2nd chapter of the study ($p=-0.85$). This was also supported by the study based on fresh pasta, including inulin from globe artichoke roots (10 and 15%) with high DP, suggesting the hypothesis that the rearrangement of the pasta structure (Difonzo et al., 2022). Inulin incorporation (4%) into Turkish noodles made from whole wheat flour also increased the firmness values in uncooked samples ($p<0.05$), although no significant differences were recorded in the hardness values of cooked samples ($p>0.05$) (Saraç, 2021). Similarly, commercial inulin (Fibruline® XL) incorporation into gluten-free noodles prepared from tiger nut flour causes a significant increase in both hardness and chewiness values in contrast to samples in the absence of inulin ($p<0.05$) (Gasparre and Rosell, 2019). The firmness values of gluten-free noodles based on rice native autoclaved resistant starch in the presence of XN were increased, as well, with the inclusion of inulin (5%) into the formulation ($p<0.05$) (Raungrusmee et al., 2020). In other respect, an adverse relation between hardness with water absorption/holding ability/capacity (Wang et al., 2012; Duta et al., 2019; Garcia-Valle et al., 2021; Oduro-Obeng et al., 2021; Odabas and Cakmak, 2022; Aydin, 2022; Weber et al., 2023), and moisture (Brennan and Tudorica, 2007) and also SI were coherent with our results as summarized in the first phases of cooking (0-5 min), and detailed in the section 4.4.1 (Table 4.25), and section 4.4.3 (Table 4.27), respectively.

Table 4.32. Texture properties of cooked gluten-free pasta with/without inulin

Texture parameter	Time (min)	20-PGS	4GR	8GR	4HPX	8HPX
Hardness (N)	0	4.34±0.16^{Ac}	6.00±0.42^{Ab}	7.39±0.25^{Aa}	6.14±0.60^{Ab}	8.06±0.13^{Aa}
	5	3.49±0.05^{Bc}	5.39±0.52^{Ba}	4.67±0.15^{Bab}	5.02±0.64^{Bab}	4.14±0.12^{Bbc}
	10	2.86±0.13^{Ca}	2.75±0.05^{Ca}	2.40±0.11^{Cb}	2.47±0.02^{Cb}	2.35±0.03^{Cb}
	15	2.42±0.02^{Da}	2.38±0.11^{Ca}	2.26±0.03^{Ca}	2.36±0.13^{Ca}	2.02±0.07^{Db}
Chewiness (N)	0	2.60±0.06^{Ab}	4.04±0.31^{Aa}	4.42±0.85^{Aa}	3.93±0.49^{Aa}	4.21±0.12^{Aa}
	5	1.54±0.13^{Bcd}	2.34±0.17^{Ba}	1.73±0.05^{Bbc}	1.92±0.07^{Bb}	1.42±0.08^{Bd}
	10	0.97±0.12^{Ba}	0.84±0.05^{Cab}	0.53±0.05^{Ccd}	0.70±0.06^{Cbc}	0.49±0.04^{Cd}
	15	0.65±0.03^{Ca}	0.58±0.02^{Db}	0.42±0.03^{Cc}	0.55±0.01^{Cb}	0.36±0.02^{Cc}
Cohesiveness	0	0.66±0.01^{Ab}	0.74±0.02^{Aa}	0.67±0.02^{Ab}	0.72±0.01^{Aa}	0.64±0.01^{Ab}
	5	0.53±0.01^{Ba}	0.57±0.04^{Ba}	0.54±0.01^{Ba}	0.56±0.03^{Ba}	0.52±0.01^{Ba}
	10	0.47±0.02^{BCa}	0.47±0.01^{Ca}	0.40±0.01^{Cb}	0.45±0.02^{Ca}	0.39±0.01^{Cb}
	15	0.44±0.01^{Ca}	0.43±0.02^{Da}	0.37±0.01^{Db}	0.42±0.01^{Ca}	0.38±0.01^{Cb}
Springiness	0	0.90±0.01^{Aa}	0.90±0.03^{Aa}	0.89±0.12^{Aa}	0.89±0.03^{Aa}	0.81±0.05^{Aa}
	5	0.83±0.05^{ABa}	0.76±0.03^{Bab}	0.68±0.01^{ABbc}	0.69±0.03^{Bbc}	0.65±0.02^{Bc}
	10	0.72±0.02^{Ba}	0.65±0.02^{Bb}	0.55±0.01^{Cc}	0.62±0.02^{Cb}	0.53±0.03^{Cc}
	15	0.61±0.03^{Ca}	0.57±0.03^{Cab}	0.50±0.04^{Cbc}	0.56±0.03^{Cab}	0.47±0.01^{Dc}
Resilience	0	0.51±0.01^{Aa}	0.48±0.05^{Aa}	0.43±0.03^{Aab}	0.47±0.05^{Aa}	0.38±0.01^{Ab}
	5	0.42±0.03^{Aa}	0.38±0.02^{Aab}	0.36±0.03^{Bab}	0.36±0.02^{Bab}	0.34±0.01^{Bb}
	10	0.33±0.01^{Ba}	0.32±0.01^{Bab}	0.31±0.01^{BCb}	0.31±0.01^{Cab}	0.28±0.01^{Cc}
	15	0.30±0.01^{Ca}	0.29±0.02^{Bab}	0.25±0.03^{Cb}	0.27±0.01^{Dab}	0.25±0.01^{Db}

Means followed by the different uppercase letters in the same column regarding cooking time for each texture parameter were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same row at the same cooking time for each texture parameter were significantly different ($p<0.05$).

Nonetheless, lower hardness and chewiness values were obtained in inulin-included GFP when compared with the 20-PGS within the last phase of cooking (10-15 min), as shown in Table 4.32. It was announced that the further swelling of starch granules owing to inulin addition into the protein-starch matrix may lead to softening of the pasta structure (Saraç, 2021). This could be explained by the disruptive influence of inulin on the starch-protein matrix, which let starch granules for rupturing while cooking, and thus high amounts of amylose releasing into the cooking medium (Tudorică et al., 2002). The reason behind this was ascribed to the dietary fiber attributes of inulin, which would result in higher moisture and SI but lesser firmness (Foschia et al., 2015a).

Moreover, the negative association between hardness/firmness with CL (Wang et al., 2012; Alinovi et al., 2023) and turbidity (Park et al., 2022), chewiness with CL (Milde et al., 2020), and amylose leaching, as well (Palavecino et al., 2017; Milde et al., 2020), were compatible with our results of the last phases of cooking (10-15 min), demonstrated in the section 4.4.4 (Table 4.28), and section 4.4.6 (Table 4.30), respectively. Therefore, a negative correlation between hardness and CL was recorded in the 2nd chapter of this study (r=-0.68). The hardness/firmness reduction with introducing inulin into pasta/noodle formulation was also recorded in several studies in the literature, where 5% proportion wheat flour substituted with either short-chain- or long-chain inulin in in fresh noodle (Chen et al., 2023), durum wheat semolina replacing up to 15% (w/w) with both long- and short-chain for pasta production ($p<0.05$) (Peressini et al., 2020), partially replaced with short-chain inulin (15%) in semolina pasta, in contrast to control durum wheat semolina pasta without fiber (Foschia et al., 2015a), substituted bread wheat flour with inulin (Orafti HP) at different rates 2.5, 5, 7.5, and 10% in pasta (Bustos et al., 2011), inulin-incorporated (Frtutafid, HD) durum wheat pasta at different amounts (2.5, 5, 7.5, and 10%) (Brennan et al., 2004; Brennan and Tudorica, 2007), and spelt pasta fortified with inulin (Orafti, HPX) at different levels (5 and 10%) and (5, 10, and 20%) (Filipović et al., 2014; Filipović et al., 2015b, respectively).

The hardness and chewiness decrement were more prominent in the GFP with higher proportions (8%) of inulin within prolonged cooking times (10-15 min) (Table 4.32). This could be interpreted that inulin used at excessive levels may weaken and/or disrupt internal connections, and thus decrease the ability of resistance to stress (Chen et al., 2024b). This can also be reasonably linked to higher CL values of GFP samples in the case of including 8% inulin, as given in section 4.4.4 (Table 4.28). In a similar vein, while a progressive increase was recorded when inulin level was raised from 2.5% to 5%, a gradual decrease after 5% at higher proportions (7.5 and 10%) was monitored in durum wheat semolina pasta (Brennan and Tudorica, 2007), that coherent with our results (Table 4.32). In another study, a reducing trend in firmness was detected when the inulin (Frutafit-HD) amount was increased from 2.5% up to 10% in spaghetti made from commercial durum semolina flour (Brennan et al., 2004), which could be disclosed to a decrease in starch gelatinization and gluten matrix discontinuity (Zarroug et al., 2022). Controversial results regarding hardness/firmness increase when higher rates of inulin are introduced into the formulation were detected in wheat flour-based fresh noodle, involving more than 6% phosphorylated long-chain inulin (8 and 10%), although a softening effect, namely reduction in hardness, was observed in lower proportions (2 and 4%). This was attributed to forming large polymers between gluten via strong cross-linking (Che et al., 2024). Similarly, athough the softening effect was oberved in pasta made from durum wheat semolina with addition of low concentrations (5%) of commercial inulin (average DP $\geq$ 23 from chicory roots), coarser texture was obtained in pasta blended with higher ratios of inulin (10%), thereby an increment was recorded in pasta firmness with increease in the inulin level (Zarroug et al., 2022). In another study, the firmness of cooked pasta samples, including inulin extracted from globe artichoke roots, was increased as the inulin level was increased from 5% to 15% ($p<0.05$). Although a similar pattern was observed in corresponding raw samples, the differences were not statistically significant between the incorporation levels (5, 10, and 15%) ($p>0.05$) (Difonzo et al., 2022). The hardness/firmness increment with the increase of inulin amount from 2.5% to 10% was also recorded in pasta made by bread wheat flour. However, inulin-fortified pasta samples also had the lowest both hardness and chewiness values among the other pasta samples consisting of different dietary fibers such as oat bran, resistant starch II, and resistant starch IV (Bustos et al., 2011). In another controversial study, the hardness values were increased in the glass noodle as the Jerusalem artichoke level rose (Singthong and Thongkaew, 2020).

When evaluating the impact of inulin in terms of their DP, pasta enriched with long-chain length inulin (high DP) had higher firmness values in comparison with that of short-chain length (low DP) (Aravind et al., 2012; Foschia et al., 2015a; Peressini et al., 2020), which agrees with our results obtained at the initial of cooking (0 min). On the other hand, GFP, including low DP, had generally higher hardness values than those of corresponding samples with high DP at the same incorporation levels in the following cooking times, in our study.

The inclusion of inulin into GFP formulation led to a reduction in springiness and resilience throughout the entire cooking process when compared with 20-PGS (Table 4.32). It was associated with the disruption of starch-protein matrix as observed with the addition of oat bran-incorporated pasta, which interferes with the pasta structure (Bustos et al., 2011). Similarly, elasticity values generally declined numerically with inulin addition into pasta formulation (Brennan et al., 2004; Brennan and Tudorica, 2007; Raungrusmee et al., 2020). This decrease was more explicit in higher levels of inulin (8%) in the case of the same DP degrees of inulin in our study (Table 4.32). An analogous behavior based on a springiness reduction was also reported when inulin was used at higher rates (7.5 and 10%) ($p<0.05$), even though lower proportions (2.5 and 5%) did not make a significant difference in comparison to the control with no inulin ($p>0.05$). This was ascribed leaching of inulin to the cooking medium, as a result of forming holes in the starch-protein matrix (Bustos et al., 2011), which aligns with our corresponding SEM results (section 4.4.12, Figure 4.20f, h). On the other hand, there was no statistically significant difference regarding springiness (Singthong and Thongkaew, 2020), resilience (Gasparre and Rosell, 2019), and cohesiveness (Bustos et al., 2011) values of inulin-enriched cooked pasta ($p>0.05$) in the literature.

As given in Table 4.32, a continued reduction was observed in all textural features of control with no inulin and both short- and long-chain inulin-added-GFP samples as the cooking progressed. In a similar vein, while the firmness of globe artichoke root-blended (5, 10, and 15%) uncooked fresh pasta was nearly 20N, the corresponding cooked samples were in the range of approximately 6-7 N, changed against to inulin ratio (Difonzo et al., 2022).

In the literature regading the evaluate influence of inulin on texture characteristics of pasta/noodle, firmness peak height was in the range between 447-622 g and 548-830 g in durum wheat semolina spaghetti including inulin (5%) with DP of 7–8 and 12-14, respectively (Aravind et al., 2012), nearly 0.94-1.0 N peak force and 8-9 N in firmness area in pasta produced with durum wheat semolina partially replacing with inulin at diferent levels (7.5, 10, 12.5, and 15%) (Tudorică et al., 2002), nearly average of 1 N firmness in inulin-added (2.5, 5, 7.5, and 10%) fresh durum wheat pasta used as a soluble non-starch polysaccharides (Brennan and Tudorica, 2007), 1.06-1.22 N hardness in pasta for a portion of bread wheat flour substituted with inulin (2.5, 5.0, 7.5 and 10.0%) (Bustos et al., 2011), 1.03-1.33 N in spaghetti based on durum wheat semolina partially substituted (15%) with inulin has different DP used as a soluble dietary fiber source (Peressini et al., 2020), almost 1.3 N and 2.8 N firmness for short-chain- and long-chain inulin-fortified (15%) functional durum wheat semolina pasta, respectively (Foschia et al., 2015a), 6744 g hardness and 22.1 g firmness in gluten-free cooked noodle prepared by tiger nut flour including inulin used as a hydrocolloid (Gasparre and Rosell, 2019), nearly 6N harndess in in wholemeal durum wheat spaghetti comprise of commercial inulin (2 and 4%) with low DP produced from chicory and inulin extracted from cardoon roots with a high DP (Padalino et al., 2017), proximately 6-7 N and 20N in cooked and raw fresh semolina pasta, respectively, where inulin extracted from globe artichoke roots used at 3 concentration (5, 10, and 15%) (Difonzo et al., 2022), 34.6 N hardness and 2206 N firmness in Turkish noodle made from wheat flour partially replaced with inulin (4%) utilized as a dietary fiber (Saraç, 2021), 8816 g and 9061 g in fresh noodle based on 5% proportion wheat flour substituted with short-chain- or long-chain inulin, respectively (Chen et al., 2023), and 61-85 N hardness in noodle based on wheat flour enriched with long-chain inulin change in according to NaCl and kansui proportion (Yue et al., 2023). The discrepancy between our results and literature may have arisen from not only differences in formulation together with the type form of of pasta/noodle, but also differences in the source, chain length/DP, and concentration of inulin.

4.4.9. Optimum Cooking Time

The quality of pasta regarding its cooking properties is commonly characterized by the capability to maintain its shape and favored texture after the cooking process at its OCT with fewer losses of solids without changing into a sticky and thick mass (Poshadri and Deshpande, 2024; Achinna and Deshpande, 2025). It determines how long pasta should be in contact with boiling cooking medium, and OCT-cooked pasta eventuates in denaturation, gelatinization, and granule transformation, causing structural loss with no granule rupture (Poshadri and Deshpande, 2024). The OCT values and representative visuals of all fresh GFP with inulin, composed of raw and cooked samples at 4 different times (1, 5, 10, and 15 min), were reflected in Figure 4.19 and Appendix 5 (e-h), respectively.

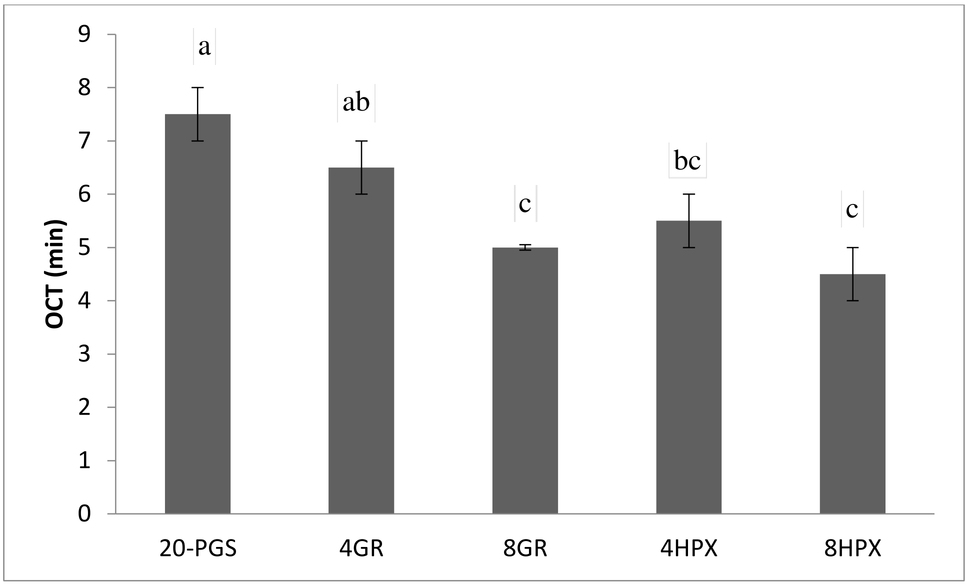

Figure 4.19. Optimum cooking time of gluten-free pasta with inulin

The OCT of inulin-fortified GFP was in the range of 4.5-6.5 min (Figure 4.19). Therefore, a significant reduction in OCT was observed when inulin was incorporated into the GFP formulation, regardless of its DP value and level ($p<0.05$), as demonstrated in Figure 4.19. This could be interpreted as inulin-added GFP samples were insufficient to withstand boiling/cooking stress (Bresciani et al., 2021) when compared to 20-PGS because of possibly their higher CL values (section 4.4.4, Table 4.28), which accelerates softness and loses the maintaining ability of mechanical features towards water (Milde et al., 2020). In a similar vein, the commercial inulin (Raftilose® Synergy 1), added at 12% (w/w) into pasta made by durum wheat semi-whole-meal flour, was reduced OCT from 11.5 min to 7.5 min in comparison with control pasta without inulin (Bavaro et al., 2024). Analogically, semolina replacement, which was conducted with inulin individually or combined with psyllium or barley balance, made a significant decline in OCT from 8 min to 5.5 min and 6.3 min for short- and long-chain inulin, respectively ($p<0.05$) (Peressini et al., 2020). A similar OCT reduction effect was recorded in noodle samples, including different dietary fibers (apple fiber, carrot fiber, pea fiber, and inulin), in contrast to the control group without any fiber sources, corresponding to 8 min. Among them, significantly lower OCT values were obtained in noodles with apple (5.35 min) and carrot fiber (5.2 min), but higher values were obtained in pea fiber (7.37 min) when compared with inulin (6.15 min) ($p<0.05$) (Saraç, 2021).

Furthermore, the OCT values of GFP with inulin were progressively decreased with an increase in its concentration, as shown in Figure 4.19. This behaviour obtained in the present study agreed with studies reported in the literature regarding a reduction in cooking times with increasing levels of inulin (Bustos et al., 2011; Aravind et al., 2012; Padalino et al., 2017; Zarroug et al., 2022; Cardullo et al., 2022). This was explained by the reason of gluten physical disruption (Zarroug et al., 2022; Difonzo et al., 2022) and/or dilution (Foschia et al., 2015a; Padalino et al., 2017;

Peressini et al., 2020; Zarroug et al., 2022) resulting in the generation of a weaker gluten structure (Foschia et al., 2015a) via alterations in protein-starch matrix (Peressini et al., 2020) that led to more easier penetration of water into starch granules (Difonzo et al., 2022; Suo et al., 2022). In a study, the OCT was decreased in chickpea flour-fortified GFP based on the combination of corn flour and rice flour that was announced with the the different structural organisation and modified composition of the amorphous matrix, eventuating in a rapid softness of the pasta matrix in return for the water uptake, likely driven by the higher content of dietary fibre (Suo et al., 2022). In another study where rice flour was substituted with sweet potato flour in GFP production, shorter OCT was defined, and this pattern was related to faster water absorption ability of potato starch granules and their fiber content, possibly mediated by their unremoved skin prior to the milling process. This was reputed to be a weakening of the starch network in response to the enhancement of moisture penetration because of fibers in pasta (Marengo et al., 2018). Non-starch polysaccharides could also cause a compact network loss that helps heat transfer while cooking, which reduces OCT as encountered with whole unripe plaintain flour mixture with semolina in spaghetti (Patiño-Rodríguez et al., 2018), and compared with Hi-Maize 260 containing spaghetti, as well (Agama-Acevedo et al., 2019). Conversely, a slight prolonging effect on OCT was recorded in 10% and 15% inulin-fortified pasta samples from 6.30 min to 6.45 min (Difonzo et al., 2022). In another study, not only using short- and long-chain inulin individually but also combined with psyllium or oat material for partial replacement (15%) of durum wheat semolina was demonstrated a significant increase in OCT ranged between 8-12 min in comparison with the control sample, which include no additive (6.5 min) ($p<0.05$) (Foschia et al., 2015a). The OCT increase with inulin application into pasta formulation was also supported by other studies such as inulin incorporation (5%) into gluten-free noodles made by resistant rice starch in the presence of XN increased OCT 13.0 min to 27.5 min (Raungrusmee et al., 2020), from 4.75 min to 5.75 min in gluten-free noodle prepared by tiger nut flour including inulin used as a hydrocolloid (Gasparre and Rosell, 2019). The reason behind this could be disclosed by having less available water for starch gelatinization in the case of pasta, which included higher protein and dietary fiber content, absorbed more water from the cooking medium, which caused longer cooking times (Rachman et al., 2019). An increment in cooking time duration was also obtained in resistant starch blended pasta, likely linked to playing a texture modification role (Foschia et al., 2017). Although it was not elucidated entirely yet and thus needs further research, hydrocolloids may also likely slow the water absorption rate of fibres according to the composition and structure of fibres (Padalino et al., 2013).

When OCT is considered from the view of inulin chain length, in general, higher OCT values were specified in long-chain length inulin-enriched pasta samples (Aravind et al., 2012; Padalino et al., 2017; Peressini et al., 2020; Foschia et al., 2015a), which disagrees with our results (Figure 4.19). It was remarked that inulin with low-DP reduced OCT more than high-DP at the same levels because of possibly able to mix more homogenous through (Aravind et al., 2012;

Peressini et al., 2020) the matrix, and also being more soluble (Aravind et al., 2012). This incompatibility may have arisen from that those corresponding studies were conducted based on pasta made from durum wheat semolina.

The OCT of inulin-added pasta/noodle in the literature were in the range of 5.5 min and 6.3 min in spaghetti based on durum wheat semolina partially substituted (15%) with inulin has short- and long chain length, respectively, used as a soluble dietary fiber source (Peressini et al., 2020), 6.15 min in Turkish noodle made from wheat flour partially replaced with inulin (4%) (Saraç, 2021), 6.30-6.45 min in where inulin extracted from globe artichoke roots used at 3 concentration (5, 10, and 15%) in fresh semolina pasta (Difonzo et al., 2022), 7.5 min in pasta enriched with 12% inulin from chicory (Bavaro et al., 2024), 8.5 min, 7.5 min, ad 7 min in pasta where bread wheat flour was replaced with inulin a portion of 2.5, 5-7.5 and 10%, respectively (Bustos et al., 2011), 8-8.5 min and 8.5-9.0 min in wholemeal durum wheat spaghetti comprise of commercial inulin (2 and 4%) with low DP produced from chicory and inulin extracted from cardoon roots with a high DP, respectively (Padalino et al., 2017), 9.3 min and 9.8 min in pasta made with wholemeal flours at 2 different substitution levels (2 and 4%) with low-chain chicory- and long-chain topiinambur-inulin, respectively (Cardullo et al., 2022), 10 min and 15 min for short-chain- and long-chain inulin-fortified (15%) functional durum wheat semolina pasta, respectively (Foschia et al., 2015a), 8-11 min in spaghetti produced with 4 wholemeal durum wheat flour which fortified with 4% inulin from cardoon roots with high-DP and chicory with low-DP (Garbetta et al., 2020), and 10.5-14.0 min and 9.5-14.5 min for durum wheat semolina spaghetti including inulin (5%) with DP of 7–8 and 12-14, respectively (Aravind et al., 2012). These incompatible results may be ascribed to the different production processes of pasta/noodle, and also include fibres and their intrinsic attributes, which have an impact on the interaction with other ingredients (Difonzo et al., 2022).

4.4.10. Differential Scanning Calorimetry

The DSC parameters are influenced by differences in amylose:amylopectin ratio, molecular scale amylopectin organization, levels of damaged starch, and also the presence of non-carbohydrate residues (Rakhshi et al., 2024). Therefore, the DSC thermal properties (T_o, T_p, T_c, and ΔH) of raw fresh GFP samples based on 20-PGS, including short- and long-chain inulin, at different proportions (4 and 8%), were given in Table 4.33.

Owing to overlapping endotherms based on starch gelatinization and protein denaturation (Kim et al., 2014), and also possibly amylose-lipid complexes**,** as previously mentioned in section 4.2.11, all of the GFP pasta samples, including inulin with different DP, displayed a single endotherm peak between nearly 60-102°C. The corresponding values were substantially lower in comparison with values obtained in gluten-free noodles made from native autoclaved rice resistant starch (type III), including 2.5% XN and 5% inulin (≈138-147°C) (Raungrusmee et al., 2020). This incompatibility possibly arose from differences in GFP formulation. Besides, comparable T_o and T_p between around 55-65°C but lower T_c (≈62-72°C) values of inulin-incorporated wheat flour/semolina-based pasta samples were declared in previous studies (Tudorică et al., 2002; Aravind et al., 2012; Xu et al., 2021), probably differences come from the raw material diversities.

Table 4.33. Thermal properties of gluten-free pasta with/without inulin

Sample	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
20-PGS	63.89±1.63^{a}	73.79±0.11^{a}	111.21±2.06^{a}	5.94±0.89^{a}
4GR	62.84±0.55^{ab}	77.41±1.73^{a}	101.91±1.73^{b}	4.47±0.30^{b}
8GR	59.62±1.55^{b}	73.04±0.78^{a}	97.10±0.17^{c}	2.15±0.13^{c}
4HPX	60.77±0.24^{ab}	74.39±4.27^{a}	99.79±1.06^{bc}	3.44±0.20^{b}
8HPX	59.92±1.43^{b}	70.68±3.17^{a}	96.66±0.86^{c}	1.75±0.46^{c}

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$).

The onset and endset temperatures of inulin-included GFP samples, regardless of chain length, were lower compared to GFP without inulin (20-PGS). Nevertheless, this reduction was more prominent when the prebiotic concentration was doubled in GFP samples (Table 4.33). Along similar lines, inulin inclusion gave rise to a decrease in all transition temperatures in gluten-free noodle formulation. This state could be disclosed with loss of rigidity and compactness of inulin molecules by absorbing water, which plays a role as a plasticizer introduced into the inulin chains, increasing the intermolecular distance or free volume and influencing the molecular mobility (Raungrusmee et al., 2020). In contrast, a slight increase in T_o, T_p, and T_c was screened when the higher molecular weight inulin (DP: 12-14) (≈T_o: 57°C, T_p: 62-63°C, and T_c: 68°C), and inulin with lower DP of 7-8 (≈T_o: 56-59°C, T_p: 62-64°C, and $T_{c:}$ 66-70°C) were incorporated into wheat spaghetti (Aravind et al., 2012).

On the other hand, a slight increase was recorded in 4% level of inulin-blended GFP samples in T_p values with regard to 20-PGS GFP without inulin ($p>0.05$) (Table 4.33). Similarly, a non-significant increase was observed in gelatinization temperature of durum wheat pasta, with incorporation of inulin in the range of 7.5-15% (≈61-64°C), in contrast to the control sample without inulin (≈61°C) ($p>0.05$). This behavior was announced with the competition between starch and soluble fiber for water absorption, and thus restricting swelling and gelatinization of starch causes higher endset temperature values (Tudorică et al., 2002). An increase to 88°C was recorded for gelatinization temperature in fresh noodles based on 5% proportion of wheat flour substituted with either short-chain or long-chain inulin, in contrast to the control group without inulin (≈69°C) (Chen et al., 2023). In another study, T_{endset} values between the control without inulin (≈73°C) and the inulin-added (2.5-10%) noodle (≈72°C) were not significant ($p>0.05$). T_o show a similar pattern when its amount was increased from 2.5% to 7.5% (≈57°C). However, a slight growing trend was observed in T_o at 10% (≈58°C), and a significant increase in T_p values at all inulin concentrations (≈62-63°C) with regard to control with no inulin (≈61°C). This increase was explained by the formation of many hydrogen bonds within inulin and water, which limit the free water molecules' migrations, and thus hinder them from the entrance of starch granules, which makes the starch hydration difficult and influences the starch gelatinization process (Xu et al., 2021). In other respects, the difference between onset and endset temperatures obtained from DSC thermograms displays an index regarding the length of the gelatinization process (Dib et al., 2018). In this context, the lower length of transition in 8% inulin-fortified GFP samples may also be an indicator for their shorter OCT values, as given in the section 4.4.9 (Figure 4.19).

On the other hand, a lower transition (melting) enthalpy was obtained in inulin-added GFP samples in contrast to the 20-PGS-based control group with no inulin (Table 4.33). A similar approach was recorded in inulin-fortified (5%) gluten-free noodle, and this gelatinization enthalpy decrease was expounded with higher moisture content and presence of non-starch compounds in rice flour used as a raw material (i.e., such as protein, lipids, fiber, and ash) (Raungrusmee et al., 2020). An analogue behavior was observed in another study, where gelatinization enthalpy was declined by nearly 28% in inulin-added noodle samples. This was ascribed to the inhibiting effect of inulin regarding heat uptake of starch granules via preventing water acquisition by the starch granules (Xu et al., 2021). A decrease in enthalpy values was also detected in durum wheat pasta with the inclusion of inulin until a certain level (Tudorică et al., 2002). Moreover, this reduction was increased in our study as the inulin concentration was raised (Table 4.33). Along these lines, gelatinization enthalpy was reduced from approximately 10 J/g in 2.5% to 8 J/g in 7.5% with an increase in inulin levels. However, this decreased enthalpy pattern was continued until a certain level of inulin inclusion is reached (10%) into noodle composition (Xu et al., 2021). In a similar approach, a gradual decreasing trend accounted for 0.8 and 0.4 J/g was recorded in durum wheat pasta, where inulin level was grown from 7.5% to 12.5%, respectively, but increased in 15% inclusion concentration without reaching the enthalpy values of control pasta without fiber. This was associated with the idea that while inulin, as a soluble fiber, could encapsulate the starch granules within the pasta when used at lower rates, fewer encapsulated individual starch granules occurred by forming pockets of cross-linked structures at higher inulin incorporation levels (Tudorică et al., 2002). Reduction in the gelatinization enthalpy indicates less energy is required for melting (Marti et al., 2010), and loss of crystalline structure in starch granules (Seetapan et al., 2019). Therefore, melted and unbend double helices fractions could decrease within gelatinization (Dib et al., 2018) by leaching out the cooking medium, thereby bringing about increased CL values (Jia et al., 2023) that were aligned with our incremental CL values as the inulin was introduced into GFP formulation (section 4.4.4, Table 4.28). In this regard, a negative correlation was defined in ΔH between T and AL in the 2nd chapter of the present study (r=-0.80, r=-0.81, respectively). In addition to this, a negative association was found between ΔH and SME, previously (Jia et al., 2023), which was coherent with the outputs of this study, as given in the section 4.3.1 (Table 4.22). Therefore, a negative correlation was registered between SME and ΔH in the 2nd chapter of this study (r=-0.84).

4.4.11. X-ray Diffraction Pattern

The commercial inulin could be in the nature of a semi-crystalline or amorphous structure, and water plays a crucial effect on its glass transition behaviour and physical state, and thus crystallisation behavior. Therefore, based on the hydration conditions, two crystalline allomorphs of inulin were defined as hydrated and a semihydrated form (Aravind et al., 2012). In order to research the crystalline structure assessment of the raw and cooked (5 min) GFP with low (GR) and high-DP (HPX) at 2 different concentrations (4 and 8%), XRD analysis was performed (Table 4.34, Appendix 6e-h).

Table 4.34. Relative intensities in X-ray diffraction patterns of raw and cooked gluten-free pasta with inulin (%)

Pos. [°2θ]	4GR		8GR		4HPX		8HPX	
	Raw	5 min	Raw	5 min	Raw	5 min	Raw	5 min
15	86.04	-	77.70	-	77.11	-	76.86	-
17	89.05	100.00	85.44	100.00	84.82	100.00	83.43	100.00
18	100.00	-	100.00	-	100.00	-	100.00	-
20	18.53	85.52	22.22	68.84	23.61	86.54	23.46	65.25
23	82.56	-	74.58	-	75.68	-	74.53	-

- not defined at the corresponding angles (2θ).

The diffraction peaks of raw GFP with different types and amounts of inulin were demonstrated at the angles (2θ) of 15, 17, 18, and 23^{o} (Table 4.34, Appendix 6e-h), representing a typical A-type crystalline pattern as evidenced by these corresponding peaks in the literature(Marti et al., 2010; Detchewa et al., 2016; Seetapan et al., 2019; Zweifel et al., 2000; Yan et al., 2023; Yue et al., 2023). This A-type starch is typical of the corn starch-based food products (Hernandez-Hernandez et al., 2023), which is in line with used as a raw material in our GFP formulations (section 3.2.1, Table 3.1). Major new diffraction peaks were not observed in raw inulin-fortified GFP samples in comparison with the control GFP with no inulin (20-PGS). Therefore, the main diffraction doublet (diffraction peak at about 18^{o} followed by 17^{o}) and other peaks (diffraction peak at about 15^{o} and 23^{o}) together with their relative intensities remain homologous between the inulin-fortified raw GFP samples with 20-PGS. Similarly, it was stated that the diffractograms of the pasta samples, including inulin, could not be ascribed only to the inulin ingredient and XRD signal (Aravind et al., 2012).

After 5 min of cooking, the A-type crystalline peaks were generally decreased or disappeared at the end of the 5 min cooking, as monitored in Table 4.34, demonstrating that starch granules were gelatinised (Aravind et al., 2012). Instead of this, the main diffraction peaks with the highest relative intensity values were observed at 2θ~17^{o} and followed by 20^{o,} which indicate B- and V-type crystal structure, respectively, corresponding to retrograded starch and single helical amylose, respectively, in cooked pasta (Aravind et al., 2012). The gelatinized starch with relatively higher moisture contents (≈60-90%) has a greater tendency to B-type crystals, but lower moisture levels account for about 30-33% are favored for generating A-type crystals that align with our moisture results (section 4.4.1, Table 4.25) for cooked and raw inulin-added GFP samples, respectively. On the other side, one of the major peaks detected at 2θ of 20^o is typical for V-type crystal structure, referring to generating more ordered single helical amylose-lipid complexes (Jia et al., 2023).

On the other side, as the inulin ratio was increased, the relative intensity values of peaks at 2θ of 20^o were decreased (Table 4.34). This narrowing influence suggests that less organized helices need a lower melting energy, which could be supported by DSC analysis (Marti et al., 2010) with lower ΔH values, as given in the section 4.4.10 (Table 4.33). In a study, as the inulin addition was increased from 5% to 15%, the degree of crystallinity was reduced from 0.22% to 0.18%, and this change was attributed to lowering the structural order level but did not originate in significant changes in the chemical bonds (Manno et al., 2009). Additionally, melting of V-type crystals generally needs more higher temperature values (90–110°C) (Yan et al., 2023), and thus our results of DSC (section 4.4.10, Table 4.33) were consistent with our XRD results. The 8% inulin-enriched GFP, which had the lowest relative intensities situated at 20^o supported by having the highest amylose leaching values throughout the cooking process, as mentioned in section 4.4.6 (Table 4.30), as well.

4.4.12. Microstructure

In this study, the morphology of 5 min-cooked 20-PGS-included GFP enriched with short- and long-chain inulin at 2 concentrations (4 and 8%) was observed by analyzing the cross-sectional microstructure of corresponding samples under SEM at *x*3000 magnitude, and presented in Figure 4.20.

Inulin-addition, regardless of its chain length, may disrupt the starch-protein matrix (Aravind et al., 2012), similarly observed in this study when compared with micrographs of 20-PGS-included GFP without inulin, as shown in section 4.2.13 (Figure 4.9c). In a similar vein, fiber inclusion, such as inulin and pea fiber into durum wheat semolina pasta, causes a less developed and open appearance with discrete “uncovered” starch granules (Tudorică et al., 2022). However, a more dense, smooth, and homogenous structure was monitored in lower levels (4%) of inulin-enriched pasta microstructure (Figure 4.20e, g), in contrast to the higher levels of inulin (8%). In a study, it was revealed that 5% inulin addition led to covering as an amorphous substance of the cooked pasta surface (Aravind et al., 2012). Similarly, the lower concentrations of inulin may enhance a more porous protein network with a high ability to entrap and retain water by hydrogen bonds (Chen et al., 2024b), also aligning with their higher WAC values than those of 8%-inulin-added GFP (section 4.4.2, Table 4.26).

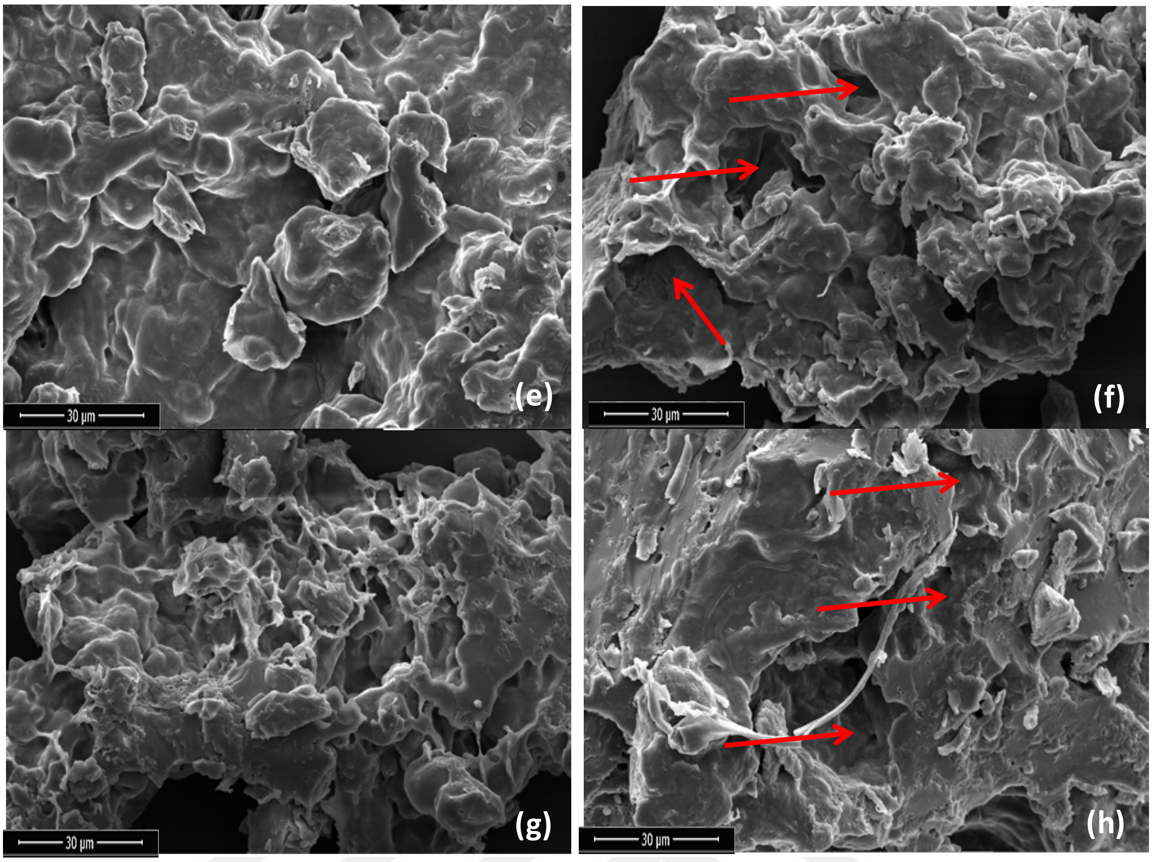

Figure 4.20. Scanning electron microscopy micrographs of cooked gluten-free pasta with inulin at a magnification level of *x*3000: (e) 4GR, (f) 8GR, (g) 4HPX, (h) 8HPX

On the other side, incorporation of high concentrations of inulin into GFP formulation was related to more open structures (Aravind et al., 2012), which align with our SEM images belonging to GFP with higher concentrations of inulin (Figure 4.20f, h). These fewer intact starch granules with low hydration incorporated into protein-gum matrix disrupt the protein–starch matrix continuity, and thus, pores or spaces (Zarroug et al., 2022) resulted in probably higher CL values (section 4.4.4, Table 4.28). In this context, higher inulin levels (8%) had more apparent holes between protein and starch granules, showed a weaker network, and a not completely embedded structure (Bustos et al., 2011). These results were paralleled by the data obtained that the increase in inulin and pea fiber amounts in pasta production caused an increase in disruption degree. However, although the starch-protein network may be disrupted, the starch-protein matrix was still more continuous in inulin-fortified semolina pasta than that of including pea fiber (Tudorică et al., 2002). Similarly, in another study, a fewer integrated structure with many holes was observed in the pasta surface with higher amounts of inulin incorporation (20%). This was attributed to inulin's potential protective action to starch granules, which may acompetition with starch for protein binding, causing a weak protein-starch (Foschia et al., 2015a). In a similar vein, significant morphological alterations, such as less prevalent starch granules, were declared above 10% inulin addition into pasta formulation (Manno et al., 2009). In contrast, the microstructure images demonstrated that higher levels of inulin (10 and 15%) cause more immerged starch granules in a more compact and dense structure in durum wheat semolina-based fresh pasta (Difonzo et al., 2022).

In other respects, particularly, the microstructure of the short-chain inulin-added pasta (4GR) presented a more dense protein-polysaccharide-starch matrix in internal structure (Bustos et al., 2011), which possibly aided to enhance the cohesiveness and elasticity, which supported by corresponding texture values (section 4.4.8, Table 4.32), as similarly declared for phosphorylated long-chain inulin-added fresh noodles (Che et al., 2024). However, long-chain inulin addition could increase pore size, apart from the thickening effect on the matrix wall (Aravind et al., 2012). In a controversial study, SEM images revealed that lower-DP of inulin incorporated pasta had a more disrupted and open structure of the gluten network owing to interference with the starch-gluten matrix, consistent with its greater swelling ability of starch granules (Peressini et al., 2020).

4.4.13. Sensory Evaluation

The sensorial evaluation in GFP with inulin was carried 3 main steps in this study based on cooking degree characterization in different cooking time (1, 5, 10, and 15 min) (Table 4.35), the cooking time preference rank of each inulin-enriched GFP samples, individually (Table 4.36 and Table 4.37), and consequently the evaluation of sensorial features (structural integrity, color, texture, taste, and overall acceptability) of inulin-fortified GFP with different DP and rates (4 and 8%) (Table 4.38, Figure 4.21) defined at determined cooking time in the 2nd step.

Table 4.35. Cooking degrees of gluten-free pasta with inulin determined by sensory analysis

Sample	**Cooking time (min)**			
	1	**5**	**10**	**15**
4GR	Al-dente	Medium-cooked	Well-cooked	Well-cooked
8GR	Al-dente	Cooked	Over-cooked	Over-cooked
4HPX	Al-dente	Medium-cooked	Well-cooked	Well-cooked
8HPX	Al-dente	Cooked	Over-cooked	Disintegrated

In the sensory analysis, the GFP samples including inulin were identified on a wide scale from “al-dente” to “disintegrated” during 15 min of cooking. The most unstable cooking degree, as disintegrated”, was recorded at the 15-min cooked 8% long-chain inulin-added GFP samples (8HPX), which could be attributed to its higher CL values at the corresponding cooking cycle (section 4.4.4., Table 4.28). On the other hand, all GFP samples with different types and concentrations of inulin were characterized as “al-dente” within 1 min of cooking by panelists, similar to the control with no inulin (20% PGS), as given in the section 4.2.14 (Table 4.15). However, this was considerably lower than the technologically identified OCT values (section 4.4.9, Figure 4.19). This could arise from that the corresponding method, which is the total white core disappearance in non-cooked starch in pasta samples after compression between 2 transparent glasses (Detchewa et al., 2022), may not be very suitable for application on gluten-free pasta/noodle samples. Furthermore, while no salt addition into distilled water was applied in the technological OCT determination, sensory evaluation was carried out with salt incorporation (12.7g/L according to "International Pasta Organisation") into tap water, which was applied to the cooking medium (Bianchi et al., 2019). Moreover, panelists' preferences may not coincide with the "al-dente" sensation in pasta, and thus, too early cooking times were characterized as "al-dente" for them. On the other hand, the differences in cooking degrees between GFP with short- and long-chain inulin-added samples at 2 different ratios (4 and 8%) were not sufficiently recognizable by panelists, likely owing to slight differences in technologically determined OCT values recorded between them (section 4.4.9, Figure 4.19).

Table 4.36. Cooking time preferences of gluten-free pasta with inulin by sensory analysis

Sample	**Cooking time (min)**			
	1	**5**	**10**	**15**
4GR	2.45 ± 1.04^{Ba}	1.36 ± 0.50^{Ca}	2.45 ± 0.93^{Ba}	3.55 ± 0.69^{Aa}
8GR	1.64 ± 0.81^{Cab}	1.55 ± 0.52^{Ca}	3.00 ± 0.63^{Ba}	3.82 ± 0.40^{Aa}
4HPX	2.36 ± 0.92^{Ca}	1.27 ± 0.65^{Da}	2.73 ± 0.79^{Ba}	3.64 ± 0.67^{Aa}
8HPX	1.27 ± 0.47^{Db}	1.73 ± 0.47^{Ca}	3.09 ± 0.30^{Ba}	3.91 ± 0.30^{Aa}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$).

With respect to the preference ranking test in the second step of this sensory evaluation, the least preferred cooking time in inulin incorporated GFP samples, irrespective of their type and amount, was determined as 15 min (Table 4.37). On the other hand, 1 min of cooking was the most would rather in the 8% long-chain inulin-added GFP, which could be associated with its gradually increased CL values throughout the cooking process (section 4.4.4, Fig. 4.28). Furthermore, in general, the preference rank was generally negatively affected by the increase in inulin level, except for 1 min of cooking (Table 4.37). Therefore, the last chosen, namely the fourth-ranked sample, was the 8HPX during the entire cooking process except for 1 min of cooking, aligning with the description of it as a "disintegrated" structure at the end of the cooking. On the other hand, the most preferred cooking time was defined as 5 min of cooking in all GFP samples with inulin, except 8HPX (Table 4.36). This attitude may be clarified by the panelist's choice, which is usually not comprised of neither "al-dente" nor "excessively-cooked" pasta. In this regard, the cooking time was assigned as 5 min in all inulin-added GFP samples for the third step of sensory evaluation, which was also aligned with technologically specified OCT values of inulin-enriched GFP, previously (section 4.4.9, Figure 4.19). This manner was also in agreement between the approaches, including that weight gain should be doubled in highly qualified pasta /noodle (Pessanha et al., 2023), as previously discovered in section 4.4.2 (Table 4.26).

Table 4.37. Preference ranking of cooking time in gluten-free pasta with inulin by sensory analysis

Rank	Cooking time (min)			
	1	5	10	15
1	8HPX	4HPX	4GR	4GR
2	8GR	4GR	4HPX	4HPX
3	4HPX	8GR	8GR	8GR
4	4GR	8HPX	8HPX	8HPX

The sensory features consisting of structural integrity, color, texture, taste, and overall acceptability in all inulin-incorporated and 5 min-cooked GFP samples were presented in Table 4.38 and Figure 4.21. In this context, the lowest scores of all sensorial attributes except color were recorded in the inulin-enriched GFP with the highest DP and concentration (8HPX), as expected, which may be related to their high CL values (section 4.4.4, Table 4.28). As the inulin rate was increased in GFP formulation, structural integrity, taste, texture, and overall acceptability were adversely influenced ($p>0.05$), although they were not statistically different between them ($p>0.05$). Similarly, an increase in inulin incorporation level from 5% to 20% negatively influenced the color, firmness, and taste scores, but still increased overall quality above the threshold of acceptability in gluten-free pasta based on maize flour (Mastromatteo et al., 2012). On the other hand, statistically no difference with the control group without inulin was recorded in some sensory attributes, such as the appearance of raw pasta (Saraç, 2021), elasticity (Padalino et al., 2017), adhesiveness, bulkiness of cooked pasta (Difonzo et al., 2022), and break to resistance in dry pasta (Padalino et al., 2017).

Table 4.38. Sensory properties of gluten-free pasta with/without inulin

Sample	Structural integrity	Color	Texture	Taste	Overall acceptability
20-PGS	7.18±0.50[a]	7.45±0.47[a]	6.64±0.64[a]	7.36±1.12[a]	7.00±0.59[a]
4GR	7.45±0.41[a]	7.36±0.59[a]	6.91±0.58[a]	7.27±0.60[a]	7.55±0.47[a]
8GR	7.09±0.56[a]	7.64±0.54[a]	6.27±0.65[a]	7.09±0.53[a]	6.91±0.48[a]
4HPX	7.36±0.43[a]	7.91±0.94[a]	6.82±0.60[a]	7.18±0.48[a]	7.36±0.45[a]
8HPX	6.55±0.56[a]	8.09±1.04[a]	6.18±0.67[a]	7.00±0.57[a]	6.82±0.60[a]

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$).

Moreover, the sensory color scores of GFP samples were slightly increased as the inulin level augmented ($p>0.05$), which was also in agreement with the values confirmed with the instrumental method regarding especially the yellowness index and color score obtained in 5 min of cooking (section 4.4.7., Table 4.31). Regarding color scores, while an increment was observed in Turkish noodle prapared from whole wheat flour substituted 4% inulin (Saraç, 2021) and fresh tagliatelle based on durum wheat semolina substituted with inulin (5, 10, and 15%) extracted from globe artichoke roots (Difonzo et al., 2022), statistically no significance was declared in dried and cooked spaghetti based on whole-meal durum enriched with inulin with low- and high-DP at 2 different addition levels (2 and 4%) (p>0.05) (Padalino et al., 2017).

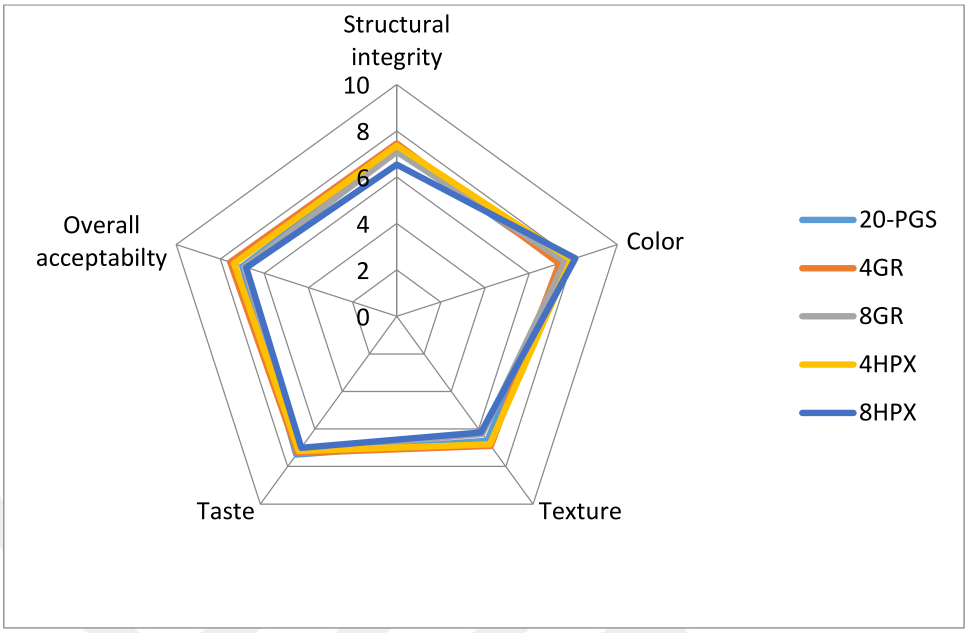

Figure 4.21. Radar plot of sensory properties in gluten-free pasta with/without inulin

The taste scores slightly differentiate between them, and thus it could be interpreted that inulin differences in terms of types and usage level did not distinguish properly for the panelists. This behavior may be associated with the limited amounts of inulin in the total content of GFP formulation, in contrast to corn starch and PGS (section 3.2.1, Table 3.1). The taste value was adversely influenced incorporation of inulin at 4% replacement ratio with whole wheat flour in Turkish noodle (Saraç, 2021), and at different levels (5, 10, and 15%) into fresh tagliatelle formulation (Difonzo et al., 2022). On the other hand, no significant difference was also recorded in taste values regarding not only concentration level (2 and 4%) but also chain length (short- or long-) in whole-meal durum wheat spaghetti, in contrast to the control group with no inulin (Padalino et al., 2017).

In addition, there could be a positive link between the scores of structural integrity and instrumentally defined cohesiveness values, which are reduced as the inulin level increases (section 4.4.8, Table 4.32). On the other hand, while 20-PGS GFP had the lowest hardness values determined by TPA within 5 min of cooking, the lowest texture scores as a sensory parameter belonged to GFP, where inulin was added at higher ratios (8%). This could be attributed to higher CL values of 8GR and 8HPX, and thus, probably sticky nature may probably adversely influence panelists' perception. In contast, while lower incorporations levels was generally reduced hardness/firmness scores such as 4% inulin-added Turkish noodle (Saraç, 2021), 5% inulin-enriched fresh tagliatelle (Difonzo et al., 2022), 2% and 4% inulin-added durum wheat spaghetti (Padalino et al., 2017), or not significantly influenced such as durum wheat spaghetti fortified with

2.5% short-chain length inulin (Aravind et al., 2012); but utilizing higher proportions of inulin (10 and 15%) increased the corresponding values such as fresh durum wheat semolina-based tagliatelle (Difonzo et al., 2022).

Interestingly, totally same formulation of 20-PGS was used in sensory evaluation at first, and in the 2nd chapter of this study, for evaluating the PGS effect as a pillar, and inulin influence as a control GFP, higher structural integrity, color, hardness, and taste scores were defined in this chapter in contrast the 1st chapter (section 4.2.14, Table 4.15). This could be associated with the physiological and psychological characteristics of consumers, including hedonic and emotional factors, which have a substantial impact on food choice and consumer behavior (Zerbini et al., 2024). Therefore, it could be interpreted that while in the presence of alternatives with higher sensory parameters except overall acceptability, such as control based on semolina and 10-PGS included GFP, was negatively influenced and hit its weight, towards worse alternatives, such as higher proportions of inulin (8%), incorporated GFP positively affected consumers' choice regarding 20-PGS.

4.5. The Effect of Probiotic Incorporation on Extrusion Parameters of Gluten-free Pasta

4.5.1. Energy-related Properties

The recorded energy-related values during mixing (G_{mixing}) and extrusion processes ($G_{extrusion}$), and also SME values of probiotic-incorporated GFP with/without inulin, were shown in Table 4.39. According to the acquired results, G_{mixing}, $G_{extrusion,}$ and SME values of all probiotic-incorporated GFP samples in the presence or not inulin were in the range of 23.00-31.00 W, 72.17-80.07 W, and 77.67-86.67 J/g, respectively (Table 4.39).

Table 4.39. Energy-related values of probiotic-incorporated gluten-free pasta with/without inulin

Sample	G_{mixing} (W)	$G_{extrusion}$ (W)	SME (J/g)
P0	23.00±0.80^{e*}	72.17±1.21^{c*}	77.67±1.09^{c*}
P-4GR	24.90±0.40^{d**}	73.90±1.20^{bc*}	79.17±0.58^{c*}
P-8GR	29.20±0.70^{b**}	77.85±0.75^{ab*}	83.46±1.14^{ab*}
P-4HPX	27.00±0.60^{c**}	76.43±1.25^{abc*}	80.14±1.35^{bc*}
P-8HPX	31.00±0.40^{a**}	80.07±1.47^{a*}	86.67±1.40^{a*}

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$).
*not significant ($p>0.05$), **significant ($p<0.05$) with probiotic inclusion.

In this context, the $G_{extrusion}$ values of all probiotic-included GFP samples were about 2 times increased in comparison to those of G_{mixing} values, as a similar tendency was seen in previous chapters of this study (section 4.1.1, section 4.3.1). In addition, the *B.coagulans* incorporation into GFP formulation with/without inulin caused a slight augmentation in the $G_{extrusion}$ and SME values ($p>0.05$), whereas the increment was significantly higher regarding G_{mixing} values in probiotic-added GFP, including inulin ($p<0.05$). This behaviour may be linked with the lower raw moisture values accepted as similar to feed moisture of both probiotic- and inulin-enriched GFP (section

4.6.1, Table 4.42), than that of the corresponding samples without probiotic, as priorly presented (section 4.4.1, Table 4.25). This may be ascribed to the lower moisture content, which could bring about an anti-lubricant effect, leading to more shearing stress, thereby a higher energy transfer (Giménez et al., 2013; Bouasla et al., 2017a). In addition, a further increase in all energy-related extrusion parameters was monitored with the incorporation of inulin and which was more prominent as the inulin level was doubled in GFP with probiotics compared to P0 with no inulin ($p<0.05$) The negative association between moisture and SME was supported previously by several studies (Manthey et al., 2004; Giménez et al., 2013; de la Peña et al., 2014; Ryu and Ng, 2001; Bouasla et al., 2017a), align with our results, as well (section 4.6.1, Table 4.42). The greater SME values refer to the overall operational cost (Sinaki and Koksel, 2024) of both inulin- and probiotic-enriched GFP during the whole pasta-making process, which would probably be higher when compared with that of GFP made by 20-PGS without dietary fiber.

4.5.2. Efficiency-related Properties

The monitored efficiency-related values of probiotic-incorporated GFP with/without inulin were shown in Table 4.40. In this research, the EY was in the range about from 53% to 71% in *B. coagulans*-added GFP samples, plus or minus inulin with different chain lengths and concentrations (4 and 8%), as summarized in Table 4.40.

Table 4.40. Extrusion efficiency values of probiotic-incorporated gluten-free pasta with/without inulin

Sample	RT (min)	EY (%)	EFR (g/min)
P0	$7.07\pm0.10^{c*}$	$53.06\pm1.47^{c**}$	$62.19\pm1.26^{a**}$
P-4GR	$7.33\pm0.22^{c*}$	$56.72\pm2.01^{bc**}$	$57.72\pm0.78^{ab**}$
P-8GR	$8.24\pm0.38^{ab*}$	$66.81\pm0.88^{a**}$	$50.65\pm1.01^{bc**}$
P-4HPX	$7.69\pm0.68^{bc*}$	$63.76\pm2.55^{ab**}$	$60.06\pm1.50^{ab*}$
P-8HPX	$8.69\pm0.17^{a*}$	$70.74\pm0.88^{a**}$	$46.55\pm4.25^{c*}$

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$).
*not significant ($p>0.05$), **significant ($p<0.05$) with probiotic inclusion.

The probiotic incorporation into GFP with/without inulin led to a significant reduction in EY ($p<0.05$), although the corresponding RT values were increased ($p>0.05$), which was contrary to the results obtained in the first and second chapters of this present study (section 4.1.2, section 4.3.2). This could probably be related to the excessive stickiness of probiotic–included GFP dough, which resulted in less output with prolonged RT, owing to an increase in feed adhesion and resistance to flow. However, an EY increment was observed with inulin addition into probiotic-fortified GFP, in contrast to P0 without inulin, and further enhanced as the inulin amount was increased ($p<0.05$) (Table 4.40). This may have originated from the increase in the total feed amount, due to the incorporation of dietary fiber, achieved by enrichment of the GFP formulation.

The EFR values of both inulin- and probiotic-fortified GFP samples were varied between nearly 47-60 g/min as seen in Table 4.40. Based on the acquired results, *B. coagulans* resulted in a reduction in EFR values, and then blended with inulin led to a further decline in the EFR values in GFP with both probiotic and inulin, which was more significant in the GFP with low-DP inulin (GR) ($p<0.05$). In this context, a negative correlation between EFR and RT was monitored in the 3rd chapter of this study ($r=-0.69$). Moreover, this decrease was more prominent as the inulin level doubled ($p<0.05$) in the probiotic-enriched GFP (Table 4.40). This phenomenon may be explained by the adverse relation between extruders' screw speed with RT, but a positive association between mass flow rate (Rosentrater et al., 2005). As a result, the probiotic-added GFP, including 8% long-chain inulin (8HPX), had the highest RT and EY, but the lowest EFR (Table 4.40), which may imply having the likely lowest screw speed.

4.5.3. Dimensional Properties

The dimensional attributes of probiotic-incorporated GFP with/without inulin were shown in Table 4.41. The ER and SEI values of probiotic-enriched GFP plus or minus inulin were varied in the range from 1.28-1.52 and 1.64-2.44, respectively, as presented in Table 4.41.

Table 4.41. Dimensional values of raw probiotic-incorporated gluten-free pasta with/without inulin

Sample	ER	SEI	UD (g/cm^3)	SL (cm/g)
P0	$1.28±0.03^{c**}$	$1.64±0.08^{d**}$	$1.68±0.04^{a**}$	$18.83±0.20^{a**}$
P-4GR	$1.33±0.02^{c**}$	$1.78±0.06^{cd**}$	$1.64±0.03^{ab**}$	$17.30±0.02^{b**}$
P-8GR	$1.47±0.03^{ab*}$	$2.16±0.09^{ab*}$	$1.49±0.02^{c**}$	$16.49±0.07^{c**}$
P-4HPX	$1.41±0.03^{b**}$	$1.98±0.09^{bc**}$	$1.59±0.01^{b**}$	$16.94±0.26^{b**}$
P-8HPX	$1.52±0.04^{a*}$	$2.44±0.21^{a*}$	$1.42±0.03^{c**}$	$16.03±0.08^{d*}$

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$).
*not significant ($p>0.05$), **significant ($p<0.05$) with probiotic inclusion.

On that note, a decline in expansion-related parameters regarding both ER and SEI values was recorded in regard to probiotic incorporation into GFP based on 20-PGS. On the other hand, an increment was monitored with inulin addition and further rose with an increase in inulin proportions when compared with P0 ($p<0.05$) (Table 4.41). It was announced that need for more SME inputs for greater expansion in extrudates (Ryu and Ng, 2001). Herein, while a similar positive link between SEI and SME was also recorded in this study in terms of inulin addition into GFP with probiotic, an adverse relation was obtained regarding probiotic fortification in GFP (section 4.5.1, Table 4.39). On the other side, a negative association between moisture amount and radial expansion index was also indicated (Sinaki and Koksel, 2024). Hence, an increase in the inulin ratio, thereby a reduced raw moisture level, accepted equal to feed moisture (section 4.6.1, Table 4.42), gave rise to an increase in the diameter of probiotic-included GFP samples, increasing both ER and SEI values (Table 4.41).

The UD values of both inulin- and probiotic-added GFP samples were in the range between 1.42-1.64 g/cm^3, as seen in Table 4.41. In this regard, the inclusion of *B. coagulans* into GFP formulation causes a significant increase in the UD values ($p<0.05$). On the other side, incorporation of inulin into GFP with probiotics led to a reduction in the UD, and also a further decrease was recorded as the inulin ratio was increased (p<0.05) (Table 4.41). This decline could be attributed to the negative impact of high feed moisture ratios during extrusion on UD (Varsha and Mohan, 2016), aligned with our results (section 4.6.1, Table 4.42). Moreover, a strong negative correlation between UD and ER was found out in the 3rd chapter of this study (r=-0.95).

The SL values of all probiotic-included GFP samples with or without inulin were given in Table 4.41. Based on Table 4.41, the SL values of both inulin and probiotic-added GFP samples were over the range of 16.03-17.30 cm/g (Table 4.41). The probiotic addition into GFP formulation, either with inulin or not, led to a significant increase in SL values (p<0.05). However, a decrement was observed with inulin incorporation and further decreased as the inulin proportions were increased in GFP with *B. coagulans* (p<0.05). This agrees with the phenomenon based on the adverse relationship between SL and SME (Ryu and Ng, 2001). Therefore, a strong negative correlation (r=-0.95) was defined between SME and SL in the 3rd chapter of this study, even though there was some reverse research in the literature, as well (Anand, 2020). Moreover, a strong negative correlation was also identified between ER and SL in the 3rd chapter of this study (r=-0.91).

4.6. Effect of Probiotic Incorporation on Cooking Parameters of Gluten-free Pasta

4.6.1. Moisture

The moisture levels raw and cooked during 15 min with 5 different time intervals (0, 1, 5, 10, and 15 min) of all probiotic-enriched fresh GFP samples with/without inulin used at 2 different concentrations (4 and 8%) and types (GR, HPX) were presented in Table 4.42.

Table 4.42. Moisture contents of raw and cooked probiotic-incorporated gluten-free pasta with/without inulin (%)

Time (min)	P0	P-4GR	P-8GR	P-4HPX	P-8HPX
Raw	34.08±0.98^{Ea**}	33.11±0.85^{Fa**}	30.04±1.63^{Ea*}	31.36±1.39^{Ea**}	30.91±1.13^{DEa**}
0	56.76±0.81^{Da**}	54.24±0.58^{Ea**}	51.35±3.51^{Da*}	52.54±0.47^{Da**}	40.67±2.26^{Db**}
1	62.43±1.47^{Ca**}	62.78±1.00^{Da*}	60.34±0.97^{Da*}	61.85±1.70^{Ca*}	53.88±1.79^{Cb**}
5	73.98±1.77^{Ba*}	71.14±1.28^{Cab*}	69.55±1.59^{Cab*}	70.52±1.07^{Bab*}	65.80±1.70^{Bb**}
10	77.37±0.11^{Ba**}	76.38±1.01^{Ba**}	75.13±0.94^{Bab**}	76.28±1.30^{Aa**}	72.95±1.40^{Ab*}
15	80.20±0.88^{Aa*}	79.01±0.54^{Aab**}	77.31±0.41^{Ac**}	77.90±0.14^{Abc**}	74.57±0.07^{Ad**}

Means followed by the different uppercase letters in the same column regarding cooking time were significantly different (p<0.05). Means followed by the different lowercase letters in the same row at the same cooking time were significantly different (p<0.05). *not significant (p>0.05), **significant (p<0.05) with probiotic inclusion.

A reduction in moisture content was mostly recorded with *B. coagulans* addition into GFP formulation, in the case of inulin inclusion or not (section 4.4.1, Table 4.25). In a study, the moisture content of probiotic-added (*B. coagulans* Unique IS-2) gluten-free vermicelli made from a pseudocereal flour blend (consisting of amaranth, buckwheat, and quinoa) accounts for about 8-

10% which was lower towards that of wheat pasta as a control group (Poshadri and Deshpande, 2024). The dramatically lesser moisture values of this study than those of its counterpart in our study may originate from drying fresh pasta samples at 50°C for 12h before the cooking process. On the other side, the hydration values in probiotic-enriched gluten-free vermicelli samples consisting of pseudocereal flours were nearly doubled with respect to control pasta produced from totally wheat flour (Achinna and Deshpande, 2025) and tripled (Poshadri and Deshpande, 2024). However, no significant differences were established in the moisture content of 1% probiotic-enriched (*B. coagulans* GBI 6068) pasta and a control sample made with white wheat flour without probiotics (García-Moncayo et al., 2024). Additionally, the moisture amounts of probiotic-enriched GFP samples were commonly more declined with the incorporation of inulin and further reduced when its amount was doubled, particularly inulin with high DP (Table 4.42).

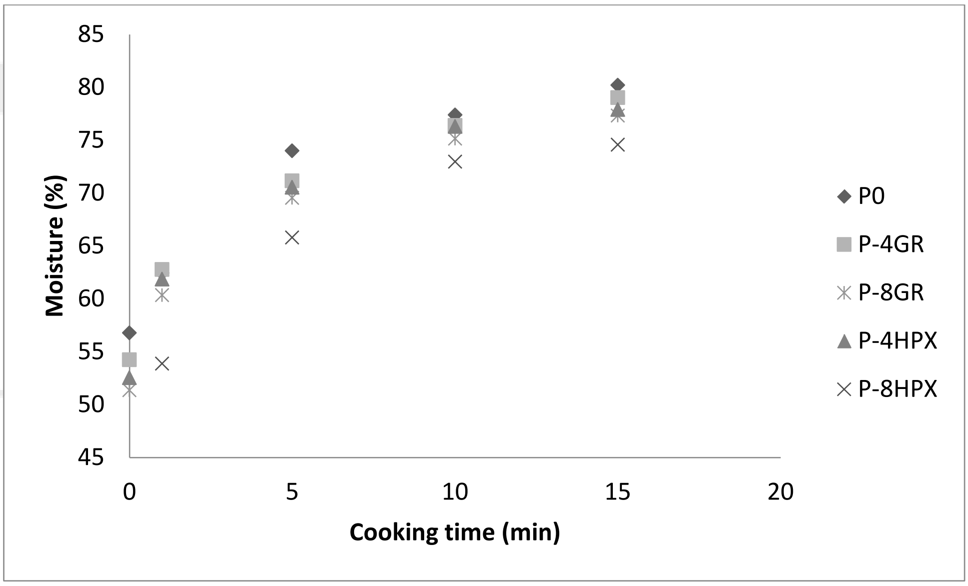

Figure 4.22. Moisture content of probiotic-incorporated gluten-free pasta with/without inulin during cooking

According to the obtained results, the moisture contents of all cooked probiotic-fortified GFP with/without inulin were between about 41-57% at the beginning of cooking (0 min) with reaching 75-80% at the end of the cooking (15 min) (Table 4.42). Therefore, a gradual rise in moisture contents was defined throughout all cooking process in all GFP samples including probiotic with/without inulin, as seen in Figure 4.22. Similar behaviour was also reported in the first and second chapters of this present study regarding the examination of the effect of pregelatinized starch and inulin in section 4.2.1. (Figure 4.1) and section 4.4.1. (Figure 4.12), respectively. In a similar vein, the moisture content of raw pasta with encapsulated probiotic with FOS-DWPI combination was nearly doubled after cooking, but the corresponding values were higher than those of control pasta without probiotic (Rajam et al., 2015).

4.6.2. Water Absorption Capacity

The WAC values of all probiotic-included fresh GFP pasta samples with/without inulin incorporation, throughout the 15-min cooking process with 5 different time intervals (0, 1, 5, 10, and 15 min), were presented in Table 4.43. According to Table 4.43, the WAC values of GFP samples, including inulin or not, were negatively influenced by probiotic addition throughout cooking when compared with the GFP samples without *B. coagulans*, as compared to the results given previously in section 4.4.2 (Table 4.26).

Table 4.43. Water absorption capacity of probiotic-incorporated gluten-free pasta with/without inulin (%)

Sample	Cooking time (min)				
	0	1	5	10	15
P0	53.44±1.59^{Ea**}	75.17±0.72^{Da**}	126.91±0.85^{Ca**}	161.70±0.60^{Ba**}	181.79±3.75^{Aa**}
P-4GR	51.33±1.11^{Ea**}	71.55±0.83^{Db**}	116.25±0.76^{Cb**}	136.33±1.89^{Bb**}	154.31±0.79^{Ab**}
P-8GR	39.12±1.34^{Db**}	60.18±1.20^{Cd**}	85.64±1.64^{Bd**}	128.50±3.68^{Ab**}	137.67±3.56^{Ac**}
P-4HPX	51.56±0.89^{Ea**}	66.74±0.30^{Dc**}	107.33±2.68^{Cc**}	133.20±1.55^{Bb**}	140.40±0.47^{Ac**}
P-8HPX	22.53±1.04^{Dc**}	45.96±1.18^{Ce**}	75.00±0.62^{Be**}	102.56±3.74^{Ac**}	108.82±2.40^{Ad**}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different (p<0.05). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different (p<0.05). *not significant (p>0.05), **significant (p<0.05) with probiotic inclusion.

A similar kind of significant reduction was reported in both fresh and dried spaghetti and noodles made from durum wheat semolina, including *Bacillus coagulans* GBI-30 (p<0.05). They maintained lower water content than the control samples without probiotics, indicating that there is less moisture uptake owing to their decreased water-holding capacity (Wang et al., 2025b). In another study, the WAC values of synbiotic gluten-free vermicelli, based on a composite pseudocereal blend with different rates (amaranth, buckwheat, quinoa), and including *B. coagulans* IS2 spores, were lower than the control group made by 100% wheat flour without any dependent variables. This manner was linked with GFP structure with less compactness and high porosity, which enables faster water penetration in contrast to gluten-containing counterparts (Poshadri and Deshpande, 2024). Similar behavior was also examined in durum wheat semolina-based dried pasta consisting of *B. coagulans* GBI-30, although the higher WAC values were obtained in comparison with the results of our study (Konuray and Erginkaya, 2020). In another study, the considerably lower average WAC, corresponding to nearly 52%, was defined in *B.coagulans*-fortified 10-min cooked pasta based on a composite flour (Piyadasa and Perera, 2019). The WAC variations could be derived from the pasta formulation, type and/or shape of pasta, extruder type, process conditions, ratio of amylose/amylopectin, and chain length (Achinna and Deshpande, 2025). Therefore, the diversities may originate from the differences in types (fresh, dried), composition (gluten-free, gluten-counterpart), and cooking time durations (1-15 min, 7 and 10 min, respectively) with corresponding pasta samples (Piyadasa and Perera, 2019; Konuray and Erginkaya, 2020) and our results. On the other hand, GFP samples including *B. coagulans* IS2 spores as a probiotic source, and produced by composite pseudocereal flour, had significantly greater hydration in contrast to the 100% wheat vermicelli as a control pasta. This was attributed to a higher compact structure because of high pressure through the extrusion process in pasta-making, and also the gluten network of the control group (Achinna and Deshpande, 2025). In other respects, the WAC of probiotic-enriched GFP samples was commonly more declined with the addition of inulin and further when its level was doubled, particularly with high-chain length (Table 4.43).

The WAC of probiotics incorporated all GFP samples, either inulin-added or not, was in the range of nearly 23-53% at the beginning (10 sec) and 109-182% at the end of the cooking process (15 min), as summarized in Table 4.43. Therefore, generally a significant increase was recorded in the WAC values of probiotic-added GFP with/without inulin in prolonged cooking duration (Figure 4.23), as a similar tendency was explained previously (section 4.2.2, Table 4.5; section 4.4.2, Table 4.26). In particular, WAC values of all GFP enriched with probiotic and including inulin with different DP (low- and high-) and levels (4%), were more than doubled at 5 min of cooking (Table 4.43), which could meet the criteria consisting of the minimum 100% increase should be observed in the weight gain of cooked pasta with hih quality (Fiorda et al., 2013b; Pessanha et al., 2023).

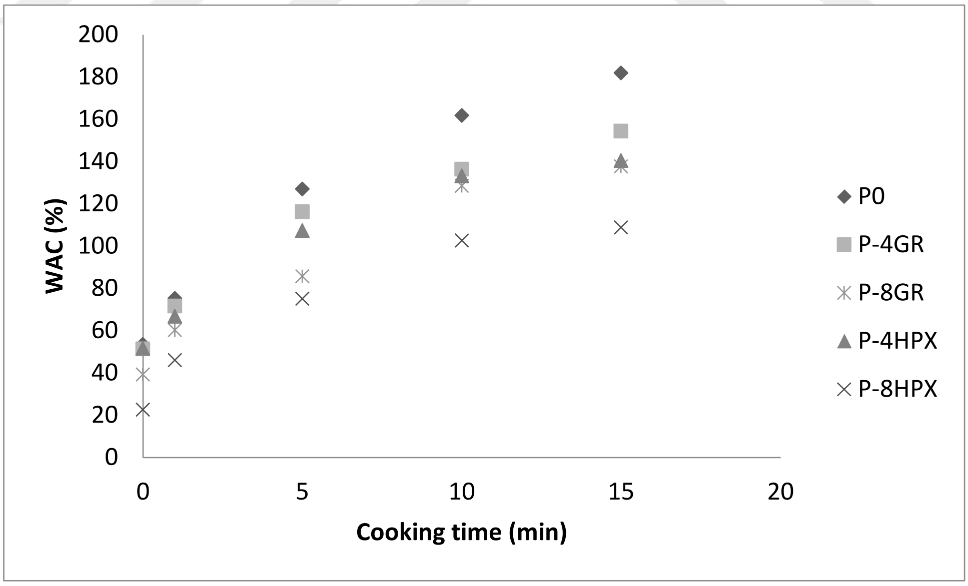

Figure 4.23. Water absorption capacity of probiotic-incorporated gluten-free pasta with/without inulin during cooking

4.6.3. Swelling Index

The SI values of all probiotic-fortified fresh GFP pasta samples blend with/without inulin, along with a 15-min cooking process with 5 different time intervals (0, 1, 5, 10, and 15 min), were summarized in Table 4.44.

Table 4.44. Swelling index values of probiotic-incorporated gluten-free pasta with/without inulin (g/g)

Sample	Cooking time (min)				
	0	1	5	10	15
P0	1.56±0.06 Ea*	2.05±0.16 Da**	2.95±0.07 Ca**	3.51±0.02 Ba**	4.06±0.17 Aa*
P-4GR	1.46±0.02 Eab*	1.88±0.01 Dab**	2.83±0.04 Cab**	3.44±0.03 Ba**	3.90±0.10 Aab**
P-8GR	1.19±0.02 Dc**	1.66±0.04 Cbc**	2.62±0.21 Bbc*	3.19±0.11 Bbc**	3.63±0.07 Abc**
P-4HPX	1.35±0.07 Eb*	1.82±0.07 Dab**	2.75±0.02 Cabc**	3.32±0.11 Bab**	3.84±0.05 Aab*
P-8HPX	1.17±0.04 Dc**	1.57±0.07 Cc*	2.52±0.10 Bc*	3.01±0.03 Ac**	3.39±0.24 Ac*

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different (p<0.05). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different (p<0.05). *not significant (p>0.05), **significant (p<0.05) with probiotic inclusion.

Probiotic addition into GFP samples in the case of either consisting of inulin or not, negatively affected SI values, when compared with the results of GFP with no probiotic, as shown in section 4.4.3 (Table 4.27). On the other side, inulin incorporation further decreased the SI values in probiotic-enriched GFP compared to P0 without inulin ($p<0.05$) (Table 4.44). Moreover, the SI of GFP samples with *B.coagulans* was commonly more declined when the inulin level was increased from 4% to 8%, particularly in high chain length (HPX) inulin (Table 4.44). A slight reduction in swelling volume was associated with inadequate water absorption (Aydin, 2022), which, in turn, the determination of a decrement in SI in all probiotic-added GFP samples with/without inulin was consistent with their propensity for WAC values, as priorly declared (section 4.6.2, Table 4.43).). Therefore, a strong positive correlation was discovered between WAC and SI in the 3rd chapter of this study (r=+0.97).

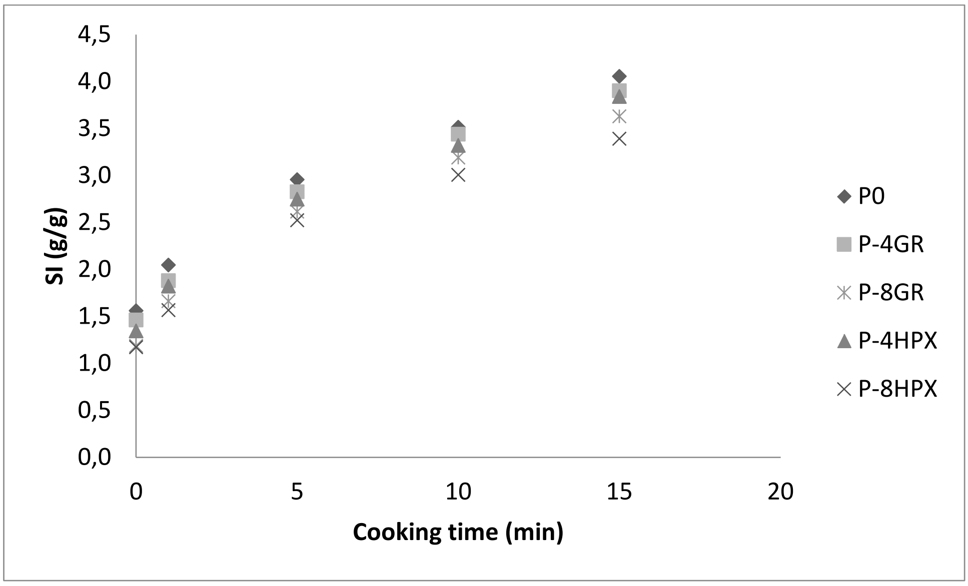

Figure 4.24. Swelling index of probiotic-incorporated gluten-free pasta with/without inulin during cooking

According to the obtained results, the SI of the probiotic-enriched GFP samples including inulin with different types in terms of their chain length and usage levels (4 and 8%), were in the range of about 1.17-3.90 g/g from the top on down of cooking (Table 4.44). An analogous tendency with WAC was also recorded as cooking time continued, regarding a 1-2 times increase for SI in all *B. coagulans*-added GFP samples with/without inulin cooked for 15 min in comparison to preliminary SI values determined in 10 sec (0 min) (Figure 4.24).

4.6.4. Cooking Loss

The CL of fresh GFP made from corn starch and PGS (80:20) supplemented probiotic with/without 2 different types (short-, long-chain) and concentration (4 and 8%) of inulin, cooked during the 15 min with 5 different time intervals (0, 1, 5, 10, and 15 min) was seen in Table 4.45.

Table 4.45. Cooking loss values of probiotic-incorporated gluten-free pasta with/without inulin (%)

Sample	Cooking time (min)				
	0	1	5	10	15
P0	3.62±0.13^{Ec**}	4.90±0.04^{Dd**}	9.73±0.72^{Cc**}	15.14±0.39^{Be**}	23.80±0.25^{Ac**}
P-4GR	4.15±0.04^{Ebc**}	6.55±0.60^{Dc**}	11.32±0.43^{Cc**}	17.77±0.48^{Bd**}	24.56±0.54^{Ac**}
P-8GR	4.79±0.08^{Ea**}	7.63±0.16^{Dab**}	15.70±0.24^{Cab**}	23.28±0.44^{Bb**}	28.13±0.93^{Ab**}
P-4HPX	4.50±0.46^{Eab**}	7.23±0.05^{Dbc**}	13.96±0.67^{Cb**}	20.33±0.67^{Bc**}	26.75±0.72^{Ab**}
P-8HPX	4.33±0.18^{Eab*}	8.17±0.17^{Da**}	17.03±0.97^{Ca**}	25.64±0.91^{Ba**}	30.08±0.61^{Aa**}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$). *not significant ($p>0.05$), **significant ($p<0.05$) with probiotic inclusion.

It was revealed that probiotic incorporation had an adverse impact on CL values when compared with the GFP samples, including inulin with no *B. coagulans*, as demonstrated in section 4.4.4 (Table 4.28). This could be interpreted as solubilization of the starch and non-starch polysaccharides and the weak structure of GFP, resulting in leaching out from pasta to the cooking medium, and becomes more cloudy (Achinna and Deshpande, 2025). Therefore, a negative correlation was defined between CL and water absorption/mass gain in the literature (Li et al., 2020; Bento et al., 2021). Similarly, probiotic-included (*B. coagulans* Unique IS2 spores) GFP based on pseudocereal flour blend had higher CL values against to control pasta made by 100% wheat (Poshadri and Deshpande, 2024; Achinna and Deshpande, 2025). In contrast, *Bacillus coagulans* GBI-30 incorporation into spaghetti and noodles made from durum wheat semolina led to a significant decline in CL values regarding both fresh and dried types ($p<0.05$), which is more prominent in noodles, indicating better structural integrity than that of extruded pasta (Wang et al., 2025b). Another controversial study revealed that CL values of durum wheat semolina-based pasta were reduced with the addition of *B. coagulans*, although an increment was recorded in those values with 6 months of storage. This was linked with a negative relationship between WAC and CL (Konuray and Erginkaya, 2020). When compared with our results, drastically lower CL values account for approximately 5% were recorded in pasta made from composite flour blended with *B.coagulans* cooked at OCT (≈10 min) (Piyadasa and Perera, 2019), which could arise from the differences in type and composition of the corresponding samples.

In addition to this, the inulin addition with an increase in usage ratio and especially with high DP into probiotic-incorporated GFP, further increased CL values, in comparison with the probiotic-included GFP without inulin (P0) (Table 4.45), which was also aligned with our previous (section 4.4.4, Table 4.28). Nevertheless, almost none of the probiotic-enriched GFP samples (except for P0) could meet the acceptable CL limit for a good quality pasta requirement, corresponding to 6%, since 1 min of cooking. It was also declared that pasta had more than 10% CL is not favorable by consumers (Poshadri and Deshpande, 2024), and thus this could be interpreted as none of the both probiotic-and inulin-fortified GFP may be preferred by them.

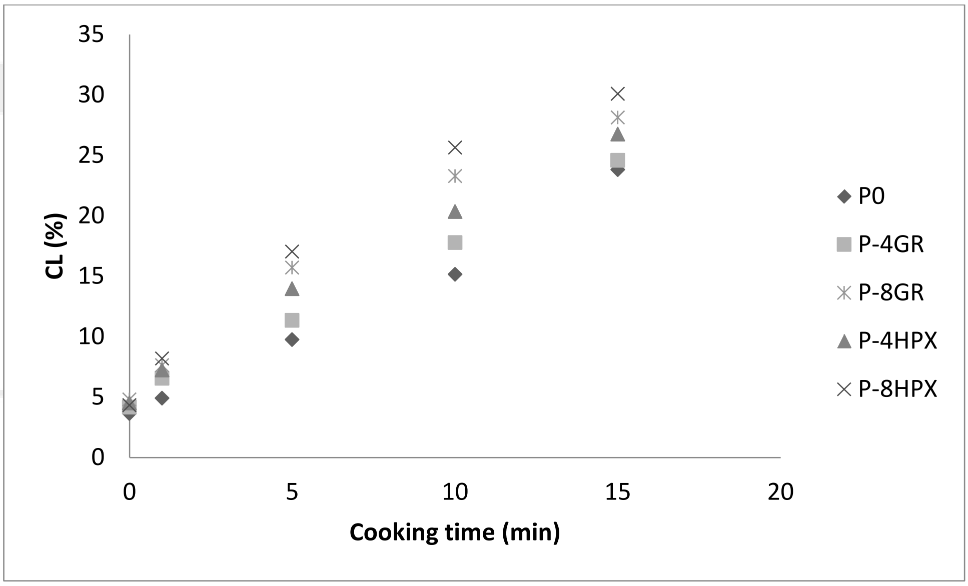

Figure 4.25.Cooking loss of probiotic-incorporated gluten-free pasta with/without inulin during cooking

According to the results, the CL of probiotic-added all GFP samples, including or not inulin, was between the range of about 3.62-4.79% to 23.80-30.08% (Table 4.45). Extreme CL values and prolonged cooking durations are unfavorable, because of bring in poor quality and more energy consumption in the pasta-making process (Achinna and Deshpande, 2025). Approximately a 5-6 times increase was recorded in CL values of both probiotic and prebiotic incorporated GFP samples at the end of the cooking (Figure 4.25). Therefore, excessive CL values with extended cooking times were undesirable in terms of technological and possibly nutritional characteristics of GFP with probiotics and inulin together.

4.6.5. Volume Increase

The VI values of *B. coagulans*-fortified GFP without (P0) and with inulin used at 2 different amounts (4 and 8%), and types such as short- (GR), and long-chain (HPX), were given in

Table 4.46. When the impact of the probiotic incorporation on VI values in GFP samples was evaluated, a significant reduction was ascertained ($p<0.05$) (Table 4.46). This may have arisen from the predominance of excessive CL on water absorption and swelling values compared to as previously screened in section 4.6.4 (Table 4.45).

Table 4.46. Volume increase values of probiotic-incorporated gluten-free pasta with/without inulin (%)

Sample	**Cooking time (min)**			
	1	**5**	**10**	**15**
P0	111.11±4.08^{Ca**}	138.89±3.21^{Ba**}	166.67±12.83^{ABa*}	194.44±3.21^{Aa**}
P-4GR	100.00±5.50^{Cab**}	138.10±4.27^{Ba**}	157.14±4.58^{ABab**}	176.19±3.37^{Aab**}
P-8GR	80.56±2.78^{Cbc**}	111.11±6.42^{Bb**}	133.33±3.00^{Bab**}	166.67±6.42^{Ab**}
P-4HPX	87.50±3.09^{Cbc**}	119.64±3.09^{Bab**}	146.43±6.19^{Bab**}	173.21±3.09^{Ab**}
P-8HPX	73.08±6.66^{Cc*}	101.92±3.33^{Cb**}	131.49±0.72^{Bb**}	162.50±2.88^{Ab**}

Means followed by the different uppercase letters in the same row regarding cooking time were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column at the same cooking time were significantly different ($p<0.05$). *not significant ($p>0.05$), **significant ($p<0.05$) with probiotic inclusion.

This decline was increased with inulin addition in probiotic-included GFP samples and further increased with an increment of its amount, particularly in high-chain length ones. However, our VI outputs of probiotic-blended GFP were comparable volume expansion values, with *B. coagulans*-added composite flour-based pasta, which corresponded to approximately 145%, although including gluten-containing raw materials as wheat flour and semolina (Piyadasa and Perera, 2019).

The VI values of GFP, including *B.coagulans* were ranged between nearly from73-111% to 163-194% throughout the entire cooking process. An increase was recorded in all probiotic-blended GFP formulations with/without dietary fiber sources during 15 min of cooking, as viewed in Figure 4.26. This expansion in volume is carried out in consequence of the absorption and swelling of water by starch granules during cooking (Steglich, 2013), as observed in our study. Therefore, a strong positive correlations between WAC and SI with VI were recorded regarding the 3rd chapter of this study ($r=+0.96$ and $r=+0.94$, respectively). However, the VI values should be in the range between 200-300% in high-quality pasta/noodle based on wheat flour (Pessanha et al., 2023). In line with this information, none of the probiotic-fortified GFP samples, in terms of either including inulin or not, could provide this necessity throughout the cooking process (Table 4.46).

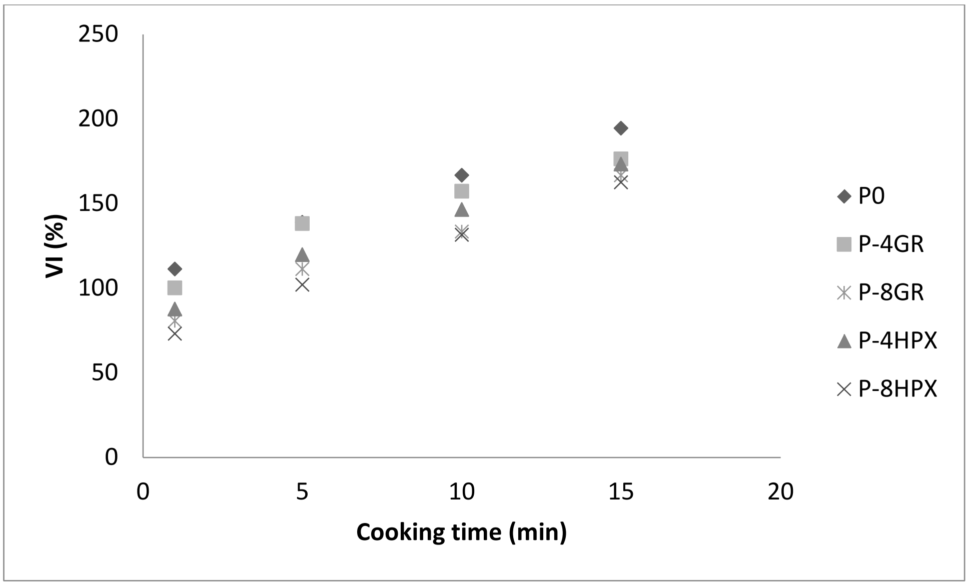

Figure 4.26. Volume increase of probiotic-incorporated gluten-free pasta with/without inulin during cooking

4.6.6. Turbidity and Amylose Leaching

The turbidity (T) and amylose leaching (AL) of *B. coagulans*-included GFP samples in the presence or not of different types (GR, HPX) and concentrations (4 and 8%) of inulin were declared in Table 4.47.

The probiotic inclusion into GFP formulation with or without inulin resulted in higher both T and AL values in contrast to those that are not present in *B.coagulans* in GFP, as formerly discussed in section 4.4.6 (Table 4.30). Furthermore, all probiotic-enriched GFP samples with inulin had higher T and AL values than those of P0 and increased more with long-chain used at higher concentrations, within the 15 min duration of cooking (Table 4.47). This may be explained by the positive relationship of T with CL, as mentioned in several studies in advance (Heo et al., 2013; Jeon et al., 2014; Moon and Kweon, 2021; Park et al., 2022; Bak et al., 2023; Li et al., 2024) that highlighted that extreme CL values could give rise to severe leaching of soluble compounds during the pasta cooking process (Kim et al., 2014). In terms of evaluation with inulin level, an increase in both T and AL values during the cooking process was recorded as the inulin addition level rose, as indicated in Table 4.47.

The T and AL values of GFP fortified with probiotics were over the range of 0.12 -1.48 abs and 0.09-1.85 abs, respectively, as represented in Table 4.47, The T and AL of cooking water in all probiotic-fortified GFP samples including inulin regardless of their DP and amount, were increased as the cooking process sustained, which is in agreement with their corresponding CL values throughout the whole boiling process, as previously stated (section 4.6.4, Table 4. 45). In addition to this, this could also be aligned with the diminishing cell viability of probiotics in GFP (section 4.6.11, Table 4.51). Moreover, there was a positive relation between the T and AL in the 3rd chapter of this study (r=+0.62) because of being one of the reasons for turbidity in cooking water.

Table 4.47. Turbidity and amylose leaching values in the cooking medium of probiotic-incorporated gluten-free pasta with/without inulin (abs at 675 nm and 650 nm, respectively)

Sample	Cooking time (min)	T (abs)	AL (abs)
P0	0	0.15±0.01^{Eb**}	0.09±0.01^{Ec**}
	1	0.18±0.01^{Dd**}	0.30±0.01^{Db**}
	5	0.44±0.02^{Ce**}	0.60±0.10^{Cb*}
	10	0.85±0.04^{Bc**}	1.17±0.07^{Bd*}
	15	1.23±0.02^{Ac**}	1.48±0.01^{Ad**}
P-4GR	0	0.12±0.01^{Ee**}	0.14±0.01^{Ed**}
	1	0.21±0.01^{Dc**}	0.30±0.01^{Db**}
	5	0.51±0.02^{Cd**}	0.75±0.03^{Ca**}
	10	0.93±0.02^{Bb**}	1.25±0.04^{Bcd**}
	15	1.27±0.02^{Ac*}	1.52±0.02^{Ad**}
P-8GR	0	0.14±0.01^{Ec**}	0.18±0.01^{Eb**}
	1	0.28±0.01^{Db**}	0.39±0.01^{Da**}
	5	0.58±0.01^{Cc**}	0.82±0.01^{Ca**}
	10	0.99±0.01^{Bab**}	1.45±0.01^{Bb**}
	15	1.38±0.01^{Ab*}	1.70±0.01^{Ab*}
P-4HPX	0	0.13±0.01^{Ed**}	0.16±0.01^{Ec**}
	1	0.22±0.01^{Dc**}	0.33±0.02^{Db**}
	5	0.66±0.01^{Cb**}	0.78±0.01^{Ca**}
	10	0.95±0.03^{Bab**}	1.34±0.03^{Bc**}
	15	1.42±0.01^{Ab**}	1.59±0.01^{Ac**}
P-8HPX	0	0.16±0.01^{Ea**}	0.21±0.01^{Ea**}
	1	0.32±0.01^{Da**}	0.41±0.01^{Da**}
	5	0.71±0.01^{Ca**}	0.84±0.01^{Ca**}
	10	1.01±0.02^{Ba*}	1.57±0.01^{Ba**}
	15	1.48±0.01^{Aa**}	1.85±0.03^{Aa**}

Means followed by the different uppercase letters in the same column regarding cooking time for each parameter were significantly different ($p<0.05$). Means followed by the different lowercase letters in the same row at the same cooking time for each parameter were significantly different ($p<0.05$). *not significant ($p>0.05$), **significant ($p<0.05$) with probiotic inclusion.

4.6.7. Color

The color parameters composed of L*, a*, b*, C*, h°, and as well as to their derivatives (yellowness index, color score, $\Delta E_{cooking}$) of both raw and cooked probiotic-fortified GFP samples with/without inulin were summarized in Table 4.48 and Appendix 4. In this context, probiotic inclusion generally led to a slight decrease in L* values both raw and during the cooking process in GFP samples with inulin. In a similar vein, while *B. coagulans* BC30 GBI 6068 addition causes a significant decrease in L* values but an increase in a*, b*, and yellowness index values in fresh pasta based on wheat white flour ($p<0.05$) (García-Moncayo et al., 2024). In another study, while L* values decreased, and a* values did not significantly influence ($p>0.05$), b* and yellowness index were significantly increased by *B. coagulans* BC30 GBI 6068 incorporation into cooked pasta made from white wheat flour ($p<0.05$) (García-Moncayo et al., 2024). Conversely, L* values were increased in probiotic-enriched GFP based on a combination of pseudocereal flours during the cooking process compared to counter-uncooked samples (Poshadri and Deshpande, 2024; Achinna and Deshpande, 2025). In another controversial study revealed that higher L* and b* but lower a* values were declared in cooked pasta prepared by a composite flour (wheat flour, semolina, corn flour, pumpkin seed flour) enriched with *B.coagulans* GBI-30, 6086, in contrast to that of the uncooked version (Piyadasa and Perera, 2019). The differences in results with our study could originate from uncooked pasta samples were dried at 65°C for 5-7 h in the corresponding study (Piyadasa and Perera, 2019). However, commonly an increase in L*, b*, and color score values was figured with inulin inclusion to probiotic-added GFP samples throughout cooking (Table 4.48), aligning with inulin impact as previously mentioned in section 4.4.7 (Table 4.31).

In general, a slight increase was recorded in YI values with probiotic inclusion into cooked GFP formulations, which was more prominent with inulin and further with higher DP, particularly within the first 5 min of cooking (Table 4.48). In contrast, although no significant differences were seen in yellow pigment concentrations of durum wheat flour used for pasta production with and without probiotic culture ($p>0.05$), a statistical decrease was monitored in yellow pigment in both raw and cooked pasta samples with the addition of *B.coagulans* BC30 to the formulation which is more prominent in cooked verison, as expected ($p<0.05$) (Fares et al., 2015).

On the other side, a decline in color values such as L*, a*, b*, YI and color score whereas increase in $\Delta E^*_{cooking}$ in all probiotic-added GFP samples either including inulin or not were determined as the cooking proccess continued (Table 4.48), observed along the lines of the first and second chapters of present study based on the evaluation of effect of PGS and inulin on GFP color, respectively (section 4.2.8, Table 4.11; section 4.4.7, Table 4.31).

Particularly, the decrease of b* throughout the cooking process of probiotic-incorporated GFP could arise from pigments mainly immanent carotenoids such as zeaxanthin and lutein, which possess a heat-labile manner (Motta Romero et al., 2017), dissolution by thermal degradation, resulting in leaching of the cooking media out (Giuberti et al., 2015; Bouasla et al., 2017b), which agrees with prior studies (Giuberti et al., 2015; Motta Romero et al., 2017; Bouasla et al., 2017b; Ungureanu-Iuga et al., 2020; Süfer, 2022). In alignment with a study determined that L*, a*, and b* values of cooked *B. coagulans*-included pasta made by durum wheat semolina were statistically lower than those of uncooked dried pasta (Konuray and Erginkaya, 2020). A similar trend was also noted for a decline in a* and b* values with cooking of *B. coagulans*-included GFP made by pseudocereal flour mixture (Poshadri and Deshpande, 2024; Achinna and Deshpande, 2025), and was also associated with not only solid loss but also water absorption (Achinna and Deshpande, 2025).

Table 4.48. Color values of raw and cooked probiotic-incorporated gluten-free pasta with/without inulin

Color	Time (min)	P0	P-4GR	P-8GR	P-4HPX	P-8HPX
L*	Raw	75.45±0.52^{Ac*}	76.88±0.13^{Ab**}	77.00±0.04^{Ab**}	77.43±0.41^{Ab**}	78.71±0.30^{Aa*}
	1	74.34±0.90^{Bc*}	75.43±0.05^{Bbc*}	75.82±1.15^{ABbc*}	76.57±0.46^{Aab*}	78.15±0.26^{Aa*}
	5	73.49±0.53^{Bc**}	74.64±0.48^{Bbc*}	75.49±0.25^{Bb*}	75.75±0.37^{Bb*}	77.39±1.02^{ABa*}
	10	69.99±1.42^{Bb*}	74.15±1.54^{BCab*}	74.45±0.19^{BCa*}	75.20±0.11^{Ba*}	75.87±0.50^{BCa*}
	15	64.77±1.65^{Cb**}	73.90±0.41^{Ca*}	74.04±0.09^{Ca*}	73.98±0.45^{Ca*}	74.72±0.13^{Ca**}
a*	Raw	0.74±0.02^{Ac*}	1.28±0.05^{Aa*}	1.20±0.01^{Aa**}	1.03±0.08^{Ab*}	0.81±0.07^{Ac**}
	1	-1.31±0.10^{Ba**}	-2.42±0.05^{Bb**}	-2.75±0.14^{Bc**}	-2.81±0.03^{Bcd**}	-3.08±0.15^{Bd*}
	5	-1.91±0.38^{BCa**}	-3.10±0.16^{Cb*}	-3.31±0.05^{Cb*}	-3.47±0.13^{Cb*}	-3.51±0.03^{Bb**}
	10	-2.18±0.10^{Ca**}	-3.57±0.07^{CDb*}	-3.60±0.04^{Db*}	-3.68±0.09^{Cb*}	-3.72±0.04^{Cb**}
	15	-2.30±0.08^{Ca**}	-3.70±0.01^{Db**}	-3.80±0.02^{Ebc**}	-3.82±0.06^{Dc**}	-3.90±0.01^{Dc**}
b*	Raw	24.14±2.46^{ABCa*}	27.84±0.82^{Aa*}	28.02±0.29^{Aa*}	28.33±0.09^{Aa*}	29.36±0.59^{Aa**}
	1	20.06±0.18^{Bc**}	21.47±1.10^{Bbc*}	22.15±0.57^{Bb*}	22.94±0.15^{Bb*}	24.95±0.93^{Ba*}
	5	17.43±0.13^{Cb*}	17.90±1.43^{BCDab*}	18.71±0.19^{Cab*}	19.79±0.25^{Cab**}	20.82±0.63^{Ca**}
	10	15.40±0.01^{Dc**}	15.51±0.40^{Cc*}	16.08±0.13^{Db**}	16.18±0.12^{Db*}	17.75±0.10^{Da**}
	15	14.22±0.07^{Ed**}	14.41±0.02^{Dd*}	14.93±0.06^{Ec*}	15.50±0.16^{Eb**}	16.31±0.33^{Ea**}
YI	Raw	45.64±4.47^{ABCa*}	51.73±1.62^{Aa*}	51.99±0.52^{Aa*}	52.27±0.11^{Aa**}	53.30±0.87^{Aa**}
	1	38.55±0.12^{Bc**}	40.65±1.18^{Bbc*}	41.75±0.98^{Bbc*}	42.80±0.53^{Bab*}	45.60±0.89^{Ba*}
	5	33.89±0.49^{Ca*}	34.29±2.85^{BCa*}	35.41±0.48^{Ca*}	37.32±0.30^{Ca**}	38.46±0.96^{Ba**}
	10	31.48±1.13^{Dab**}	29.95±1.07^{Cb*}	30.85±0.17^{Dab**}	30.73±0.18^{Db*}	33.41±0.04^{Ca**}
	15	31.42±0.88^{Da**}	27.85±0.13^{Cc*}	28.81±0.15^{Ebc*}	29.93±0.48^{Eab**}	31.18±0.58^{Da**}
Color score	Raw	6.19±0.46^{ABCb*}	6.63±0.08^{Aab*}	6.65±0.03^{Aab**}	6.70±0.03^{Aab**}	6.87±0.08^{Aa*}
	1	5.72±0.07^{Bd*}	5.92±0.11^{Bcd*}	6.01±0.01^{Bbc*}	6.12±0.01^{Bb*}	6.40±0.11^{Ba*}
	5	5.42±0.02^{Cc*}	5.52±0.23^{BCbc*}	5.65±0.01^{Cbc**}	5.77±0.05^{Cab**}	5.95±0.01^{Ca**}
	10	5.04±0.12^{Dc*}	5.26±0.09^{Cb*}	5.33±0.02^{Db*}	5.38±0.02^{Db*}	5.57±0.04^{Da*}
	15	4.66±0.14^{Ec*}	5.14±0.03^{Cb**}	5.20±0.01^{Eab**}	5.25±0.01^{Eab**}	5.37±0.04^{Ea**}
$\Delta E^*_{cooking}$	1	4.77±0.32^{Dc**}	7.53±0.91^{Da*}	7.28±0.20^{Dab**}	6.69±0.05^{Dab*}	5.96±0.62^{Dbc*}
	5	7.50±0.11^{Cb**}	11.18±1.18^{Ca*}	10.46±0.11^{Ca**}	9.81±0.22^{Cab**}	9.72±0.40^{Cab**}
	10	10.92±1.20^{Bb**}	13.79±0.20^{Ba*}	13.12±0.17^{Ba**}	13.22±0.10^{Ba**}	12.79±0.19^{Ba*}
	15	15.03±1.15^{Aa*}	14.63±0.10^{Aa**}	14.32±0.04^{Aa**}	14.15±0.05^{Aa**}	14.44±0.34^{Aa*}.

Means followed by the different uppercase letters in the same column regarding cooking time for each color parameter were significantly different (p<0.05). Means followed by the different lowercase letters in the same row at the same cooking time for each color parameter were significantly different (p<0.05). *not significant (p>0.05), **significant (p<0.05) with probiotic inclusion.

4.6.8. Texture

The textural attributes of 5 min-cooked GFP, including *B. coagulans* consists of whether inulin or not, were demonstrated in Table 4.49. According to the results, probiotic inclusion into GFP comprising inulin generally induces a significant reduction in textural characteristics when compared with the results of 5 min-cooked GFP without *B.coagulans* ($p<0.05$) (in section 4.4.8, Table 4.32). Similarly, significantly lower firmness, chewiness, and springiness but as distinct from higher cohesiveness values were detected in synbiotic GFP based on pseudocereal composite flour (amaranth, buckwheat and quinoa) including *B. coagulans* Unique IS2 spores and psyllium seed husk, as a probiotic and prebiotic source, respectively ($p<0.05$), in contrast to control made from wheat flour (Achinna and Deshpande, 2025). A study revealed that while a hardness reduction was identified with *Bacillus coagulans* GBI-30 into durum wheat semolina pasta, cohesiveness, springiness, and resilience were not influenced substantially by probiotic fortification (Konuray and Erginkaya, 2020). In another study, no significant difference was recorded in firmness values between control penna rigata pasta made from durum wheat flour without probiotic source and the ones enriched with 1% of probiotic culture (*Bacillus coagulans* GBI-30, 6086) (Fares et al., 2015). In contrast, 1% inclusion of *B. coagulans* GBI 6068 into pasta based on wheat flour made a significant increase in hardness and chewiness values and was interpreted as enhancing the strength of the gluten network ($p<0.05$), although no substantial differences were obtained in cohesiveness, springiness, and resilience values ($p>0.05$) (García-Moncayo et al., 2024).

The lowest values in almost all texture parameters were attained in the P0 as the control probitoic-enriched GFP with no inulin (Table 4.49). This could be attributed to their higher WAC and SI values than those of the ones including inulin; additionally, as described previously in section 4.6.2 (Table 4.43) and section 4.6.3 (Table 4.44), respectively. However, a significant increase was recorded in almost all textural features, except for cohesiveness, in both inulin and probiotic-included GFP ($p<0.05$) (Table 4.49). Therefore, results obtained in this study suggest that inulin incorporation into probiotic GFP may enhance the textural structure of the end-product in contrast to P0 as a probiotic-added control GFP (Table 4.49).

Table 4.49. Texture properties of probiotic-incorporated gluten-free pasta with/without inulin

Sample	Hardness (N)	Chewiness (N)	Cohseiveness	Springiness	Resilience
P0	$2.17±0.32^{c**}$	$0.64±0.16^{c**}$	$0.53±0.03^{a*}$	$0.55±0.03^{b**}$	$0.24±0.03^{d**}$
P-4GR	$3.65±0.20^{a**}$	$1.18±0.12^{a**}$	$0.46±0.02^{b**}$	$0.70±0.01^{a**}$	$0.38±0.01^{ab*}$
P-8GR	$3.13±0.48^{ab**}$	$0.78±0.06^{bc**}$	$0.41±0.02^{bc**}$	$0.61±0.03^{ab**}$	$0.35±0.01^{bc*}$
P-4HPX	$3.35±0.03^{ab**}$	$0.96±0.05^{ab**}$	$0.45±0.02^{b**}$	$0.64±0.06^{ab*}$	$0.42±0.02^{a**}$
P-8HPX	$2.74±0.41^{bc**}$	$0.62±0.12^{c**}$	$0.38±0.03^{c**}$	$0.59±0.02^{b**}$	$0.31±0.01^{c**}$

Means followed by the different lowercase letters in the same column were significantly different ($p<0.05$). *not significant ($p>0.05$), **significant ($p<0.05$) with probiotic inclusion.

4.6.9. Optimum Cooking Time

The OCT values of all *B. coagulans*-enriched GFP with/without inulin were given in Figure 4.27 and representational imaginatives composed of raw and cooked samples at 4 different time intervals (1, 5, 10, and 15 min) visualised in Appendix 5 (i-m).

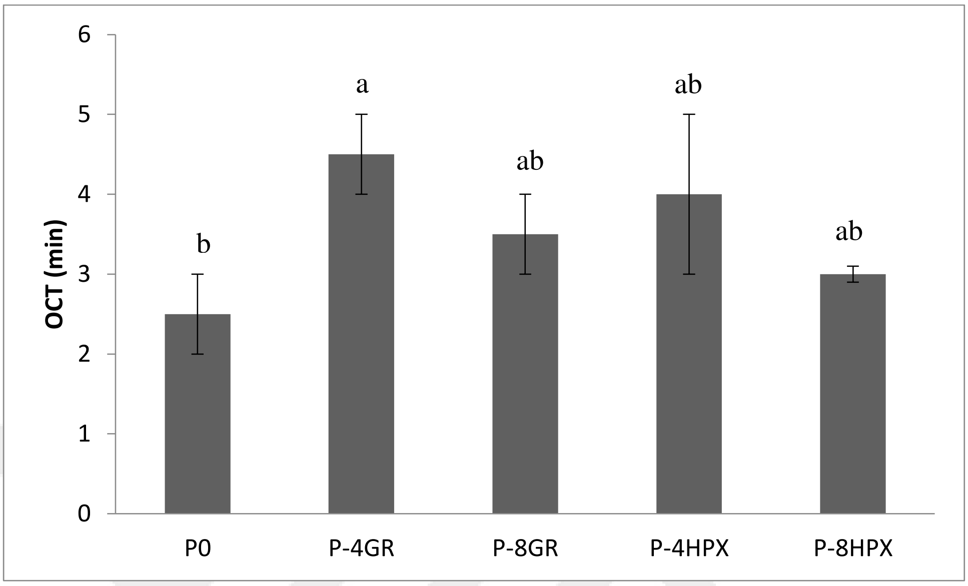

Figure 4.27. Optimum cooking time of probiotic-incorporated gluten-free pasta with/without inulin

The OCT of probiotic-fortified GFP in the event of either inulin addition or not was between 2.5-4.5 min (Figure 4.27). Therefore, a decrease in OCT was monitored when the probiotic was incorporated into the GFP formulation. In this regard, this could be paraphrased with probiotic-added GFP samples, which were not sufficient to retain for cooking stress (Bresciani et al., 2021) owing to having a poor structure as a result of quicker water transfer and diffusion/penetration into central portions (Rachman et al., 2020). In a similar vein, a decline was determined in OCT values of probiotic (*B.coagulans* Unique IS2 spores) admixed-GFP based on a composite of pseudocereal flours in comparison with 100% wheat pasta. This was dedicated to the absence of gluten network, which caused the loss of mechanical attributes in the cooking water, resulting in accelerated softening (Poshadri and Deshpande, 2024; Achinna and Deshpande, 2025). In another study, the OCT of wheat semolina-based dried pasta including *B. coagulans* GBI-30 as a probiotic source was determined as 7 min, and was not influenced by undergoing 6 months of storage after drying (Konuray and Erginkaya, 2020). Substantially higher OCT was also recorded, accounting for nearly 10 min, in another study based on *B.coagulans*-included pasta prepared by a gluten-containing composite flour (Piyadasa and Perera, 2019). The higher OCT values in the literature, in contrast to our study, could originate from being gluten-containing counterparts and being exposed to drying before the cooking process (Piyadasa and Perera, 2019; Konuray and Erginkaya, 2020). On the other side, the lowest OCT was monitored in P0, which is the *B. coagulans*-enriched GFP based on 20-PGS without inulin. Therefore, inulin incorporation into probiotic-added GFP extended the OCT (Figure 4.27). This could be enlightened by having less available water for starch gelatinization in the presence of dietary fiber, absorbed water from the cooking medium (Rachman et al., 2019). However, an increase in the inulin concentration causes a reduction in OCT in probiotic-blended GFP, and this reduction was more prominent in GFP including both probiotic and inulin with high chain length (HPX), as observed similar attitude in section 4.4.9 (Figure 4.19).

4.6.10. Microstructure

In this study, the morphology of 5 min-cooked probiotic-incorporated GFP based on 20-PGS with/without inulin was observed under SEM at *x*5000 magnification by analyzing the cross-sectional microstructure of corresponding samples, and presented in Figure 4.28.

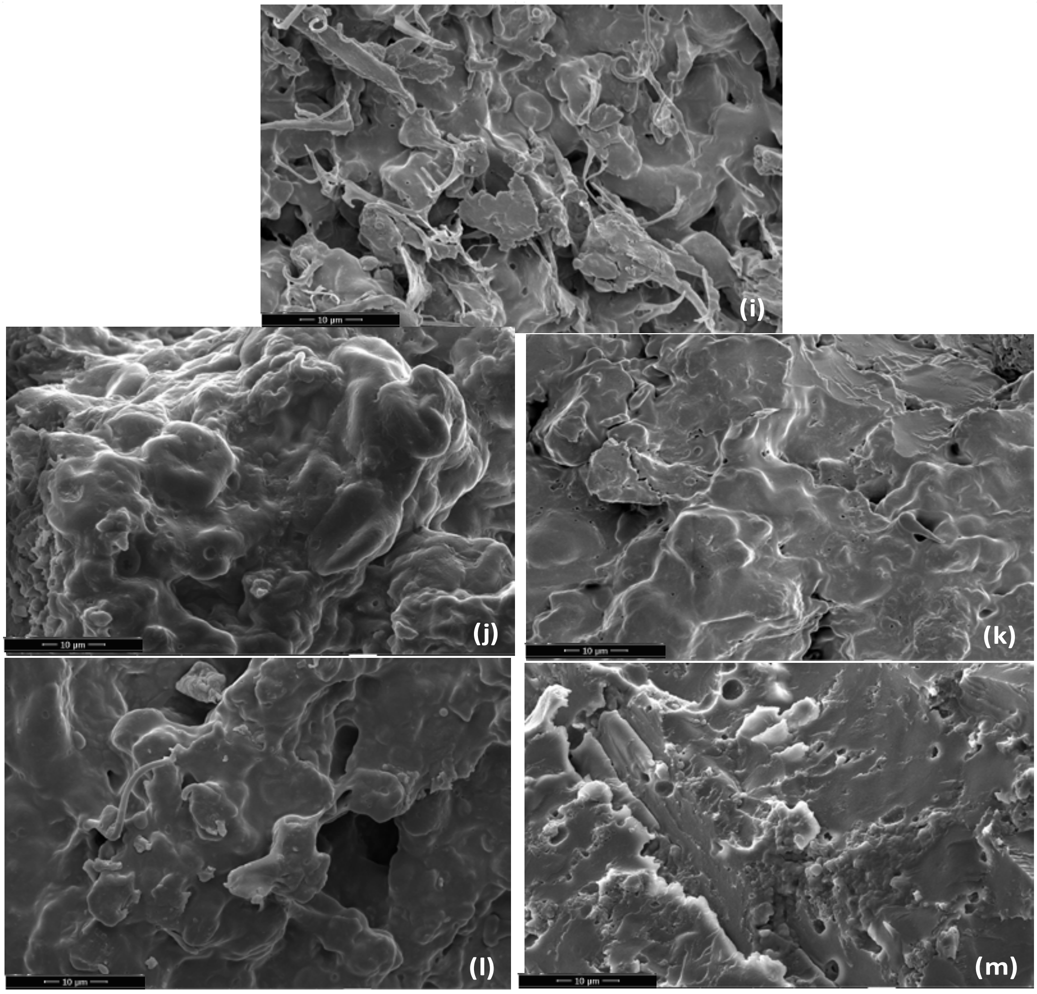

Figure 4.28. Scanning electron microscopy micrographs of cooked probiotic-incorporated gluten-free pasta with/without inulin at a magnification level of *x*5000: (i) P0, (j) 4GR, (k) P-8GR, (l) P-4HPX, (m) P-8HPX

The probiotic-enriched GFP samples with/without dietary fiber (P0) showed the presence of empty spaces and likely reticulated structure (Figure 4.28a), when compared with the 20-PGS without probiotic (4.2.13, Figure 4.9c). The presence of a reticulated structure, which likely gives rise to a form interaction between inulin and protein network, may strengthen the structure of pasta supported with high firmness values by instrumental analysis (Difonzo et al., 2022). However, in the present study, P0 with no inulin had the lowest hardness values (section 4.6.8, Table 4.49), owing to its higher moisture, WAC, and SI than those of inulin-added counterparts of GFP, including probiotic.

On the other hand, particularly the surface of probiotic-enriched GFP with higher levels of inulin (8%), irrespective of its chain length, becomes excessively smooth after 5 min hydration in boiled water. In this case, it could be that the evidence for starch granules could not be encapsulated and probably because of leaching unbound components out from pasta to the cooking medium (Bouasla et al., 2017b). This was agreed with their highest CL (section 4.6.4, Table 4.45), turbidity, and amylose leaching values (section 4.6.6, Table 4.47), together with lower probiotic cell viability (section 4.6.11, Table 4.51).

4.6.11. Viability of Probiotic Cells in Cooked Gluten-free Pasta

It was highlighted that mechanical stress while processing, drying, and boiling as a high-temperature heat treatment had a detrimental effect on probiotic viability (Wang et al., 2025b). In this context, the cell count of viable *B. coagulans* in 5 min and 10 min-cooked GFP samples without (P0) and with inulin in 2 different types (as short- and long-chain length) and concentration (4 and 8%) as a prebiotic source were presented in Table 4.50. According to the results, slight differences were found between the enumerated and Otsu threshold applied segmented probiotics (Appendix 7) in all probiotic-enriched GFP samples subjected to both 5 min and 10 min of boiling.

Table 4.50. Enumeration of probiotic cells with different methods in cooked gluten-free pasta with/without inulin (log CFU/g)

Sample	5 min		10 min	
	Plating	ImageJ	Plating	ImageJ
P0	7.41±0.04^{c**}	7.44±0.02^{b**}	5.35±0.17^{d**}	5.43±0.02^{d**}
P-4GR	8.58±0.18^{a**}	8.44±0.10^{a**}	7.12±0.17^{a**}	6.91±0.14^{a**}
P-8GR	7.82±0.15^{b**}	7.76±0.14^{b**}	6.65±0.05^{ab**}	6.72±0.13^{ab**}
P-4HPX	7.76±0.21^{bc**}	7.68±0.30^{b**}	6.43±0.27^{bc**}	6.34±0.36^{bc**}
P-8HPX	7.65±0.05^{bc**}	7.57±0.01^{b**}	6.01±0.10^{c**}	5.96±0.12^{c**}

Means followed by the different lowercase letters in the same column for each enumeration method were significantly different ($p<0.05$). *not significant ($p>0.05$), **significant ($p<0.05$) regarding cooking times in the same enumeration method.

As expected, the viable cell count of the probiotic was decreased with the cooking process compared with the initial concentration in the GFP formulation ($p<0.05$). This could be explained by thermal processes, such as boiling/cooking, which cause a decline in cell counts of viable probiotic microorganisms (Fares et al., 2015). Similarly, the number of *B. coagulans* was reduced to 5.23 log CFU/g from the 13 log CFU/g initial concentration in wheat flour pasta after 8 min of cooking (García-Moncayo et al., 2024). In another study, the viable cell counts of *B. coagulans*

GBI-30 in noodles retained a relatively high viability, accounting for 5.75 log CFU/g after 7 min cooking, which meets the suggested probiotic efficacy threshold (Wang et al., 2025b). On the other hand, although the cooking time was shorter than that of the present study, lower viable probiotic cell numbers were determined in the literature contrast to our results (Fares et al., 2015; Rajam et al., 2015; García-Moncayo et al., 2024; Wang et al., 2025b), as declared in Appendix 8. Therefore, the differences in acquired results could originate from different probiotic genera and strains, pasta ingredients and their proportions, pasta-making methods, and cooking duration.

Table 4.51. Viability of probiotic cells in cooked gluten-free pasta with/without inulin (%)

Sample	5 min	10 min
P0	82.32±0.43^{c**}	59.41±1.01^{d**}
P-4GR	95.34±1.17^{a**}	79.12±1.07^{a**}
P-8GR	86.89±0.95^{b**}	73.89±0.54^{ab**}
P-4HPX	86.17±1.31^{bc**}	71.48±1.70^{bc**}
P-8HPX	85.03±0.52^{bc**}	66.82±1.04^{c**}

Means followed by the different lowercase letters in the same column were significantly different (p<0.05). *not significant (p>0.05), **significant (p<0.05) in cooking times between the same cell numaration method.

It was declared that the greater loss of viable probiotic cells was recorded as the cooking time was prolonged (Wang et al., 2025b). Therefore, the extent of viable cell count reduction correlated with cooking duration (Fares et al., 2015). As expected, a further reduction was observed as the GFP cooking duration was increased, in our study (Table 4.51). In this regard, the viability of probiotics was above approximately 85% and 67% at 5 min and 10 min of cooking in GFP consisting of prebiotics with different DP and levels, respectively (Table 4.51). In a similar vein, a gradual decrease from 7.20 log CFU/g to 6.08 log CFU/g was detected when the cooking duration was increased from 5 min to 11 min, in durum wheat semolina pasta (Konuray and Erginkaya, 2020). The number of *B. coagulans* GBI-30 in uncooked probiotic- and barley flour-added pasta samples was nearly 7 log CFU/g, which is consistent with quantitative PCR data. While the number of viable *B. coagulans* was 6.91 log CFU/g at 5 min, it decreased to 6.72 log CFU/g after 11 min of cooking. However, the satisfactory pasta cooking quality was obtained with 7 min of cooking time. Therefore, the consumption of a standard serving size of 100 g of the 7 min cooked pasta, including nearly 8-9 log CFU probiotic bacteria, was accepted sufficient to exert potential health benefits related to gut microbiota modulation (Fares et al., 2015).

The GFP samples consisting of inulin had higher counts of viable *B. coagulans* than P0 without inulin in both cooking durations (5 min and 10 min), owing to its prebiotic activity (Table 4.50). Therefore, the significantly lowest number of viable probiotic cells was recorded in P0 as the probiotic-enriched GFP without prebiotics (p<0.05), as expected. However, except for P0 cooked at 10 min, the results obtained in both 5 min and 10 min of cooking were still above the acceptable threshold, which corresponds to at least 10^6 CFU/g of live probiotic strains to show a potential positive impact on host health (Bultosa, 2016). Nevertheless, it could be interpreted that prolonged cooking durations of more than 10 min would not meet the probiotic efficacy in GFP with/without inulin. Similar to P0 in our study, lower viability was recorded in the case of the pasta, including *B. coagulans* without prebiotic sources (García-Moncayo et al., 2024; Wang et al., 2025b). The increase in GFP with *B. coagulans* was prominent in probiotic-enriched GFP-incorporated inulin with short-chain (GR), in contrast to that of long-chain length (HPX), in the present study (Table 4.50). Interestingly, the viable probiotic cell count was reduced as the prebiotic concentration was increased in GFP (Table 4.50). This could likely have originated with their higher CL values, as mentioned in section 4.6.4 (Table 4.45).

When the effect of mechanical stress while processing on *B. coagulans* in pasta/noodle was assessed in the literature (Appendix 8), the *B. coagulans* was incorporated at a $1x10^9$ CFU/g level into the formulation of the synbiotic gluten-free pasta made by a combination of pseudocereal flour mixture-psyllium husk, and thanks to its spore-forming nature and high thermal stability, the viable count was 7.0 log CFU/g in cooked pasta at OCT. Moreover, the viability of the probiotic also met the accepted threshold level for probiotic foods (≥6 log CFU/g) after 6 months of ambient temperature storage. Under *in vitro* simulated gastrointestinal conditions, the survivability of synbiotic pasta was 90.42%, referring to high digestion stability, as well (Achinna and Desphande, 2025). In another study, the onset concentration of probiotic (*B. coagulans* Unique IS2 spores) was 9.68 log CFU/g in a synbiotic GFP formulation based on pseudocereal flour blend. The viability of those pasta samples after extrusion and drying was 8.76 log CFU/g and 8.31 log CFU/g, representing a survivability rates of 90.67% and 85.98%, respectively. Although the viable *B. coagulans* count was decreased by 7.53 log CFU/g at the end of the cooking, this level was still above the acceptable threshold for probiotic efficacy. The viable probiotic count corresponding to 8.09 log CFU/g (83.66% survival) was sustained after 180 days of storage at room temperature. Lastly, the survival rate was 90.83%, equal to 6.83 log CFU/g after exposure to 6h of gastric and intestinal conditions. To sum up, per serving size (≈50 g), this synbiotic pasta enables a functionally relevant amount of probiotics, after processing and digestion, as well (Poshadri and Desphande, 2024). To provide a better preservation opportunity with drying, throughout storage, and in the simulated conditions of acidic and bile environment, the fructooligosaccharides (FOS) with denatured whey protein isolate (DWPI) were chosen (compared to using FOS individually or combined with WPI) for use in noodle production as an encapsulation wall material for encapsulation of *Lactobacillus plantarum* (MTCC 5422). The remaining viable probiotic cells account for 93.63%, 80.29%, and 64.74% in raw and dried noodles at low and high temperatures, respectively. The decrease in probiotic viability because of drying was dedicated to heat stress throughout dehydration, resulting in damage to the cell wall and membrane. On the other side, the cell viability of encapsulated *Lactobacillus plantarum* (MTCC 5422) was lost in dried noodles after cooking, which was referred to as thermal inactivation that occurred throughout rehydration of dried cells because of leakage of fundamental cellular compounds. This could be attributed to differences in probiotic genera owing to the better heat tolerance of *B. coagulans* than *Lactobacillus plantarum*. However, 62.42% encapsulated cell viability was still retained in noodles after cooking. This was dedicated to the initial moisture content of raw noodles with encapsulated probiotics, which preserves the cell membrane from osmotic shock and thermal damage throughout rehydration. Moreover, partial preservation of probiotic cells was provided by encapsulation in cooked raw noodles (Rajam et al., 2015). On the other hand, the better preserved cell viability of *L. rhamnosus* GG was acquired in sheeting-based noodle production than that of *B. coagulans* GBI-30 in extrusion-based production, which relates to both mechanical and thermal stress, indicating a strain-specific processing tolerance (Wang et al., 2025b). Therefore, total probiotic cell viability was diminished in dried noodles, and thus recommended for serving as fresh noodles would be a more appropriate carrier system for ensuring viable probiotic cells (Wang et al., 2025b), which was a similar approach to our study.

4.6.12. Sensory Evaluation

The sensory properties comprised structural integrity, color, texture, taste, and overall acceptability in all fresh 5 min-cooked probiotic-added GFP samples with/without inulin, were evaluated, and then represented in Table 4.52 and Figure 4.29.

Table 4.52. Sensory properties of probiotic-included gluten-free pasta with/without inulin

Sample	Structural integrity	Color	Texture	Taste	Overall acceptability
P0	4.36±0.70^{a**}	5.91±0.51^{a**}	2.45±1.13^{b**}	5.09±0.48^{a**}	4.00±0.60^{a**}
P-4GR	5.82±0.55^{a**}	6.09±0.49^{a*}	4.45±0.51^{a**}	5.45±0.47^{a**}	5.64±0.47^{a**}
P-8GR	5.64±0.67^{a**}	6.18±0.46^{a*}	4.27±0.41^{a**}	5.36±1.12^{a**}	5.09±0.39^{a**}
P-4HPX	5.73±1.01^{a**}	6.36±0.39^{a**}	4.73±1.01^{a**}	5.55±0.49^{a**}	5.55±0.49^{a**}
P-8HPX	5.55±0.73^{a*}	6.82±0.35^{a**}	4.36±1.03^{a**}	5.27±0.69^{a*}	5.36±0.68^{a*}

Means followed by the different lowercase letters in the same column were significantly different (p<0.05). *not significant (p>0.05), **significant (p<0.05) with probiotic inclusion.

The taste scores of probiotic-enriched GFP were slightly differentiate between them (p>0.05), thereby could not be commonly discriminated by panelists. This manner could be related to the finite concentrations of inulin in the whole composition of *B.coagulans*-enriched GFP formulation. The sensory attributes, except color, were negatively affected with an increase in inulin rate (p>0.05), which could be associated with their excessive CL values of probiotic-added GFP in comparison with lower inulin levels (4%) (section 4.6.4, Table 4.45).

On the other side, the color scores of GFP samples, including both prebiotic and probiotic, were increased as the inulin amount augmented, which was as well as to line with the values found with the instrumental method (section 4.6.7, Table 4.48). In consequence, a significant reduction was generally recorded in all scores of organoleptic properties as a result of probiotic incorporation into GFP ($p<0.05$) (Table 4.52). Therefore, instrumentally determined overall negative influence of probiotic addition into GFP with/without inulin was also confirmed with sensory analysis by panelists. On the other hand, the lowest color, taste, and overall acceptability were discovered in the probiotic-fortified GFP with no inulin (P0). This could be dominated by its lowest texture scores (Table 4.52) aligned with its lower hardness values, which were detected instrumentally (section 4.6.8, Table 4.49), as well. Therefore, it could be interpreted that particularly lower levels of inulin incorporation have the potential to enhance sensorial attributes of probiotic-enriched GFP.

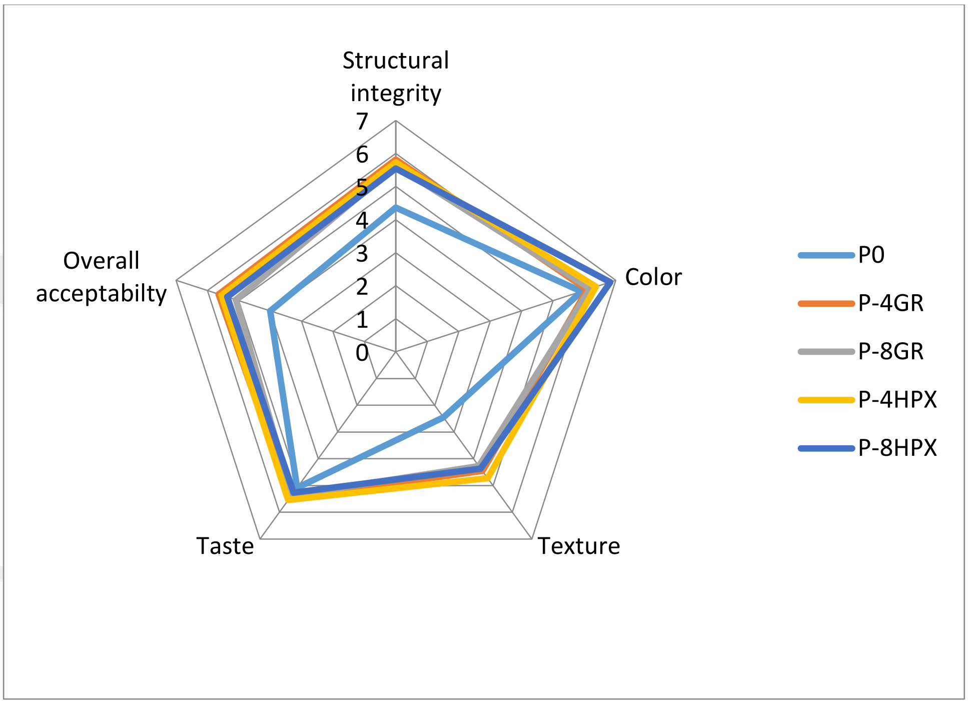

Figure 4.29. Radar plot of sensory properties in probiotic-incorporated gluten-free pasta with/without inulin

5. CONCLUSIONS

There is no study in the literature based on evaluating the influence of the combination usage of inulin with different DP and *B.coagulans* on extrusion and cooking properties of gluten-free pasta, as far as we know. Therefore, the major goal of this present study was assess the extrusion properties as energy-related (G_{mixing}, $G_{extrusion}$, specific mechanical energy), effeciency-related (residence time, extrusion yield, extrusion flow rate), dimensional (expansion ratio, cross-sectional expansion index, unit density, specific length) and also cooking properties (moisture, water absorption capacity, swelling index, cooking loss, volume increase, turbidity and amylose leaching in cooking medium, color, texture, optimum cooking time, DSC, XRD, SEM and sensory analysis), at different time intervals (0, 1, 5, 10, and 15 min) of functional fresh gluten-free pasta (GFP) partially subsituted 10, 20 and 30%) with pregelatinized starch (PGS), selection of the best fresh GFP formulation by hybrid multi-criteria decision making (HMCDM) consisting of analytical hierarchical process (AHP) and technique for order preference by similarity to ideal solution (TOPSIS), respectively, according to extrusion (extrusion yield), technological (cooking loss, color, texture), and sensorial (taste) attributes for the following chapters.

According to the AHP method, the criteria weights of extrusion yield, cooking loss, yellowness index, hardness, and taste were defined as 0.34, 0.22, 0.05, 0.11, and 0.29, respectively. These results indicated that the efficiency of production related to economic issues and providing continuity with satisfying consumers' requests were the main drivers of decision-making in GFP selection. The consistency ratio with regard to experts' viewpoints was found to be 0.05, which was less than 0.1, thereby accepted as reliable for further analysis as TOPSIS. The control pasta based on durum wheat semolina was the first-ranked pasta sample with the nearest distance to the positive ideal solution and also the farthest distance to the negative ideal solution, according to the TOPSIS results of MCDM, followed by 20% PGS incorporated fresh GFP as the best alternative to the control group. Therefore, the incorporation of inulin with two different polymerization degrees (DP≥10 and DP≥23), at different concentrations (4 and 8%), and then probiotic (*Bacillus coagulans* GBI-30, 6086) inclusion was conducted in the 2nd and 3rd chapters of this present study, respectively, for functional and potential synbiotic fresh GFP based on native corn starch was partially replaced with 20% PGS.

In general, the $G_{extrusion}$ values of all fresh GFP samples with/without inulin and/or *Bacillus coagulans* were almost tripled in regards to being those of the G_{mixing} values. The energy-related extrusion properties (G_{mixing}, $G_{extrusion}$, SME) were increased as the substitution level of PGS was raised and gradually further increased with the incorporation of inulin and probiotics, respectively, without reaching the values obtained in durum wheat semolina-pasta. This was attributed to the gluten network and the reduction in feed moisture content, bringing about an anti-lubricant effect, causing more shearing stress, which in turn a higher energy transfer sourced from a discontinuity in the formed structure with a higher flow viscosity and higher friction between the interior barrel surface with pasta dough. Therefore, this could be interpreted as higher overall operational costs during the extrusion process could be achieved in control pasta production. The extrusion yield (EY) of fresh GFP with different ratios of PGS were in the range of approxiamtely 64-86%, the inulin incorporation was positively influenced the process efficiancy which also prolonged to retention time because of the increased in the total feed material but decreased flow rate, whereas although the residence time was increased, the probiotic addition had a negative impact on EY of fresh GFP probably due to excessive stickness in interior barrel surface. As the replacement ratios of native corn starch with PGS were increased, a significant increase in radial expansion, but a reduction in unit density and specific length, was observed in fresh GFP. The viscosity might have dominated the expansion, which in turn reduced the moisture could be one of the major reasons influencing the expansion under the circumstances of cold-extrusion in this study. The greater geletainization degree is related to higher expansion and has a positive relationship with SME, and thus a similar pattern was observed as the PGS replacement rate was increased in fresh GFP.

Lower moisture content, water absorption capacity (WAC), swelling index (SI) and volume increase (VI) were determined within the first 5 min of cooking in all fresh inulin-incorporated GFP in contrast to 20-PGS GFP, whereas higher values were recorded in fresh GFP incorporated with lower ratio (4%) of inulin with short-chain length (GR, DP≥10) at the extended cooking (10-15 min), and thus a decreasing trend was generally recorded in turbidity and amylose leaching in the cooking medium, hardness and chewiness as texture characteristics. Although inulin has a highly hydrophilic character and could interact with greater amounts of water molecules via hydrogen linkages absorbs water preferentially owing to the competition with other hydrophilic molecules for water, which may cause a restriction to their water absorption and/or holding and swelling ability by making a physical barrier around the starch granule by encapsulating it within the reticule of fiber. This uneven water distribution could be attributed to differences in the speed of water uptake of PGS and inulin, and thus influenced the end-product quality throughout the cooking process. Moreover, even though inulin could better bind water via hydrogen bonds at lower concentrations, owing to continuity disruption caused by the incompatibility of macromolecular biopolymers, which in turn led to a decline in water-binding properties at higher levels of inulin. Greater cooking loss (CL) values in inulin-enriched fresh GFP were monitored in contrast to GFP with no inulin, which were statistically higher ($p<0.05$) when used at a higher concentration (8%) throughout the whole cooking process due to the water-soluble nature of the fiber. A reduction in the optimum cooking time (OCT) was also determined with inulin incorporation and further shortened gradually with probiotic addition into the fresh GFP formulation, which could be interpreted as higher CL values, which accelerate softening due to the increased diffusion/penetration rate of water to the central point, and therefore a lower resistance to cooking stress.

The probiotic inclusion into fresh GFP with/without inulin had generally negatively affected the end-product quality, regarding a decline in moisture, WAC, SI, and VI, together with an increase in CL, turbidity, and amylose leaching. However, the viability of probiotics was generally above approximately 85% and 67% at 5 min and 10 min of cooking in fresh GFP consisting of prebiotics with different DP and levels as prebiotic source, respectively. Therefore, the results were still above the acceptable threshold, which corresponds to at least 10^6 CFU/g of live strains to show a potential positive impact on host health, except for probiotic-enriched fresh GFP with no inulin (P0) at 10 min of cooking.

The moisture, WAC, SI, CL, VI, turbidity, and amylose leaching in cooking medium were increased whereas color (L*, a*, b*, yellowness index, and color score) and texture profile properties (hardness, chewiness, cohesiveness, springiness, and resilience) were generally decreased as the cooking time prolonged in fresh GFP with/without inulin and/or probiotic source. The WAC values of GFP, where native corn starch was partially (10, 20, and 30%) replaced with PGS, and enriched with inulin and/or probiotics, were approximately 2-3 times increased at the end of the cooking (15 min). However, to achieve high-quality pasta, the minimum weight gain of cooked pasta should increase by 100%, and thus, in general, 5 min of cooked fresh GFP with different levels of PGS, including or/not inulin with different DP and concentrations, and/or the probiotic could meet this criterion. In general, a 1-2 times increase in SI values of GFP based on partial substitution with PGS with/without inulin and/or *B. coagulans* was observed at the end of cooking. In general, pasta with more than 10% CL was not generally preferred by consumers, and the maximum limit of CL for a high-quality pasta was generally accepted as 6%. Therefore, 5 min cooked fresh GFP with excessive amounts of PGS (30%) and higher incorporation levels of inulin (8%) and probiotic addition could not meet this requirement in these conditions. On the other side, in general, the VI should be in the range between 200-300% in high-quality pasta; in view of this, while GFP, except for the replaced wit highest levels of PGS (30%) and including inulin with high DP (HPX) at high concentrations (8%), could meet this requirement at the end of cooking (10-15 min), none of the probiotic-included fresh GFP could throughout the cooking process. In conclusion, it was interpreted that the addition of inulin with high concentration and polymerization degree and/or probiotics to GFP may have a negative impact not only on technological properties but also on sensory characteristics and nutritional role during prolonged cooking times.

According to differential scanning calorimetry (DSC) analysis results, both the durum wheat semolina-based control group and the GFP with/without inulin showed an endothermic peak representing different reactions, primarily starch gelatinization, protein denaturation, and amylose-lipid complex formation. The highest enthalpy value (ΔH) was observed in the durum wheat semolina-based control group, which was attributed to embedded starch granules within the strong gluten network. Increasing the PJN substitution rate and inulin concentration resulted in a decrease in the ΔH values of the GFP samples. According to X-ray diffraction (XRD) analysis results, the uncooked GFP samples with/without inulin were characterized by an A-type crystalline structure of starch, with the highest relative diffraction intensities detected peaks at 2θ=17° and 18°. On the other hand, in cooked fresh GFP samples with /without inulin, the highest relative diffraction intensities were observed peaks at the 2θ=17° and 20°, indicating a B-type and V-type crystalline structure of starch, respectively. According to the microstructural examination performed on 5-minute cooked pasta samples using scanning electron microscopy (SEM), the gluten network in the durum wheat semolina-based control group has a complex structure, encapsulating elliptical starch granules, which is consistent with having a lower CL, turbidity, and amylose leaching, as well as higher OCT and ΔH values. A morphological structure with distinct apparent holes with a more open structure allowing for excessive water access and penetration was observed in GFP enriched with a high concentration (8%) of inulin, and this effect was further progressed with the addition of probiotics, resulting in a highly smooth surface consistent with their highest CL values.

The fresh GFP had different levels of PGS, with/without inulin were generally defined in a wide range from "al-dente" to "over-cooked" through 15 min of cooking by the panelists. However, the time for acquiring "al-dente" GFP with/without inulin in terms of sensory evaluation was defined in 1 min of cooking, which was considerably lower than the technologically determined OCT. This could be mainly stemmed from the commonly used OCT method for semolina/wheat flour-based pasta may not be convenient to apply to gluten-free counterparts, and also panelists' preferences may not coincide with the "al-dente" sensation. In general, while the least preferred cooking time was 15 min, the most chosen cooking time was defined as 5 min, except for the GFP with higher PGS replacement levels (30%) and including higher amounts of long-chain length inulin (8HPX). Moreover, the cooking time preference rank was generally negatively affected by the increase in PGS and inulin ratio at the latest stage of cooking (10-15 min) and nearly the whole cooking duration (except 1 min), respectively. Therefore, the specified sensory characteristics (structural integrity, color, texture, taste, overall acceptability) were evaluated in 5 min of cooked in all GFP with/without inulin or probiotics. In this context, sensory parameters declined as the replacement ratio of native corn starch with PGS increased in GFP formulation, and increased usage amounts in inulin, except for color scores. Furthermore, the technologically determined overall negative influence of probiotic addition into GFP with/without inulin was also confirmed by panelists with lower scores for all organoleptic properties.

To sum up, fresh potential symbiotic GFP had acceptable technological and sensory qualities that were produced within limited cooking times (5 min), with a sufficient amount of viable probiotic strain to demonstrate the potential positive effects on host health. However, future studies should focus on determining and kinetically characterizing the time-dependent cooking properties of functional dried gluten-free pasta samples subjected to different drying temperatures and times, examining nutritional value particularly regarding dietary fiber content, starch digestion fractions (slowly-, rapidly-digestible) and predicted glycemic index based on *in vitro* kinetics of starch digestion, and assessing the number of viable probiotic strains *in vivo* during digestion in the gastrointestinal tract.

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CURRICULUM VITAE

Gamze Nil YAZICI received her bachelor’s degree in 2014 from Ankara University, Department of Food Engineering, Ankara, Türkiye. During her BSc, she was at the Hochschule Geisenheim University in Germany for three months via the Erasmus program. She successfully completed her master's degree at the Cukurova University Institute of Natural and Applied Science, Department of Food Engineering, Adana, Türkiye. She was admitted to the PhD program in 2019 at Cukurova University Institute of Natural and Applied Science, Department of Food Engineering, Adana, Türkiye. She worked as a research and teaching assistant in her professional career at Cukurova University's Department of Food Engineering in the Cereal/Bakery Science and Technology laboratory from April 2017 until October 2024. During this period, she conducted the dissertations of final-year undergraduate student teams from idea generation to presentation. She performed 5 research projects, contributing to resource planning, and carried out conceptualization, investigations, coordination, formal analysis, and methodology, and wrote project reports. She was responsible for the Department’s accreditation for engineering programs in alignment with the standards of MUDEK (Association for Evaluation and Accreditation of Engineering Programs). She was awarded with her team projects for the 1st place at the Final Pitch competition in the EIT Food RIS Inspire Summer School Programme: “Targeted Nutrition” in 2021, 2nd place at the Final Pitch contest in the EIT Food Food RIS Inspire Programme: “Sustainable Food Production and Consumption” in 2023, and 3rd place at the Final Pitch contest in the EIT Food RIS Inspire Programme Summer School Programme: “Circular Business Models” in 2021.

APPENDICES

Appendix 1. The major effects of using inulin on the technological properties of pasta/noodles in the literature

Inulin types and properties	Usage ratio (%)	Pasta shape/ type	Major findings in technological properties ↑	↓	↔	References
Commercial (ND)	4%[a]	Turkish noodle	Moisture, volume increase, WA, Color(Raw): *L**, b*, Color(Cooked): a*, b*, Texture(Raw): firmness, total shearing force, Texture(Cooked): adhesiveness, Sensory(Raw): fragility, Sensory(Cooked): color	CT, CL, Color(Raw): a*, Color(Cooked): *L**, Sensory(Raw): color^, Sensory(Cooked): hardness^, chewability^, taste^	Texture(Cooked): hardness, Sensory(Raw): apperance	Saraç, 2021
Commercial (average DP≥23)	5[a] and 10%[a]	–	CL^, Color(Raw): *L**, *b**, Texture(Cooked): firmness: ^(10%)	CT^, SI^, Color(Raw): *a**↓, Texture(Cooked): firmness^(5%),		Zarroug et al., 2022
Commercial (average DP≥23)	0.5%	Gluten-free noodle	Diameter, CT, WA, SI, Color(Cooked): L*, a*, b*↑, Texture(Raw): firmness, work of shear, chewiness, resilience; Texture(Cooked): firmness, adhesiveness	CL↓, Color(Raw): L*, b*; Texture(Raw): hardness, adhesiveness,	extrusion force, Color(Raw): a*, Texture(Cooked): work of shear, hardness, resilience	Gasparre and Rosell, 2019
Extracted from artichoke roots	5a, 10a, and 15%[a]	Fresh tagliatelle	CT^, CL, Color(Raw): *a**, Color(Cooked): *a**, Texture(Raw): firmness, Texture(Cooked): firmness, Sensory: color, firmness, odour	Moisture (except 15%), SI, Color(Raw): *L**, *b**, Color(Cooked): *L**, *b**, Sensory: taste	WA, Sensory: bulkiness, adhesiveness	Difonzo et al., 2022
Extracted from cardoon roots (H-DP), Commercial (L-DP= average DP: 20-25)	2 and 4%	–	SI(H-DP), WA(4% H-DP), CL, Texture(Cooked): adhesiveness(except 2% H-DP),	CT^, SI(L-DP), WA(L-DP), Texture(Cooked): hardness, Sensory(Cooked): firmness, bulkiness, adhesiveness, overall quality (except 4% H-	WA(2% H-DP), Sensory(Dried): color, break to resistance, overall quality; Sensory(Cooked): color, elasticity, taste	Padalino et al., 2017

DP)

Appendix 1(*continued*). The major effects of using inulin on the technological properties of pasta/noodles in the literature

Inulin types and properties	Usage ratio (%)	Pasta shape/ type	Major findings in technological properties ↑	↓	↔	References
Commercial (average DP≥10, average DP≥23)	15[a]		Moisture, CT^, WA, SI, CL, Color(Raw): a*(DP≥10), Color(Cooked): L*, a*,	Color(Raw): L*, b*(DP≥10), Color(Cooked): b*, Texture(Cooked): firmness(DP≥10); maximal breaking strength (DP≥10)	Color(Raw): a*(DP≥23), b*(DP≥23); Texture(Cooked): firmnesss(DP≥23), maximal breaking strength(DP≥23)	Foschia et al., 2015a
Commercial (H-DP: average 12-14; L-DP: average 7-8)	2.5, 5, 7.5, 10, and 20	Spaghetti	CT(2.5%, 5% H-DP), CL	CT(7.5, 10, 20% H-DP; 5, 7.5, 10% L-DP), WA(except 2.5% L-DP), Texture(Cooked): firmness (5, 7.5, 10% L-DP)	WA(7.5, 10, 20% H-DP), SI(2.5,20%H-DP; 2.5, 5% L-DP), Texture(Cooked): firmness (2.5% L-DP; except 20% H-DP)	Aravind et al., 2012
Commercial (average DP: ≈8-13)	2.5, 5, 7.5, and 10	–	Dry matter	WA^, SI, Texture(Cooked): firmness^, elasticity(except 5%), SI (7.5, 10% H-DP and L-DP),	CL, Texture(Cooked): adhesivenes	Brennan et al., 2004
Commercial (average DP≥23)	2.5, 5, 7.5, and 10		CL, Color(Cooked): L*,	WA, SI, Color(Cooked): b*, Texture(Cooked): hardness, chewiness, springiness(7.5%, 10%), Sensory: firmness(except 2.5 L-DP), chewy(except 2.5% L-DP)	Color(Cooked): a*(except 2.5%), Texture(Cooked): springiness(2.5%, 5%), cohesiveness	Bustos et al., 2011
Commercial (average DP>20)	5[a], 10[a], and 15[a]	Macaroni	Moisture(Dried)^(except 5%)			Manno et al., 2009
Commercial (average DP: 8-13)	7.5[a], 10[a], 12.5[a], and 15 [a]	Spaghetti	Dry matter(raw), dry matter(cooked)(15%), SI (7.5 and 10%); CL, Texture(Cooked): adhesiveness(except 7.5%), stickiness(12.5 and 15%), DSC:	dry matter(cooked) (7.5 and 10%), SI(15%), Texture(Cooked): stickiness(7.5 and 10%), elasticity, DSC: enthalpy	Dry matter(cooked) (12.5%); SI(12.5%), Texture(Cooked): firmness (peak force)↔, DSC: gelatinization temperature(except 15%)	Tudorică et al., 2002

			Tonset^(except15%), Tendset^,			

Appendix 1(*continued*). The major effects of using inulin on the technological properties of pasta/noodles in the literature

Inulin types and properties	Usage ratio (%)	Pasta shape/ type	Major findings in technological properties			References
			↑	↓	↔	
Commercial (average DP≥10, DP≥23)	15[a]	Spaghetti	CL, Texture(Cooked): stickness	Moisture, Color(Raw): L*:(DP≥10),; a*, b*, Texture(Cooked): firmness,	Color(Raw): L*(DP≥23),	Peressini et al., 2020
Commercial (average DP: 8-11)	2.5[a], 5[a], 7.5[a], and 10[a]	Spaghetti	CL^, Texture(Cooked): stickiness^, adhesiveness^,	SI^, Texture(Cooked): firmness^, elasticity^		Brennan and Tudorica, 2007
Commercial (average DP≥23)	10[a], and 20[a]	Spaghetti	Color: L*, b*,	Color: a*, Texture: hardness(20%), adhesiveness, work of shear	Texture: hardness(10%)	Filipović et al., 2015a
Commercial (average DP≥23)	5[a], 10[a], and 20[a]	–	Texture(Cooked): adhesiveness	Texture(Cooked): hardness, work of shear	Color(Cooked): L, a*, b*,	Filipović et al., 2015b
Extracted from Jerusalem artichoke tubers:	1, 2, and 3	–	Color: a*^, b*^, Texture: hardness^,	Color: L*^, Texture: cohesiveness^,	Texture: springiness^	Singthong and Thongkaew, 2020
Commercial (average DP>10)	5[a], 10[a], and 20[a]	–	Texture(Cooked): adhesiveness	Texture(Cooked): hardness, work of shear	Color: L, a*, b*,	Ivkov et al., 2018
Commercial (average DP≥23)	5[a] and 10[a]	–	Color: L*(10%), b*, Texture(Cooked): adhesiveness,	Texture(Cooked): hardness, toughness	Color: L*(5%);	Filipović et al., 2014[c]

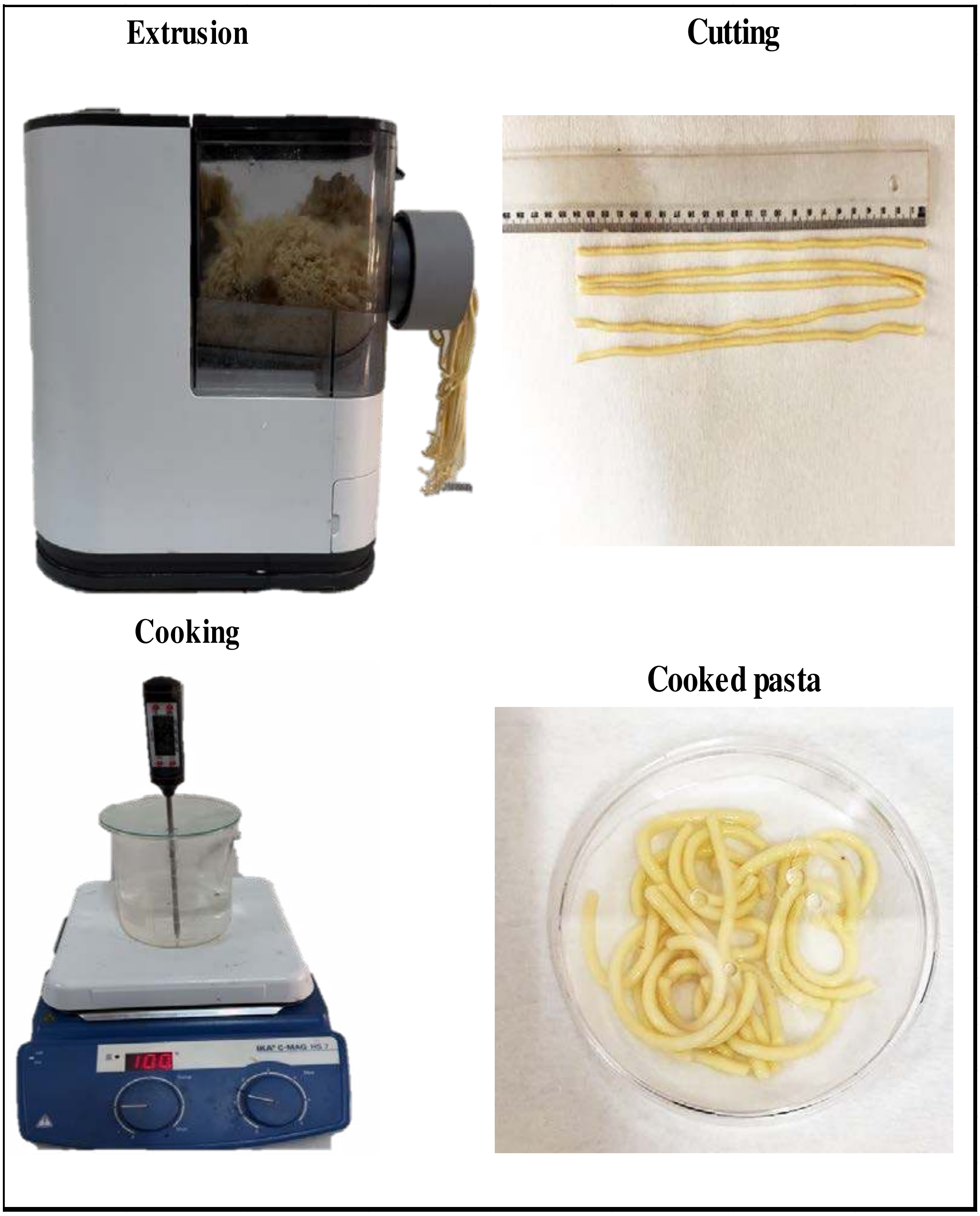
a: concentration was given as a flour replacement ratio, CL: cooking loss, CT: cooking time, H-DP: high polymerization degree, L-DP: low polymerization degree; ND: not defined SI: swelling index, WA: water absorption, ↓ indicates increment is statistically different, ↑ indicates decrease is statistically different, ↔ indicates increment or decrease is not statistically different, ^Results were not given statistically

Appendix 2. The major effects of using inulin on the nutritional properties of pasta/noodles in the literature

Inulin types and properties	Usage ratio (%)	Pasta shape/ type	Major findings in technological properties			References
			↑	↓	↔	
Commercial (ND)	4%[a]	Turkish noodle	Ash, dietary fiber			Saraç, 2021
Extracted from artichoke roots	5[a], 10[a], and 15[a]	Fresh tagliatelle	Ash(except 5%), total dietary fiber	Protein, lipid, carbohydrate (except 5%), predicted glycemic index		Difonzo et al., 2022
Extracted from cardoon roots (H-DP), Commercial (L-DP= average DP: 20-25)	2 and 4	–	Lipid(except 4% L-DP), total dietary fiber, soluble dietary fiber, insoluble dietary fiber	Protein (except 2% H-DP), available carbohydrate, starch digestibility		Padalino et al., 2017
Commercial (average DP: ≈8-13):	2.5, 5, 7.5, and 10	–			Glycemic index	Brennan et al., 2004
Commercial (average DP>20)	5[a], 10[a], and 15[a]	Macaroni		Protein^, starch^, ash^(except 10%)		Manno et al., 2009
Commercial (average DP: 8-1)	2.5[a], 5[a], 7.5[a], and 10[a]	Spaghetti	Total non-starch polysaccharide^	Protein^, carbohydrate^, starch^,		Brennan and Tudorica, 2007
Commercial (average DP≥23)	10[a] and 20[a]	Spaghetti	Cellulose, total dietary fiber	Protein, lipid↓, sugar, starch,	ω-3 fatty acids content	Filipović et al., 2015a
Commercial (average DP≥23)	5[a], 10[a], and 20[a]	–	Sugar, cellulose, dietary fiber, non-digestible carbohydrates	Protein(except 5%), lipid, starch, energy^		Filipović et al., 2015b
Commercial (average DP>10):	5[a], 10[a], and 20[a]	–	Sugar, cellulose, dietary fiber, non-digestible carbohydrates	Protein(except 5%), lipid, starch, energy^		Ivkov et al., 2018
Commercial (average DP≥23)	5 and 10	–	Sugar, cellulose	Protein, starch	Lipid	Filipović et al., 2014

↓ indicates increment is statistically different, ↑ indicates decrease is statistically different, ↔ indicates increment or decrease is not statistically different, ^Results were not given statistically, a: concentration was given as a flour replacement ratio, CL: cooking loss, CT: cooking time, H-DP: high polymerization degree, L-DP: low polymerization degree; ND: not defined SI: swelling index, WA: water absorption

Appendix 3. Representative images for the production and cooking of fresh pasta samples

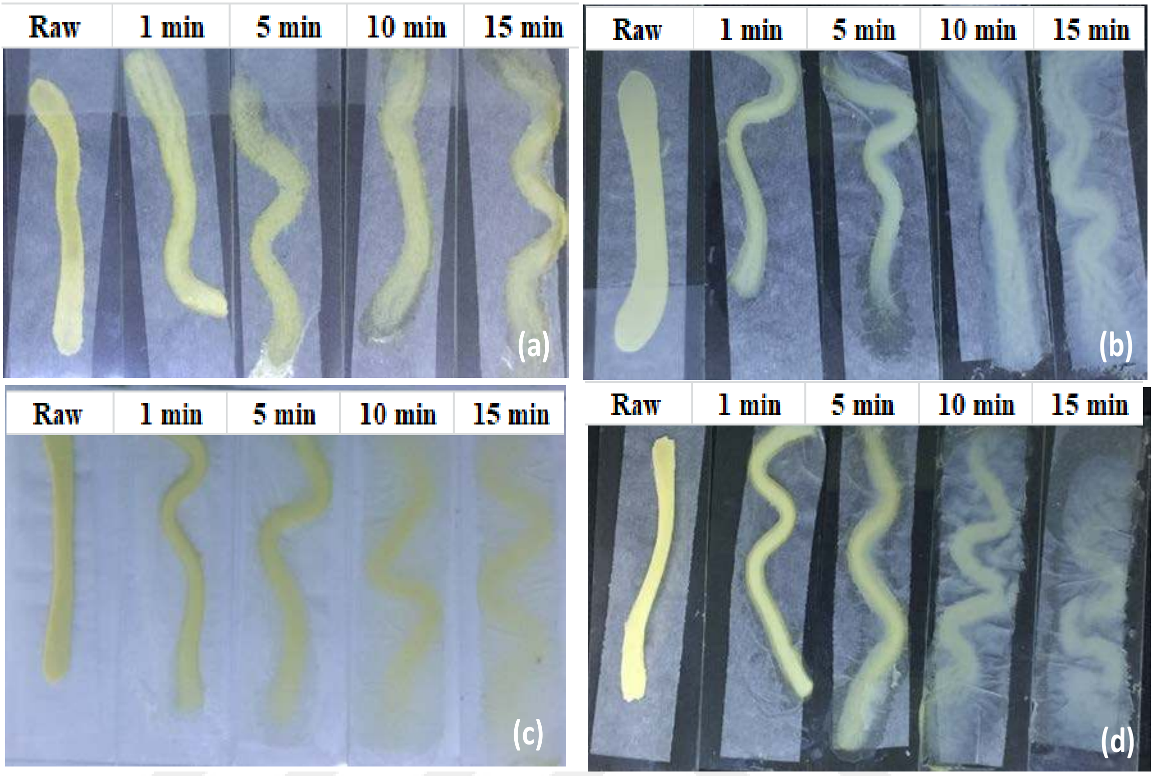


Appendix 4. Chroma (C*) and hue (h°) values of semolina- and gluten-free pasta with/without inulin and probiotic

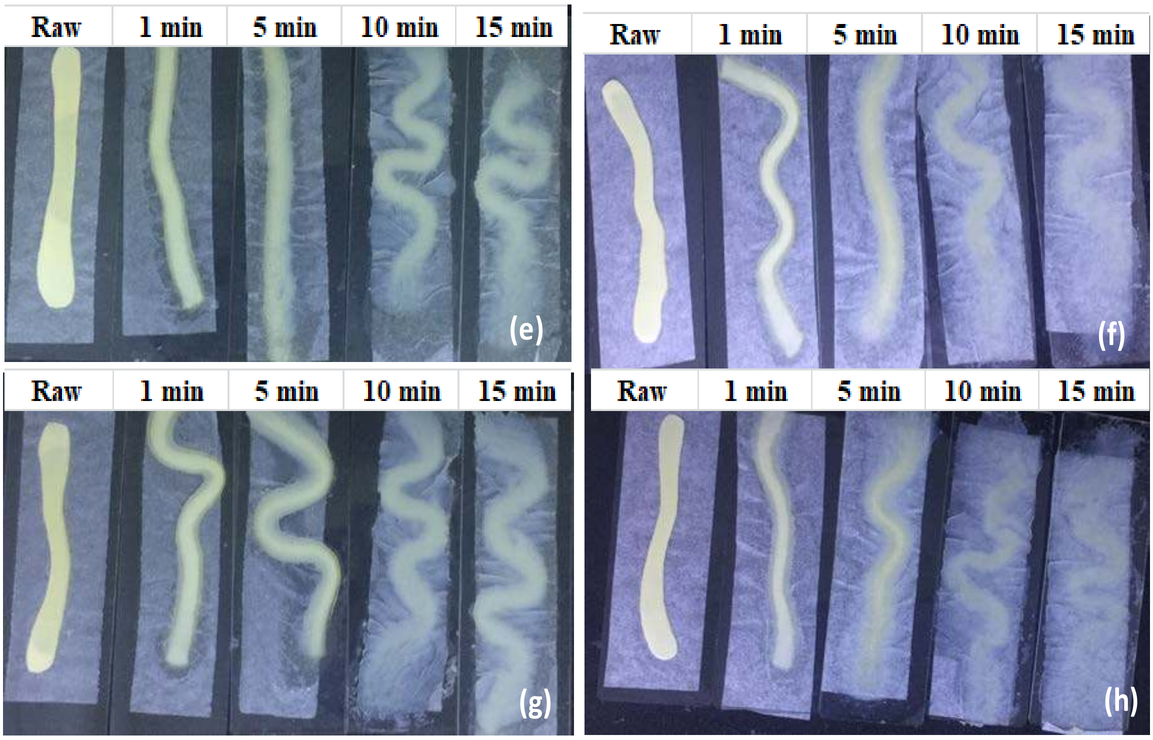
Sample	Color parameter	Raw	Cooking time (min)			
			1 min	5 min	10 min	15 min
C	C*	33.33±1.46^{Aa}	27.32±1.18^{ABa}	23.25±0.66^{BCa}	22.49±0.27^{Ca}	20.16±0.33^{Da}
	h°	86.00±0.09^{Eb}	95.00±0.07^{Db}	96.79±0.05^{Cc}	98.90±0.02^{Bb}	100.85±040^{Ab}
10-PGS	C*	31.20±0.35^{Aa}	22.65±2.06^{Bb}	19.36±0.20^{Bb}	15.91±0.21^{Cb}	14.54±0.13^{Db}
	h°	88.67±0.19^{Da}	97.67±1.05^{Ca}	100.07±0.03^{Cb}	104.02±0.06^{Ba}	106.25±0.03^{Aa}
20-PGS	C*	26.69±0.54^{Ab}	21.63±0.20^{Bb}	17.82±0.42^{Cc}	14.95±0.22^{Dc}	14.03±0.25^{Db}
	h°	88.27±0.28^{Da}	97.44±0.31^{Ca}	100.36±0.18^{Ba}	104.02±0.26^{Aa}	105.42±0.33^{Aa}
30-PGS	C*	24.86±0.39^{Ab}	21.13±0.12^{Bb}	16.73±0.28^{Cc}	14.26±0.50^{Dc}	13.36±0.19^{Dc}
	h°	86.00±0.09^{Eb}	95.00±0.07^{Db}	96.79±0.05^{Cc}	98.90±0.02^{Bb}	100.85±0.40^{Ab}
4GR	C*	27.65±0.54^{Aab}	22.07±0.24^{Bb}	18.17±0.43^{Cbc}	15.29±0.18^{Dcd}	14.44±0.32^{Ebc}
	h°	86.99±0.33^{Ec}	97.46±0.19^{Da}	100.48±0.14^{Ca}	103.28±0.11^{Ba}	104.50±0.19^{Abc}
8GR	C*	28.89±1.07^{Aa}	22.27±0.22^{Bb}	18.67±0.18^{Cab}	15.80±0.11^{Dbc}	14.80±0.58^{Dbc}
	h°	87.54±0.07^{Ebc}	98.00±0.16^{Da}	100.68±0.35^{Ca}	103.32±0.03^{Ba}	104.69±0.51^{Aab}
4HPX	C*	27.92±0.39^{Aab}	23.50±0.37^{Ba}	18.89±0.15^{Cab}	16.33±0.16^{Db}	15.26±0.14^{Eab}
	h°	87.70±0.19^{Eab}	97.78±0.03^{Da}	100.72±0.13^{Ca}	102.67±0.13^{Bb}	104.05±0.01^{Abc}
8HPX	C*	28.34±0.25^{Aab}	24.10±0.29^{Ba}	19.21±0.31^{Ca}	17.38±0.30^{Da}	15.95±0.16^{Ea}
	h°	87.91±0.24^{Eab}	97.94±0.27^{Da}	100.71±0.20^{Ca}	102.11±0.13^{Bc}	103.78±0.03^{Ac}
P0	C*	24.15±2.46^{Aa*}	20.10±0.19^{Ac**}	17.54±0.09^{Ab*}	15.55±0.01^{Bc**}	14.40±0.08^{Ce*}
	h°	88.20±0.27^{Cab*}	93.74±0.26^{Bc**}	96.24±1.27^{ABb**}	98.06±0.37^{Ac**}	99.17±0.25^{Ac**}
P-4GR	C*	27.87±0.83^{Aa*}	21.60±1.09^{Bbc*}	18.17±1.42^{BCDab*}	15.92±0.41^{Cc*}	14.87±0.01^{Dd*}
	h°	87.38±0.02^{Ed*}	96.43±0.21^{Db**}	99.93±0.86^{Ca*}	102.96±0.08^{Ba**}	104.41±0.06^{Aa*}
P-8GR	C*	28.04±0.29^{Aa*}	22.31±0.58^{Bb*}	19.00±0.20^{Cab*}	16.47±0.12^{Db**}	15.41±0.06^{Ec*}
	h°	87.55±0.05^{Ecd*}	97.06±0.17^{Da**}	100.03±0.05^{Ca**}	102.62±0.24^{Ba**}	104.28±0.13^{Aa*}
P-4HPX	C*	28.35±0.09^{Aa*}	23.11±0.15^{Bb*}	20.09±0.27^{Cab**}	16.59±0.13^{Db*}	15.96±0.14^{Db**}
	h°	87.92±0.16^{Ebc*}	96.98±0.03^{Da*}	99.94±0.24^{Ca**}	102.80±0.20^{Ba*}	103.83±0.33^{Aab**}
P-8HPX	C*	29.37±0.59^{Aa**}	25.14±0.94^{Ba*}	21.12±0.62^{Ca**}	18.13±0.10^{Da**}	16.77±0.32^{Ea**}
	h°	88.43±0.16^{Ea*}	97.04±0.09^{Da*}	99.56±0.22^{Ca*}	101.82±0.05^{Bb*}	103.45±0.30^{Ab*}

Means followed by the different uppercase letters in the same row regarding cooking time are significantly different ($p<0.05$). Means followed by the different lowercase letters in the same column within each sample at the same cooking time were significantly different ($p<0.05$). *not significant ($p>0.05$), **significant ($p<0.05$) with probiotic inclusion.

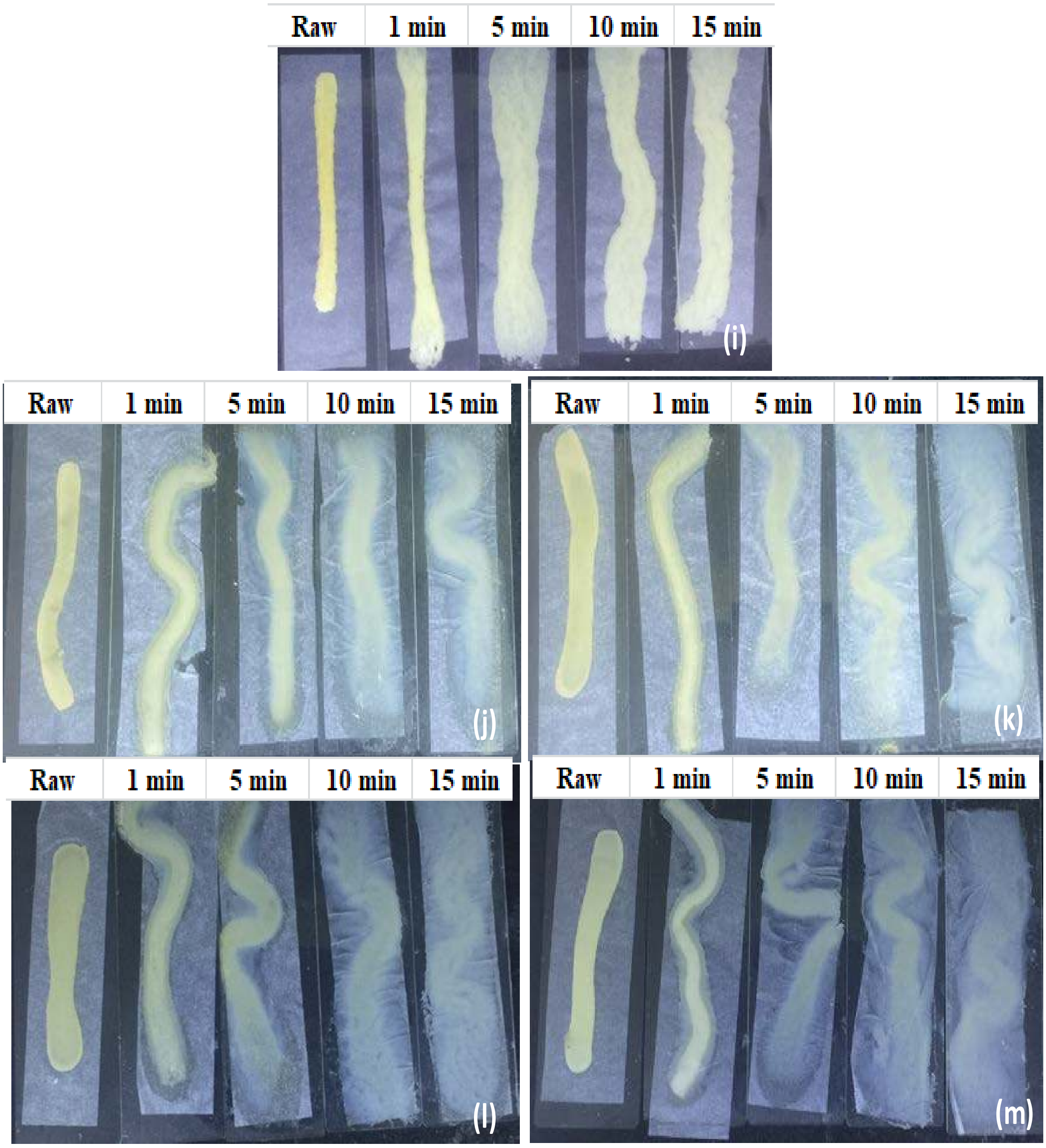
Appendix 5. Representative images of raw and cooked semolina- and gluten-free pasta with/without inulin and probiotics; e: 4GR, f: 8GR, g: 4HPX, h: 8HPX



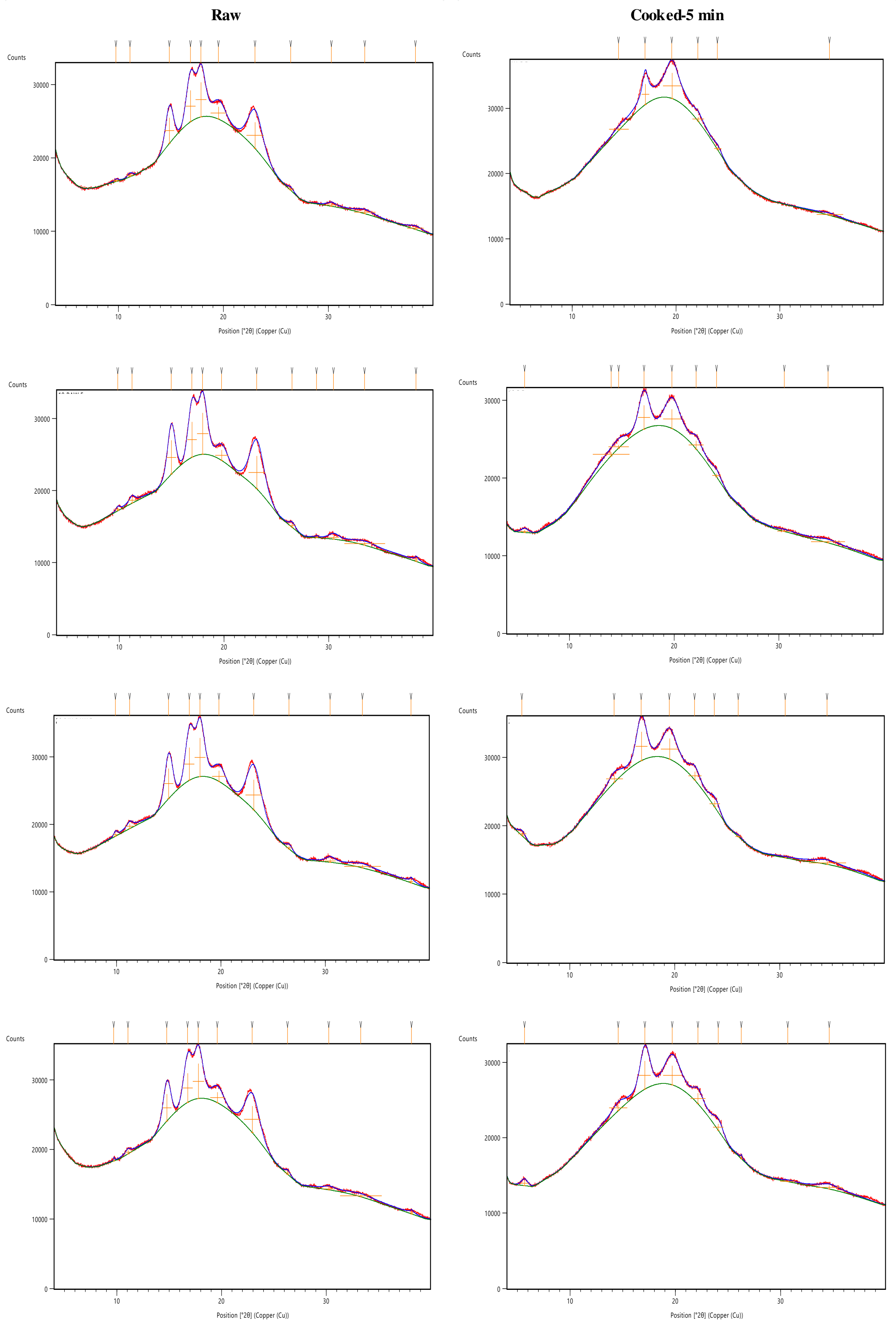
a: Control with semolina, b: 10-PGS, c: 20-PGS, d: 30-PGS



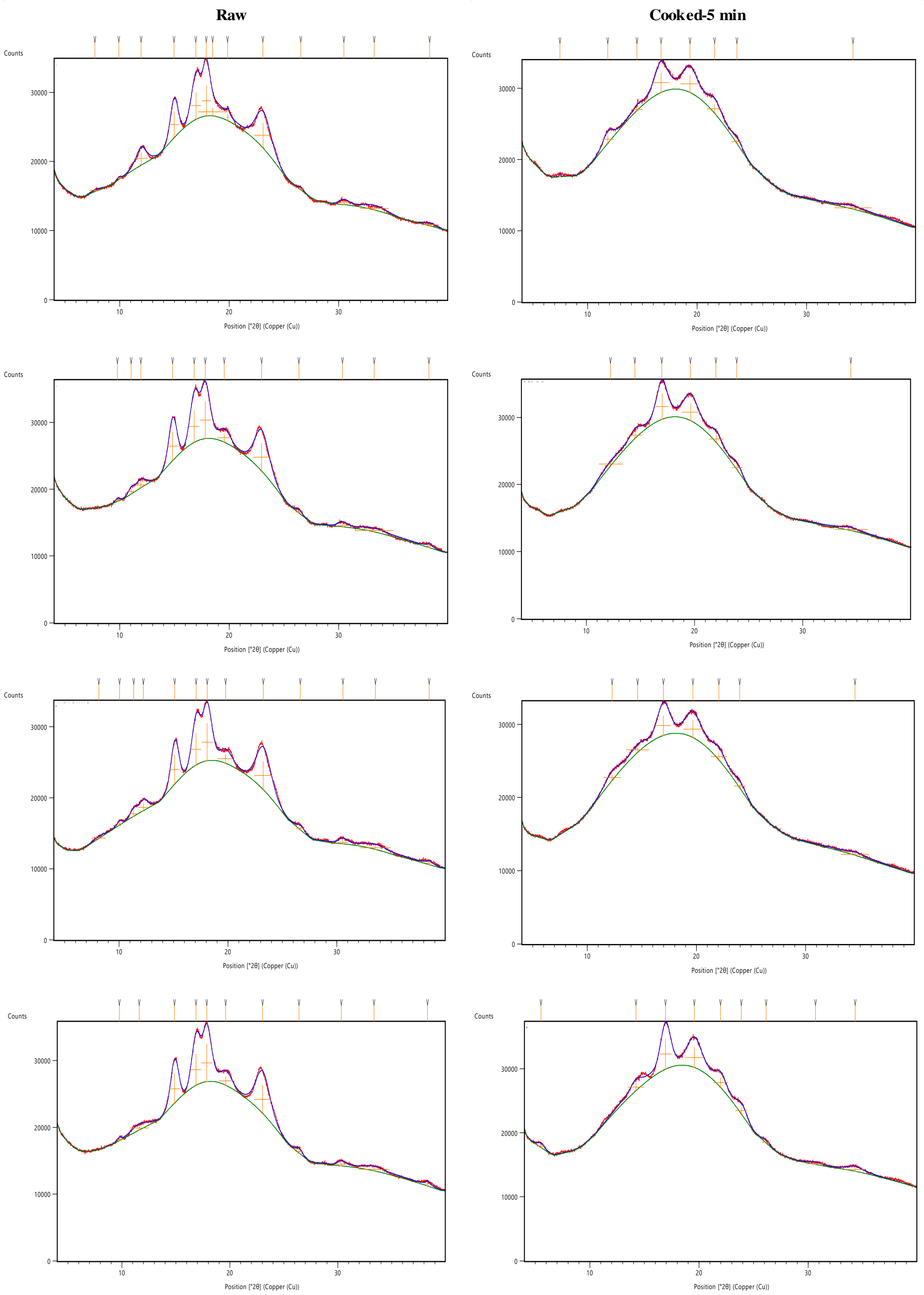
Appendix 5 *(continued)*. Representative images of raw and cooked semolina- and gluten-free pasta with/without inulin and probiotics; i: P0, j: P-4GR, k: P-8GR, l: P-4HPX, m: P-8HPX



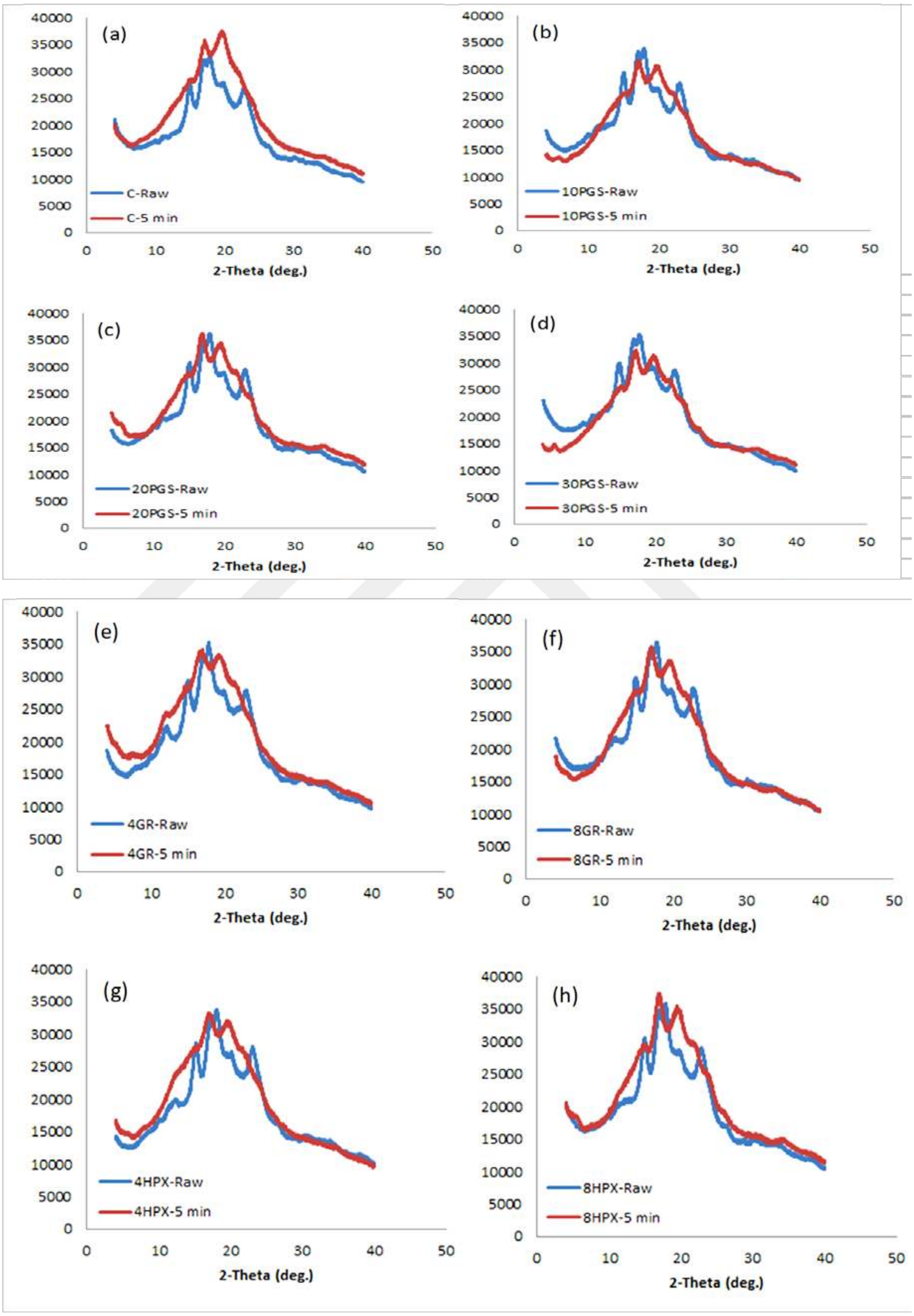
Appendix 6a. X-ray diffraction patterns of raw and cooked semolina- and gluten-free pasta with/without inulin; a: Control with semolina, b: 10-PGS, c: 20-PGS, d: 30-PGS



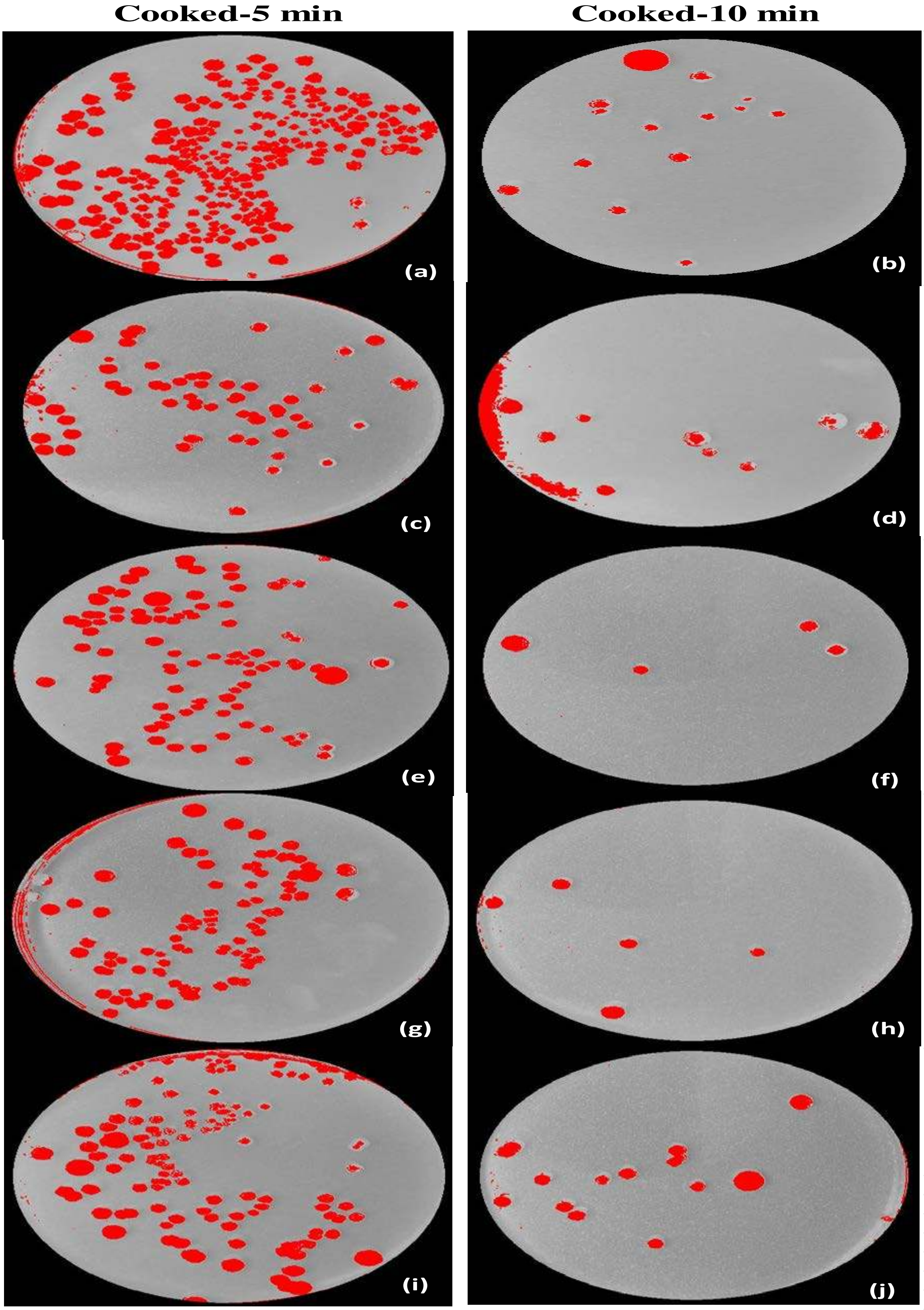
Appendix 6 (*continued*). X-ray diffraction patterns of raw and cooked semolina- and gluten-free pasta with/without inulin; e: 4GR, f: 8GR, g: 4HPX, h: 8HPX



Appendix 6 (*continued*). X-ray diffraction patterns of raw and cooked semolina- and gluten-free pasta with/without inulin; a: Control with semolina, b: 10-PGS, c: 20-PGS, d: 30-PGS, e: 4GR, f: 8GR, g: 4HPX, h: 8HPX



Appendix 7. Representative segmentation images of Image J Otsu threshold for enumaration of probiotic cells; a: P0, 10^{-5}dilution; b: P0 10^{-4}dilution; c: P-4GR, 10^{-7}dilution; d: P-4GR, 10^{-6}dilution; e: P-8GR, 10^{-6}dilution; f: P-8GR, 10^{-6}dilution; g: P-4HPX, 10^{-6}dilution; h: P-4HPX, 10^{-6}dilution, i: P-8HPX, 10^{-5}dilution;j: P-8HPX, 10^{-5}dilution



Appendix 8. The cooking effect of probiotic-added pasta/noodle with/without prebiotic on cell viability in the literature

Prebiotic or potential prebiotic source	*B. coagulans* concentration	Time	Cell viability (log CFU/g)	Viability/ survivability (%)	Reference
–	9.69 log CFU/g	7 min*	5.75	–	Wang et al., 2025b
–	13 log CFU/g	8 min*	5.23	–	García-Moncayo et al., 2024
Psyllium husk	10^9 CFU/g	≈5 min*	7.52	79.46	Achinna and Deshpande, 2025
		0**	7.25	100.00	
		3h**	7.14	95.02	
		6h**	6.80	90.50	
Psyllium husk	10^9 CFU/g	0h**	7.53	100.00	Poshadri and Desphande, 2024
		3h**	716	95.10	
		6h**	6.83	90.83	
–	10^8 CFU/g	5 min*	7.20	–	Konuray and Erginkaya, 2020
		7 min*	6.57		
		9 min*	6.27		
		11 min*	6.08		
–	–	1 min*	6.78	–	Majeed et al., 2021
		5 min*	7.20		
		7 min*	6.57		
Wall material: FOS	9.85 log CFU/g	3.45 min*	–	62.42	Rajam et al., 2015
–	–	1 min*	–	91.70	Piyadasa and Peera, 2019
		2 min*	–	88.80	
		3 min*	–	88.60	
		4 min*	–	87.50	
Barley flour	–	5 min*	6.91	–	Fares et al., 2015
		7 min*	6.72		

*: duration in conventional pasta cooking, **: duration in GIT