

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE
ENGINEERING AND TECHNOLOGY

**FUNCTIONAL PROPERTIES AND QUALITY PARAMETERS OF GRAPE
EXTRACT POWDER SUBSTITUTED BREAD AND EXTRUDED PRODUCTS**

Ph.D. THESIS

Zeynep TACER CABA

Department of Food Engineering

Food Engineering Programme

MARCH 2015

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MARCH 2015

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To my son Taner Demir CABA,

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ABBREVIATIONS

GA1	: Hard wheat bread flour substituted with 7% (w/w) CGEP
GA2	: Hard wheat bread flour substituted with 7% (w/w) CGEP and 10% (w/w) HACS
GA3	: Hard wheat bread flour substituted with 7% (w/w) CGEP and 20% (w/w) HACS
ABTS	: 2,20-azinobis-3-ethylbenzo-thiazoline-6-sulphonic acid diammonium salt
GP1	: Hard wheat bread flour substituted with 7% (w/w) CGEP
GP2	: Hard wheat bread flour substituted with 6.5% (w/w) WPI and (w/w) CGEP
GP3	: Hard wheat bread flour substituted with 13% (w/w) WPI and 7% (w/w) CGEP
A1	: 100% (w/w) hard wheat bread flour
A2	: Hard wheat bread flour substituted with 10% (w/w) HACS
A3	: Hard wheat bread flour substituted with 20% (w/w) HACS
CGEP	: Concord Grape Extract Powder
P1	: 100% (w/w) hard wheat bread flour
P2	: Hard wheat bread flour substituted with 6.5% (w/w) WPI
P3	: Hard wheat bread flour substituted with and 13% (w/w) WPI
Cy-3-GI	: Cyanidin-3-O-glucoside
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
DSC	: Differential Scanning Calorimetry
DW	: Dry Weight
RVA	: Rapid Visco Analyzer
GAE	: Gallic Acid Equivalent
HACS	: High Amylose Corn Starch
HPLC	: High Performance Liquid Chromatography
SEM	: Scanning Electron Microscope
SME	: Specific Mechanic Energy
TEAC	: Trolox Equivalent Antioxidant Capacity
TROLOX	: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
WPI	: Wheat Protein Isolate

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FUNCTIONAL PROPERTIES AND QUALITY PARAMETERS OF GRAPE EXTRACT POWDER SUBSTITUTED BREAD AND EXTRUDED PRODUCTS

SUMMARY

Cereal products are commonly considered as staple foods for human beings and have been significant for nations' nutrition for ages. They play an important role in diets of humans throughout the world.

This thesis involves eight chapters consisting of introduction, literature review, materials and methods followed by 4 separate chapters of results and discussions consisting of: proximate analysis, physicochemical properties and functional properties of flour mix samples, CGEP added bread, CGEP added extrudates, comparison of functional properties for those cereal products, and finally conclusion chapter. Following the introduction on the scope of the study, a short literature review about the current knowledge on cereal products especially bread and extruded products and phenolic compounds commonly found in grapes and recent studies on cereal products involving different fruit sources were covered.

In Chapter 3, all materials and methods used in the study were explained in detail. Six different flour mix samples were prepared to evaluate the interactions of CGEP and high amylose corn starch (HACS) and six other flour mixes were prepared to evaluate the interactions of CGEP and wheat protein isolate (WPI). Amylose substituted flour mix samples comprised of hard wheat bread flour substituted at three different levels of HACS together with 7% CGEP and without CGEP. For preparation of protein substituted samples three different levels of WPI together with 7% CGEP and without CGEP were used.

Results of proximate analysis and physicochemical tests were given In Chapter 4, and results revealed that, ash content in samples with CGEP and protein content in WPI substituted samples were significantly higher than the other samples ($p < 0.05$). Resistant starch content of HACS substituted samples were higher than the rest of the samples. No significant difference was detected in the total starch contents

In Chapter 5, first rheological dough characteristics and bread-making qualities of bread samples baked for protein treatment were evaluated to elicit possible interactions mainly due to WPI and CGEP. Bread samples with only CGEP (as in GP1 breads) were firmer (3-4 times firmer), while together with CGEP and WPI addition, breads became softer in texture. Increases in WPI content within control samples made significant increases in loaf volumes. Same parameters were also evaluated with bread samples baked using CGEP and HACS. According to the results, firmness of the bread samples increased with CGEP and HACS to higher levels. Volume and specific volume measurements decreased significantly with the use of CGEP and CGEP + HACS in combination ($p < 0.05$). The functional properties

of bread samples started with the results on functional properties of ingredients. CGEP (7%) substitution increased the amount of total phenolics around to a 3 times higher level. Besides, flour with CGEP (7%) had also a significantly higher antioxidant activity, flavonoid and anthocyanin contents with respect to control wheat flour after substitution. In the last part of the chapter, the functional properties of individual parts of crust, crumb and dough of bread samples with CGEP and together with WPI and HACS were evaluated. Total phenolics and total antioxidant activity (both DPPH and ABTS radical scavenging activity) significantly increased after CGEP substitution in comparison to no CGEP samples. Loss (%) of phenolics and antioxidant activities due to baking in comparison to unprocessed flour samples mixes were higher in samples without CGEP, although HACS or WPI substitution made no significant recoveries in % losses in samples with CGEP. In CGEP substituted samples, phenolic content of dough samples were significantly higher than that of crumb and crust parts ($p>0.05$). Increasing levels of WPI substitution decreased the total flavonoids in crumb and dough significantly ($p<0.05$). Gallic acid was also dominant phenolic acid in CGEP substituted bread samples. Syringic acid, p-coumaric acid, sinapic acid and epigallocatechin were also present in CGEP added bread samples but the content of these compounds were much lower (around 10-15 $\mu\text{g}/100\text{ g DW}$). Rutin was the most abundant flavonol in CGEP added bread samples, although it was lost as much as 25 % during baking. For rutin and quercetin-3- β -glucoside, increase in WPI level had a preserving effect. On the other hand, significant decreases in delphinidin-3-o-glucoside, delphinidin, cyanidin-3-o-glucoside, and cyanidin-3,5-diglucoside in particularly crust of bread samples were evident. No change in cyanidin was detected. Level of HACS substitution made no significant effects on measured phenolic content of these samples ($p>0.05$). Level of HACS substitution made no significant effect on the antioxidant activity of those samples ($p>0.05$). Before HACS substitution, bread sample with only CGEP seemed to have an insignificant difference in flavonoid content between parts of bread samples and dough ($p>0.05$). However, HACS seemed to decrease the measured amount of flavonoids in the crust and crumb. Therefore, amylose substitution decreased flavonoids significantly ($p<0.05$) in crust and crumb but made no effect for the dough. Flavonoid contents of the crust and crumb decreased with the increase in HACS level, whereas for dough no change was seen. Although the anthocyanin content of bread samples with only CGEP substitutions were statistically similar ($p>0.05$), dough had significantly higher total anthocyanins than crust and crumb of bread after HACS addition. For some phenolic compounds; such as procatechuic acid, epigallocatechin, catechin hydrate, vanillic and p-coumaric acids, crumb had higher concentrations than dough. Flavonols displayed no significant differences among crumb, crust and dough ($p>0.05$). However significant differences were observed between the anthocyanin profiles of crust, crumb and dough of bread samples. Generally crusts had lower amounts of anthocyanin compounds, as temperature during baking was the highest one among different parts. On the other hand; HACS substitution seemed to have no effect on the remaining flavonols and anthocyanins. Only exception was malvidin, since the malvidin content increased with the increase in HACS substitution.

In the next chapter (Chapter 6) first of all the effects of CGEP and WPI on quality parameters of hard wheat flour extrudates were investigated. Results revealed that the only parameter effective on quality was the temperature; especially at 150°C, differences between formulations were more distinct for (diametric expansion, bulk

density) when compared to lower die temperatures (90 and 120°C). However, pasting properties were detrimentally affected by the single addition of CGEP with respect to single WPI substitution. Presence of both CGEP and WPI made the pastes even weaker (34.1 to 61.1% decrease in peak viscosity). Therefore, in extrudates, loss of protein content by means of CGEP substitution did not exert a significant quality loss and even by increasing the protein level no improvement in quality parameters was obtained. Chapter 6 also comprised the effects of CGEP, HACS, and their combinations on quality parameters of extruded products. Extrusion temperature was found to have more distinct effect on physical quality parameters of extrudates than the substitution level of ingredients. Both CGEP and HACS additions negatively affected pasting properties, slightly affected resistant starch content and prevented gelatinization. However retardation of retrogradation was more evident when substitution was with CGEP alone rather than its combination with HACS. The last part of Chapter 6 was on the evaluation of the functional properties of the extrudates. Measured amount of phenolics increased significantly with the increase in WPI level. In CGEP substituted samples the total antioxidant activity of samples increased significantly. Extrusion temperature made a significant decrease in the antioxidant activity only at 150°C ($p < 0.05$). Substitutions with WPI made a significant increase of ABTS radical scavenging activity only in samples extruded at 90°C ($p < 0.05$). In samples with CGEP, antioxidant activity was significantly lower ($p < 0.05$) at 150°C than the other extrusion temperatures. Substitutions of WPI and gradual increase of substitution level seemed to increase the total flavonoid content in all different extrusion temperatures, although the differences were not statistically significant ($p > 0.05$). Increase in extrusion temperature had a detrimental effect on phenolics and flavan-3-ols, generally. Gallic acid increased significantly with the WPI level in CGEP substituted samples. HACS substitutions also decreased the antioxidant activity significantly ($p < 0.05$). Neither the effect of HACS levels, nor the extrusion temperature was found significant on the anthocyanin level measured.

In Chapter 7, the results of comparison of bread crumb samples and extrudate samples with CGEP revealed that anthocyanin compounds were better retained in bread samples. HACS substitution better retained the phenolic compounds in extrudates, while WPI gave better results in bread samples. After HACS or WPI substitutions flavonoid content of bread crumbs decreased about 50%. For samples extruded at 150°C on the other hand, flavonoid contents were better retained as about 20 % increase was observed. WPI substitutions had detrimental effects on total anthocyanin content, whereas HACS act as an improver for the measured anthocyanin contents both for bread crumb and extruded product samples

As a conclusion of this thesis (Chapter 8) it was obtained that addition of CGEP increased the total phenolic content of extrudates about 10-fold, whereas for bread it provided 10-14 fold increase with respect to no-CGEP added products. So it can be said that addition of CGEP increased the functionality of the cereal products in a significant manner. However WPI substitution besides CGEP gave better quality in bread samples whereas for extrudates CGEP addition had not so much detrimental effect on the quality. WPI level of 6.5% seemed to preserve the bioactive compounds in a better way with respect to other levels of substitution. Whereas for HACS substitutions no specific was evident for different substitution levels.

FONKSİYONEL ÜZÜM EKSTRAKTI İÇEREN EKMEK VE EKSTRÜDE ÜRÜNLERİN FONKSİYONEL ÖZELLİKLERİ VE KALİTE PARAMETRELERİ

ÖZET

Hububat ürünleri, başta ekmek olmak üzere, insanlar için yüzyıllardır çok yüksek önem teşkil eden ürünler arasındadır. Bu ürünler ayrıca uygun yapıları nedeniyle farklı birçok formülasyon kullanılarak üretilmekte ve sağlık için daha da faydalı hale gelebilmektedir. Concord üzüm ekstraktı tozu (CGEP), benzer diğer meyve kaynakları gibi çok sayıda farklı fenolik madde içermekte olup, özellikle içeriğindeki antosiyanin, flavanol ve flavonol bileşikleri dikkat çekmektedir. Buna bağlı olarak, bu önemli kaynağın hububat ürünlerinde kullanımı ile fonksiyonel özelliklerin artırılması hedeflenmektedir. Hububat ürünleri birbirinde farklı çok sayıda ürünü içerdiğinden, çalışma kapsamında tek bir hububat ürünü yerine farklı üretim yöntemleri ile üretilmiş ve birbirinde tamamen farklı yapılardaki iki ürünün ele alınması hedeflenmiştir. Bu amaçla, ekmek ve ekstrüde atıştırmalık ürünler aynı un karışımları kullanılarak üretilmiştir. Ürünlerin fonksiyonel özellikleri yanında, kalite özelliklerindeki değişimler ve hububat ürünlerinde bulunan iki önemli makromolekülün (protein ve yüksek amiloz içeren nişasta) CGEP ile etkileşimi ve fonksiyonel özellikler üzerindeki etkilerinin incelenmesi hedeflenmiştir.

Tez giriş, literatür özeti, materyal metot bölümleri ile temel bileşen analizleri, un karışımı örneklerinin fizikokimyasal ve fonksiyonel özellikleri, CGEP eklenmiş ekmek, CGEP eklenmiş ekstrüde ürün ve hububat ürünlerinin fonksiyonel özelliklerinin karşılaştırılması olmak üzere dört ayrı tartışma bölümünden ve sonuç bölümünden oluşmaktadır. Girişte çalışmanın kapsamıyla ilgili genel bir giriş yapılmasının ardından, literatür özeti ile ekmek ve ekstrüde ürünler başta olmak üzere mevcut hububat ürünleri, üzümde bulunan fenolik bileşikler ve meyve kaynaklarını içeren hububat ürünlerine ilişkin güncel çalışmalar özetlenmiştir.

Bölüm 3'te, çalışmada kullanılan tüm materyal ve metotlar özetlenmiştir. Yüksek düzeyde amiloz içeren mısır nişastası (HACS) ve CGEP etkileşimin anlaşılması amacıyla altı farklı un karışımı ve CGEP ve protein etkileşimin incelenmesi amacıyla üç farklı düzeyde buğday proteini izolatu (WPI) bir diğer altı un karışımı örnekleri ve bu örneklerle %7 oranında CGEP eklenmesiyle elde edilmiş örneklerden oluşmaktadır. Çalışmada kullanılan ekmekler AACCI 10-10.2 yöntemi kullanarak üretilirken, ekstrüzyon denemelerinde çift vidalı ekstrüder, sabit vida hızında (360 RPM) ve %25 giriş nem miktarında ve farklı ekstrüzyon sıcaklıklarında (150°, 120° ve 90°C) çalıştırılmıştır. Un örneklerinin temel bileşen analizleri kapsamında toplam nem, kül ve protein miktarları belirlenmiştir. Ayrıca toplam nişasta, toplam dirençli nişasta ve amiloz/amilopektin miktarı enzimatik/gravimetrik yöntemlerle belirlenmiştir. Un karışımı örneklerinin su kaldırma özellikleri farinograf kullanılarak, jel oluşturma özellikleri ise RVA Hızlı Visko Analizörü (RVA) yardımıyla belirlenmiştir. Daha sonra pişmiş ekmeklerde son ürün kalitesinin

belirlenmesi amacıyla ekmek içi elastikiyet ölçümü için doku analizörü kullanılmış, ekmek hacmi ve ekmek özgül hacmi ölçümleri ile kromametre ile renk özellikleri ve Taramalı elektron mikroskobu (SEM) ile mikroskobik görünüş özellikleri incelenmiştir. Ekstrüde ürünlerde ise, spesifik mekanik enerji (SME), jel oluşturma özellikleri (RVA ile), sertlik (doku analizörü ile), yığın yoğunluğu, genleşme, su kaldırma, dirençli nişasta ve termal özellikler (Diferansiyel Taramalı Kalorimetre (DSC) kullanılarak) ölçümleri yapılarak, kalite parametreleri test edilmiştir. Ekmek ve ekstrüde ürünlerin fonksiyonel özelliklerin belirlenmesi tezin bir sonraki aşamasını oluşturmaktadır. Buna göre, toplam fenolik madde tayini için Folin-Ciocalteu metodu, toplam antioksidan tayini amacıyla ise DPPH ve ABTS radikal yakalama yöntemleri uygulanmıştır. Bunun yanında toplam flavonoid ve toplam antosiyanin miktarı da belirlenmiştir. Spektrofotometrik yöntemlerin yanında, kromatografik yöntemler (RP-HPLC-PDA) kullanılarak fenolik bileşenlerin daha ayrıntılı olarak incelenmesi de mümkün olmuştur. Örnekler arasındaki farklılıklar tek yönlü varyans analizi (ANOVA) ve Duncan's New Multiple Range Test ile ölçülmüştür.

Bölüm 4'teki sonuçlara göre, CGEP eklenmiş örneklerdeki toplam kül içerikleri ile WPI eklenen örneklerin protein içerikleri diğer örneklerle göre önemli düzeyde yüksek bulunmuştur. HACS içeren örneklerde ise toplam dirençli nişasta miktarı diğer karışımlara oranla yüksek bulunmuştur ($p < 0.05$). Örneklerin toplam nişasta miktarları birbirinden farklı değildir ($p > 0.05$). Amiloz miktarları ise, beklendiği üzere, HACS içeren örneklerle korale olarak yüksek olup, sert buğday unu (A1), ve sert buğday unu ve sadece CGEP içeren (GA1) örnekleri için sırasıyla, %26.5 ve %20.6 düzeyinde iken, HACS ikamesi ile sırasıyla %41.7 ve %31.0 düzeylerine yükselmiştir. HACS ve WPI içeren örneklerin su kaldırma miktarları diğer örnek karışımlarına göre yüksektir. CGEP ikamesi onların su kaldırma yüzdelerini önemli düzeyde etkilememiştir ($p > 0.05$) (%53.6 ve %55.7). CGEP'in un örneklerinin jel yapısını bozan en önemli etken olduğu belirlenmiştir. WPI veya HACS ilavesi ile yapıda herhangi bir iyileşme görünmezken, en zayıf yapı CGEP ve HACS'ın birlikte kullanılmasıyla elde edilmiştir.

Bölüm 5'te öncelikle, protein denemelerinde CGEP ve WPI arasındaki etkileşimler esas alınarak değişen düzeylerde (%0, 6.5 ve 13) WPI ikamesi yapılan un karışımı örnekleriyle üretilen hamur ve ekmeklerin kalite özelliklerindeki değişiklikler incelenmiştir. Protein miktarındaki artışlar kontrol ekmeklerinde önemli bir etki yaratmazken, CGEP eklenmesiyle ekmek sertliği önemli düzeyde artmaktadır ($p < 0.05$). Sadece CGEP ikamesi içeren ekmek örnekleri (GP1) daha sert iken (3-4 kat), CGEP ve WPI kullanımı ekmeklerde bir miktar yumuşama sağlamıştır. Ekmek hacimlerinin birbirinden önemli düzeyde farklı oldukları bulunmuş olup artan WPI ikamesi ekmeklerin protein miktarını arttırırken aynı zamanda hacimleri de (803, 886 ve 1073 cm³, sırasıyla) artmıştır. CGEP eklenen örneklerde ise hacimler önemli düzeyde düşüktür. Buna göre CGEP içeriğindeki fenolikler hacimdeki artışların önündeki en önemli engel olmuş, eklenen WPI ise bu durumda bir miktar iyileştirme gerçekleştirmiştir. Benzer etkiler özgül hacim ölçümlerinde de gözlenmiştir. Bir değer deneme serisi de CGEP ve HACS içeren örneklerle uygulanmış ve bu un karışım örnekleri kullanılarak üretilen ekmekler de aynı özellikler irdelenmiştir. Buna göre, CGEP ve CGEP + HACS ikamelerinin birlikte yapıldığı örneklerde ekmek hacmi önemli düzeyde düşmüş olup ekmeklerin kabul edilebilirlikleri de düşmüştür. Buna ek olarak ekmek sertliği de önemli düzeyde artmıştır. Bu bölümün devamında öncelikle hammaddelerin fonksiyonel özellikleri ele alınmaya

başlamıştır. CGEP ve ekmeklik sert buğday unundaki toplam fenolik madde içerikleri 34623.8 ± 2934.7 mg GAE/ 100 g kuru madde ve 721.8 ± 6.5 mg GAE/100 g kuru madde, olarak bulunmuştur. CGEP ikamesi (7%) ile, CGEP içerek sert buğday unundaki toplam fenolik madde miktarı 2374.2 ± 89.2 mg GAE/100 g kuru madde olmuştur. Böylelikle, CGEP (7%) ikamesinin toplam fenolik madde içeriğini % 330 civarında arttırmış olduğu görülmüştür CGEP ikamesi ayrıca antioksidan aktivite, toplam flavonoid ve antosiyanin miktarlarını da kontrol ununa göre önemli düzeyde attırmıştır. Bu bölümün son kısmında ise CGEP ile WPI veya HACS eklenen ekmeklerin kabuk, ekmek içi ve fermente hamur (pişirilmemiş) kısımlarındaki fonksiyonel özellikleri incelenmiştir. Elde edilen sonuçlara göre, CGEP ilavesiyle ölçülen toplam fenolik madde ve antioksidan aktivite miktarları en az 7-8 kat artmış olup, artışlar istatistiksel olarak önemli bulunmuştur ($p < 0.05$). Sadece WPI eklenen ancak CGEP eklenmeyen örneklerde, fenolik madde içeriğinde ve antioksidan aktivite miktarlarında pişme sonrası kayıplar (%) daha yüksek iken, HACS veya WPI ikamesinin kayıpların azaltılmasında önemli bir rolü olmadığı belirlenmiştir. CGEP içeren örneklerin hamur kısmındaki toplam fenolik madde miktarı ekmek içi ve kabuğundan önemli düzeyde yüksektir ($p > 0.05$). Buna karşın, WPI miktarının artması ile ekek içi ve hamurdaki toplam flavonoid miktarları azalmıştır ($p > 0.05$). CGEP içermeyen kontrol örnklerinde HPLC-PDA sonuçlarına öore, fenolik madde olarak protokateşuik asit, gallik asit, sinapik asit, vanillik asit, p-kumarik asit ve ferulik asit belirlenmiştir. Gallik asit ve vanillik asit miktarlarının pişme sonrası önemli düzeyde arttığı tespit edilmiştir (yaklaşık 2-kat). Buna gore, CGEP içermeyen örneklerde WPI ikamesindeki artışın ekmek kabuğu, içi ve fermente hamurdaki fenolik maddeler üzerinde önemli bir etkisi bulunmadığı belirlenmiştir. Gallik asit CGEP içeren ekmek örneklerinde de en önemli fenolik asit olmuştur. Ayrıca sirinjik asit, p-kumarik asit, sinapik asit ve epigallokateşin de tespit edilmiştir ancak bu bileşenlerin miktarı çok daha düşüktür (yaklaşık 10-15 $\mu\text{g}/100$ g kuru madde). Rutin bu örneklerdeki en önemli flavonol olmasına karşın pişme sonra % 25 düzeyinde kayba uğramıştır. WPI ikamesindeki artışın rutin ve kuersetin-3- β -glukozid üzerinde bir koruyucu etkisi olduğu görülmüştür Buna karşın, özellikle ekmek kabuğunda bulunan delfinidin-3-o-glukozid, delfinidin, siyanidin-3-o-glukozid, ve siyanidin-3,5-diglukozid miktarlarında önemli düşüşler olmuştur. Siyanidinde ise önemli bir fark görülmemiştir. HACS ikamesi örneklerin toplam fenolik madde içeriklerinde önemli bir fark oluşturmamıştır ($p > 0.05$). Sadece HACS ikamesi içeren kontrol örnekleri, HACS and CGEP içeren örneklere göre daha düşük miktarda fenolik madde içermelerine karşın ($p < 0.05$), HACS miktarı fenolik madde düzeyinde önemli bir etki oluşturmamıştır ($p > 0.05$). Ayrıca, HACS miktarı bu örneklerin antioksidan aktivite düzeylerinde de önemli bir etki oluşturmamıştır ($p > 0.05$). Pişmeye bağlı kayıp (%) CGEP içermeyen ekmek içi örneklerinden yüksektir (%77). HACS ikamesi genel olarak antioksidan aktivite kaybının (%) önlenmesinde önemli bir etki yaratmasına karşın, %20 HACS içeren hamur ve ekmek kabuğu örneklerinde istisnai bir durum gözlenmiştir. Örneklerin flavonoid miktarları incelendiğinde, sadece CGEP içeren ancak HACS ikamesi içermeyen örneklerde ekmeklerin farklı kısımlarındaki flavonoid düzeylerinde fark bulunmadığı belirlenmiştir ($p > 0.05$). Buna karşın, HACS ikamesi ekmek kabuğunda ve ekmek içinde bir azalmaya neden olmuştur. Bu nedenle, HACS ikamesinin ekmek kabuk ve içindeki flavonoid miktarını önemli düzeyde azaltırken ($p < 0.05$), hamurdaki miktarı etkilemediği belirlenmiştir. Sadece CGEP içeren farklı ekmek kısımlarında antosiyanin miktarları birbirinden farklı değilken ($p > 0.05$), HACS ikamesi sonrasında hamurdaki antosiyanin miktarının diğer kısımlara göre yükseldiği görülmüştür ($p < 0.05$). HPLC sonuçlarına göre,

CGEP içermeyen kontrol örneklerinde en fazla gallik asit (2.4 to 18 µg/100 g kuru madde) ve vanillik asit (3.38 to 12.39 µg/100 g kuru madde) bulunmaktadır. Protokatesuik asit, epigallokateşin, kateşin hidrat, vanillik ve p-kumarik asit gibi bazı fenolik maddelerin miktarları ekmek içinde hamurdan daha fazladır. HACS ikamesi CGEP eklenmeyen ekmeklerde önemli bir fark oluşturmamıştır. CGEP eklenen ekmeklerde kuersetin-3-β-glukozid, delfinidin 3-O-glukozid, siyanidin 3-O-glukozid ve bunların diglukozidleri ile, delfinidin, siyanidin ve malvidin antosiyanidinleri tespit edilmiştir. Flavonoller farklı kısımlardan elde edilen ekmek örneklerinde değişkenlik göstermemiştir (p>0.05). Ancak, farklı ekmek kısımlarının antosiyanin profillerinde önemli farklar gözlenmiştir. Genel olarak ekmek kabuğunda daha az antosiyanin bulunmuştur. Buna karşın, HACS ikamesi pişme sonrası kalan flavonol ve antosiyanin miktarı üzerinde etkili olmamıştır. Bu durumdan farklı tek bileşik mavidin olup, malvidin miktarı HACS ikamesi ile bir miktar artış göstermiştir.

Sonraki bölümde (Bölüm 6) öncelikle WPI ve CGEP ikamesiyle üretilen ekstrudatların kalite özellikleri belirlenmiştir. Elde edilen sonuçlara göre CGEP ekstrüde ürünlerin kalite özelliklerinde önemli düşüşlere neden olmazken, bu ürünlerdeki kalite parametreleri için esas kritik olan değişkenin ekstrüzyon sıcaklığı olduğu görülmüştür. Bu ürünlerde özellikle 150 °C’de, genel olarak tüm kalite özelliklerinde önemli kayıplar kaydedilmiştir. Ancak önemli düzeydeki düşüşler özellikle genleşme ve yığın yoğunluğunda ölçülmüştür. Jel özellikleri ise önemli düzeyde kaybedilmiştir, sadece CGEP eklenen örneklerde pik viskozitede % 66.7 düzeyinde bir düşüş ölçülmüşken, CGEP ve WPI eklenen ürünlerde jel daha da zayıflamıştır. Ürünlerin bayatlama özellikleri ise CGEP ve WPI varlığından etkilenmemiştir. Buna göre CGEP kullanımı sebebiyle gerçekleşen protein ikamesinin ekstrüde ürünlerde önemi bir kalite kaybına yol açmadığı görülmüş olup, ekmekten farklı olarak protein miktarının ekstrüde ürünleri kalite özellikleri için çok kritik bir özellik olmadığı belirlenmiştir. Aynı bölümüm devamında ise CGEP ve HACS ikamelerinin ekstrüde ürün kalitesi üzerindeki etkileri incelenmiştir. İlk kısma benzer biçimde, su kaldırma, yığın yoğunluğu, genleşme ve sertlik gibi fiziksel özelliklerin yanında, termal özellikler, jel oluşum özellikleri ve dirençli nişasta miktarı ölçümleri gerçekleştirilmiştir. 90, 120 ve 150°C ekstrüzyon sıcaklıklarında ortalama sertlik; 0.916, 0.987 ve 0.467 N; su kaldırma, 2.12, 4.07 ve 5.12 ml su/g; genleşmeler 1.35, 2.09 ve 2.51 ve yığın yoğunlukları 1286.6, 723.6 and 311.1 kg/m³ olarak ölçülmüştür. Ekstrüzyon sıcaklığı bu örneklerin kalitelerinde de formülasyon etkisinde daha belirleyici olmuştur. CGEP ve HACS’ın birlikte kullanımı jel oluşturma özelliklerin daha olumsuz etkilerken, dirençli nişasta miktarında bir miktar değişiklik oluşturmuş ve örneklerde jelatinizasyonu engellemişlerdir. Ancak bayatlamada amilozun bir miktar olumlu etkisi olduğu gözlemlenmiştir. Bu bölümün son kısmında ise WPI ve CGEP içeren ekstrüde ürünler ise HACS ve CGEP içeren ekstrüde ürünlerde toplam fenolik madde, toplam antioksidan aktivite, toplam flavonoid ve toplam antosiyanin miktarları belirlenmiş ve RP-HPLC PDA kullanılarak ürünlerin fenolik madde karakterizasyonları gerçekleştirilmiştir. Gallik asit örneklerdeki en önemli fenolik asit olmuştur. Gallik asit yanında, protokatesuik asit, ellajic asit, siringik asit, vanilik asit ve klorojenik asit de belirlenmiştir. Ayrıca, 312 nm’de kaffeik asit p-coumaric asit, ve ferulic asit) bulunmuştur. Flavan-3-ollerden (+)-kateşin ve izomer (-)-epicatechin, (-)-epigallokateşin, ve (-)-epikateşin-3-O-gallat da belirlenmiştir. Kuersetin ve kuersetin-3-β-glukozid, myrisetin ve rutin flavonolları ile siyanidin, malvidin, delfinidin aglikonları ve delfinidin-3-glukozid,

siyanidin-3-o-glukozid, siyanidin-3,5-diglukozid,delfinidin-3,5-diglukozid, malvidin-3,5-diglukozid de belirlenmiştir.

Çalışma sonuçlarına göre, CGEP önemli bir fonksiyonel kaynak olmasına karşın, hamur ve ekmek kalitesinde önemli bir bozulma faktörü olarak rol almaktadır. HACS kullanımı ekmek kalite özelliklerinde olumsuz anlamda daha fazla etki yaratmıştır. CGEP ve WPI kullanımı ise bir iyileştirme alternatifi olarak dikkat çekmiştir. Ekstrüde ürünlerinin kalite özellikleri ise ekmekten farklı olarak CGEP varlığından daha az etkilenmiştir. HACS kullanımı bu ürünlerde bayatlamayı geciktirme ve dirençli nişasta miktarını arttırma anlamında önemli bir rol oynarken, WPI belirgin bir etki yaratmamıştır. Fenolik maddelerin yapılarına bağlı olarak ekmek ve ekstrüde ürünlerde farklı düzeylerde korunduğu belirlenmiştir. Antosiyanin bileşenleri ekstrüde ürünlerde HACS varlığında daha iyi korunmuş olmalarına karşın, ekmeklerde pişme sonrası kalan miktarlar ekstrüde ürünlerden (150°C) önemli düzeyde yüksektir. Ekstrüde ürünlerde ekstrüzyon sıcaklığı toplam fenolik madde miktarı ve antioksidan aktivite üzerindeki en önemli değişkendir. Genel anlamda 150°C’de elde edilen ölçümler diğer sıcaklıklardan farklı iken, HACS ikamesi ile 120 °C ve 150°C’deki fark azalmıştır.

1. INTRODUCTION

Cereal products are commonly considered as staple food for human beings and have been significant for nations' nutrition for ages. They play an important role in diets of humans throughout the world.

Cereals generally require thermal processes such as baking, roasting, extrusion etc. before they are consumed. Commercially available cereal products include bread, specialty bread, pasta, noodles, cakes and muffins, biscuits and cookies, and extruded snacks, breakfast cereals, etc. Various physical, chemical, and biological changes such as starch gelatinization, protein denaturation and component interactions take place during processing and storage of such products. By means of processing, organoleptic properties improve and nutrients become bioavailable in addition to inactivation of heat labile toxic compounds and enzyme inhibitors (Rosell, 2011).

Cereal products are complex systems that are composed of various ingredients that are interacting with each other. Starch serves as the main storage carbohydrate in cereal crops and exists in an insoluble granular form in the cereals. Another key constituent in cereal flour, by means of its functional properties and nutritional value, is protein. Detailed evaluation of the manipulating ingredients, such as proteins and starch, in addition to processing conditions has been significant for cereal products in many different perspectives. These two ingredients found in cereal products remarkably affect the textural and sensory qualities of the final product.

Current food research has moved progressively toward the development of novel functional foods as alternatives to traditional and/or natural ingredients. Functional foods are considered as food alternatives which possess specific benefits for health among their nutritional aspects by enhancing one or more physiological functions and/or providing direct reduction of a disease risk. A functional food product might be defined as (Ashwel, 2002):

“(1) a food product containing enhanced levels of one or more of certain components; (2) a food product from which undesirable, unhealthy components have been removed; (3) a food product in which the properties of one or more of the potentially health-inducing ingredients have been modified to improve bioavailability and/or bioactivity; and (4) a combination of one or more of these three options (1) to (3)” although official channels such as European legislation still does not consider functional foods as specific food categories, but rather a concept (Siro et al., 2008). Therefore as a result of the consumer demands toward functional foods in recent years, a very significant expectation is for functionality of the cereal products (Hayta and Özüğür, 2011). Thus, cereal products are the focus of research as they enhance many positive attitudes on the prevention of many chronic diseases such as cardiovascular diseases and diabetes. Moreover they enable the incorporation of functional components into the product formulations. These functional components are most abundantly found in colored fruits and vegetables.

Bread is a popular staple food consumed globally, which includes various types of products having different shapes, sizes, texture, crust color, softness, taste, and flavor. Bread ingredients remarkably affect the textural and sensory qualities of the final bread product. Attempt for increasing the functional properties of bread is a very common approach for the food industry.

Extrusion is another common approach for the production of various products by food industry, in which a rotating screw applies shear energy while the extruder barrel is heated, enabling the temperature of the food material to increase. The food becomes “liquefied” as it is conveyed through a die or series of dies under high pressure and changes into a final extrudate which is a different product from the raw material in terms of its novel texture and desirable sensory attributes. Therefore, extrusion is of particular interest as the products may have various different properties when compared to the baked products. Main cereal components; starch and protein are assumed to be easily applicable in extrusion processes, however control over the end product characteristics and quality requires much effort. In addition, nature of extrusion process also enables the easily incorporation of functional components into the product formulations.

According to the above information obtained from previous studies data obtained from different studies might not reflect the similar results neither for other bakery

products nor for other processes. Moreover, in previous studies, possible interactions of large molecules in the food system, which might be important for future applications, were not determined. Accordingly, further research on the interactions between regular and functional ingredients during extrusion under different processing parameters and quality changes of the cereal products was suggested (Brennan, 2013). Elucidating the interaction of baking process with phenolics and other phytochemicals is necessary as it is not still certain. It can only be possible if the studies include different bakery products as much as possible. Moreover, specific properties of distinct phytochemicals (such as instability of anthocyanins) might give different results as well, since these components are also widely added into cereal products. However, the consensus about interaction mechanisms of those ingredients in such products and the way they are affected from the processes are still not clear. Therefore investigating the effects of such ingredients in the simplest food systems possible and on basic elements is scientifically important.

Therefore, in this thesis, it is hypothesized that addition of anthocyanins and other bioactives from Concord grape extract powder during the production of extruded products and bread, at same proportions, will be beneficial for understanding the interactions between the bioactive components of fruits and structural changes occurring in the product matrix. Although some studies about cereal products are available in literature, they contain some inconsistencies about the effects of bioactives. Therefore, trials consisting of bread and extruded products using same raw materials will be helpful for better understanding of the significant mechanisms between healthy components and cereal products. Moreover, in previous researches possible interactions related to large molecules in the food system, which might be important for future applications, were not determined. Accordingly, further research on the interactions between regular and functional ingredients during extrusion under different processing parameters was conducted and quality changes of the extruded products were evaluated.

With respect to the above remarks and the hypothesis, two objectives given below were selected. The first objective of this thesis is the characterization and quantification of the remaining bioactives in the bread and extruded product and to compare the effects/interactions of amylose and WPI substitutions on the remaining bioactive components. The second objective will comprise how the anthocyanins

and other bioactive components affect the rheological and quality properties of the bread/dough properties and extruded product. The final objective will be the comparison of these two functional cereal products by means of interactions, functionality and quality.

This research thesis is presented as;

1. Introduction
2. Literature Review
3. Materials and Methods
4. Results and Discussion on Proximate Properties of Flour Mixes
5. Results and Discussion on Concord Grape Extract Powder (CGEP) Added Bread
 - 5.1. Effect of protein on CGEP added bread quality
 - 5.2. Effect of amylose on CGEP added bread quality
 - 5.3. Evaluation of CGEP added bread as a functional food
 - 5.3.1. Effect of CGEP
 - 5.3.2. Interaction of CGEP with protein
 - 5.3.3. Interaction of CGEP with amylose
6. Results and Discussion on CGEP Added Extrudate
 - 6.1. Effect of protein on CGEP added extrudate quality
 - 6.2. Effect of amylose on CGEP added extrudate quality
 - 6.3. Evaluation of CGEP added extrudate as a functional food
 - 6.3.1. Effect of CGEP
 - 6.3.2. Interaction of CGEP with protein
 - 6.3.3. Interaction of CGEP with amylose
7. Comparison of functional properties of cgep added bread and extrudate samples
8. Conclusion

2. LITERATURE REVIEW

This chapter of this thesis comprised a short literature review about the current knowledge on cereal products especially focusing on bread and extruded products. Moreover, it involves a short presentation of different phenolics commonly found in grapes and summarizes recent studies on cereal products involving different fruit sources.

2.1 Cereal Products

Cereals and cereal products are the most abundant components of human diet for ages. Generally, cereal grains are subjected to different processes to convert them into suitable products for human consumption and those processes usually influence their chemical and nutritional properties. Cereals are important sources of dietary proteins, carbohydrates, B group vitamins, vitamin E, iron, other trace minerals, and fibers. According to FAO (2007), they provide approximately 45% of protein and energy requirement of the human diet, whereas wheat alone is able to provide as much as 20% of protein and energy requirement of the human diet. Wheat and rice are the two most significant cereal grain commodities in the world, although they are produced at lower amounts than corn (Rosell, 2011).

2.2 Bread

2.2.1 General information

Bread is considered and consumed as a staple and convenient food by almost all nations around the world and consumed in relatively large amounts for more than 4000 years (Barrett, 1975; Cauvain, 2004). It is in the base of the food pyramid, and its consumption is recommended in all dietary guidelines. Bread is a complex system, in which a dough typically composed of flour, water, yeast and salt are mixed, kneaded, proofed and baked (Sluimer, 2005). However, bread types vary

widely depending on the traditional preference, raw materials and baking techniques. Although bread making include stages of mixing the ingredients, dough resting, dividing, shaping, proofing, and baking, mainly; stages between mixing and baking steps are the most critical dependents for the types of bread. These differences primarily affect gluten and thus change bread dough structure besides starch, which is important for mainly the final bread textural properties and stability. Breads are supplying energy but also they are sources of dietary fiber, nutritious protein and lipids rich in essential fatty acids. Wheat (*Triticum aestivum*) is by far the dominant grain used for bread making because of its supreme baking performance in comparison with all other cereals, although other sources are also used.

2.2.2 Functional breads with phenolics

Attempt for increasing the functional properties of cereal products and particularly bread is a very common approach for the food industry, as the consumer demand has a tendency toward functional foods in recent years. This trend is also not so difficult to reach from the economic point of view. That's why so much research focused on this particular aim.

However, the interaction between phytochemicals and rheological properties is double sided, most studies in literature were interested in the changes in phenolic acid levels as a result of baking (Gelinas and McKinnon, 2006; Koh and Ng, 2009; Sivam et al., 2011) in addition to differences in dvarious steps of baking (Moore et al., 2009) or different parts of the final product (e.g. crust and crumb) (Gelinas and McKinnon, 2006).

Generally thermal processing of food products is expected to lower antioxidant activity due to the heat instability of the food ingredients, although contradictory results were obtained for tomatoes and carrots in previous studies (Dewanto et al., 2002; Patras et al., 2009) which reported that heat processing had been considered as a factor to increase the antioxidant activity. This increase in antioxidant activity is through the breakdown of cell walls and other cellular constituents. In this way, bound phenolics are released and contribute to total antioxidant capacity (Dewanto et al., 2002). Browning reactions and other reactions such as Maillard reaction, caramelization reactions and chemical oxidation of phenols are also among contributors to the total phenolic content and antioxidant activity in these products.

Particularly browning reactions could help in dissociation of conjugated phenolic moiety during thermal processing followed by some polymerization and/or oxidation reaction and the formation of phenolics other than those endogenous in the grains (Ragae et al., 2014).

Maillard reaction is the most common browning reaction occurring as a result of the interaction between reducing sugars and protein during the baking process, which makes lysine unavailable and reduces the quality of the protein, but it was found to produce a dramatic increase in compounds that possess free radical scavenging properties in cookies (Bressa et al., 1996; Ragae et al., 2014).

Thermal processing is generally known as a detrimental factor for phenolics in bread (Friedman, 2004), although the results are contradictory for many products (Dewanto et al., 2002; Friedman, 2004; Gelinas and McKinnon, 2006). Most studies about the effects of phenolic acids in baking processes used the bread making process since it may be considered as the fundamental practice for bakery production. The action of phenolic acids during bread making is proposed to be similar to ferulates (phenolic groups present in wheat bran, which are responsible for the negative effect of wheat bran on dough rheology together with the soluble and insoluble fiber constituents of the bran). Since the nature of bread making process is composed of many specific determinants such as the presence of gluten and fermentation process, the action of ingredients is highly dependent on those phenomena. Fermentation, not like the gluten-related mechanisms, hydrolyses the bonds in antioxidants and releases them to scavenge more radicals (Saulnier et al., 2001). The occurrence of dough with a reduced volume mechanism for antioxidants is generally due to their reducing reaction on gluten disulfide cross-linkages. This activity reduces the dough stability by generating dough breakdown during mixing (Awika, 2011). Sivam et al., (2011) worked on different bread formulation models for understanding the conformational changes occurred after the addition of polyphenol rich fruit extracts, pectin and bread ingredients. They used a control bread sample without added functional ingredients, in addition to samples produced by changing the same amount of flour with a combination of pectin and a fruit extract. Also water content of the samples was increased by 20% in the second group, whereas the rest of formulation differences were retained. Their result also showed that significant amount of polyphenols retained in the finished products after baking, while increase in water content

decreased the total phenolics detected in the products, although varying between different fruit extracts. Many possible reasons such as degradation by temperature or complex formation with either wheat proteins or polysaccharides were related to these changes. Their results by FTIR spectroscopy revealed a modification in the secondary protein conformation (Amides I and II). And it may be supposed that those changes detected by both of the FT-IR and Raman results had an impact on extractability and stability of the added polyphenols in the bread matrices (Sivam et al., 2011).

The change in the antioxidant activity of the phenolic acids during baking process is also very interesting, since not only the process conditions but also the type of the phenolic acid involved is significant. A very recent study (Han and Kohb, 2011) focused on how the individual phases of bread making process (mixing, fermentation and baking) influenced the antioxidant activity of certain phenolic compounds added (syringic acid, gallic acid, ferulic acid and caffeic acid) both in the flour dough and bread. Those compounds were also evaluated for their antioxidant potential in flour before the baking process. The mixing had a decreasing effect on the antioxidant activity (attributed to the physical destruction effects on bonds, interactions between the thiol free radicals of gluten and phenolic acids, and the action of the phenolic acid as a reducing reagent by Han and Kohb (2011) and other researchers (Mac Ritche et al., 1991)). On the other hand, fermentation and baking increased the antioxidant activity of the phenolics (Han and Kohb, 2011). Moreover, baking may cause the formation of novel compounds having antioxidant property, (for example Maillard reaction products); another possibility may be the formation of pro-oxidants lowering the total antioxidant capacity of the products (Nanditha and Prabhasankar, 2009).

In literature, a number of studies existed to evaluate the effects of chosen phenolic compounds on dough rheology. In their recent study, Han and Koha (2011) investigated caffeic, ferulic, syringic and gallic acids addition to hard wheat flour at a level of $4.44 \mu\text{mol L}^{-1} \text{g}^{-1}$ flour. They grouped the phenolic acids used in the study as the ones having high antioxidant activity (caffeic acids and ferulic acids) and low antioxidant activity (syringic acid and gallic acid) and supposed that their effects on dough rheology were consistent with their antioxidant activity. Therefore caffeic acid caused the most significant decrease in elasticity and extensibility of the dough. In

contrast, the effects of syringic and gallic acids, which are hydroxybenzoic acids, were less than the others. The effect of distinct phenolic acid added, on bread volume reduction was also in good correlation with their effect on elasticity (Han and Kohb, 2011). Moreover, it was also revealed that phenolics also influenced wheat proteins by rearranging them. Their results were in agreement with some previous findings, as well (Koh and Ng, 2009).

According to the study by Peng et al. (2010) who added grape seed extract, into bread formulations in three different levels (300 mg, 600 mg and 1 g), thermal processing decreased the antioxidant activity in grape seed extract added breads for 30-40% in comparison to standard solutions of grape seed extract with the same concentrations. The researchers reported that this decrease might be related with effect of heat on the proanthocyanidins of grape in addition to the interactions of the protein and starch macromolecules of bread samples. Another possibility they suggested might have been the thermal degradation of proanthocyanidins (Peng et al., 2010).

Therefore, it might be said that the structure of phytochemicals is an important factor for determining the mechanism and should be investigated in detail.

2.2.3 Specific quality parameters of breads

As the bread quality is highly depend on the subjective preferences, the evaluation its quality might also be considered as a reflection of consumer preferences in the target market. Loaf attributes such as loaf volume, crumb texture, grain character, flavor etc. are among important parameters. Volume is one of the most significant properties and defined as the space occupied by the loaf of bread. Marked deviations in the loaf volume (too large or too small) are not desirable. Crust color normally rages between a deep glden brown on the top crust to light golden brown on the sides and bottom. Among internal cahracteristics crumb firmness is the major quality parameter. How the crumb feels with touch may generally be defined using sensory terms such as smooth, velvety, silky, soft etc. or harsh, crumbly, lumpy, course etc. Another common approach on the other hand, is use of devices such as texture analyzer. Texture is generally influenced by the grain or cell structure of the crumb (Pyler and Gorton, 2008).

2.3 Extrusion and Extruded Products

2.3.1 General information

Extrusion is a common approach for the food industry. In this process, the rotating screw applies the shear energy in addition the barrel, which is heated, enabling the temperature of the food material to increase. The food therefore becomes “melted” as it is conveyed through a die or series of dies under high pressure and changes into a final extrudate which is a very different product than the raw material. The change occurs both physically and chemically, since the applied mechanical stress is in the form of heat and shear (White et al., 2010).

Since the food material used in the extrusion is exposed to high temperature for a very short time under high pressure (the cooking temperature could be around 180–190°C, but residence time is usually 20–40 seconds, varying according to the types of extruders and speed of the shaft), extrusion is a HTST (High Temperature-Short Time) process and beneficial for protecting some of the heat-labile components of food materials (Moscicki and Zuilichem, 2011).

Extrusion technology is commonly applied in different industries for years. Single-screw extruders were first used in 1935, for plasticizing thermoplastic materials. The pasta industry for the production of spaghetti and macaroni-type products became the first application area for single-screw extruders in food industry. However, the first use of twin-screw extruders, both co-rotating and counter-rotating, for food products was after the mid-1930s. In 1946, in US the single-screw extruder had been used to cook and expand corn- and rice-snacks. This process for this type of snacks produced with single-screw extruder is, in principle, still the same; although the flavorings and equipment have been improved (Moscicki and Zuilichem, 2011).

The chemical reactions occurring during the extrusion process and rheology of the extruded products which are generally considered to be a non-Newtonian behavior are among the specific properties of the extrusion (Moscicki and Zuilichem, 2011). Moreover, its features such as to process a variety of products via even minor ingredient or process condition changes, energy efficiency and low cost (since automation increases the productivity and its scale up is easy to manage) makes extrusion an important process for the food industry (Riaz, 2006).

Extruded cereal products are becoming more and more popular because of consumers' increasing demands for tasty, convenient and healthy cereal products. Extrusion is a technology in which a liquid or semi-liquid food material is forced to flow through a die with the aid of mixing, heating, and shearing in an integrated process to form the finished products (Rossen and Miller, 1973). Extrusion of food materials to produce snack foods can date back to late 1940s. This technology combines different unit operations of food engineering including conveying, mixing, thermo-mechanical changes, and shaping (Bhattacharya, 2012). Common extruded cereal products are; pasta, extruded snacks, extruded food ingredients, and ready-to-eat extruded breakfast cereals. Advantages of extrusion over conventional processing methods are; shorter processing time and lower production cost.

Before extrusion, all the ingredients are blended and fed into the extruder. The blending may require hours to properly condition all the ingredients. Proper formulation and blending of the ingredients are the most important steps for producing extruded products.

The nature of extrusion technology makes it possible to produce numerous new products by simply changing process parameters such as moisture, screw configuration, screw speed, temperature, die shape, and specific mechanical energy. Moreover, another reason for the wide variety of products is the use of different treatments after the extrusion, such as drying, frying, baking, and toasting. Although extrusion may be considered as an economic way of transforming the raw material into various palatable products by using the thermal and shear energy, good knowledge and skills for proper operations are required.

The feed in the extruder is mainly a particulate/powdered material. The first physical change during extrusion is the change of this particulate material into viscoelastic dough after combination with water and increase in temperature. As temperature, pressure, and shearing increase during the conveying, particles form viscoelastic dough. Feed moisture content also play a vital role in this change as a plasticizing agent to change the phase of starch granules from a semi-crystalline stage to an amorphous stage during extrusion. The second step of physical change is the formation of the puffed structure. While moving through the die, sudden evaporation of water in the dough causes the formation of the puffed structure of the extruded products.

The low moisture content of the food materials and the high barrel temperature in the extrusion process also promote Maillard reaction. Cereals are rich sources of carbohydrates. Moreover, shearing on starch and sucrose during extrusion generates more reducing sugars to react with proteins for the Maillard reaction (Yaylayan et al., 1992).

Extrusion has become a widely used operation for processing increasing numbers of food products over the years. However, it is still considered a complicated procedure with “multiple input-output” variables. During the process, protein and starch interact with each other under high temperature and pressure conditions within the barrel. Protein and starch available in cereal products are thermo-mechanically transformed into new food products in the extruder barrel with a number of textural advantages, such as increased expansion and crispiness, in addition to extrusion’s economic advantages of high productivity, low operating costs, and energy and time savings (Brennan et al., 2011).

Extrusion process is an either a technological and chemical aspect for the bakery products. Therefore, it requires particular interest as it is related to the products having different properties when compared to products of baking process. The nature of extrusion enables the corporation of functional components into the product formulations and this approach has a wide use in various food commodities. However the effects of the added functional components are not clear.

Moreover extrusion makes clear differences on the raw material physiochemical and functional properties as it involves heat, pressure and shear forces simultaneously. The product properties are determined by both raw material properties and extrusion process variables such as feed rate, feed moisture, screw speed and configuration, etc. (Sarawong et al., 2014; Ragaei and Abdel-Aal, 2014). Extrusion is considered as a hydrothermal process as it is a high-temperature short-time process that works with low-moisture raw materials. Hydrothermal process of cereals may be a factor for liberating the bound forms of phenolic compounds from the plant cell walls by breaking in addition to forming changes in their structure (Dewanto et al., 2002; Khan and Ungar, 1986).

2.3.2 Functional extrudates with bioactives

Fruits have been added into extrudate formulations either as fresh, pomace or powder forms. Application of fruit powders in extrusion feed is an easy way of incorporating fruit into extrusion processing, though only a small number of reports have focused on powder substitution (Camire et al., 2007; Potter et al., 2013). Fruit powder substitution could provide potential health benefits in terms of antioxidant activity and anthocyanin content in extruded samples, and substitution level could be increased to yield more significant health benefits (Camire et al., 2007). Published studies are generally limited to either determination of phenolic compounds and antioxidant activity or to evaluation of basic physical quality parameters of extrudates (Camire et al., 2007; White et al., 2010; Stojceska et al., 2009). Some studies have mainly focused on the effects of extrusion conditions on the properties and quality of functional extrudates (Karkle et al., 2012; Altan et al., 2009; Yağcı and Göğüş, 2008). These studies investigated the effects of increasing substitution levels of health-promoting substances; however the combined effects of adding phenolic compounds/ingredients and basic cereal components at the same time, particularly proteins and starch, were not evaluated in detail to see the interactions.

Only a few number of studies investigated the effects of extrusion on total phenolic content and antioxidant activity. Extrusion caused changes in particular phenolics; however the effects of the added functional components are not clear. Most commonly found phenolic acid in cereals, ferulic acid, was detected to decrease insignificantly during the twin-screw extrusion of germinated brown rice (Ohtsubo et al., 2005). Anthocyanin content was generally reported to decrease after extrusion, particularly above 100°C (Camire et al., 2002; Khanal et al., 2010; Chaovanalikit et al., 2003). However, according to some other studies, although total amount of anthocyanins declined after extrusion of corn starch and cranberry pomace, total flavonols, especially quercetin 3-rhamnoside was found to increase in the range of 30%–34% (White et al., 2010). Antioxidant activity of dark buckwheat flour after extrusion at 170°C (Sensoy et al., 2008) displayed no change, whereas barley extrudates declined in both antioxidant activity and total phenolics (Altan et al., 2009; Ragaei et al., 2014). According to Hirth et al. (2014), although total phenolics and antioxidant activity was reported to be unaffected by the differences in extruder conditions; extent of anthocyanin retention varied depending on the conditions.

A recent research was mostly related to the effects of extrusion process conditions on the final product and the functional compound. In their study Chang and Ng (2009) investigated the changes in the ginseng and ginsenosides components in extruded snack formulations. A wheat flour-ginseng powder blend (10% ginseng powder, w/w) in a twin-screw extruder using feed moisture (25, 30, and 35%), screw speed (200 and 300 rpm), and zone 5 barrel temperature (110, 120, 130, and 140 °C) was studied. Their results revealed that extrusion caused an increase in the amounts of ginsenosides (ginseng saponins, main active components of ginseng). Also in some samples new ginsenosides-Rg2 and Rg3 were formed and the optimum conditions for this formation were determined (Chang and Ng, 2009). Therefore the extrusion process might be considered as a beneficial tool for producing functional products.

White et al. (2010) studied the changes in the bioactive components (procyanidins, anthocyanins and flavonols in addition to total antioxidant activity) of cranberry pomace after the extrusion when different amounts were incorporated into corn starch for extrusion. They revealed that extruder barrel temperature and percent pomace extrusion were important factors on the loss of total anthocyanins, whereas screw speed did not affect the total anthocyanin content. According to their study, the amount of anthocyanin loss during extrusion was less when the ratio of corn starch in the mixture was higher. Therefore they suggested that starch may act like a barrier to preserve anthocyanins, although the mechanism is not determined (White et al., 2010). Interestingly, same study revealed that the total amount of thirteen flavonols detected in cranberry pomace were 30-40% higher in extruded samples when compared to unextruded control (White et al., 2010). However the effects of anthocyanins or flavonols on dough structure were not determined. Moreover, the possible mechanisms related to major components in the food system, which might be important for future applications were not determined. Therefore further research about the effect of extrusion on phytochemicals, beneath process parameters is surely necessary.

Sensoy et al. (2005) revealed that extrusion changed only the polar compounds, whereas roasting changed the structure of both the polar and non-polar compounds. Mechanical energy applied by the extruder is a factor on the formation of some complexes between the flour components while also a factor on the degradation of larger molecules such as starch.

Previous studies on extrusion of fruits have been aimed at detecting effects of extrusion processing conditions, such as die temperature, screw speed and the level of pomace added, on the final product parameters while incorporating fruit pomace (Altan et al., 2009) or fruit by-products (blends of orange peel, grape seed and tomato pomace) (Yağcı and Göğüş, 2008). These studies were primarily interested in the effects of incorporating fiber-rich substances during extrusion. Other researchers (Stojceska et al., 2009) evaluated the effects of two different levels of feed moisture and barrel temperature conditions during extrusion of wheat flour and corn starch substituted with brewer's spent grain and red cabbage in four different formulations, and concluded that fiber content, total phenolics and antioxidant activity all increased in samples containing red cabbage.

It is known that the physicochemical aspects of plasticized starch matrix are modified during extrusion depending on the processing conditions (Alvarez-Martinez et al., 1988). Some other studies also exist which evaluated the effects of starch or amylose on the physical properties of the extrudates (Della Valle et al., 1997; Blanche and Sun, 2004; Zhu et al., 2010). However, in the literature, scarce knowledge is available about the combined effects of anthocyanin-rich fruit-based sources and high amylose starch during extrusion applications.

2.3.3 Specific quality parameters of extrudates

Specific mechanical energy (SME) is a common measure of the work input from the extruder to the extrusion material and is an indicator of the effect of molecular breakdown or degradation and enables a good characterization of the final product (Guerrero et al., 2012). It is considered to be highly related to the extrusion process parameters of screw speed, barrel temperature, feed moisture, and feed composition, all of which affect the viscosity and flow of the extrusion melt (Schaich and Rebello, 1999).

Water absorption index (WAI) is a common way of measuring the condition of starch in selected samples. High temperature and thermos-mechanical force applied during extrusion process damage the starch granules of native flour. The increase in damaged starch content after extrusion increases swelling and water retention capacity. Therefore, WAI increases up to a point with respect to the damage starch content and then decreases with the increase in temperature as the feed moisture

decreases. This is probably because dextrinization and starch melting become prevailing factors over gelatinization as the temperature increases (Maskan and Altan, 2011).

Expansion is one of the primary purposes for extrusion process. An ideal expansion and texture is made generally possible by starches having 5-20% amylose content and by degermed corn/grits and rice. If a lower expansion accepted, other kinds of cereals might be used in extrusion process, as well (Riaz, 2006). The physicochemical aspects of plasticized starch matrix during extrusion, the effects of process conditions and porosity of the material are among the main parameters that determine the expansion mechanism of extruded cereal products (Robin et al., 2011; Robin et al., 2012).

Bulk density is an important parameter to evaluate the degree of puffing of extrudates and it is highly related with the expansion ratio (Asare et al., 2004).

2.4 Cereal Flour Ingredients (with Emphasis on Starch and Protein Biopolymers) and Their Interactions with Fruit Phytochemicals

Solubility, stability, absorption and bioavailability properties of the target components are the important factors for determining their bioactive molecular activity (Tapal and Tikku, 2012). Therefore careful examination of these properties is important for the food systems. In complex food systems such as cereal products, interactions of molecules is even more determinant since these interactions are able to lead to structural and behavioral changes that are effective on the molecular stability (Awika et al., 2009). Moreover, food systems are generally composed of many minor bioactive compounds instead of major compounds. Therefore the synergistic interactions between them might be an important indicator for determining their real effects. Synergy might be defined as “the ability of two or more functional components, such as antioxidants in a phytochemical background, to mutually enhance their functionalities” (Vattem and Shetty, 2005).

Cereal flour is composed of many different constituents, although two large biopolymers (starch and protein) having the most important roles in products. In literature, the interactions between phytochemicals and other molecules were rarely studied resulting in some inconsistent findings. Tapal and Tikku (2012) tried to

investigate the complexation reactions between curcumin and soy protein isolates to increase the stability of this functional constituent. Fluorescence spectrophotometry was used as the instrument to follow and measure the extent of formation. Their results showed that curcumin molecules were able to make complexes with soy protein isolate probably due to hydrophobic interactions. Study by Heo et al. (2007) revealed that different phenolic acid standards combined in different combinations of two or three, showed no more antioxidant activity than they presented individually (Hoe et al., 2007). In contrast, Reber et al. (2011) indicated that synergistic effects existed in three combinations of the two-phenolic compounds and five combinations of the three-phenolic compounds of strawberry (Reber et al., 2011). Wang et al. (2011) used a number of model systems to investigate the inhibition potential of sugar-free extracts of some berries (from blueberries, blackberries, strawberries, raspberries, cranberries, and Noble muscadine grapes) on the formation of advanced glycation end products (AGE)s. The different antiglycation models included BSA (bovine serum albumin)-fructose, BSA-methylglyoxal, arginine–methylglyoxal, and also a model for methylglyoxal scavenging were used. These models helped to follow the different reaction phases.

Protein–polyphenol binding consists of both hydrogen and hydrophobic bondings. In addition, covalent interactions are also possible for some commodities. Binding mostly depends on the chemical (polarity) and structural properties of interacting molecules (Hagerman et al., 1998; Roopchand et al., 2012).

Another study focused on the binding activity of anthocyanins to different flour types (Roopchand et al., 2012). Defatted soybean flours (DSF) were found to absorb the highest amount of anthocyanins from blueberry and cranberry juices when compared to wheat, rice and corn flours, respectively (Roopchand et al., 2012). The anthocyanins and proanthocyanidins in the cranberry polyphenol-enriched DSF were between 2.5–17 mg/g and 21–101 mg/g, respectively. Although the defatted soybean flour had the highest content of protein among all sample flours, its protein content did not correlate with the binding activity. Therefore the carbohydrates were also found as important indicators for polyphenol binding in flours. Moreover, the protein-rich particles having large surface area in defatted soy flours might be the reason for their non-covalently binding to flavonoids in addition to the electrostatic interactions. It was another observation that the blueberry and cranberry polyphenols

managed to retain their activity in the flour matrices for as long as 22 weeks in their intact forms (according to LC-MS data) (Roopchand et al., 2012).

2.4.1 Starch

Starch is the main storage carbohydrate in cereal crops and exists in an insoluble granular form in the grains. It is a food reserve to supply a bulk nutrient and energy source in the human diet. The main classification of carbohydrates comprises the available and unavailable fractions. Available carbohydrates are those digested and absorbed by humans, which include (non-resistant) starch and soluble sugars. In contrast, unavailable carbohydrates (dietary fiber) are not digested the human digestion system (Southgate, 1991). Dietary fiber includes the resistant starch, cellulose and other complex polysaccharides, such as arabinoxylans, β -glucans, pectins and arabinogalactans, together with lignin. Starch granule is composed of two main constituents: amylose and amylopectin. Amylose is an essentially-linear starch molecule, whereas amylopectin is a highly-branched molecule consisting of about 5% α -1, 6 branch linkages (Hizukuri, 1986). The amylose/amylopectin ratio is one of the most important factors determining starch properties.

During cooking and cooling of cereals with water, many processes, such as swelling, deformation, fragmentation, disintegration, solubilization, and reaggregation, that take place in a very complex media primarily governed by starch granule behavior. Pasting properties of starch are affected by its amylose and lipid contents and by branch chain length distribution of amylopectin. Amylopectin contributes to swelling of starch granules and pasting, whereas amylose and lipids inhibit the swelling because the release of amylose from the interior of starch granules is retarded (Tester and Morrison, 1990). Moreover, starch is also very effective on bread baking. Because this biopolymer is the source of fermentable sugars to yeast and has a significant contribution to dough rheology (Cauvain, 2003).

During extrusion, subject to many changes with the applied processing conditions while forming the extrudate, starch granules becomes swollen and even gelatinized (Eliasson and Gudmundsson, 2006). The main change during extrusion is the breakdown of the crystalline structure of starch through gelatinization, but crystallinity can again form during storage. Retrogradation of starch during extrusion is mainly attributed to the B-type starch crystals. On the other hand, formation of

amylose-lipid complexes is commonly related to the V-type crystals in starch (Van Soest and Knooren, 1998; Chanvrier et al., 2007). Similar to all other cereal products, starch also contributes to the structure, texture, and mouthfeel of the extrudate. Thus, the control of starch gelatinization and expansion during extrusion is crucial to obtain final products of good quality. Amylose content affects the expansion ratio of cereal products, although the impacts appear to be controversial between different cereals and depend on other parameters as well (Chinnaswamy and Hanna 1988; Gomez et al., 1988; Mohamed 1990; Chen and Yeh, 2001).

Starch requires thermal treatment in the presence of water to gelatinize. Therefore, hydration during extrusion is an effective factor on starch gelatinization. Starch gelatinization temperature is mainly determined by the origin of the starch; however, the presence of ingredients such as sugars, fats, and salts has significant impacts on the gelatinization property of starch (Jin et al., 1994; Abd Ghani et al., 1999; Edwards, 2007). Stoichiometry between water molecules and each hydroxyl group requires at least 25% moisture content for complete starch gelatinization. The rate of starch gelatinization tends to increase with a higher mass temperature and greater shearing and pressure. Particle size also affects the extent of starch gelatinization. When the feed particles become smaller, less thermal energy is needed for the gelatinization.

2.4.2 Protein

Protein is a key constituent in cereal flour by means of its functional properties and nutritional value. Proteins found in cereal grains vary in the amino acid composition and molecular weight. During heating, the native protein structure is destabilized, and unfolding may facilitate sulfhydryl disulfide interchange reactions and oxidation together with hydrophobic interactions, leading to the association of proteins and, consequently, to the formation of large protein aggregates. Protein denaturation is the loss of its secondary, tertiary, and quaternary structure (Rosell, 2011).

During bread production, when wheat flour is mixed with appropriate amount of water, glutenin and gliadin fractions of gluten interact and contribute to the formation of a viscoelastic dough to retain gas, which makes wheat flour suitable for the preparation of bread. Gluten is the primer quality contributor to the unique properties of wheat dough properties, affecting dough characteristics and final product.

Extrusion is another process to cause numerous changes in protein structure and these changes are related to the temperature, hydration, and shearing stress applied through the extrusion process. Protein digestibility increases as more enzyme-accessible sites are revealed after extrusion. Changes in protein solubility in water or aqueous solutions are analyzed to determine the extent of protein denaturation (Camire, 1991). During extrusion, enzymes may be the mostly influenced protein moieties by denaturation. Thus, extrusion can be applied to destroy anti-nutritional factors in raw food materials. For example, the levels of anti-nutritional factors such as trypsin inhibitors and lectin can be efficiently reduced by extrusion (Singh et al., 2007).

Cereal proteins are highly affected by processing conditions. Temperature may be considered one of the most important factors, as high temperature has irreversible effects on proteins, including protein unfolding and loss of functionality, as a result of aggregation and/or covalent bond breaking (Leon et al., 2003). In contrast, the effect of high pressure on protein denaturation is different as it leaves some parts of the molecule unchanged (Knorr et al., 2011). However, as proteins are exposed simultaneously to multiple extreme conditions, such as high temperature, high pressure, and mechanical shear during extrusion, changes occur in proteins, including conformational disassembling and reassembling through different interactions of disulfide, hydrogen and non-covalent bonds. This results in extrusion products that are completely different in comparison to their raw materials. Protein based sources (legumes and soy products as blends with cereals, being the most common) were generally added into extruded products to help nutritional balance (Bhattacharya, 2012).

Proteins are known for their ability to react with other food components, including polyphenols, which lead to changes in structural, functional and nutritional properties both of the food components. The interactions between proteins and polyphenols during extrusion are mostly governed by temperature which can affect hydrogen bonding and cause the formation of hydrophobic interactions (Ozdal et al., 2013).

2.5 Polyphenols

Most of the colorful and multifunctional phytochemicals (plant chemicals) or nutraceuticals existing in nature are found in fruits. As naturally occurring antioxidants, phenolic compounds have been reported to possess diverse beneficial bioactivities, including anti-allergic, antiviral, anti-inflammatory and anti-mutagenic properties (Yao et al., 2004). Polyphenols are a major group of phytochemicals and are sub-classified into two main groups: phenolics and flavonoids. A wide range of phenolic compounds and flavonoids are reported in fruit and vegetables and they are mostly found in the peels, rather than the pulp of the fruit and vegetables (George et al., 2004; Pande and Akoh, 2009).

Phytochemicals are generally detected using chromatographic methods and high performance liquid chromatography (HPLC) is probably the most commonly used system among all separation methods. Different separation modes (types of column) have been used to separate and analyze different phytochemicals. The adsorption/desorption based columns including the normal phase (NP) and reversed phase (RP) columns are most frequently used columns among them. The majority of the food-originated phytochemicals are analyzed by RP HPLC. For the separation of polyphenols, the stationary phase is almost exclusively C18, which is coupled with a binary mobile phase system containing acidified water (solvent A) and a polar organic solvent (solvent B), where solvent A usually includes aqueous acids or additives such as phosphate, and solvent B pure or acidified methanol or acetonitrile (Tsao and Deng, 2004).

2.5.1 Flavonoids

There are numerous colorful and multifunctional phytochemicals (plant chemicals) or nutraceuticals (food/parts of food providing medical or health benefits) in nature. Flavonoids and their derivatives (flavonols, flavones, flavanols, flavanones, anthocyanidins and isoflavones) are the largest group of phenolic compounds or polyphenols with approximately 2000 individual members, which are compounds that display various functional properties originating from their phytochemical structure (Motohashi and Sakagami, 2009). The flavonoids are the largest class of polyphenols. Their common structure is composed of diphenylpropanes (C6-C3-C6), consisting of two aromatic rings (A and B) linked through three carbons, usually in

the form of a heterocyclic ring. Variations in substitution patterns to C ring result in the major flavonoids classes, including flavones, flavonols, flavanones, flavanols, flavanonols anthocyanidins, and isoflavones (Ross and Kasum, 2002; Hallman and Katan, 1997). The members of flavonoid group compounds are generally potent antioxidants, metal chelators and effective in chronic diseases, as is typical for phenolic compounds (Lin and Weng, 2006).

2.5.2 Anthocyanins

Anthocyanins are natural pigments (giving the red, blue, purple, and orange color of many fruits and vegetables) used in food, nutraceutical and pharmaceutical industries. They are mostly found in the epidermal tissues of fruits and vegetables (grape skin and red cabbage being the predominant concentrated sources of anthocyanins) (Manach et al., 2004; Francis, 2000). They are one of the most important subclasses of flavonoids with more than 500 different structures identified in nature. In plant tissues they have different biological functions, such as protection against solar exposure and UV radiation, pathogen attacks, oxidative damage and attack by free radicals; attracting animals for seed dispersal and confusing signals (Flamini et al., 2013). Anthocyanins might be considered as specific compounds both in terms of their health (different effects through numerous pathways of antioxidant, antimicrobial and anti-carcinogenic and cardio protective) and technology (various effects during food processing) related properties (Pascual-Teresa and Sanchez-Ballesta, 2008).

Flavonols are normally glycosylated at the C-3 position of the C ring of the flavonoids while anthocyanins are synthesized from anthocyanidins by glycosylation at the 3 and 5 positions of the C ring. Depending on the glycosylation at the C-3 and C-5 positions of the molecule, the color of the anthocyanin pigment is affected, 3-glucoside derivatives being more intensely colored than 3, 5-diglucosides (Flamini et al., 2013). They are mainly found in the form of heterosides in nature. The aglycone form of anthocyanins (anthocyanidin) is structurally based on the flavilium ion or 2-phenylbenzopyrylium, and presents hydroxyl and methoxyl groups in different positions. The number and position of the hydroxyl and methoxyl substituents and degree of methylation of these OH groups are the main determinants for different anthocyanidins (Mazza and Brouillard, 1987; Mazza and Miniati, 1993; McGhie and

Walton, 2007). Because glycosylation induces intramolecular H-binding within the anthocyanin molecule generally anthocyanidin glycosides are more stable than the corresponding aglycones (Borkowski et al., 2005). Six anthocyanidins that are commonly found in fruits and vegetables are: cyanidin, pelargonidin, delphinidin, petunidin, peonidin and malvidin. Cyanidin is the most widespread anthocyanidin found in foods. The structures of common anthocyanidins were given in Figure 2.1. Delphinidin derivatives of anthocyanins are associated with blue color and cyanidin derivatives are reddish (Flamini et al., 2013).

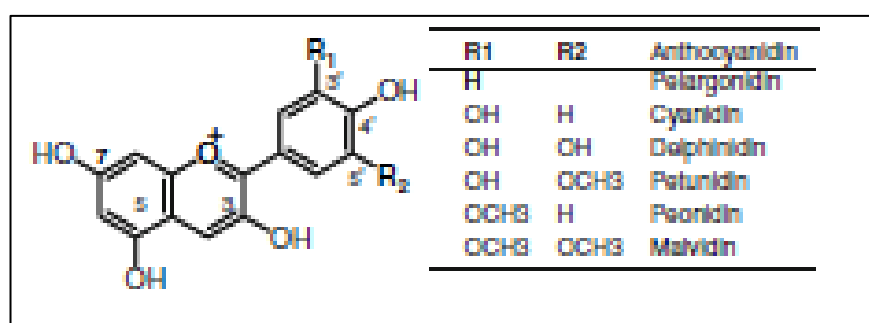


Figure 2.1: The anthocyanidins commonly found in fruits and vegetables (Flamini et al., 2013).

Anthocyanins are highly soluble in water and alcoholic solutions however they are not very stable under processing conditions as their stability is highly dependent on factors such as oxygen, light and pH changes (Hirth et al., 2014). Anthocyanidins in fruits and vegetables are structurally linked to one or more glycosidic units. Sugars are attached to the 3-hydroxyl position of the anthocyanidin (sometimes to the 5 or 7 position of flavynium ion) (McGhie and Walton, 2007). Sugar may be linked as mono, di or triglycosides and may, in addition, be acylated with different organic acids. Glucose and galactose, as hexoses, and rhamnose, arabinose and xylose, as pentoses, are the most common sugars forming glycosidic structures with anthocyanidins. Variations in anthocyanins is also related to the nature and number of sugar moiety attached to the phenolic molecule and to some extent the nature and number of aliphatic or aromatic acids attached to them.

Although some other methods also exist, anthocyanin detection is generally achieved by using two common methods. The first one is the spectrophotometric pH differential method (Lee et al., 2005) giving the total anthocyanins and the second one is the HPLC/DAD (HPLC coupled with a diode array detector) or MS detection.

A considerably high correlation ($R \geq 0.925$, $p \leq 0.05$) was reported between the two methods (Lee et al., 2008).

Philpott et al. (2004) worked on the antioxidant potential of sweet potato by both *in situ* and *in vitro* methods. Their findings have revealed a distinct correlation between anthocyanin contents and antioxidant activity of the samples; however the presence of the correlation even without anthocyanin components directed their studies through the examination of bioactive components individually by a chromatographic method. Therefore, according to their findings, it might be concluded that hydroxycinnamic acids have a potential synergistic effect with antioxidant activity of anthocyanins (Philpott et al., 2004).

Li et al., (2011) hypothesized that different ovens having different heat transfer coefficients (impingement, reel oven and convection oven) and different acidulents (citric and lactic acids and glucono- δ -lactone) may be important indicators for the residual total anthocyanin contents of blue corn cookies, since anthocyanins are sensitive to a number of factors such as temperature, exposure to oxygen and pH. The decrease in pH gave an increase in the residual total anthocyanin content in cookies, Citric acid having the best results (supposed to be because of its structure it has three replaceable hydrogen ions). For all oven types, the combination of low temperature and short time gave the highest retention of anthocyanins. The best combination was achieved in cookies baked with citric acid in the convection oven. The study was important as it incorporated knowledge on the anthocyanin retention in a bakery system as heat and pH are important factors (Li et al., 2011).

2.5.3 Stilbene derivatives

Stilbenes are phytoalexins naturally occurring low-molecular-mass compounds in a number of plants, including grapes.

cis- and *trans*-resveratrol (3, 5, 4'-trihydroxystilbene) are widely found in skins of grapes, in addition to resveratrol-3-O- β -D-glucopyranoside (piceid), piceatannol (3, 4, 3', 5'-tetrahydroxy-*trans*-stilbene) and resveratrol dimers (viniferins) (Careri et al., 2003). They are higher in red grapes and are among the main polyphenols associated with the beneficial effects of grapes and drinking wine (Virac et al., 2005). *trans*-Resveratrol is an antioxidant, anticarcinogenic and inhibitor of platelet aggregation

and low-density lipoprotein (LDL) oxidation (Fremont et al., 1999; Pace-Asciak et al., 1995).

2.5.4 Flavonols and flavan-3-ols

Flavonols are secondary metabolites under the group of flavonoids and found in many plants. They are placed in the outer epidermis of grape skin and their main role in plants is to absorb strongly at both UV-A and UV-B wavelengths and act as UV- and photo-protectors. In general, flavonols are C₆-C₃-C₆ polyphenolic compounds, in which two hydroxylated benzene rings, A and B, are joined by a three-carbon chain which is part of a heterocyclic C ring with a 3-hydroxyflavone backbone, and a double bond. Flavonols differ in the number and type of substitution in the B ring. Kaempferol is monohydroxylated in position 3'; quercetin is dihydroxylated in positions 3' and 4'; and myricetin is trihydroxylated in positions 3', 4' and 5'. Isorhamnetin is the methylated form of quercetin, and laricitrin and syringetin are the methylated forms of myricetin. They normally occur in grape as glucosides, galactosides, rhamnosides, rutinosides and glucuronides. Sugar is linked to position 3 of the flavonoid skeleton (Flamini et al., 2013). Quercetin and myricetin are the most important flavonols found in grapes. LDL oxidation protection (Mayer et al., 1998), in addition to antioxidant and anti-cancer properties is significant for flavonols.

Catechins are a group of low molecular weight flavan-3-ols isomers and found in grapes. Catechins include four major compounds, (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG), and (–)-epigallocatechin gallate (EGCG), and four minor compounds, (+)-catechin (C), (–)-catechin gallate, (–)-gallocatechin, and (–)-gallocatechin gallate, which are present in a variety of foods, such as tea, wine, fruits, and chocolate. Catechins are water soluble, colorless, and astringent. The basic structure of catechins is composed of two benzene rings (A and B rings) and a dihydropyran heterocycle (C ring) with a hydroxyl group on carbon 3. The concentrations of catechins in foods highly depend on the food sources and vary to a large extent.

2.6 Concord Grapes

Grapevine (*Vitis* spp.) fruits belong to *Vitis* genus in the flowering plant family of Vitaceae vines. Since the ancient Greek and Roman civilizations, grapes have been

significant for winemaking and one of the most cultivated fruit plants among the world with total global grape production in year 2012, accounts for approximately 67 million tons (FAOSTAT, 2014). Processed grape products are mainly grouped into four categories: juice; canned; raisins; and alcoholic beverages (mainly wine) and *Vitis vinifera* belongs to one of the three main species of grapes: “European grapes”, is important as it is widely used to produce winery products.

The Concord grape is another significant cultivar and belongs to the *Vitis labrusca* species and so called as “North American grapes” with *Vitis rotundifolia* species. They are mainly cultivated in US, in the area comprising the Finger Lakes District of New York, Lake Erie, Lake Ontario, Southwestern Michigan, and the Yakima Valley in Washington (Amanor-Boadu et al., 2013). Group of “French hybrids” is the last main species of grapes. The history of Concord grapes goes back until 1849 and to the Massachusetts village of Concord, by Ephraim Wales Bull. Concord grapes were first used and processed by Dr. Welch to produce an unfermented grape juice for communion in local churches (Anon., 2011).

The Concord grape is typically dark blue or purple in color, and often is covered with a lighter-colored "bloom" that can be rubbed off. It is a slip-skin variety, meaning that the skin is easily separated from the fruit. Concord grapes have large seeds and are highly aromatic and therefore not generally used in winemaking due to their strong "foxy" (sometimes described as candied-strawberry/musky) flavor. They have higher crop yields, higher sugar-acid ratios, and milder labrusca flavors than other grape varieties (Amanor-Boadu et al., 2013). Main applications of this type of grapes include grape jelly, grape juice, grape-flavored soft drinks, and candy. In Turkey they are usually defined as Foxy grape and grow in Black Sea region (Northern part of Turkey) (Celik et al., 2008).

Grapes, different parts and grape products are all rich and important sources of polyphenolic compounds of anthocyanins, flavanols, catechins and proanthocyanidins qualitatively and quantitatively. Anthocyanins, flavanols, flavonols and stilbene derivatives (resveratrol) are among the most important grape polyphenols. Xia et al. (2010) summarized the phenolic composition in different parts of grapes as following; the seeds of grapes are rich in gallic acid, (+)-catechin, epicatechin, dimeric procyanidin and proanthocyanidins (Pastrana-Bonilla et al., 2003) while skin is found to contain proanthocyanidins, ellagic acid, myricetin,

quercetin, kaempferol and trans-resveratrol mostly. In leaves myricetin, ellagic acid, kaempferol, quercetin and gallic acid were the most widely found phenolic compounds and rutin; quercetin 3-O-glucuronide, trans-resveratrol and astilbin were the most abundant compounds in stem (Xia et al., 2010). Grape pomace is also a rich source of anthocyanins, catechins, flavonol glycosides, phenolic acids and alcohols and stilbenes (Schieber et al., 2001). Cyanidin, peonidin, delphinidin, petunidin, and malvidin derivatives are most widely found anthocyanin in grapes. Glycosides and acylglycosides are the most common types. Malvidin-3-glucoside is the main type in all grape varieties (Fraige et al., 2014). Structures of some important compounds commonly found in grapes were given in Figure 2.2.

Grape and its main components; anthocyanins, flavonoids and resveratrol have various bioactivities, such as antioxidant, cardio protective (Dell Agli et al., 2005; Falchi et al. 2006; Park et al., 2004; Vinson et al., 2001; Shanmuganayagam et al., 2007); anticancer (Singletary et al., 2003), anti-inflammation, anti-aging (Shukitt-Hale et al., 2004) and antimicrobial (Krikorian et al., 2010, Smith and Stouffer, 2014) effects which are closely related to the prevention against disease and promotion of health, providing greater potential for grape in the field of food and pharmaceutical applications. Numerous studies on the incorporation of grapes into different cereal products existed in literature that focused on both addition of these fruits and/or their certain parts. Grape seed extract into bread (Peng et al., 2010), grape pomace into extruded products (Khanal et al., 2009); milled grape pomace of wine processing (Merlot and Cabernet Sauvignon grapes) into flour to produce grape seed flour as raw materials for producing cereal bars, pancakes and noodles (Soto et al., 2012) are among different uses of grapes in cereal products.

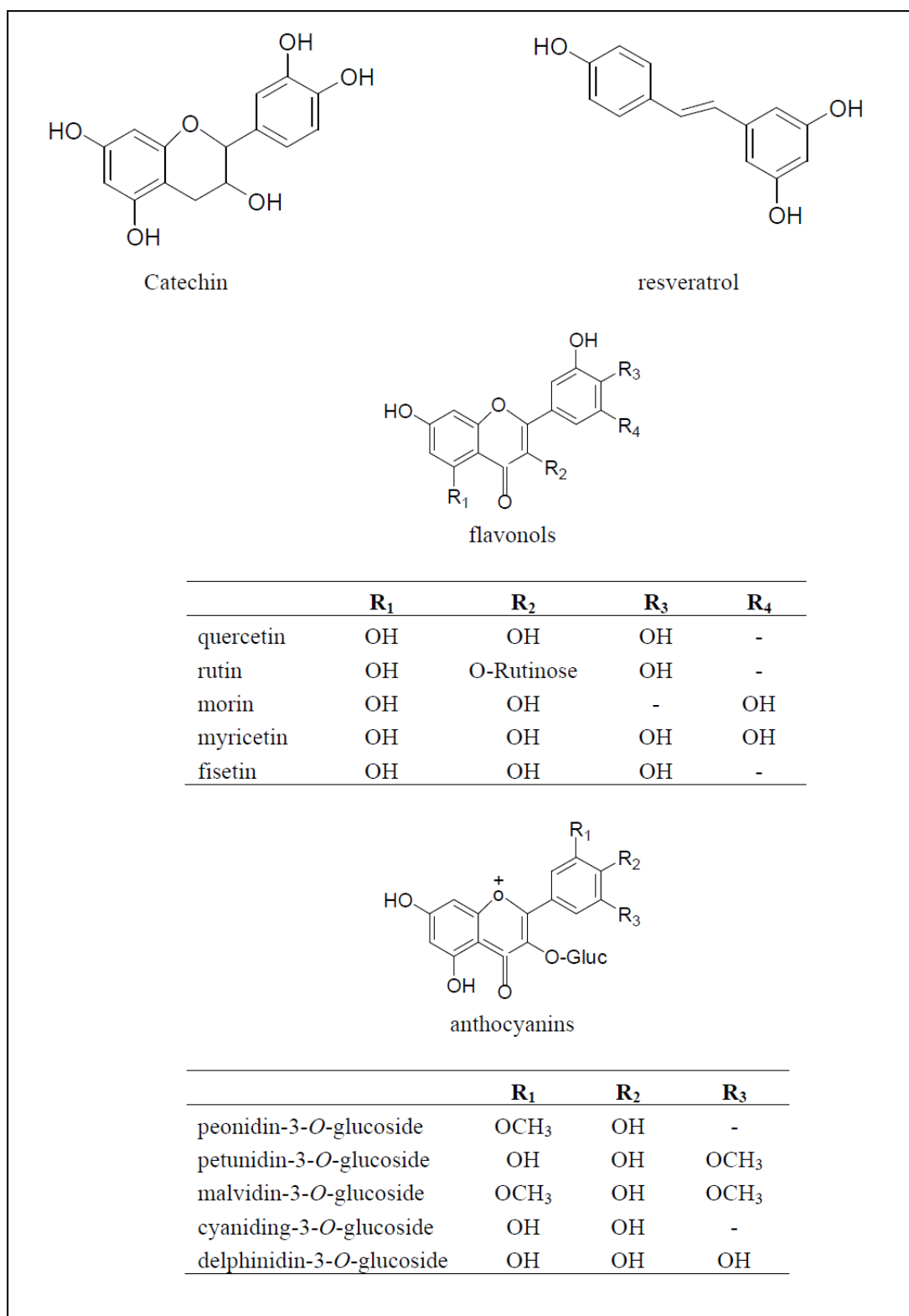


Figure 2.2 : Structures of some important phenolic compounds commonly found in grapes (Xia et al., 2010).

Concord grape juice is known for its positive effects on health with high levels of antioxidants from anthocyanins, tartarate esters of hydroxycinnamates, and flavan-3-

ols (Mullen et al., 2007; Stalmach et al., 2011). Singletary et al. (2003), Shanmuganayagam et al., (2007), Shukitt-Hale et al. (2006) specifically focused on the effects of Concord grape juice and revealed its positive effects on mammary cancer, cholesterol reduction and on reduction of cognitive and motor deficits in aging, respectively. Shanmuganayagam et al. (2007) worked on the effect of Concord grape juice on the potential of its phenolic compounds to defend the hypercholesterolemia-induced platelet aggregation and according to the results, the development of atheroma was near 30% lower than that of the control group. Stalmach et al. (2011), tried to identify and quantify the 60 polyphenolic compounds in 100% Concord grape juice and detected anthocyanin as the dominant group almost 46% of all phenolics, followed by the tartarate esters of hydrocinnamic acids (approximately 29%) and the rest as a mixture of flavan-3-ol monomers, procyanidin flavonols, gallic acid, free hydroxycinnamic acids, and *trans*-resveratrol (Stalmach et al., 2011).

3. MATERIALS AND METHODS

In this chapter the materials and methods used to evaluate the effect of CGEP (Concord grape extract powder), together with either amylose or protein, on the quality parameters and functional properties of bread and extruded products will be explained.

3.1 Materials

The following raw materials were used to produce the samples;

- Hard wheat bread flour provided from Mennel Milling Company (Fostoria, Ohio, USA)
- Wheat protein isolate (WPI) supplied from MGP Ingredients Inc. (Atchison, Kansas, USA),
- High amylose corn starch (HACS, HYLON VII) obtained from National Starch and Chemical Company (Bridgewater, NJ, USA),

Concord grape extract powder (CGEP) was provided from Milne Fruit Products (Prosser, WA, USA). Other ingredients used during bread making were sugar, salt and instant dry yeast which were obtained from the market (Meier, MI, USA) and shortening was supplied from Crisco, Orrville, OH.

- During analysis following chemicals were used: HPLC grade methanol, formic acid, and acetonitrile purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteu reagent, ABTS: 2,20-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid, DPPH: 2,2-diphenyl 1-picrylhydrazyl, potassium hydroxide (KOH), dimethyl sulphoxide (DMSO), sodium carbonate, potassium chloride, sodium nitrite, hydrochloric acid, sodium hydroxide, trifluoroacetic acid (TFA) obtained from Sigma (Saint Louis,

MO, USA). Standards of gallic acid, chlorogenic acid, syringic acid, ferulic acid, catechin hydrate, epicatechin, epicatechin gallate, epigallocatechin, cyanidin, malvidin, myricetin, quercetin, sinapic acid etc. were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

3.2 Sample Preparations

Six different flour mixes were prepared to evaluate the interactions of both CGEP and HACS or CGEP and WPI. Each mix had different formula (Table 3.1). According to formulations ingredient are weighed and then mixed for 30 minutes by a Hobart mixer (Hobart Corp., OH, USA) to obtain a batch of 3 kg. Each batch was made in duplicate and used for both making the bread and producing the extrudates. Control samples were also prepared with only CGEP and without CGEP.

3.2.1 Preparation of protein (WPI) added samples

For protein treatments, similar to amylose treatments, first of all the control samples were prepared. The control sample P1 was only hard wheat bread flour. For the further two levels, control flour was substituted with 6.5% (w/w) WPI to give approximately 20% total protein in final samples (P2) while in samples P3, substitution level was around 13% (w/w) to give approximate amount of 25% total protein content in the 3 kg batch. GP1 samples were made by substituting control flour with 7% (w/w) CGEP. Control flour substituted with 7% CGEP and 6.5% (w/w) WPI was coded as sample GP2 and control flour substituted with 7% CGEP and 13% (w/w) WPI was named as GP3. Similar to amylose treatments the mixing process was made at least 24 hours before the extrusion to ensure that the moisture level in the mix was well balanced. The mixes were stored in plastic containers in the cold storage room at 4°C until use. Formulations for the protein treatments with the codings are shown in Table 3.2.

Table 3.1 : Sample formulations for protein treatments.

Sample	Ingredients
P1	100% (w/w) hard wheat bread flour
P2	93.5% (w/w) hard wheat bread flour and 6.5% (w/w) WPI
P3	87% (w/w) hard wheat bread flour and 13% (w/w) WPI
GP1	93% (w/w) hard wheat bread flour and 7% (w/w) CGEP
GP2	86.5% (w/w) hard wheat bread flour, 6.5% (w/w) WPI and 7% (w/w) CGEP
GP3	80% (w/w) hard wheat bread flour, 13% (w/w) WPI and 7% (w/w) CGEP

3.2.2 Preparation of amylose (HACS) added samples

In amylose treatments, control hard wheat bread flour was represented as A1 and was substituted with two levels of HACS. In samples A2, A1 was substituted with 10% (w/w) HACS whereas in A3 substitution level was 20% (w/w).

In amylose treatments, control flour (A1) was further substituted by 7% (w/w) CGEP to obtain (GA1). This level of CGEP was chosen according to the trials done before. For GA2 samples control flour was substituted with 7% CGEP and 10% HACS (w/w) whereas for GA3 samples control flour substituted with 7% CGEP and 20% HACS (w/w). For each of the batch same mixing method was used. The mixing process was made at least 24 hours before the extrusion to ensure that the moisture level in the mix was well balanced. The mixes were stored in plastic containers in the cold storage room at 4°C until use. Formulations for the amylose treatments with the codings are shown in Table 3.1.

Table 3.2 : Sample formulations for amylose treatments.

Sample	Ingredients
A1	100% (w/w) hard wheat bread flour
A2	90% (w/w) hard wheat bread flour and 10% (w/w) HACS
A3	80% (w/w) hard wheat bread flour and 20% (w/w) HACS
GA1	93% (w/w) hard wheat bread flour and 7% (w/w) CGEP
GA2	83% (w/w) hard wheat bread flour, 10% (w/w) HACS and 7% (w/w) CGEP
GA3	73% (w/w) hard wheat bread flour, 20% (w/w) HACS and 7% (w/w) CGEP



Figure 3.1: Hobart mixer used for mixing the ingredients.

3.3 Methods

3.3.1 Bread making procedure

For bread making, the Optimized Straight-Dough Bread-Making Method 10-10B of AACC International (2000) was used without adding ascorbic acid. The ingredients were weighed according to recipe and the dough was prepared using the pin-type mixer (National MFG Co, Lincoln, NE). Then it is fermented for 90-min in the fermentation cabinet (National MFG Co, Lincoln, NE) at 30°C and 85% relative humidity (RH). Punching and sheeting were made using the dough sheeter (National MFG Co, Lincoln, NE) which is then followed by a proofing process for 90 min. After molding, the dough was again put into fermentation cabinet a for 33 min extra proofing.

In addition to flour mixes (approximately 100 g flour at 14% moisture basis), bread formulation contained sugar (6.0 g), salt (1.5 g), shortening (3.0 g), instant dry yeast (1.96 g) and water. Amount of water used in the formulations was calculated from the water absorption data obtained from Farinograph (Brabender GmbH & Co. KG, Germany). Rotary type oven (National MFG Co, Lincoln, NE) was used for baking and it was performed at 215°C for 24 min. For each formulation five loaves were prepared and each treatment was repeated twice. Randomly chosen three loaves were baked completely, while the other two were sampled after fermentation as the dough sample for analyzing the functional components.

Unbaked loaves were stored around 1 hour at room temperature and then cut into 1cm³ pieces and put into freezer to freeze completely for 24 hours. Baked loaves were cooled for two hours at room temperature before the physical tests. Baked loaves were also separated to crumb and crust samples and frozen in order to use in the further analyses. For this purpose, crumb samples were cut as rectangles in 1 cm distance from the sides, while crust samples were taken as 3 mm width pieces around the slices. Samples were frozen for 24 hours and then freeze dried for 16 hours using a freeze dryer. The freeze-dried bread dough, crumb and crust samples were powderized with a mortar and pester and sieved through a 500-µm sieve. Powders were stored in zipped plastic bags and stored at -20°C for further analysis.



Figure 3.2 : Fermentation cabinet, rotary type oven and pin type mixer used for breadmaking.



Figure 3.3 : Freeze-dryer used for bread crumb, crust and dough samples.

3.3.2 Extrusion process

For extrusion, a laboratory scale twin screw extruder (Model MP 19T2-25, APV Baker, Grand Rapids, MI, USA) was used. Constant feed moisture content and screw speed were 25% and 360 rpm, respectively. The low shear screw configuration was applied, where screw diameter is 19.0 mm (1 D) and one kneading paddle is 1/4 D. The details of the applied configuration were given in Table 3.3

Table 3.3 : Screw elements used for low shear extrusion configuration.

8 D Twin Lead Screws
7 x 30° Forward Kneading Elements
8 D Twin Lead Screws
3 x 60° Forward Kneading Elements
3 x 30° Reverse Kneading Elements
2 D Single Lead Screws
4 x 60° Forward Kneading Elements
3 x 30° Reverse Kneading Elements
2 D Single Lead Screws
7 x 90° Kneading Elements
2 D Single Lead Screws

The exit die had an opening of 3 mm, barrel diameter was 19 mm, and barrel length to diameter ratio (L/D) was 25:1. Distilled water was injected with an E2 Metripump positive displacement-metering pump (Bran + Luebbe, Northampton, UK) to maintain feed moisture of 25%. The extruder temperature profile was comprised of 5 different temperature zones, with zone 1 at the feed port and zone 5 at the die.

Samples were extruded at three different processing temperature profiles at a 2 kg/h feed rate. Temperature profiles of the five zones of the extruder barrel, from feed port towards exit die were: 40-60-60-70-90°C, 40-60-80-100-120°C, and 40-60-100-140-150°C. Hereafter, the three processing temperatures will be referred to as 90°, 120°, and 150°, respectively.

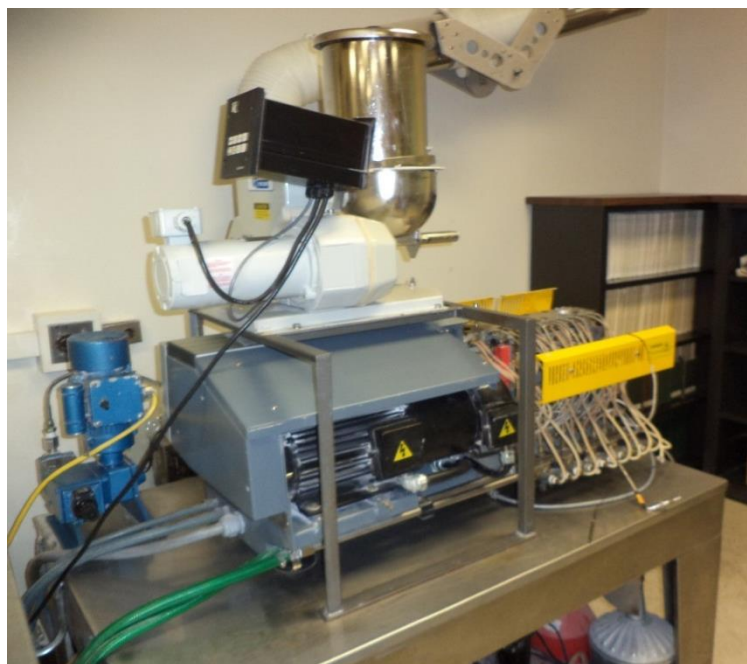


Figure 3.4 : Extruder used for extrusion process.

The extruded samples were cut into 100 mm lengths, cooled down to room temperature, and dried in an air oven at 50°C for 16 hours. A portion of each sample of dried extrudates was milled using a laboratory grinder (Thomas Scientific, Willey Lab. Mill, Model 4 3375-E45, Swedesboro, NJ, USA), sieved through a 500- μ m sieve, and stored in zipped plastic bags at -20°C for further analysis.

Flow diagram of the methods used in the thesis was shown in Figure 3.5.

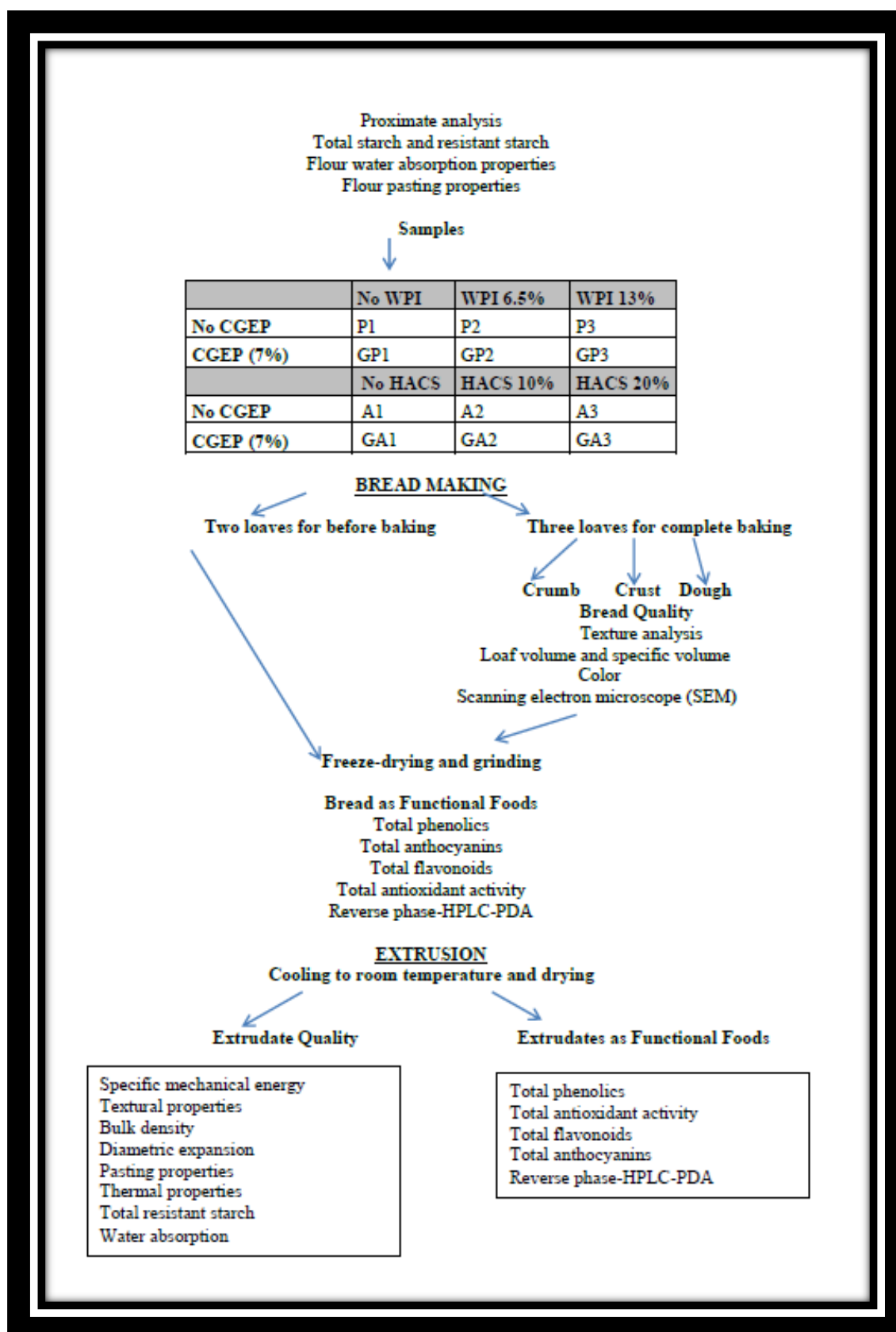


Figure 3.5 : Flow diagram of the methods used in the thesis.

3.3.3 Determination of native flour properties

3.3.3.1 Proximate analysis

Total moisture content of the samples was determined using the AACC International method 44-15 (Moisture-Air Oven) (AACC, 2000). Samples were dried for 1 hour at 130°C.

Ash content determinations of the samples were made using the AOAC International method of 923.03 by ashing in the muffle furnace for 16 h at 600°C.

For protein content of the samples, Kjeldahl method for total protein (AOAC 920.87) was used. Factor of 5.7 was used for converting the total nitrogen into total protein. Each analysis was replicated twice for all samples.

3.3.3.2 Total starch and resistant starch

Contents of total resistant starch and total starch in samples were determined according to AOAC Method 2002.02 and AACCI Method 32–40, respectively using the Resistant Starch Assay Kit (Megazyme Int. Ireland Ltd, Wicklow, Ireland). Method comprised of incubating samples in a shaking water bath with pancreatic α -amylase and amyloglucosidase (AMG) for 16 hours at 37°C, during which non-resistant starch is solubilized and hydrolyzed to D-glucose by the combined action of these two enzymes. Resistant starch is recovered as pellets of suspension after termination of the reaction using ethanol-methanol washings and centrifugation. Further treatments with addition of 2M KOH, then neutralization with acetate buffer and quantitative hydrolyzation to glucose with AMG were applied. Both of resistant starch and non-resistant starch contents were measured with glucose oxidase/peroxidase reagent (GOPOD) and summed up to give the total starch content of the samples. The analysis was replicated twice for each sample.

3.3.3.3 Total amylose

Total amylose contents of flour samples were determined by using the Amylose/Amylopectin Assay Kit (Megazyme Int. Ireland Ltd, Wicklow, Ireland). In this method, samples were firstly treated with dimethyl sulphoxide (DMSO) and ethanol to precipitate the starch in the sample. After dissolving of precipitated sample in an acetate/salt solution, amylopectin portion is complexed with concanavalin A

(Con A) to form a precipitate. Amylose in the aliquot, on the other hand, is enzymatically hydrolyzed to D-glucose, and analyzed using a GOPOD reagent. The analysis was replicated twice for each sample.

3.3.3.4 Flour water absorption properties

Farinograph parameters of different dough samples were determined using AACCI Method 54-2 (AACCI, 2000). The farinograph equipped with a 50-g bowl was used for the analyses (Figure 3.6). The analysis was replicated twice for each sample.



Figure 3.6 : Farinograph used for flour water absorption properties.

3.3.3.5 Flour pasting properties

Pasting properties of the flour mixes were determined according to AACCI Method 76-21 (2000) using a Rapid Visco Analyzer (RVA-4, Newport Scientific, Warriewood, Australia) (Figure 3.7). Each sample (corrected to 3.5 gon a 14% moisture basis) was mixed with 25 ml (adjusted to sample weight) of distilled water using the Standard 1 heating cycle of 50°C-95°C-50°C. Sample was equilibrated at 50°C for approximately 1 minute, heated to 95°C over 3.75 minutes, then held at 95°C for 2.5 minutes, cooled down to 50°C over another 3.75 minutes, and held at 50°C for 2 minutes, for a total heating cycle of 13 minutes. Pasting data was acquired

using Thermocline version 1.2 Software (Newport Scientific Inc., Warriewood, Australia). The analysis was replicated at least twice for each sample.

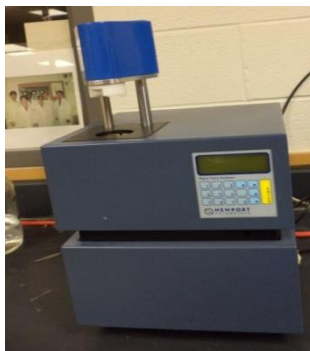


Figure 3.7 : Rapid Visco Analyzer (RVA).

3.4 Bread Quality Tests

3.4.1 Texture analysis

For evaluating crumb firmness a texture analyzer (TA Stable Microsystems, Godalming, Surrey, UK) coupled with Texture Expert Software (Version 1.22, Stable Micro Systems, Surrey, UK) was used (Figure 3.8). Two slices of 25 mm width were cut from the middle of the loaves symmetrically using a commercial electric knife. For this purpose, AACC Method 74-09 (Determination of Bread Firmness) was performed using a cylindrical probe (38 mm radius) and applying 40% strain. Each slice was measured 10 times and the results are the mean values of those measurements.

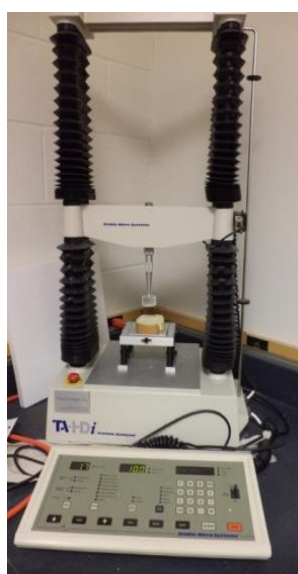


Figure 3.8 : Texture analyzer.

3.4.2 Loaf volume and specific volume

Loaf volume measurements were made using the rapeseed displacement method at a loaf volumeter (National MFG, Lincoln, Nebraska) (Figure 3.9). The analysis was replicated twice for each sample. Specific volume was calculated by dividing loaf volume (cm^3) to its weight (g) (AACC, 2000).



Figure 3.9 : Rapeseed displacement volumeter.

3.4.3 Color

Color measurements of bread dough powders and bread crumb and crust were measured using a Chromameter (CR-400, Konica Minolta Holdings Inc., Tokyo, Japan). Hunter $L^*a^*b^*$ values which describes lightness (100 for perfect white and 0 for black), red-green ($+a^*$ for redness and $-a^*$ for greenness) and yellow-blue ($+b^*$ for yellowness and $-b^*$ for blueness) color of the samples respectively were measured. The instrument was calibrated with a standard white reflector plate prior to the measurements. The results are the average values of 5 measurements from different parts of bread samples.

3.4.4 Scanning electron microscope (SEM)

Bread powders from different parts of bread (dough, crumb and crust) were fixed on aluminum pin-type stubs using carbon paste. Excess powder was removed using high-pressure air. Samples were coated with gold-palladium by a mini sputter coater (Quorum SC7620, Qorum Technologies LTD, Kent, UK). The samples were viewed

at 1500X resolution at a scanning electron microscope (FEI Quanta 250, Company, Hillsboro, Oregon, USA).

3.5 Extrudate Quality Tests

3.5.1 Specific mechanical energy (SME)

Specific mechanical energy of the extrudates was calculated according to the following equation (3.1) (Hu et al., 1993):

$$SME(kWhkg^{-1}) = \frac{Screw\ speed \times Power\ (kW) \times Torque\ (\%)}{Maximum\ screw\ speed \times Throughput\ (kg\ h^{-1}) \times 100} \quad (3.1)$$

3.5.2 Pasting properties

Pasting properties of extrudate powders were determined according to AACCI Method 76-21 (2000) using a Rapid Visco Analyzer (RVA-4, Newport Scientific, Warriewood, Australia) as described in Section 3.3.3.7 of this thesis. Pasting data was acquired using Thermocline version 1.2 Software (Newport Scientific Inc., Warriewood, Australia). The analysis was replicated at least twice for each sample.

3.5.3 Textural properties

A TA-HDi texture analyzer (Stable Microsystems, Godalming, Surrey, UK) fitted with a 50-kg load cell, coupled with Texture Expert Software (Version 1.22, Stable Micro Systems, Surrey, UK) was used for determining the hardness of extrudates. An extrudate (100 mm long) was compressed between a 3-Point Bending Rig (HDP/3PB) and a blade 90 mm long and 3 mm thick, at a crosshead speed of 2 mm/sec (Stojceska et al., 2009). The highest value of force (N) in the force – time (distance) curve was taken as the value for hardness. Measurements were made on 10 extrudates for each treatment.

3.5.4 Bulk density

Bulk density measurements of the extrudates were made as indicated by Alvarez-Martinez et al. (1988) according to equation (3.2):

$$\text{Bulk density (kg/m}^3\text{)} = 4m/\pi d^2 L \quad (3.2)$$

where m = mass of extrudate sample, d = extrudate diameter and L = extrudate length

3.5.5 Diametric expansion

For diametric expansion, 20 pieces from each extrusion run were measured with a caliper. The expansion ratio was calculated as the cross-sectional diameter of an extrudate divided by the diameter of the die opening (3 mm) (Camire, Dougherty & Briggs, 2007).

3.5.6 Water absorption

Water absorption capacities of the extruded samples were determined according to AACCI Method 56-30 (AACCI, 2000) with the following modifications. A sample (1 g) was weighed in a 50 ml centrifuge tube (pre-weighed), 30 ml of water was added, and the tube was vortexed. The tubes were left at room temperature for 30 min, intermittently vortexed, and centrifuged at 2000g for 15 min. After centrifuging, the tubes were left upside down for 10 minutes to drain the water and weighed. The difference between the combined dry sample plus tube weight and the final weight gave the amount of absorbed water. The analysis was replicated twice for each sample.

3.5.7 Total resistant starch

Content of total resistant starch in samples was determined according to AACCI Method 32–40 using the Resistant Starch Assay Kit (Megazyme Int. Ireland Ltd, Wicklow, Ireland) as explained in detail in Section 3.3.3.2. The analysis was replicated twice for each sample.

3.5.8 Thermal properties

Thermal properties were investigated using a Differential Scanning Calorimeter (DSC) (Model Q10, TA Instruments Inc., New Castle, DE, USA). For determining transition onset temperature (T_o), transition peak temperature (T_p), and transition enthalpy (ΔH), Universal Analysis 2000 Version 4.5A (TA Instruments Inc., New Castle, DE, USA) software was utilized. The calibration was made using an indium standard. According to the modified method of Kim et al., (2006), samples of about 3 mg were weighed into aluminum pans (TA Instruments, Inc., New Castle, DE, USA) and 12 μ l of distilled water were added using a micro-syringe. The sample pans were hermetically sealed and allowed to equilibrate for 2 hours at room temperature.

Samples were heated from 20 to 160°C at a rate of 10°C/min. A sealed empty pan was used as a reference. Gelatinization was evaluated by searching for the presence of any enthalpy around 60 °C.

For retrogradation determination, same samples were stored at 4°C for seven days and re-run by DSC (Jane et al., 1999). The samples were heated from 20 to 180°C at a rate of 10°C/min. The analysis was replicated twice for each sample.

3.6 Evaluation of Bread and Extrudates as Functional Foods

3.6.1 Extraction method

For extraction, 5 ml of methanol:water solution (75 :25, v/v) with 3% formic acid was added to 1 g of sample and the mixture was sonicated in ice-water for 15 minutes. Extraction method was adapted from Bino et al. (2005).

Samples were vortexed before and after sonication. After sonication, the tubes were centrifuged at 10000 rpm at 4°C for 5 min. The centrifugation was repeated until 20 ml of extract is collected in two replicates for each sample.

3.6.2 Total phenolics

Total amount of phenolic acids in non-processed and processed samples were determined using the modified Folin Ciocalteu method (Velioglu et al., 1998). Folin-Ciocalteu reagent was diluted with water (1:10). Diluted solution (750 µl) was well mixed with 100 µl of sample extract and 750 µl of sodium carbonate solution (6%). After incubation for 90 minutes in the dark the solution absorbance was measured at 750 nm against water. The analysis was replicated twice for each sample.

3.6.3 Total anthocyanins

Samples with CGEP were analyzed for total amount of anthocyanins using the modified pH differential method of Lee et al. (2005). The samples were diluted with two buffer solutions (KCl-pH 1.0 and Sodium acetate-pH 4.5) and absorbance measurements were made at 520 and 700 nm. The analysis was replicated twice for each sample.

Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L) =

$$A \times MW \times DF \times 10^3 / (\epsilon \times 1)$$

where $A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH } 4.5}$;

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu);

DF = dilution factor established in D;

l = pathlength in cm;

ϵ = 26 900 molar extinction coefficient, in $\text{L} \times \text{mol}^{-1} \times \text{cm}^{-1}$, for cyd-3-glu; and

10^3 = factor for conversion from g to mg.

3.6.4 Total antioxidant activity

Total antioxidant capacity of the samples was determined by two different methods; ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging method (Miller and Rice-Evans, 1997) and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method (Viluda-Martos et al., 2011). ABTS solution was prepared by weighing and mixing with $\text{K}_2\text{S}_2\text{O}_8$ (Miller and Rice-Evans, 1997). After incubating this solution for 14-16 hours in the dark, the analysis was done by first diluting the ABTS solution to 0.9 ± 0.2 absorbance with 0.05 M KPi buffer (pH=8) at 732 nm. Then 100 μl extract was added into 1 ml of ABTS solution in the tube and absorbance was read against water at 732 nm after 15 seconds of vortexing and 45 seconds of resting. According to the DPPH radical scavenging method, 0.1 mM of DPPH solution was freshly prepared. Sample extract (100 μl) was mixed with 2 ml of DPPH solution and after 30 minutes, absorbance was measured at 517 nm against methanol as reference. The analysis was replicated twice for each sample.

3.6.5 Total flavonoids

Determinations of the total flavonoid content in Concord grape extract powder added samples was made using the modified method of Kim et al., 2003. Sample extract (1 ml) was mixed with 0.3 ml of sodium nitrite solution (5%, w/w) and 0.3 ml of aluminum chloride solution (10%, w/w). Mixed solution was incubated at ambient temperature for 5 min. and then 2 ml of NaOH (1M) and 2.4 ml of distilled water were added respectively. Absorbance was measured at 510 nm against water as reference. The analysis was replicated twice for each sample.

3.6.6 Reverse phase-HPLC-PDA

Methanolic extracts of samples were filtered through a 0.45- μ m porous PET (polyester) syringe filter (CHROMAFIL, Düren, Germany) and 1 ml of extract was transferred into vials. The HPLC system comprised a Waters 600 control unit, a Waters 996 photodiode array (PDA) detector, and a column incubator which is heated to 40 °C. as the stationary phase Supelcosil HPLC column LC-18, (5 μ m, 25 cmx4.6 mm) (Supelco Inc., Bellefonte, Pennsylvania, USA) was used. The mobile phase was composed of solvent A; Milli-Q water with 0.1% (v/v) TFA and solvent B; acetonitrile with 0.1% (v/v) TFA. A linear gradient was used: at 0 min, 95% solvent A and %5 solvent B; at 45 min, 65% solvent A and 35% solvent B; at 47 min, 25% solvent A and 75% solvent B; and at 54 min returns to initial conditions. The flow rate was 1 ml/min. chromatograms were extracted at wavelengths of 280 nm, 312 nm, 360 nm and 520 nm. Identification was based on the retention times and characteristic UV spectra and quantification was done by external standard calibration curves. Standard calibration curves were prepared using standards prepared in methanol with changing concentrations in a linear range. All analyses were performed in duplicate.

3.7 Statistical Analysis

Factors of die exit temperature (3 levels) x CGEP addition (2 levels) x high amylose starch addition (3 levels) were tested statistically. Both the effects of extrusion barrel temperatures and the differences between the formulations were investigated by analysis of variance (ANOVA) ($p < 0.05$). Detailed examinations for significant differences were made using Duncan's New Multiple Range Test (Montgomery & Runger, 2007). Pearson's correlation matrix was applied to determine the correlation coefficients between parameters measured. SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA).

4. RESULTS AND DISCUSSION ON PROXIMATE ANALYSIS AND PHYSICOCHEMICAL PROPERTIES OF FLOUR MIX SAMPLES

4.1 Proximate Analysis and Physicochemical Properties of Flour Mix Samples

Proximate analysis of flour mix samples were determined to characterize the raw materials used in the study (Details were given in Tables 3.1 and 3.2). Total moisture, ash, and protein measurements were carried out to investigate the raw material properties. Total starch, resistant starch and amylose/amylopectin ratios of the flour mix samples were measured in all samples which are coded as:

P1- bread flour control;

P2- bread flour substituted with 6.5% wheat protein isolate (WPI);

P3- bread flour substituted with 13% WPI;

GP1- bread flour substituted with 7% CGEP;

GP2- bread flour substituted with 6.5% WPI and 7% CGEP;

GP3-: bread flour substituted with 13% WPI and 7% CGEP (details are given in Table 3.1 and 3.2).

A1- bread flour control;

A2- bread flour substituted with 10% high amylose corn starch (HACS);

A3- bread flour substituted with 20% HACS;

GA1- bread flour substituted with 7% Concord grape extract powder (CGEP);

GA2- bread flour substituted with CGEP and 10% HACS;

GA3- bread flour substituted with CGEP and 20% HACS

In addition, water absorption and pasting properties of flour mix samples were also investigated.

4.1.1 Proximate analysis of flour mix samples

Total moisture, ash and protein contents of the flour mix samples were given in Table 4.1. Moisture contents of mix samples were found to be in the range of 11.0 and 12.2 %. Wheat protein isolate (WPI) has a high water-binding capacity (Dayy et al., 2006), but made only a slight decrease in the moisture content of both P and GP samples. The moisture contents of high amylose corn starch (HACS) added samples (A2 and A3) were slightly higher than control flour (A1), but the difference was not statistically significant ($p>0.05$). CGEP addition decreased the moisture contents significantly ($p<0.05$) since it has lower moisture content (around 6%) and water absorption character (Potter et al., 2013) because of its sugar and soluble fiber content (12% dry basis).

Table 4.1 : Proximate values for flour mix samples, %

Flour mix	Moisture content	Ash content ¹	Protein content ¹
P1	12.0±0.4abc	0.51±0.00b	16.7±0.5bc
P2	11.4±0.1bcd	0.60±0.00b	22.1±1.5ab
P3	11.4±0.1cd	0.60±0.03b	25.8±0.5a
GP1	11.0±0.6d	1.49±0.17a	16.2±1.1bc
GP2	11.6±0.1abcd	1.56±0.00a	19.3±0.1b
GP3	11.1±0.1d	1.60±0.02a	20.8±0.0ab
A1	12.0±0.4abc	0.51±0.00b	16.7±0.5bc
A2	12.1±0.4ab	0.47±0.03b	11.0±0.4c
A3	12.2±0.3a	0.43±0.01b	9.7±0.3c
GA1	11.0±0.6d	1.49±0.17a	16.2±1.1bc
GA2	11.0±0.3d	1.51±0.02a	11.3±0.4bc
GA3	11.3±0.3d	1.41±0.08a	9.9±0.2c

¹Results are given on dry matter basis. Data given is the mean value± standard deviation of duplicate analyses. P1: bread flour control; P2: bread flour substituted with 6.5% wheat protein isolate (WPI);

P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% Concord grape extract powder (CGEP); GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

A1: bread flour control; A2: bread flour substituted with 10% high amylose corn starch (HACS); A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% concord grape extract powder (CGEP); GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP

Ash contents of the flour mix samples were between 0.43 and 1.60% on dry matter basis. HACS and WPI made no significant changes in the ash contents of all A and P coded samples. However, CGEP addition significantly increased ($p<0.05$) the ash contents of all GA and GP samples, approximately three times. Fruit powders are

significant sources for minerals as reported in other researchers as well (Filipčev et al., 2010).

Protein contents of flour mix samples changed between 9.7 and 25.8% on dry matter basis. Protein content of control flour (A1) gradually decreased due to both HACS and CGEP substitutions, as expected (from app. 16.7% to 9.7%). Both CGEP and HACS substituted samples had a slightly lower protein content than only HACS substituted samples. On the other hand, WPI substitution significantly increased the protein contents from 16.7 % to 25.8% in P samples and the change was found to be statistically significant ($p < 0.05$). In CGEP and WPI substituted samples (GP samples) highest level of protein content was 20.8% and the change in protein levels was statistically insignificant ($p > 0.05$).

4.1.2 Total starch and resistant starch contents of flour mix samples

Total resistant starch and total starch contents of flour mixes were given in Table 4.2. Resistant starch contents of flour mixes ranged between 0.6 and 10.4 %. Level of amylose substitution was the main determining factor for variations in resistant starch measurements. Higher amylose content after HACS substitutions lowered starch digestibility, thus amylose content and resistant starch formation are positively correlated (Singh et al., 2010). Therefore, in only HACS substituted A2 and A3 samples, the resistant starch increased gradually up to approximately 20 times and the difference was found statistically significant ($p < 0.05$). In both HACS and CGEP substituted samples of GA2 and GA3, extent of increase in resistant starch was slightly more than A samples. Previously, it was revealed that phenolic and anthocyanin fractions in fruits are important for the digestibility of starch. By means of their pertinent inhibitory effects on α -glucosidase and α -amylase enzyme activities, they increase resistant starch contents (McDougall et al., 2005). Therefore, the positive effect of CGEP on resistant starch formation in flour mixes might be related this inhibitory effect of anthocyanins/phenolics on enzymes.

In WPI substituted samples, the increase in resistant starch content was also observed after a certain level of substitution (from samples P2 to P3 and GP2 to GP3, respectively). In the available literature, similar correlation between protein and resistant starch content was also reported in various rice samples differing in amylose and protein contents and proposed to different impacts of protein on different

fractions of resistant starch (RS type I (physically inaccessible starch) rather than on RS type III (retrograded starch) (Zhang et al., 2007).

Table 4.2 : Total starch and resistant starch contents of flour mixes, %

	Total Resistant Starch¹	Total Starch¹
P1	0.6±0.1	80.9±1.6
P2	0.7±0.1	67.8±1.5
P3	9.3±1.2	58.6±1.9
GP1	0.9±0.2	71.8±1.7
GP2	0.8±0.1	63.2±1.0
GP3	5.0±0.8	56.8±0.6
A1	0.6±0.1	80.9±1.6
A2	8.8±0.9	80.7±0.3
A3	9.6±0.4	80.3±3.7
GA1	0.9±0.2	71.8±1.7
GA2	6.4±0.2	74.5±0.5
GA3	10.4±1.6	71.5±1.3

¹Results are given on dry matter basis. Data given is the mean value± standard deviation of duplicate analyses.

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

A1: bread flour control; A2: bread flour substituted with 10% HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP

4.1.3 Amylose contents of flour mix samples

Amylose/amylopectin ratios of HACS substituted flour samples changed between 20.59 and 41.71% (Table 4.3). Generally, increase in amylose content was only significant at the highest level (A3) of HACS substituted samples ($p < 0.05$). For both HACS and CGEP substituted samples, although a gradual increase was also detected, it was found statistically insignificant ($p > 0.05$). Differences in amylose/amylopectin content are mostly effective on the textural and primarily on the swelling and gelling properties of the cereal products (Graybosch, 1998).

Table 4.3 : Amylose contents of flour mix samples, %

Sample	Amylose % (w/w)
A1	26.51±0.45b
A2	31.64±1.83b
A3	41.71±1.92a
GA1	20.59±0.26c
GA2	25.96±2.61bc
GA3	31.02±0.90b

¹Results are given as % of total starch contents. Data given is the mean value± standard deviation of duplicate analyses. A1: bread flour control; A2: bread flour substituted with 10% high HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP

4.1.4 Flour water absorption properties

Water absorption and dough characteristics of flour mix samples determined by farinograph were given in Table 4.4. Water absorption of flour mix samples changed in the range of 53.6 and 62.3%. During dough mixing step of bread making, gluten film is formed between the gluten fractions of flour by disulphide bonds. Thus, the dough components are held together by forming a viscoelastic mass, but this is only possible by determining the exact mixing time of dough with exact amount of water and farinograph is an appropriate tool to determine those parameters (Mohamed et al., 2010).

WPI substituted but no-CGEP added CP samples, absorbed significantly higher levels of water as the protein substitution level has increased, since WPI worked as a water-binder (Day et al., 2007). Moreover, as water absorption was found related with the protein content of samples in the previous reports (Locken, 1972; Sarker et al., 2008; Hallen et al., 2004), increasing protein substitution of the samples caused increases in water absorption values.

The level of water absorption for C samples has increased gradually by increasing HACS substitution. This finding was in agreement with the literature (Ozturk et al., 2009; Hung et al., 2005), because the amount and water binding capacity of the starch granules has been reported as another factor effecting dough rheology in addition to the protein (Faubion and Hosene, 1989). Statistically significant ($p<0.05$) increase occurred particularly at the 20% substitution level.

On the other hand, presence of CGEP was a factor that decreased the water absorption significantly ($p<0.05$) and lowest absorption values were obtained for only CGEP added samples (GA1 and GP1). The water absorption character (Potter et al., 2013) of CGEP itself is probably the cause for low absorption values for flour mix as it decreases the available water for the flour but opposes to other fruit sources previously used in breadmaking (Mohamed et al., 2010). Besides, in either of A and AP samples, almost no significant change in water absorption was detected ($p>0.05$) by the increase in HACS and WPI substitution level.

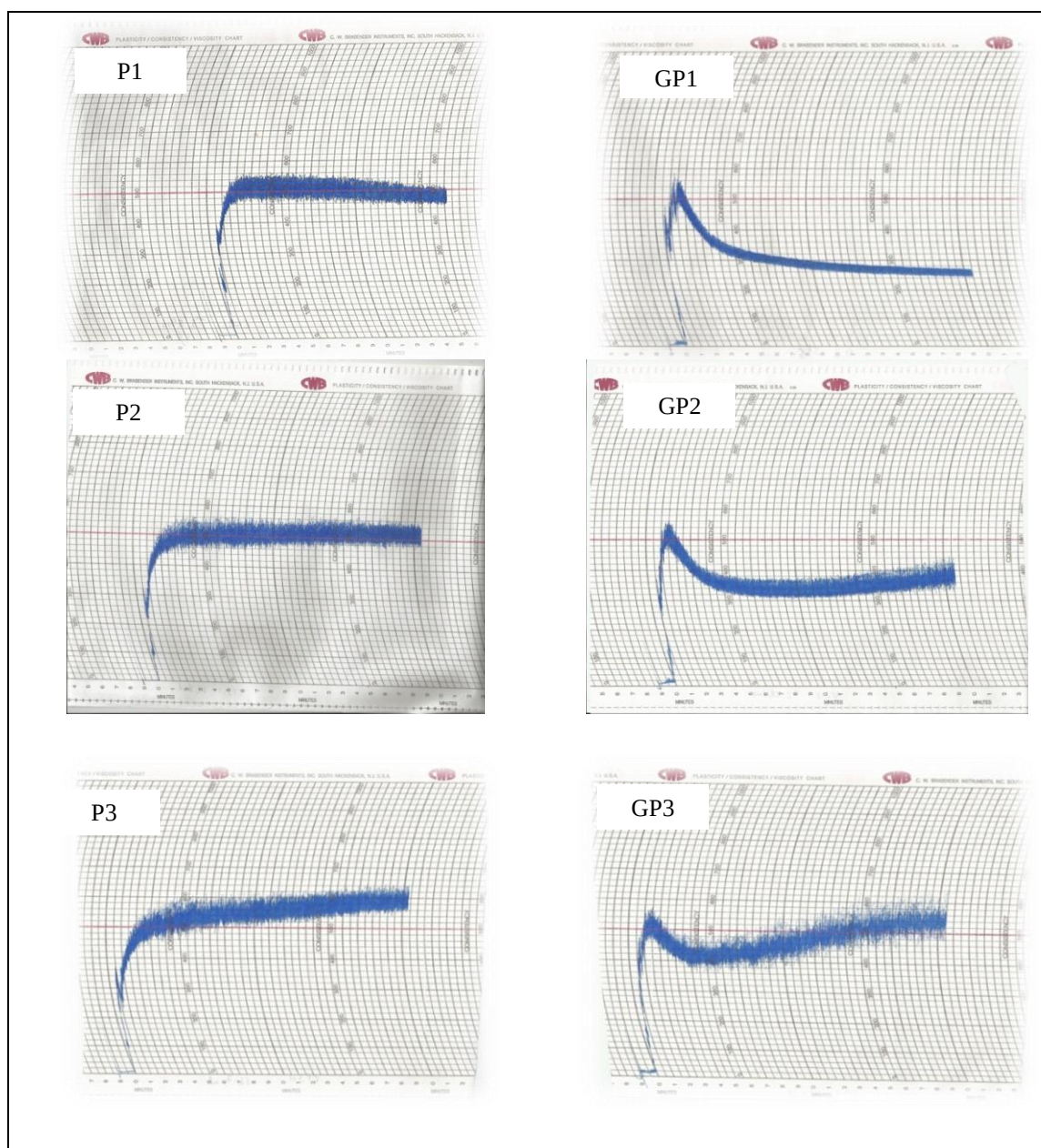
Table 4.4 : Water absorption values (%) of flour mix samples obtained by farinograph

Sample	Water Absorption (%)
P1	57.0±0.7c
P2	59.1±0.3b
P3	61.9±0.2a
GP1	53.6±1.1d
GP2	55.4±0.2cd
GP3	55.7±0.2c
A1	57.0±0.7c
A2	59.0±0.7b
A3	62.3±0.6a
GA1	53.6±1.1d
GA2	55.7±0.4c
GA3	55.4±0.4cd

Data given is the mean value± standard deviation of duplicate analyses. P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP. A1: bread flour control; A2: bread flour substituted with 10% HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

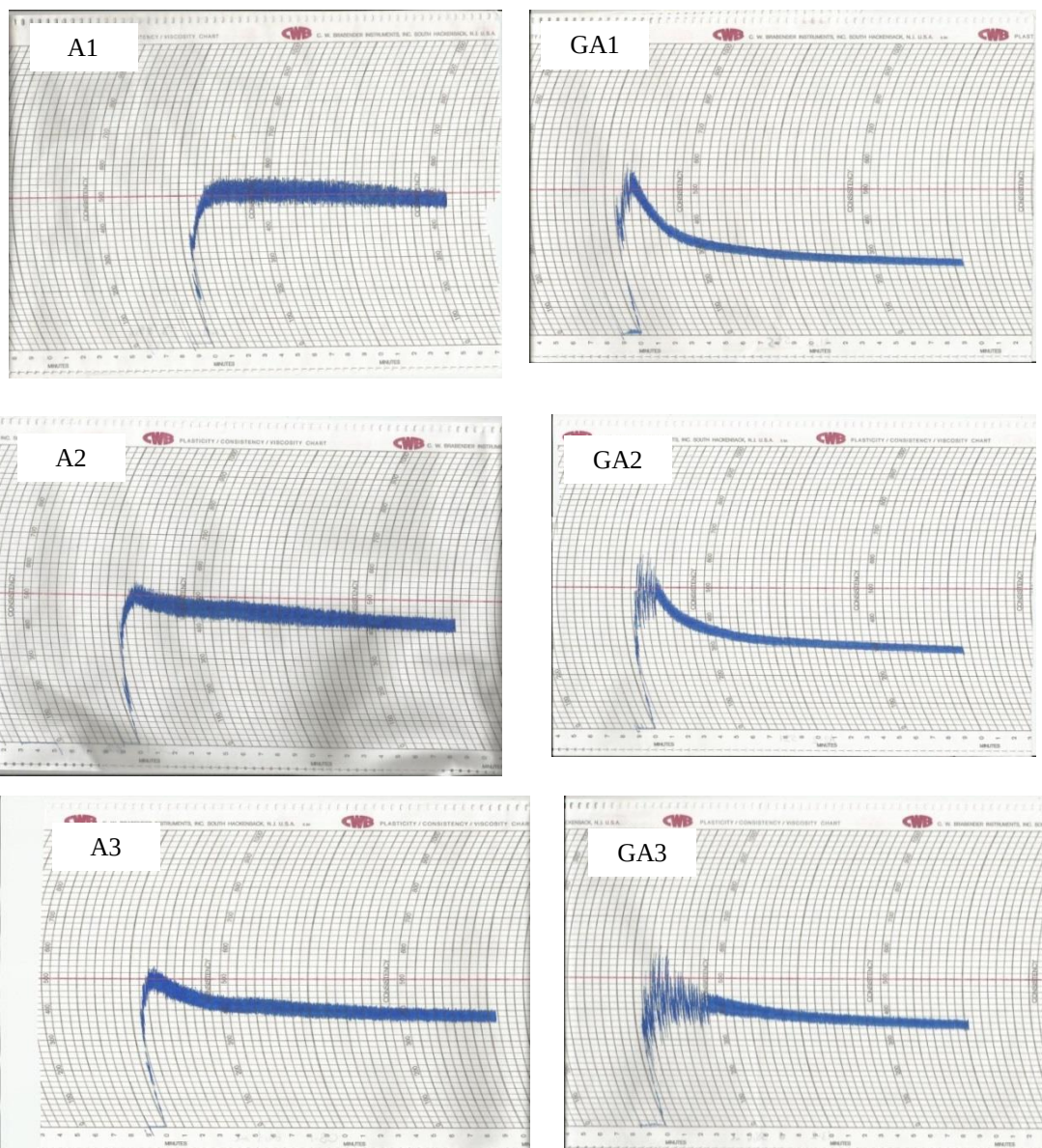
Farinograms for flour mix samples were depicted in Figures 4.1 and 4.2. In addition to water absorption, other farinogram parameters are also commonly used; perhaps the most important parameter is the dough development time or peak time (Sarker et al., 2008). The dough mixing tolerance index (MTI) is defined as the difference in Brabender Units (BU) between the top of the Farinograph-profile peak and 5 min later. Dough stability is the difference in minutes between the time when the top of the curve reaches 500 BU and the time where it leaves the 500 BU. The arrival time is also one of the dough quality parameters obtained from the Farinograph testing. It

is the time needed for the curve to reach the 500 BU mark, where longer arrival time indicates slower gluten development during mixing. Consequently, this will increase dough mixing time and delay bread production (AACC, 2001).



P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

Figure 4.1: Farinograms of WPI substituted flour mix samples.



A1: bread flour control; A2: bread flour substituted with 10% hHACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

Figure 4.2: Farinograms of HACS substituted flour mix samples.

4.1.5 Flour pasting properties

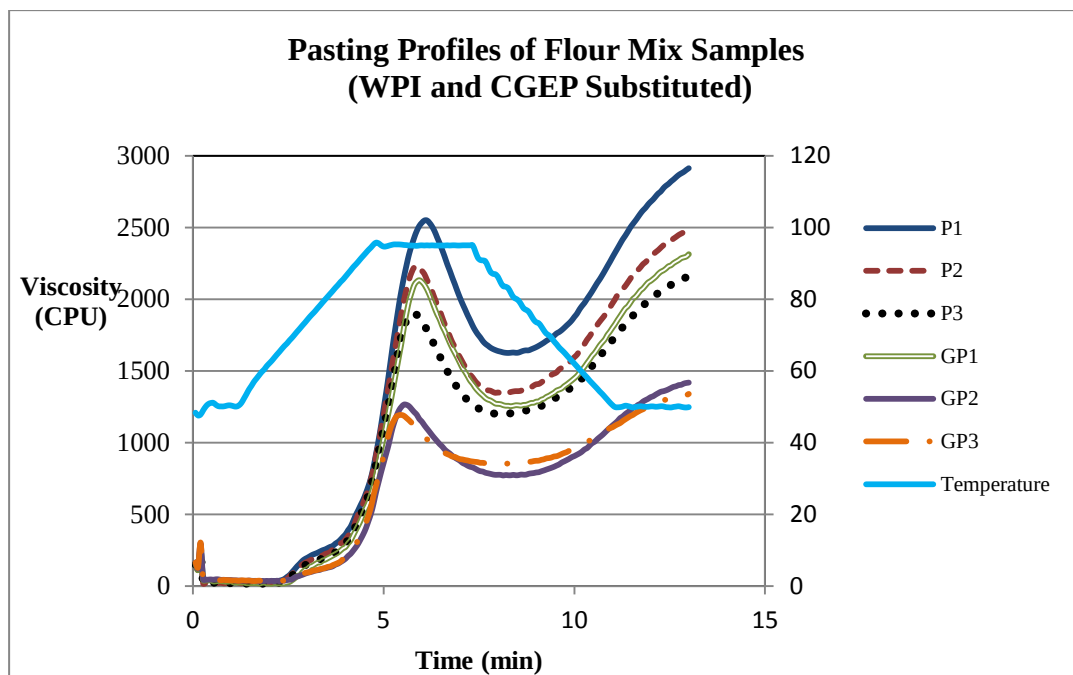
Rapid visco analyser (RVA) pasting parameters were given in Table 4.5 and 4.6 while RVA profiles of flour mix samples were given in Figures 4.3 and 4.4.

All RVA pasting parameters, except for pasting temperatures of only HACS substituted C samples, depicted a gradual and significant decrease as the HACS substitution level increased ($p < 0.05$). The effect of amylose on pasting properties is known to be different than amylopectin which contributes to swelling of starch

granules. Amylose, on the other hand inhibits the swelling (Tester and Morrison, 1990). Peak viscosity (the maximum viscosity developed during the heating of RVA sample) of A1 decreased as much as 75% in A3 flour. Incorporation of CGEP was also found to decrease the peak viscosity of the flour mix GA1 in comparison to A1 sample, significantly ($p < 0.05$). According to Núñez et al., (2009), a drop in peak viscosity might be related to the presence of hygroscopic substances as they decrease the amount of available water. According to another research, decline in peak viscosity after the incorporation of different phenolic acids into wheat flour was found to be related with the structure of the polyphenol chosen. Although some phenolicss, particularly hydroxybenzoic acids, increased the peak viscosity of the starch pastes, flavonoids and particularly flavan-3-ols generally reported as reducing factors of peak viscosity (Zhu et al., 2008). In both HACS and CGEP substituted GA2 and GA3 samples on the other hand, since the CGEP addition already declined the pasting parameters significantly, by increasing the amount of HACS, only slight decreases were observed. Although as flour contents are different in samples, it is hard to decide the real factor that affected the pasting properties.

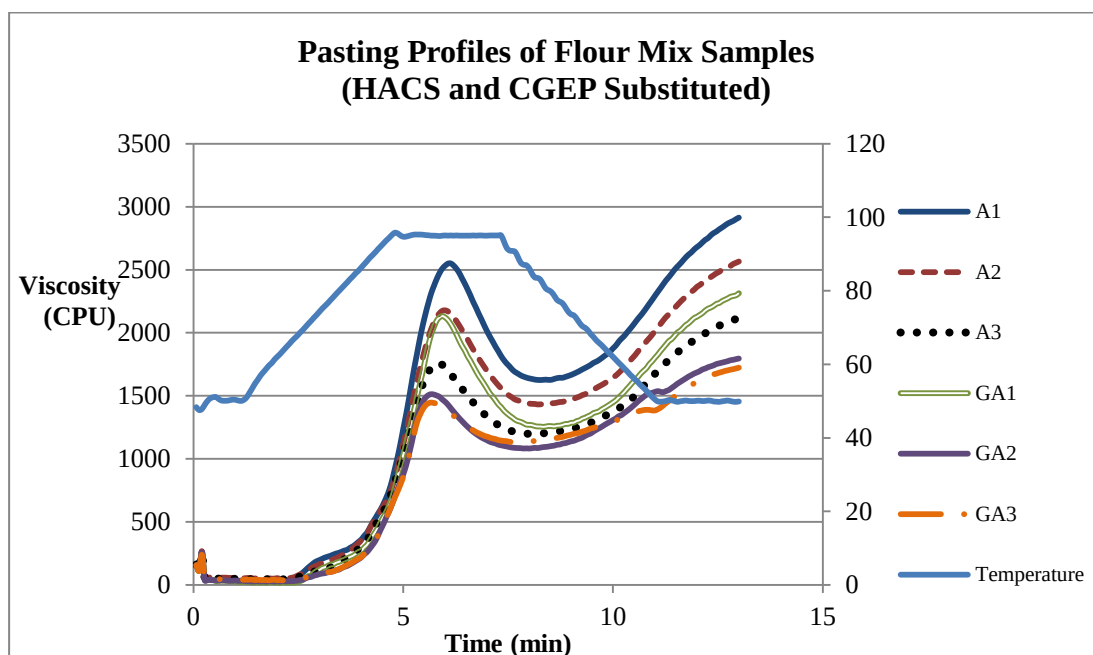
In the next phase of RVA test, after peak viscosity has been reached, by the mechanical stress applied at constant heating at high temperature (95°C), the starch granules were further disrupted; this period which ends up with a decrease in viscosity is called the breakdown viscosity (Newport Scientific, 1998). Breakdown viscosity also presented a similar trend to that of peak viscosity and breakdown viscosity and peak viscosity were found to correlate for hard and soft wheat samples in addition to their blends with resistant starch (Lei et al., 2008). The inverse relationship between amylose content and breakdown viscosity was previously revealed by other researchers as well (Noda et al., 2004). Correlation was linked to the swelling power of some starch granules that enables them to reach the maximum viscosity, as well as to easily breakdown by weak intermolecular forces that become more sensitive to rotary force when temperature is increased (Zaidul et al., 2007).

Increasing the protein content of the flour mixes by WPI substitution was found as another reason for the decrease in peak viscosity and all other viscosity parameters. Similar to the present results, gradual increase in protein levels in the formulations is considered as a weakening factor for the gel (Joshi et al., 2014). Interestingly, WPI substitution depicted similar trend with the HACS substitution.



P1: bread flour control; P2: bread flour substituted with 6.5% wheat protein isolate (WPI); P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% concord grape extract powder (CGEP); GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

Figure 4.3: RVA pasting profiles of WPI and CGEP substituted flour mix samples.



A1: bread flour control; A2: bread flour substituted with 10% HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% concord grape extract powder (CGEP); GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

Figure 4.4: RVA pasting profiles of HACS and CGEP substituted flour mixsamples.

Decrease in pasting parameters after CGEP substitution is both related to the weakening of protein network and retarding of paste formation in samples (Chaunier et al., 2007; Núñez et al., 2009) which may be attributed to the hygroscopic behavior of CGEP in formulations. Both CGEP and WPI substitution declined RVA parameters to lower levels.

Table 4.5 : RVA parameters of WPI and CGEP substituted flour mix samples.

Sample	Peak Viscosity (CPU)	Through Viscosity (CPU)	Breakdown Viscosity (CPU)	Final Viscosity (CPU)	Setback Viscosity (CPU)	Peak Time (minutes)	Pasting Temp. (°C)
P1	2558.8± 55.7a	1639.3± 6.2a	919.5± 62.0a	2923.5± 53.5a	1284.2± 59.8a	6.1± 0.1a	68.0± 0.2b
P2	2201.8± 74.8b	1341.5± 20.5b	860.3± 54.2a	2481.0± 55.0b	1139.5± 34.5b	5.8± 0.1b	68.8± 0.6b
P3	1835.0± 19.0d	1155.5± 20.5c	679.5± 1.5b	2092.0± 15.0c	936.5± 5.5c	5.8± 0.1b	68.6± 0.0b
GP1	2014.0± 22.5c	1172.0± 2.5c	842.0± 20.0a	2216.0± 12.0c	1044.0± 9.5b	5.8± 0.0b	76.7± 7.3ab
GP2	1550.3± 26.8e	899.3± 30.8e	651.0± 4.0b	1676.5± 34.5d	777.2± 3.8d	5.6± 0.0bc	84.6± 1.8a
GP3	1403.7± 22.2e	972.8± 8.3d	431.0± 14.0c	1544.3± 18.8e	571.5± 10.5e	5.5± 0.0c	84.4± 0.4a

Data given is the mean value± standard deviation of triplicate analyses. Values with different letters (a–d) within the same column differ significantly ($p < 0.05$). P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

Table 4.6 : RVA parameters of HACS and CGEP substituted flour mix samples.

Sample	Peak Viscosity (CPU)	Through Viscosity (CPU)	Breakdown Viscosity (CPU)	Final Viscosity (CPU)	Setback Viscosity (CPU)	Peak Time (minutes)	Pasting Temp. (°C)
A1	2558.8± 55.7a	1639.3± 6.2a	919.5± 62.0a	2923.5± 53.5a	1284.2± 59.8a	6.1± 0.1a	68.0± 0.2b
A2	2185.8± 40.8b	1429.5± 28.0b	756.2± 12.7b	2565.5± 40.0b	1136.0± 12.0b	6.0± 0.0b	75.49± 1.24ab
A3	1761.0± 49.5d	1196.5± 32.5c	564.5± 17.0c	2119.0± 49.5c	922.5± 17.0c	5.8± 0.0c	81.16± 0.41a
GA1	2014.0± 22.5c	1172.0± 2.5c	842.0± 20.0ab	2216.0± 12.0c	1044.0± 9.5b	5.8± 0.0b	76.7± 7.3ab
GA2	1515.0± 39.5e	1080.0± 20.5d	435.0± 19.0d	1796.0± 47.0d	716.0± 26.5d	5.7± 0.0d	84.21± 0.99a
GA3	1446.8± 30.2e	1132.8± 27.3cd	314.0± 3.0e	1723.5± 38.5d	590.8± 11.3e	5.7± 0.00d	79.99± 4.06ab

Data given is the mean value± standard deviation of triplicate analyses. Values with different letters (a–d) within the same column differ significantly ($p < 0.05$). A1: bread flour control; A2: bread flour substituted with 10% HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

5. RESULTS AND DISCUSSION ON CGEP ADDED BREAD

5.1 Effect of protein addition on CGEP added bread quality

5.1.1 Introduction

Bread has been the most significant cereal product for ages almost for all nations. Nowadays, attempts for increasing the functional properties of the cereal products have been quite common due to consumer demands and bread is one of the most widely used cereal product as the vehicle for functional ingredients. However, increased functionality is known to affect the quality of bread, generally in a negative way.

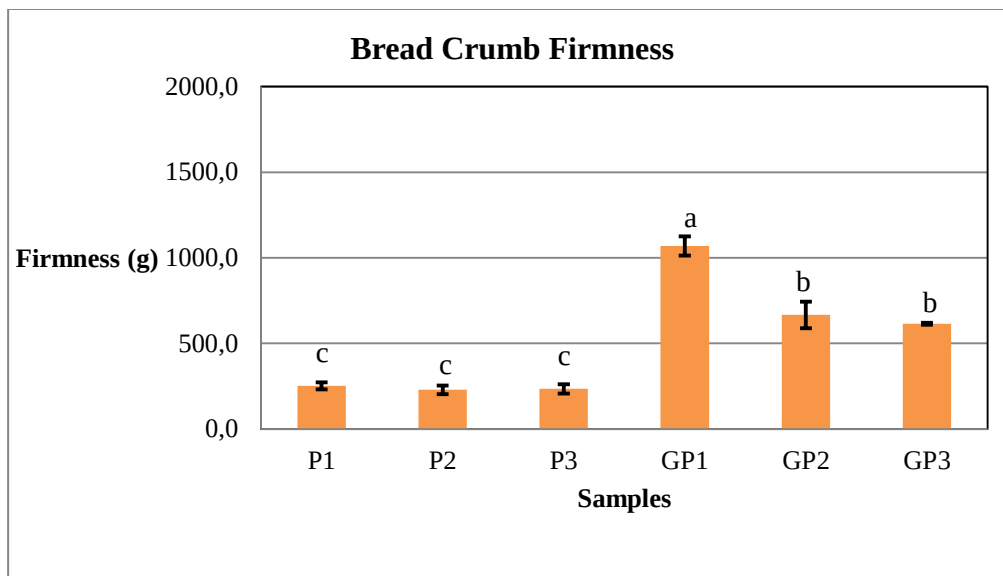
In this chapter, Concord grape extract powder was added as a phenolic and anthocyanin source, with and without wheat protein isolate (WPI) as the protein source, to evaluate the changes occurring in some quality attributes of bread samples such as textural properties, physical properties such as bread volume, specific volume, color and molecular structure (by Scanning Electron Microscopy) and thermal properties by means of CGEP interactions with WPI.

5.1.2 Texture evaluation

Texture of bread samples were evaluated by bread crumb firmness measurements and were given in Figure 5.1. Crumb firmness values ranged between 229 ± 25.3 and 252.0 ± 20.4 g for only protein added samples (CP), whereas for CGEP and WPI added bread samples (AP) values of 615.7 ± 5.7 and 1068.8 ± 56.5 g were obtained, respectively. Therefore, AP samples were found to be approximately 3 to 4-fold firmer than CP samples.

Firmness is defined as “the peak force of the first compression of the product” (Bourne, 1982). CGEP added samples became significantly softer as the protein substitution increased ($p < 0.05$). However, no significant changes were detected for CP bread crumbs by the increase in protein levels after WPI substitutions.

Hardness is an important characteristic and is commonly used as one of the main indexes to determine bread quality, because firmness changes are usually accompanied by loss of elasticity during storage (Wang et al., 2006). Therefore, firmness value gives an idea about the storage quality of the bread.



P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

Figure 5.1 : Crumb firmness values of WPI and CGEP substituted bread samples.

Bread crumb structure was reported to be related with the molecular distribution of water among starch, gluten and other components such as CGEP. During breadmaking, water moves from hydrated gluten to starch granules causing gelatinization and interactions between gelatinized starch granules and the gluten network occur in crumb, causing a loss of kinetic energy and subsequently an increase in firmness (Ottenhof and Farhat, 2004). Therefore, CGEP is a factor to affect the water mobility, gluten network development and protein secondary structure (Sun-Waterhouse et al., 2011; Baik and Chinachoti, 2000; Bollaín et al., 2005; Gray and Bemiller, 2003; Sivam et. al., 2011). Moreover, CGEP substitution in sample GP1 caused a decrease in protein content. Therefore, bread hardness has increased significantly in bread crumbs made from this (GP1) mixture of flour. Significant increase in bread hardness was detected in previous studies that used phenolic sources as ingredients (Wang et al., 2007; Wang et al., 2006; Mildner-

Szkudlarz et al., 2011) and the change has been attributed to the replacement of flour with fruit sources (Sivam et al., 2011). On the other hand, some researchers found out that different levels of grape seed extract addition was not a significant factor for bread hardness (Peng et al., 2010). Increase in protein level in GP2 and GP3 samples made a softening effect on bread crumbs significantly, although they were still significantly harder ($p < 0.05$) than CP samples.

5.1.3 Bread volume and specific volume

Bread volume and bread specific volume measurement of WPI and CGEP substituted bread samples were given in Figures 5.3 and 5.4, respectively. Loaf volumes of bread samples were found to be in the range of 456.67 cm³ to 1073.33 cm³. In P1 bread, in which no WPI and CGEP substitutions were made, the volume was measured as 803.33 cm³. Among other P samples (P2 and P3), significant increases were related to increasing WPI substitutions from P2 to P3 ($p < 0.05$).

The lowest volume was in GP1 bread, in which only CGEP substitution was made in its formula. Increase in WPI substitutions significantly increased bread volumes of AP samples at all levels (GP2 and GP3) ($p < 0.05$). CGEP added breads (AP) had about 40% lower bread volumes than CP samples.

Bread volume is one the main parameters for testing the effect of added ingredients on bread quality. A well-developed gluten network, mainly formed by inter- and intra- disulfide bonds via the SH–SS interchange reaction during dough mixing, requires oxidants such as dehydroascorbic acid to aid the oxidation of glutathione (thiol group) from its reduced form to the oxidized form, forming a strong three-dimensional protein structure which leads to good bread volume and optimum crumb texture. The main reason for reduction in volume and loss in bread quality in fruit-based ingredient added bread samples, may be the presence of antioxidants that prevent oxidation and cause improper gluten network formation (Wang et al., 2006; Cauvain, 2003; Han and Koh, 2011b). Therefore, CGEP being a fruit source can be responsible from the decrease in bread volume.

The effect of CGEP on dough rheology is similar to the other fruit-based ingredients and mainly related with its ability to compete with gluten for water, so that weaken the gluten network necessary for dough formation (Mohamed et al., 2010). Therefore, in WPI substituted samples, the negative effect of CGEP on dough

rheology has been tolerated and the loaf volume is much better (23% increase) in comparison to only CGEP added sample (GP1).

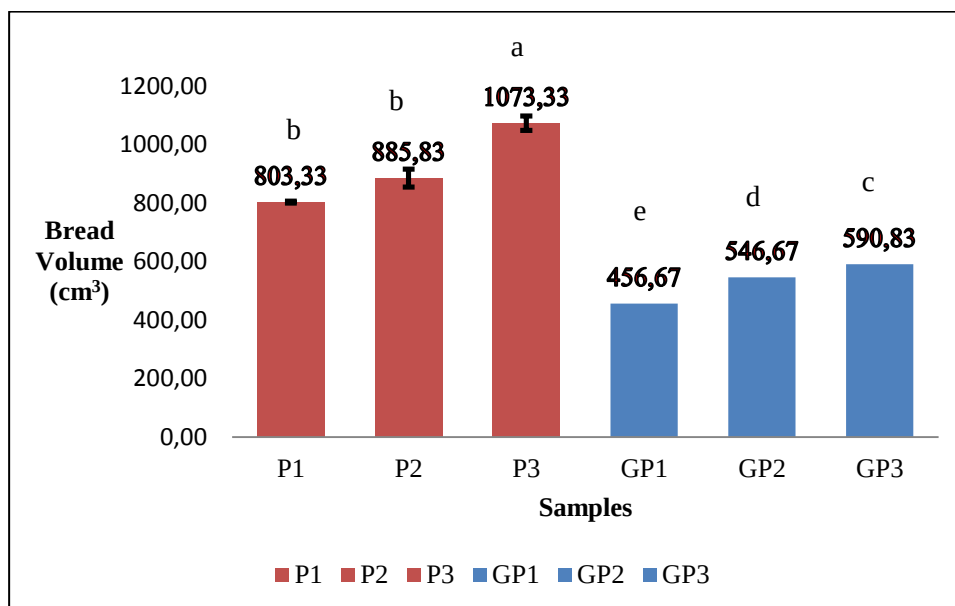


Figure 5.2 : Bread volumes of WPI and CGEP substituted samples.

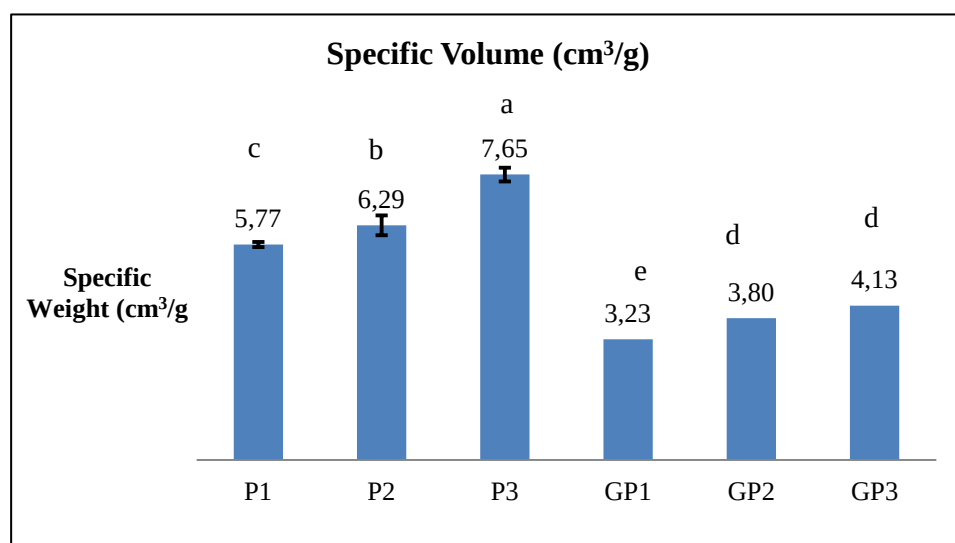


Figure 5.3 : Bread specific volumes of WPI and CGEP substituted samples.

Bread specific volumes ranged between 3.23 cm³/g and 7.65 cm³/g. In terms of specific volumes, for only WPI added bread samples (CP) increase in volume was significant at all levels ($p < 0.05$). In contrast, positive effect of WPI in CGEP substituted AP samples seemed insignificant between GP2 and GP3 bread samples ($p > 0.05$).

5.1.4 Color evaluations in bread samples and doughs

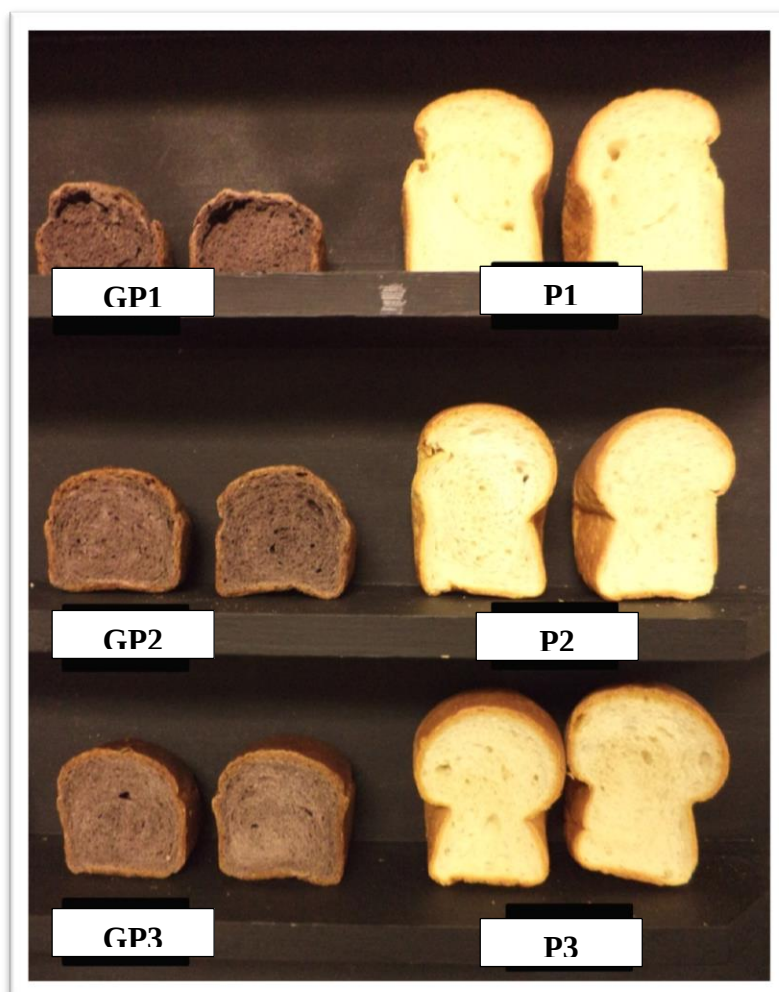
CGEP substitutions into bread flour formulations were visually evident both for the dough (Figure 5.4) as the purple color and for the breads after baking as the brown color (Figure 5.5). CGEP ingredient was dark purple in color with $L^*a^*b^*$ values 28.8, 7.51 and -9.00, respectively. Therefore; bread color measurements were expected to be different in comparison to no-CGEP added bread samples. Differences between the color measurements of the samples was found statistically significant ($p < 0.05$). Lightness ($+L^*$ 100 for perfect white and $-L^*$ 0 for black), red-green ($+a^*$ for redness and $-a^*$ for greenness) and yellow-blue ($+b^*$ for yellowness and $-b^*$ for blueness) color parameters of samples taken from three different parts of bread samples: crust, crumb and dough (taken before baking) were measured. In traditional white bread, the crust is expected to be brown as a result of both Maillard and caramelization reactions whereas, the crumb is in light cream color as temperature inside the bread is much lower and water activity is higher in the crumb (Borelli et al., 2003).



Figure 5.4 : Dough samples with CGEP (at the right side) and without CGEP (at the left side).

No CGEP added CP samples were significantly lighter in all parts than CGEP added AP samples ($p < 0.05$). L^* value decreased significantly ($p < 0.05$) in all parts of CGEP added bread samples, and this change indicated the darkening effect of CGEP by decreasing brightness. The darkening effect of Concord grapes is in agreement with other studies on different cereal products (Camire et al., 2007). Dough and crumb of CP breads were similar in color, whereas crust was darker as expected. CGEP added AP breads have significantly different brightness in different parts of the bread

samples going from lighter to darker from dough to crumb and crust and can be related with the heat sensitivity of phenolic substances in CGEP. Increase in protein content through different levels of WPI substitution made a significant darkening effect in only crust probably by the increase in Maillard reaction products. In CGEP added AP samples on the other hand, lightening effect was evident both in crumb and crust from GP1 to GP2 probably because of the interaction of protein and CGEP pigments.



P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

Figure 5.5 : CGEP+WPI substituted (GP) and WPI substituted (P) bread samples.

No CGEP added CP samples were significantly different in all parts than CGEP added AP samples. Negative a^* value in no-CGEP added CP crumb and dough samples indicated the occurrence of greenness, being significantly different than CGEP added samples because of the presence of redness (because of the purple

color of CGEP). Crust of AP samples were also significantly higher a* value (more red) than the other two parts and all AP samples had positive a* values (suggesting the presence of redness).

No CGEP added CP samples were significantly different in yellowness (b value) than from all parts of CGEP added AP samples. Among CP breads crust yellowness was significantly higher than other two parts. Differences between b* values of CGEP added AP samples were insignificant, although slightly negative b* values (indicating the presence of blueness) was detected in only some CGEP added AP samples.

Table 5.1 : Color measurements in WPI and CGEP substituted breads parts.

	L*			a*			b*		
	Crumb	Crust	Dough	Crumb	Crust	Dough	Crumb	Crust	Dough
P1	80.97 ± 2.39ax	62.48± 0.60ay	83.28 ± 1.11ax	-0.66 ± 0.01cy	8.47± 0.18cx	-0.32± 0.01cy	12.32± 0.23by	21.34± 0.52ax	11.72± 0.13cy
P2	83.0 6± 0.25ax	59.60± 0.09by	81.76 ± 0.45abx	-0.14 ± 0.01by	10.26± 0.23abx	-0.40± 0.05cy	12.79± 0.06ay	20.34± 1.14ax	13.49± 0.00by
P3	83.49 ± 0.40ax	57.57± 0.42cz	79.96 ± 0.21by	-0.57 ± 0.01cy	10.94± 0.33ax	-0.17± 0.04cy	13.10± 0.19ay	21.27± 0.40ax	14.05± 0.19ay
GP1	40.36 ± 0.01cy	28.73± 0.17ez	56.56 ± 0.45cx	4.63 ± 0.20by	7.49± 0.52cx	6.23± 0.09ax	-0.82± 0.10cx	-2.20± 0.09dy	-1.92± 0.11ey
GP2	47.41 ± 0.14by	39.06± 0.29dz	56.06 ± 0.04cx	6.99± 0.04ay	9.61± 0.51bx	5.95± 0.22ay	-0.52 ±0.02cy	1.25± 0.12cx	-0.22± 0.01dy
GP3	45.49 ± 2.36by	39.13± 0.58dz	53.58 ± 0.30dx	6.08±0.34 ay	10.72±0.0 7abx	5.51± 0.12by	-0.52± 0.04cy	3.27± 0.21bx	-1.73± 0.10ez

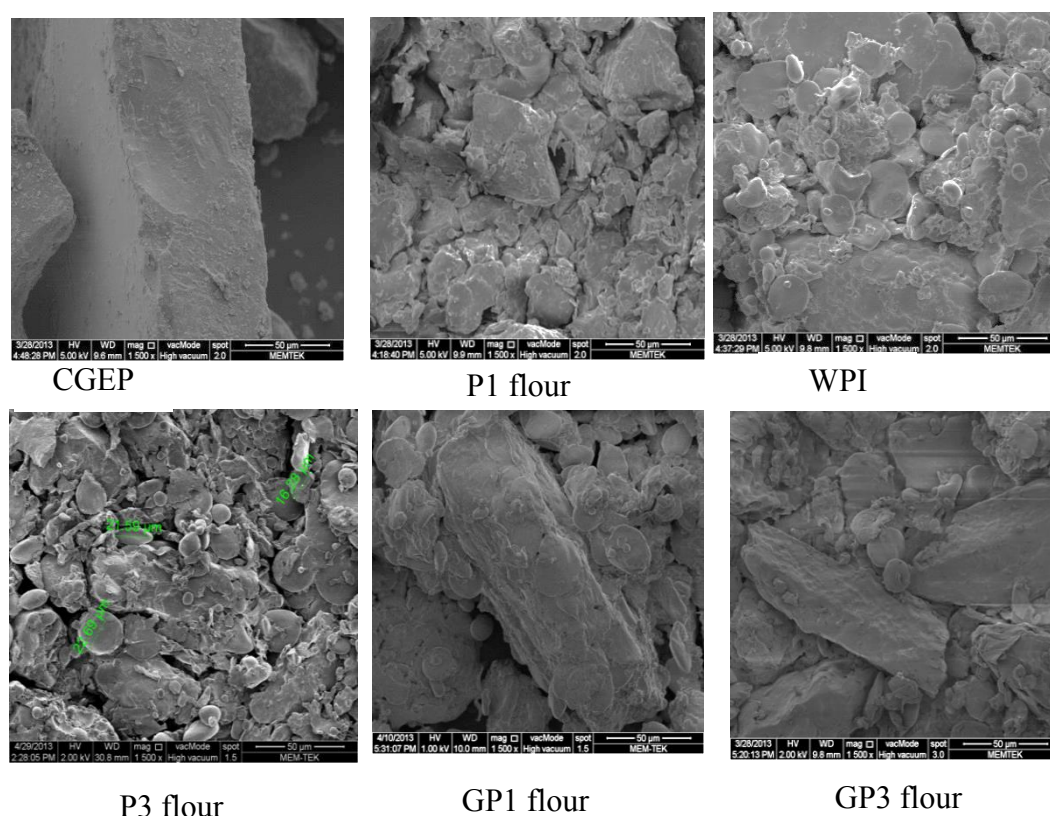
Data given is the mean value± standard deviation of triplicate analyses. Values with different letters (a–d) within the same column and different letters (x–y) within the same row differ significantly ($p < 0.05$).

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

5.1.5 Scanning electron microscope (SEM)

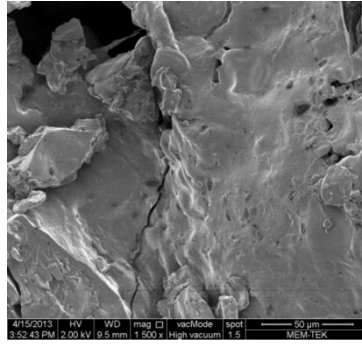
Micrographs of selected ingredients and bread samples were given in Figure 5.7 and Figure 5.8, respectively. When SEM images of ingredients are evaluated, dense protein matrix was seen in WPI, whereas for CGEP more ordered crystal-like structure was observed. In flour mixes; starch granules, protein matrix and protein adhesion to starch granules were noticed. Moreover, crystalline structure of CGEP is also well noticed. For flour samples containing WPI, a more dense protein matrix in which all other components were embedded was a characteristic micro-structure.

The crumb structure of GP1 and P1 bread samples are similar, although a smooth but homogeneous texture from soluble CGEP particle was evident. Starch granules embedded in the fibrillar protein network (Swanson, 2004) is noticed in all dough samples. In both P3 and GP3 bread dough samples, looser structure was detected and the void between particles is more than the one for P1 and GP1 dough samples. This is related with the increased volume by the well-developed gluten network.

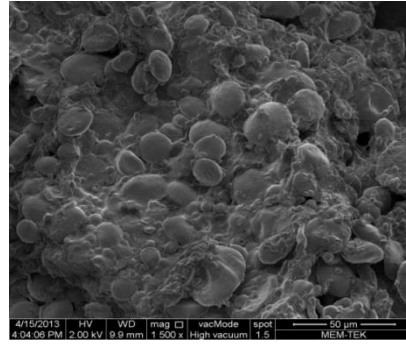


Magnification bars in SEM micrographs represents 200 µm-WPI: wheat protein isolate; CGEP: Concord grape extract powder; P1 flour: bread flour; P3 flour: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP3: bread flour substituted with 13% WPI

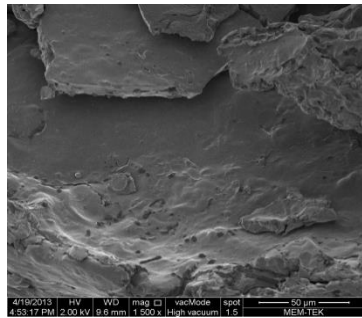
Figure 5.6 : SEM images of selected ingredients.



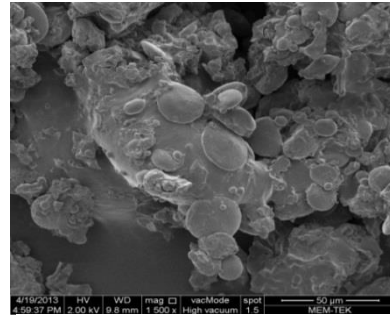
P1 Crumb



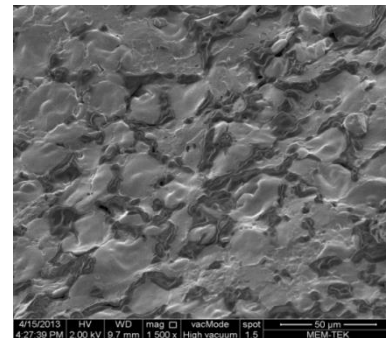
P1 Dough



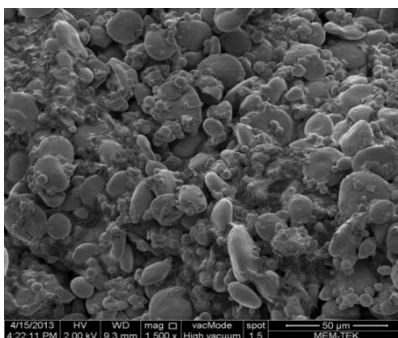
P3 Crumb



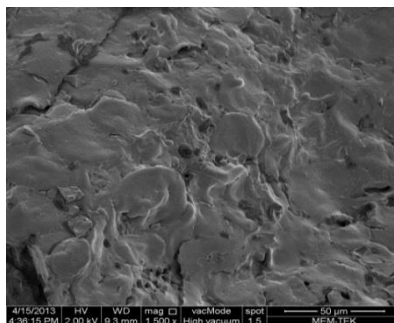
P3 Dough



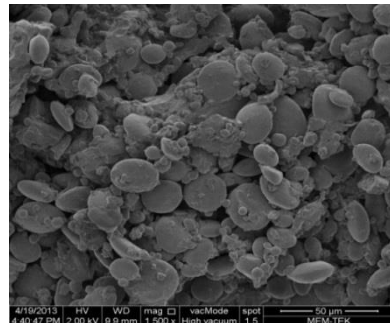
GP1 Crumb



GP1 Dough



GP3 Crumb



GP3 Dough

: **Figure 5.7:** SEM images of selected WPI and CGEP substituted bread samples.

5.2 Effect of Amylose on CGEP Added Bread Quality

5.2.1 Introduction

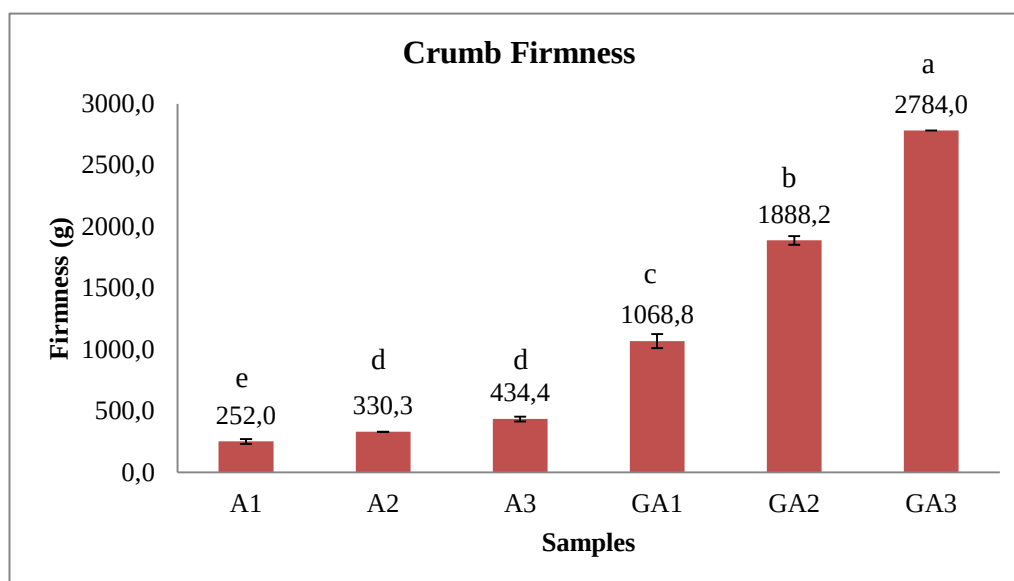
In this chapter, Concord grape extract powder (CGEP) substituted bread samples were further substituted with high amylose corn starch (HACS) to determine the changes occurring in some quality attributes of bread samples such as physical properties (bread volume, bread specific volume in bread and crumb & crust color and SEM images of the dough and finished bread) and textural properties (crumb and crust firmness) s as a result of interactions with HACS.

5.2.2 Texture analysis

Texture analysis of the HACS and CGEP substituted bread samples was made by determining the crumb firmness values of the samples. Crumb firmness is often related with bread staling together with loaf volume and crumb structure (Goesaert et al., 2005) and texture analysis is one of main methods used to measure firmness of bread (Sidhu et al., 1997). The crumb firmness results of bread samples were given in Figure 5.8. The firmness of crumbs changed between 252.0 and 2784.0 N. In only CGEP substituted bread crumb sample (GA1) was 4 fold harder than A1 crumb. In control breads (C), increase in HACS level from A1 to A2, made significant a change in crumb firmness although further increase did not affect the firmness significantly. In contrast, in CGEP substituted breads, when the HACS substitution level increased significant increases in crumb firmness more than 7 and 8 times than the GA1 crumb hardness were detected.

Bread crumb structure was reported to be related with the water molecular distribution among starch, gluten and other components such as CGEP. HACS substitutions decreased protein content in each of A samples, as the amylose content increased. Decreased protein content has been found as the reason of change in the protein secondary structure (Sivam et al., 2011) and main cause of increase in bread firmness. In addition, according to some previous research, another possibility of increased hardness in phenol-rich fruit sources, such as catechin from green tea extract (Turchetti et al., 2005; Wang et al., 2007), green tea polyphenols (Zhang and Kashket, 1998), added breads is the affecting enzyme activity and yeast activity during baking by restricted amylase activity and decreased the maltose content for

the yeasts (Wang et al., 2007; Zhang and Kashket, 1998;; Mildner-Szkudlarz et al., 2011).



Data given is the mean value± standard deviation of duplicate analyses. A1: bread flour control; A2: bread flour substituted with 10% HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

Figure 5.8 : Bread crumb firmness values of HACS and CGEP substituted bread samples.

5.2.3 Bread volumes and bread specific volumes

Loaf volumes and loaf specific volumes of bread samples were given in Figure 5.10 and Figure 5.11. Loaf volume is a way of measurement of baking performance quantitatively. Therefore, it has been regarded as the most important bread characteristics. Moreover, it is important for the consumer since their expectation for bread is its being light and not so dense (Hathorn et al., 2008). Loaf volumes ranged between 335cm³ and 803.33 cm³. Volume of only CGEP substituted GA1 bread was as low as 56% of the A1 control bread. As the HACS substitution level increased the volumes became significantly lower in both of CGEP added GA2 and GA3 (25% lower in comparison to GA1 bread volume) breads and no CGEP added A2 and A3 breads (17% lower in comparison to A1 bread volume). The effect of CGEP on dough rheology, as a gluten network weakener and competitor with gluten for water (Mohamed et al., 2010; Koh and Ng, 2009), and as a factor to decrease the relative protein content in the flour mix, have been mentioned in Chapter 5.1.3. Moreover, use of HACS is another factor on the decline in bread volumes because it had lower stability during mixing and acted like soft wheat flour (Hung et al., 2006). A

previous report on bread from starch and gluten blends with amylose content of 19.2–21.6% exhibited similar crumb structure and volume to that of bread with regular wheat starch which contained 24% amylose (Lee et al., 2002). Therefore, the current results with amylose contents of 20.59, 25.96 and 31.02, for GA1, GA2 and GA3 flour samples, with significantly lower bread characteristics might be related with the effect of CGEP and decline in protein content.

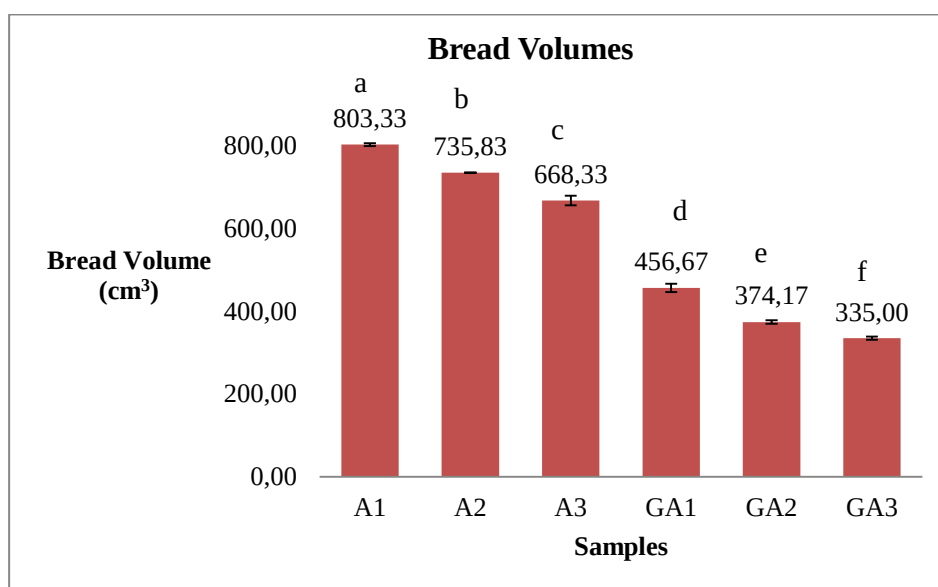


Figure 5.9 : Bread loaf volumes of HACS and CGEP substituted bread samples.

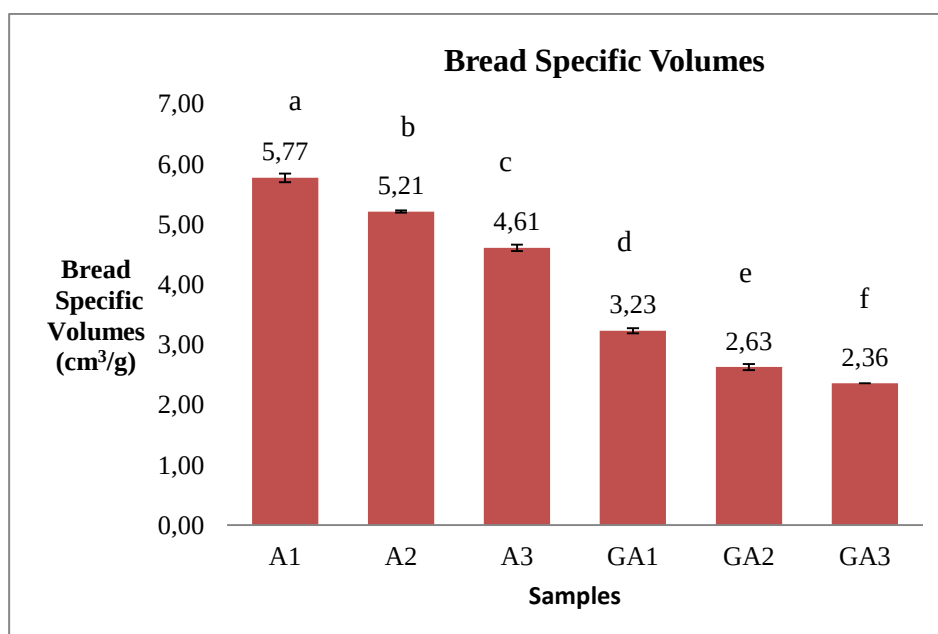


Figure 5.10 : Bread specific volumes of CGEP and HACS substituted bread samples.

Bread specific volumes had also the same tendency with bread volume results and significantly decreased with CGEP and HACS substitutions. However the reductions are comparable with results of other studies for breads fruit sources such as carob fiber, inulin and pea fiber; (Wang et al., 2002); apples, kiwifruits and berry polyphenols (Sun-Waterhouse et al., 2011), blackcurrant polyphenols and pectin (Sivam et al., 2011) and maitake mushroom powder (Seguchi et al., 2001).

5.2.4 Color

Color Color measurements of CGEP and HACS substituted bread samples in three different parts of bread samples were given in Table 5.2. Bread samples produced using CGEP and HACS substituted flour were depicted (Figure 5.11). Differences between CGEP and no-CGEP added bread samples in addition to $L^*a^*b^*$ values on different parts of the bread samples were statistically significant ($p<0.05$). As mentioned in section 5.1 this is an expected result because CGEP has a strong color.

Color attributes of breads are generally considered as a significant sensory criterion for consumers when they are making their selection and most important parameter is probably the darkness or lightness (Hathorn et al., 2008). No CGEP added C samples were significantly lighter in all parts than CGEP added A bread samples. L^* value decreased significantly ($p<0.05$) in all parts of CGEP added bread samples, and this change indicated the darkening effect of CGEP. CGEP added A breads have significantly different brightness in different parts of the bread samples ($p<0.05$). Increase in amylose content through different levels of HACS substitution made only a slight change although increasing paleness was noticed visually probably, due to the presence of starch, but not found as significant at all levels statistically ($p<0.05$). In CGEP added A samples, crust color became lighter when GP2 was compared with GP3.

a^* values determined to compare between redness and greenness. No CGEP added C samples were significantly different in crumb and dough color than CGEP added A samples. Slightly negative a^* value in no-CGEP added C crumb and dough samples indicated the occurrence of greenness being significantly different than crust suggesting the presence of redness (because of the purple color of CGEP). Crusts of all breads were found as similar in color although redness slightly decreased with the increase in HACS substitution levels. Color in different parts of the breads were not

consistent and this was probably due to the improper crust formation which caused decreased the temperature difference between crust and crumb.

No CGEP added C samples were significantly different in yellowness in all parts of CGEP added A samples. Yellowness among CP breads was significantly higher than other parts only in the crust. Although slight negative b* value was evident in all parts of CGEP added A samples, differences between different parts were insignificant.

Table 5.2 : Color measurements in HACS and CGEP substituted bread parts.

	L			a			b		
	Crumb	Crust	Dough	Crumb	Crust	Dough	Crumb	Crust	Dough
A1	80.97 ±2.39 ax	62.48 ±0.60 by	83.28 ±1.11 bx	-0.66 ±0.01 cy	8.47 ±0.18 ax	-0.32 ±0.01 cy	12.32 ±0.23 ay	21.34 ±0.52 bx	11.72 ±0.13 ay
A2	81.10 ±1.24 ax	65.17 ±0.18 ay	78.44 ±0.51 cx	-1.23 ±0.05 dy	8.23 ±0.01 ax	-0.67 ±0.03 cdy	10.75 ±0.03 by	23.03 ±0.18 ax	10.54 ±0.02 by
A3	81.03 ±0.11 ax	62.64 ±0.98 by	85.30 ±0.13 ax	-1.43 ±0.04 dy	5.48 ±0.51 cx	-0.74 ±0.06 dy	10.12 ±0.08 cy	16.39 ±0.23 cx	10.74 ±0.06 by
GA1	40.36 ±0.01 by	28.73 ±0.17 dz	56.56 ±0.45 dx	4.63 ±0.20 by	7.49 ±0.52 abx	6.23 ±0.09 ax	-0.82 ±0.10 dx	-2.20 ±0.09 dy	-1.92 ±0.11 cy
GA2	36.62 ±0.36 by	28.80 ±0.16 dz	50.69 ±0.38 ex	5.54 ±0.15 axy	7.41 ±0.66 abx	5.28 ±0.26 by	-2.00 ±0.14 ex	-2.49 ±0.31 dx	-2.66 ±0.09 dx
GA3	37.72 ±0.85 by	30.76 ±0.38 cz	49.48 ±0.48 ex	4.77 ±0.41 by	6.41 ±0.15 bcx	5.86 ±0.05 ax	-1.94 ±0.13 ey	-1.80 ±0.38 dx	-2.66 ±0.05 dz

*Data given is the mean value± standard deviation of triplicate analyses. Values with different letters (a–d) within the same column and different letters (x–y) within the same row differ significantly ($p < 0.05$).

A1: bread flour control; A2: **bread flour substituted with 10% HACS**; A3: **bread flour substituted with 20% HACS**; GA1: **bread flour substituted with 7% CGEP**; GA2: **bread flour substituted with 10% HACS and 7% CGEP**; GA3: **bread flour substituted with 20% HACS and 7% CGEP**.

5.2.5 Scanning electron microscope (SEM)

SEM images of different ingredients and some bread samples of HACS and CGEP were given in Figure 5.12. HACS contained homogenous starch granules, while CGEP is in a crystal-like form and A1 (control flour) was containing both of starch and protein commodities. A1 flour consisted of smooth, round and spherical granules of varying sizes. On the other hand, in crumb images, large and small starch granules in addition to damaged and deformed ones and also swollen and elongated granules due to gelatinization were all appeared. In CGEP added bread crumbs

(particularly GA3), the density of the bread has decreased because of the decreased loaf volume, thus the structure of those breads was more compressed than other samples. This finding was consistent with other studies that have used fruit sources in bread formula (Wang et al., 2007). Another reason is proposed as higher water holding capacity of phytochemicals (Zhu et al., 2009). According to their observations, a looser internal matrix was noticed in phytochemical added breads during storage because of higher water holding capacity of phytochemicals. All crust samples were smooth with less particles. Crusts of A1 and A3 contained same intact and non-swollen starch granules consistent with the previous observations (Pomeranz et al., 1984).

Bread dough was explained as a continuous phase containing leached starch molecules with or without lipid complexes entrapped in an elastic network of gluten molecules (Gray and Miller, 2003). Slightly rough dough structure in the bread dough samples of GA3 and A3 is because of HACS and similar observations were reported in previous studies and this rough structure was related with the irregular and discontinuous gluten sheet that is unable to cover all starch granules in those dough samples (Hung et al., 2006).

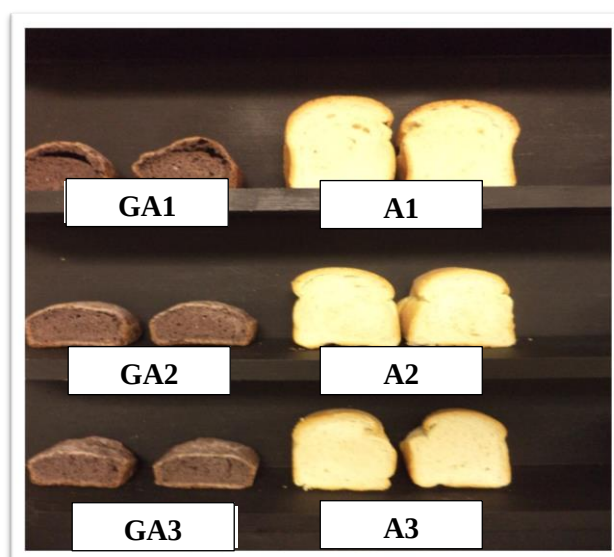
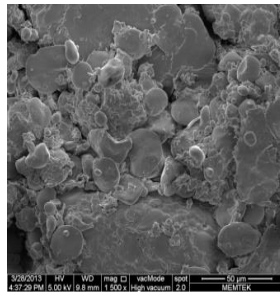
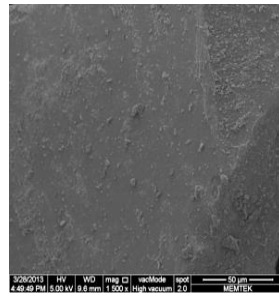


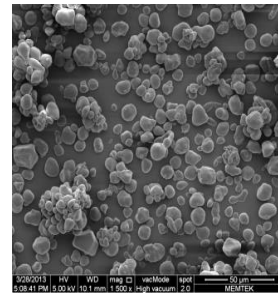
Figure 5.11 : HACS and CGEP substituted bread samples.



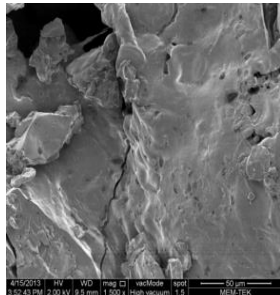
A1 flour



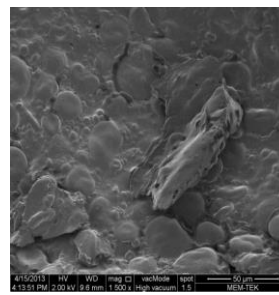
CGEP



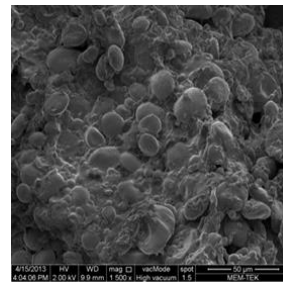
HACS



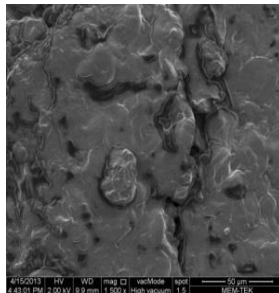
A1 Crumb



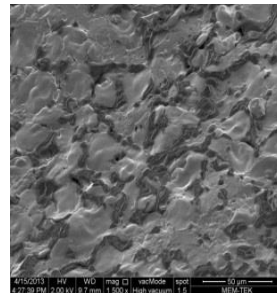
A1 Crust



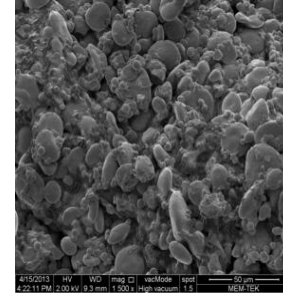
A1 Dough



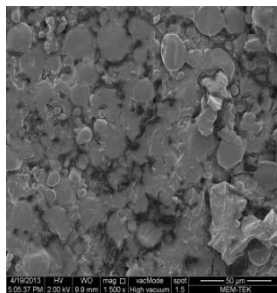
GA1 Crumb



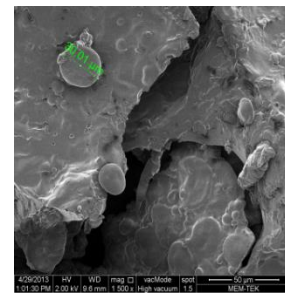
GA1-Crust



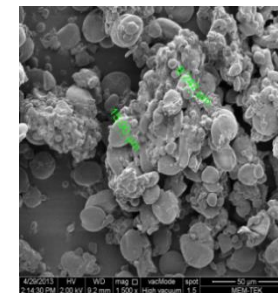
GA1-Dough



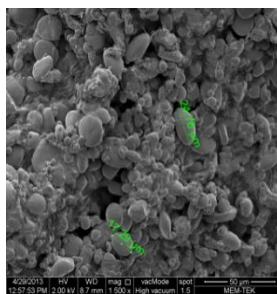
A3 Crumb



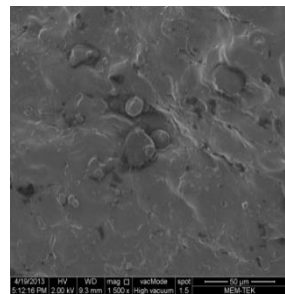
A3 Crust



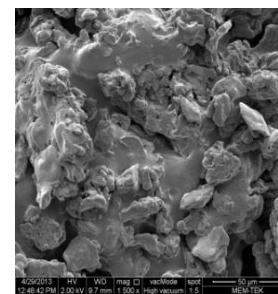
A3 Dough



GA3 Crumb

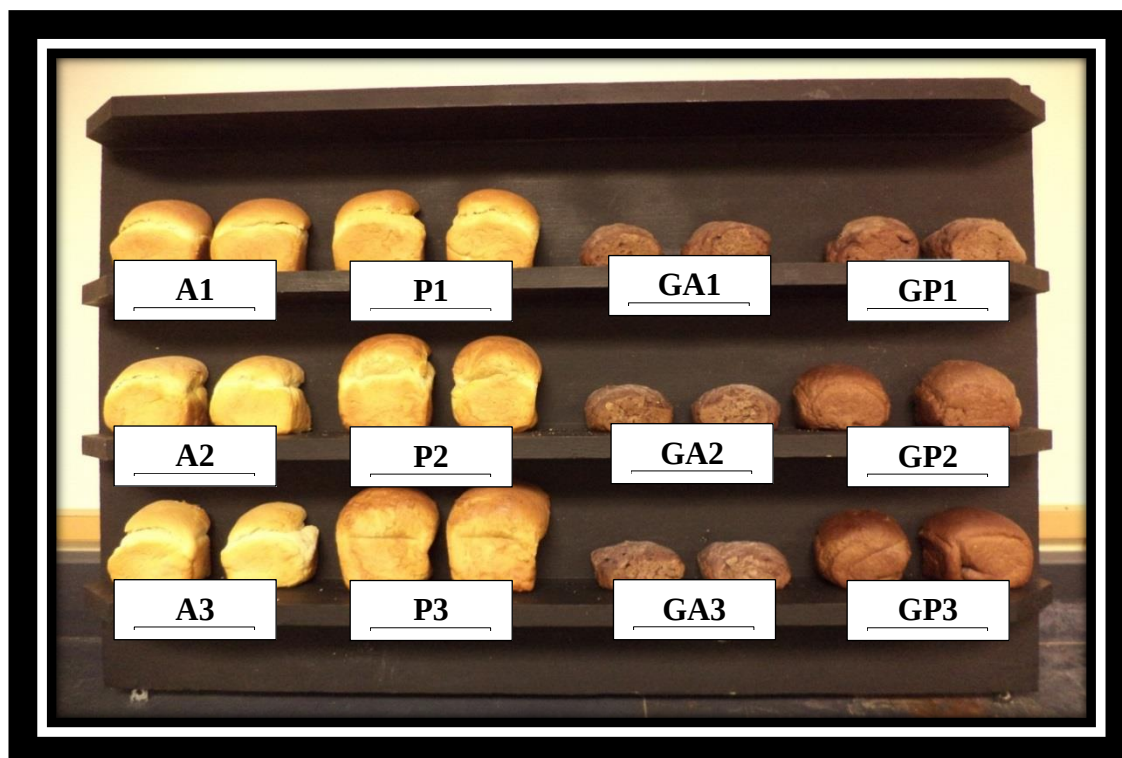


GA3 Crust



GA3 Dough

Figure 5.12 : SEM images of HACS and CGEP substituted bread samples.



A1: bread flour control; A2: bread flour substituted with 10% HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP. P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

Figure 5.13 : All bread samples

5.3 Evaluation of CGEP Added Bread As a Functional Food

5.3.1 Evaluation of main ingredients for their functionality

5.3.1.1 Introduction

Concord grape extract powder (CGEP) is a rich source of bioactives with special emphasis on phenolics and anthocyanins. This chapter aimed to investigate the functional properties of Concord grape powder and control hard wheat bread flour in addition to their combinations, as they are main ingredients of bread. Moreover, the study comprised changes in functional properties of bread and extruded products, focusing on the effect of incorporating either wheat protein isolate or high amylose corn starch, distinctly. Therefore, a detailed discussion on the changes in total phenolic contents, total antioxidant activity, total flavonoid contents, total anthocyanin contents, in addition to phenolic/anthocyanin profiles of bread samples obtained from HPLC chromatograms have been made in this chapter.

5.3.1.2 Total phenolic content

Total phenolic contents of Concord grape extract powder (CGEP), control hard wheat bread flour with no high amylose corn starch or wheat protein isolate addition (A1) and only CGEP (7%) substituted hard wheat flour before processing (GA1) were given in Table 5.3.

Total amount of phenolics in CGEP was found as 34623.8 ± 2934.7 mg GAE/ 100 g DW. This result was higher than the previous literature that reported total concentration of average phenolic compounds in five purple (Paulk, Cowart, Supreme, Ison, and Noble) muscadine grape cultivars as 1979.9 ± 493.2 , 335.0 ± 43.4 and 24.2 ± 8.6 mg GAE /100 g grape in seed, skin and pulp, respectively (Pastrana-Bonilla et al., 2003). *Cabernet Sauvignon* grape pomace had a quite lower phenolic content of 14 mg/100g than Concord grape powder as well (Zhu et al., 2013). Phenolic contents of fresh fruits among 6 raisin cultivars (*Vitis vinifera* L.) ranged from 316.3 to 1141.3 mg gallic acid/100 g DW (Breaksa et al., 2010). In Concord grape juice however, higher result were obtained and 2587.6 mg GAE/100 g was measured as total phenolic content after aging period of the juice processing. Variation in the total phenolics in grape skins was associated with variations in cultivar, soil composition, climate, geographic origin, and cultivation practices or exposure to diseases, such as fungal infections (Bruno and Sparapano, 2007; Xia, 2010). According to another study, the total phenolic content of fresh Concord grapes was reported as 84.7 mg (GAE) /100 g of grapes (Bruni et al., 2014). Generally, CGEP was superior in total phenolic content in comparison to other fruit forms; this might be related with the level used (Camire et al., 2007), its concentrated polyphenolic content and extraction method used.

Total phenolic content of control hard wheat bread flour was found as 721.8 ± 6.5 mg GAE/100 g DW. After CGEP (7%) substitution, total phenolic content of hard wheat flour with CGEP was found as 2374.2 ± 89.2 mg GAE/100 g DW. Therefore, CGEP (7%) substitution increased the amount of total phenolics around 330%. It was reported that hard and soft wheat flours contained 56.2 and 50.1 mg GAE/100 g dry matter of phenolic compounds (Ragaei et al., 2006). Sensory et al. (2006) measured the total phenolic content of white buckwheat wheat flour as 179 mg/100 g DW. The differences in the total phenolic content can be attributed to differences in the solvent

system used for extraction and the differences in the wheat cultivars (Sharma et al., 2012).

5.3.1.3 Total antioxidant capacity

Total antioxidant capacity of Concord grape extract powder (CGEP), control hard wheat bread flour (A1) and only CGEP (7%) substituted hard wheat flour before processing (GA1) were given in Table 5.3. Among the two methods used, DPPH radical scavenging activity method gave lower results for all samples. Generally, at least two different antioxidant capacity assays are suggested to be used since each method comprises different radicals and therefore the results might differentiate (Moore et al., 2009).

CGEP was found to have the highest antioxidant activity (24890.3 ± 783.2 mg TEAC /100 g DW) by both DPPH radical scavenging method and ABTS radical scavenging method (29710.2 ± 143.0 mg TEAC /100 g DW). These two results were close to each other but they were higher than the values found in the literature for fresh grapes (185.5 ± 1.6 μ mol TEAC /100 g grapes for DPPH (equivalent to appr. 46.4 mg TEAC/ 100 g grapes) and 318.5 ± 3.7 μ mol TEAC /100 g grapes for ABTS (79.6 mg TEAC/ 100 g grapes) (Burin et al., 2014). Antioxidant activity for control hard wheat flour was measured as 56.9 ± 6.5 2 mg TEAC /100 g DW and 361.3 ± 12.4 2 mg TEAC/100 g DW for DPPH and ABTS radical scavenging activity methods, respectively. DPPH results were slightly lower than the results in literature; 108.38 mg TEAC/100 g DW (Lu et al., 2014), 48.3 mg TEAC/100 g DW (Pellegrini et al., 2006) and 220.2552 mg TEAC/100 g DW (Lu et al., 2014) by ABTS radical scavenging activity method

5.3.1.4 Total flavonoids

Total flavonoid contents of CGEP, control hard wheat bread flour (A1) and only CGEP (7%) substituted hard wheat flour before processing (GA1) were given in Table 5.3. CGEP, control wheat flour and CGEP substituted flour were found to have 79787.0 ± 6451.9 , 542.0 ± 83.4 and 1748.2 ± 86.3 mg QE/ 100 g DW flavonoids, respectively. Wheat flour had the lowest flavonoid content as expected since flavonoid compounds are found in higher amounts in fruits and vegetable in

comparison to cereals (Zielinski and Kozłowska, 2000). Muscadine grape pomace was reported to have a total flavonoid content of 300 mg QE/ 100 g DW.

5.3.1.5 Total anthocyanins

Total anthocyanin contents of CGEP, and only CGEP (7%) substituted hard wheat flour before processing (GA1) were given in Table 5.3. In CGEP total anthocyanin content was 618.8 ± 24.0 mg Cy-3-glc/100 g DW. Present finding was slightly higher than some previous studies that measured the anthocyanin content in the skins of Concord grapes as 326 ± 5.9 mg Malvidin 3,5-diglucoside/100 g fresh (skin) weight. Other non-*Vitis vinifera* grape species grapes contained 258 ± 37 mg/100 g anthocyanins for Foch, and 888 ± 78 mg/100 g for Norton grapes on fresh weight basis (Munoz-Espada et. al., 2004). Previously, Concord grape powder was found to contain 234.3 mg anthocyanins/100 g (Camire et al., 2007).

Table 5.3 : Total antioxidant activity, total phenolic, flavonoid and anthocyanin contents of Concord grape powder, control hard wheat bread flour substituted with only CGEP (7%) (GA1) and control hard wheat bread flour (A1).

Analysis	CGEP (Concord grape extract powder)	Only CGEP (7%) substituted flour, before processing (GA1)	Control flour, No amylose, No WPI (A1)
TP (mg GAE/ 100 g DW)	34623.8 $\pm$ 2934.7	2374.2 $\pm$ 89.2	721.8 $\pm$ 6.5
TAA-DPPH (mg TEAC/100 g DW)	24890.3 $\pm$ 783.2	1490.7 $\pm$ 11.3	56.9 $\pm$ 6.5
TAA-ABTS (mg TEAC/100 g DW)	29710.2 $\pm$ 143.0	3436.2 $\pm$ 134.7	361.3 $\pm$ 12.4
TF (mg QE/ 100 g DW)	79787.0 $\pm$ 6451.9	1748.2 $\pm$ 86.3	542.0 $\pm$ 83.4
TAC (mg Cy-3-glc/100 g DW)	618.8 $\pm$ 24.0	30.8 $\pm$ 0.2	nd

TAA: Total antioxidant activity; TP: Total phenolics; DW: Dry weight; TEAC: Trolox Equivalent Antioxidant Capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,20-azinobis-3-ethylbenzo-thiazoline-6-sulphonic acid diammonium salt; GAE: gallic acid equivalence; TF: Total flavonoids; TAC: Total anthocyanin content; DW: Dry weight; QE: quercetin equivalence; Cy-3-glc: cyanidin-3-glucoside

Difference in the measured anthocyanin contents of different grape samples possibly arised from their expressing the results in fresh basis in addition to the effect of lower moisture content of present Concord grape powder extract (having a moisture content of 5.96%) and with the difference in chosen anthocyanin for expressing their results. The extinction coefficient of Cyanidin-3-glucoside standard generally used to measure the total anthocyanins (Cy-3-glc equivalent). Moreover, anthocyanins as

powders were proven to be more stable than they were in fresh or extracts forms, as long as their relative humidity is low, therefore present source CGEP might also possess a higher anthocyanin content for longer time periods (West and Mauer, 2013). In flour with CGEP (7%) anthocyanin content was found as 30.8 ± 0.2 TAC (mg Cy-3-glc/100 g DW).

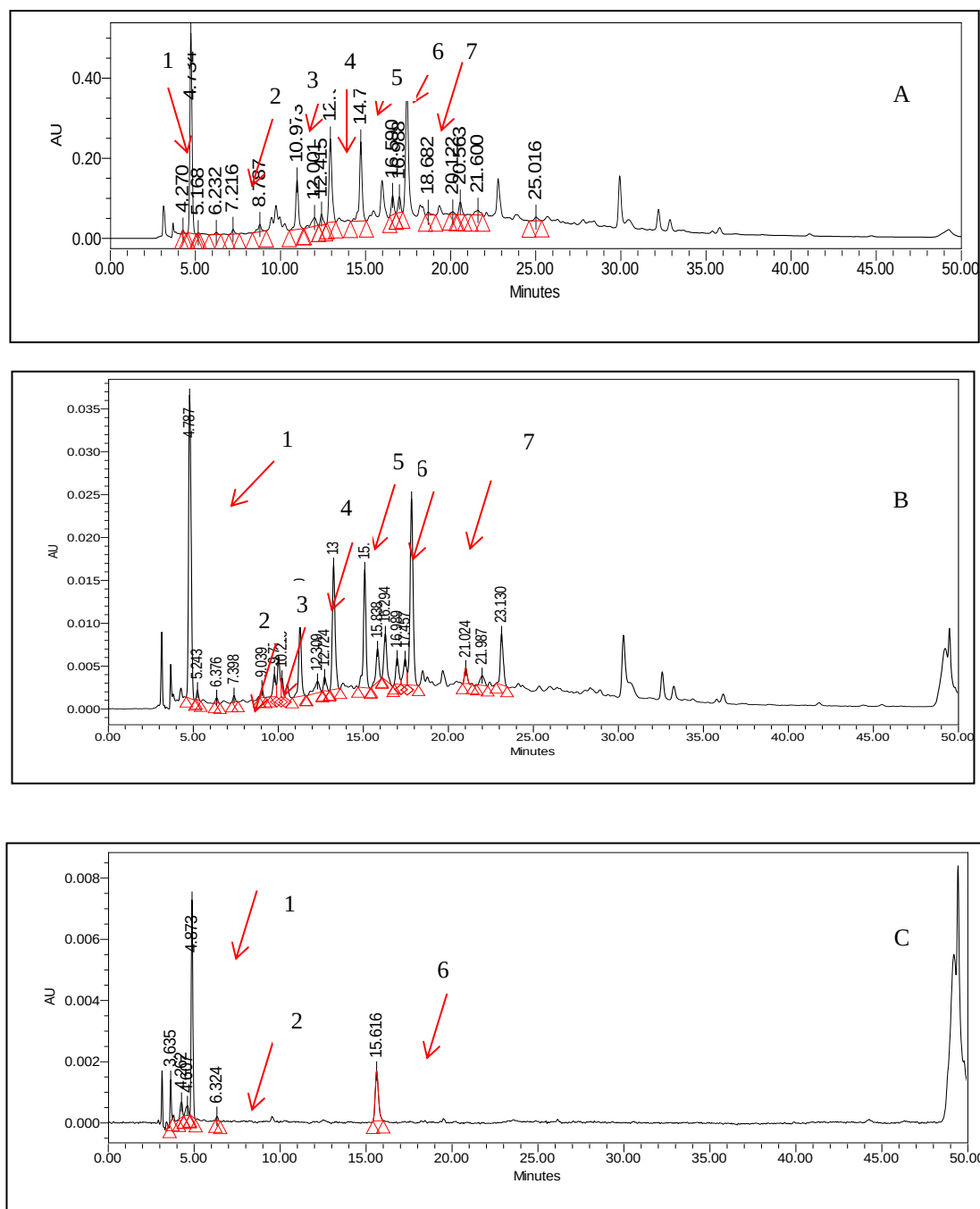
5.3.1.6 Phenolic and anthocyanin profiles and composition

HPLC-PDA data were extracted at different wavelengths of 280 nm, 312 nm, 360 nm and 520 nm. Chromatograms obtained at each wavelength for the methanolic extract of CGEP were given in Figures 5.14-5.17.

At 280 nm gallic acid (0.466 mg/100 g DW), protocatechuic acid (0.015 mg/100 g DW), ellagic acid (0.143 mg/100 g DW), syringic acid (0.239 mg/100 g DW), vanillic acid (0.116 mg/100 g DW) were detected. Other phenolic acids of chlorogenic acid (0.134 mg/100 g DW), caffeic acid (0.045 mg/100 g DW), *p*-coumaric acid (0.163 mg/100 g DW), and ferulic acid (0.004 g/100 g DW) were also identified at 312 nm. Among stilbenes, only *t*-resveratrol has also been identified (0.002 mg/100 g DW). The four of five flavan-3-ols found in grapes were detected as following: (+)-catechin (0.011 mg/100 g DW) and its isomer (-)-epicatechin (0.010 mg/100 g DW), (-)-epigallocatechin (0.149 mg/100 g DW), and (-)-epicatechin-3-O-gallate (0.051 mg/100 g). (+)-gallocatechin was the only one left as unidentified in Concord grape extract powder. Quercetin (0.011 mg/100 g DW) in addition to its glycoside quercetin-3- β -glucoside (0.265 mg/100 g DW), myricetin (0.007 mg/100 g DW) and rutin (0.003 mg/100 g DW) were the flavonols in the grape sample and identified at 360 nm. Other researchers also found also kaempferol in Concord grapes (Burin et al., 2014) and Concord grape juices (Toaldo et al., 2015). Concord grapes have been reported as rich sources of simple phenolics such as; gallic acid (0.090 mg/100 g FW), vanillic acid (0.13 mg/100 g FW), syringic acid (0.028 mg/100 g FW), protocatechuic acid (0.02 mg/100 g FW) and ellagic acid (0.63 mg/100 g FW) previously (Burin et al., 2014), in accordance with the current study. Same researchers also detected higher amount of caffeic acid (2.110 mg/100 g FW), *trans*-caftaric acid (0.020 mg/100 g FW), *p*-coumaric acid (0.288 mg/100 g FW) and ferulic acid (0.450 mg/100 g FW). In addition they also detected lower amounts of flavan-3-ols ((+)-catechin (0.254 mg/100 g FW) and (-)-epicatechin

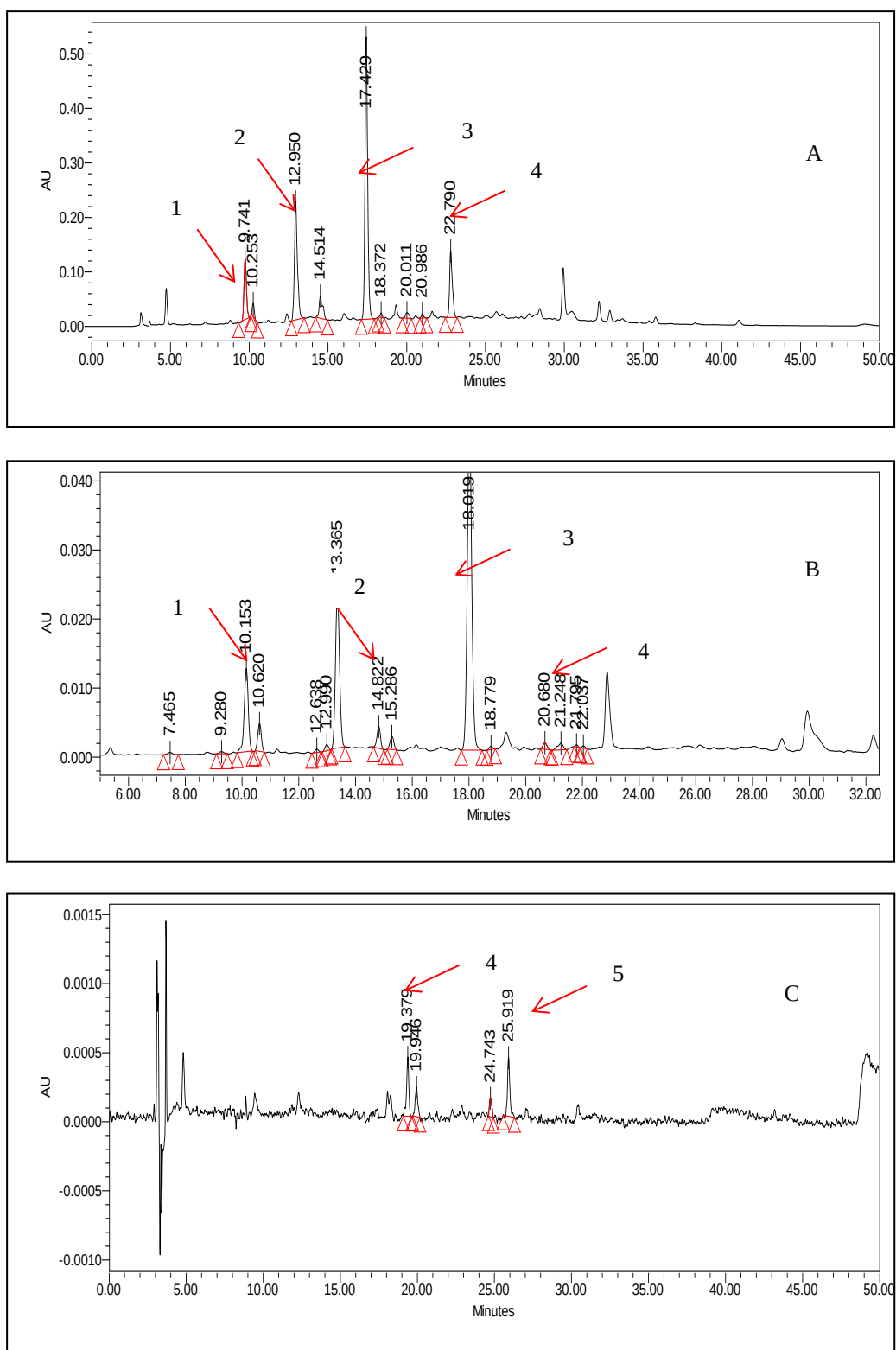
(0.206 mg/100 g FW), flavonols (kaempferol (0.009 mg/100 g FW) and quercetin (0.152 mg/100 g FW)) and also *trans*-resveratrol (0.064 mg/100 g FW) and tyrosol (0.130 mg/100 g FW) (Burin et al., 2014). However, myricetin was not detected. Most of those compounds except for *trans*-caftaric acid and tyrosol were also detected in this study. Flavonol concentration varies extensively among varieties, ranging from 1 to 80 mg per kg of grape FW (Castellarin et al., 2012). In Concord grape juice the most abundant flavonols were identified as quercetin-3-O-glucuronide and quercetin-3-O-glucoside (Stalmach et al., 2011). At 520 nm anthocyanins delphinidin-3-O-glucoside (0.057 mg/100 g DW), cyanidin-3-O-glucoside (0.022 mg/100 g DW), cyanidin-3,5-diglucoside (0.033 mg/100 g DW), delphinidin-3,5-diglucoside (0.010 mg/100 g DW) and malvidin-3-O-glucoside (0.153 mg/100 g DW) in addition to aglycones of delphinidin (0.018 mg/100 g DW), cyanidin (0.019 mg/100 g DW) and malvidin (0.011 mg/100 g DW) were identified. Aglycones of cyanidin, peonidin, malvidin, delphinidin, and petunidin have been reported to be found invariably in all varieties (Castellarin et al., 2012), although only cyanidin, malvidin and delphinidin aglycones were identified in the current study. Malvidin-3-O-glucoside (0.153 mg/100 g DW) with delphinidin-3-O-glucoside (0.057 mg/100 g DW) were identified and quantified as the most significant anthocyanins in Concord grape powder. In contrast, the most abundant anthocyanins in the skins of Concord grapes were detected as cyanidin monoglucoside in addition to lower amounts of cyanidin coumaroyl diglucoside (cyanidin 3-O-(6-O-(E)-*p*-coumaroyl- β -D-glucoside) and malvidin coumaryl diglucoside (malvidin 3-O-(6"-*p*-coumaroyl-glucoside) and deep blue-black color of these grapes were attributed to the presence of these compounds (Munoz-Espada et al., 2004). Another study revealed that delphinidin-3-O-glucoside together with cyanidin-3-O-glucoside and petunidin-3-O-glucoside as the most abundant anthocyanins in Concord grape juice (Stalmach et al., 2011), while cyanidin-3,5-diglucoside and malvidin-3,5-diglucoside were identified as the most abundant ones in organic and conventional Concord grape juice samples (Toaldo et al., 2015). Pelargonidin-3-O-glucoside and peonidin-3-O-(6-O-*p*-coumaryl)-5-O-diglucoside were reported as other anthocyanin glucosides that existed in Concord grape skin extracts (Flamini et al., 2013). Delphinidin 3-O-glucoside was reported the most abundant anthocyanin, and Peonidin 3-O-(6-O-acetyl)-glucoside, Delphinidin 3-O-(6-

O-coumaryl)-glucoside-5-O-glucoside, Cyanidin 3-O-(6-O-coumaryl)-glucoside, Cyanidin 3-O-glucoside and Cyanidin 3-O-(6-O-coumaryl)-glucoside-5-O-glucoside were also important in composition of anthocyanins in ‘Concord’ (*V. labrusca*) (Liang et al., 2008).



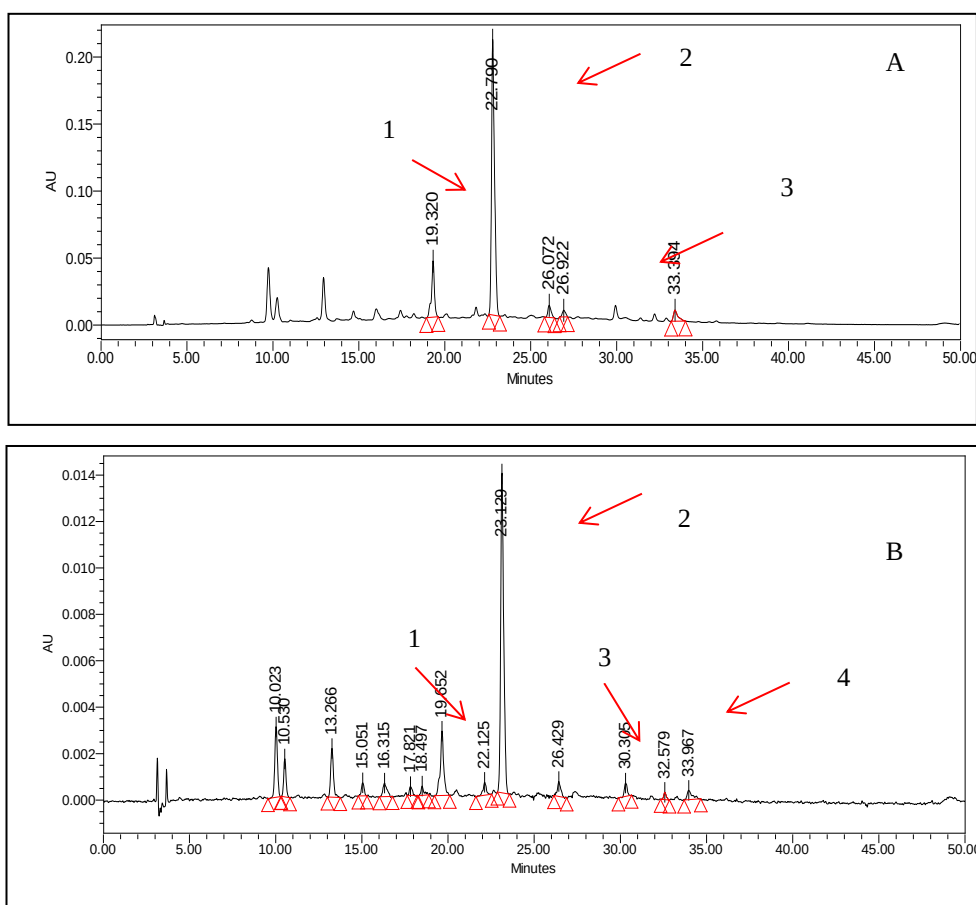
(1: Gallic acid, 2: Protocatechuic acid, 3: Epigallocatechin; 4: Catechin hydrate; 5: Epicatechin; 6: Syringic acid, 7: Vanillic acid)

Figure 5.14 : Representative HPLC-PDA chromatogram extracted at 280 nm A: CGEP, B: CGEP substituted flour, unprocessed (GA1), C: Hard wheat flour (A1).



(1: Chlorogenic acid; 2: Caffeic acid; 3: P-coumaric acid; 4: Ferulic acid; 5: nd)

Figure 5.15 : Representative HPLC-PDA chromatogram extracted at 312 nm for A: CGEP, B: CGEP substituted flour, unprocessed (GA1), C: Hard wheat flour (A1)



(1: Quercetin-3-beta-glucoside; 2: Myricetin 3: Rutin, 4: Quercetin)

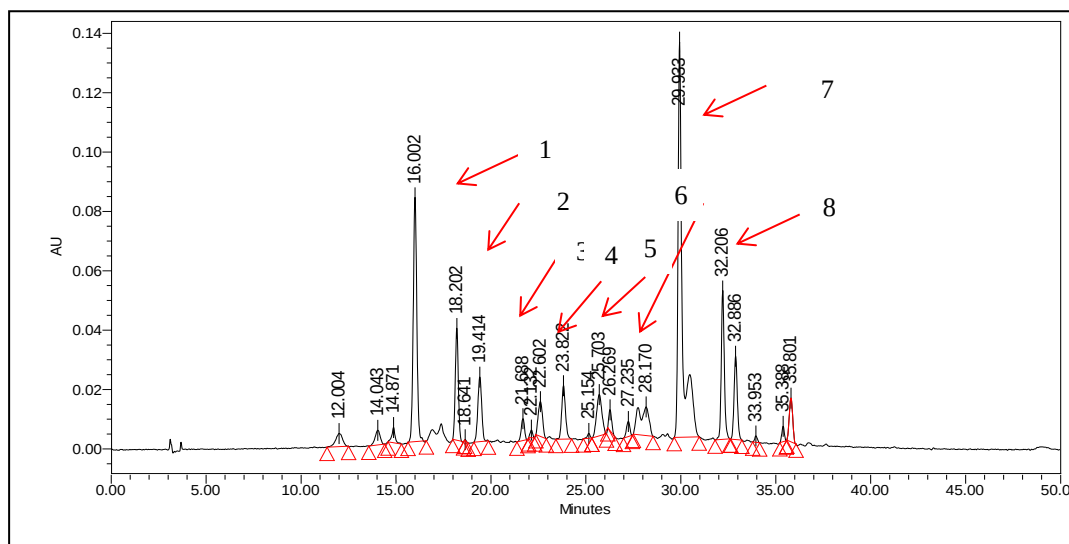
Figure 5.16 : Representative HPLC-PDA chromatogram extracted at 360 nm for A: CGEP, B: CGEP substituted flour, unprocessed (GA1).

Therefore some inconsistent findings existed about the anthocyanin content of Concord grapes. Esterification of p-coumaric acid with anthocyanin-3-glucosides can also occur in the skin of red varieties have been reported previously (Castellarin et al., 2012). Therefore, non-identified peaks are probably coumaric and caffeic acid esters (generally p-coumaroylated or caffeoylated) of malvidin-3-O-glucosides, cyaniding-3-O-glucosides and cyanidin-3-O-glucosides.

Gallic acid (6.5 $\mu\text{g}/100 \text{ g DW}$), ferulic acid (0.013 $\mu\text{g}/100 \text{ DW}$, p-coumaric acid (0.030 $\mu\text{g}/100 \text{ g DW}$) and syringic acid (2.78 $\mu\text{g}/100 \text{ g DW}$) were detected in flour sample and presented in Figure 5.14. Phenolic acids present in wheat include ferulic, p-coumaric, vanillic, caffeic, chlorogenic, gentisic, syringic, and p-hydroxybenzoic acids (Onyeneho and Hettiarachchy, 1992). On the other hand, in both in winter wheat flour and spring flour samples sinapic acid, syringic acid, ferulic acid, p-coumaric acid and vanillic acids were detected phenolics (Vaher et al., 2005). Similar

to these results, the presence of ferulic acid, p-coumaric, vanillic and sinapic acids in two types of wheat, wheat bran and flour samples were also reported (Liyana-Pathirana et al., 2006).

CGEP added raw flour samples (GA1) were the richest samples of bioactives since they are composed of both the grapes' and flour's compounds.



(1: Delphinidin-3-glucoside; 2: Cyanidin-3-o-glucoside; 3: Cyanidin-3,5-d,glucoside; 4: delphinidin-3,5-diglucoside; 5: delphinidin; 6: cyanidin; 7: malvidin; 8: malvidin-3-o-glucoside)

Figure 5.17 : Representative HPLC-PDA chromatogram extracted at 520 nm for identification of anthocyanins in Concord grape extract powder.

5.3.2 Interaction of CGEP with protein

5.3.2.1 Total phenolic content

Total phenolic contents of different parts of bread samples substituted with wheat protein isolate and Concord grape extract powder were given in Table 5.4. Phenolic contents of the samples ranged from 108.7 ± 3.3 mg GAE/ 100 g DW to 2032.7 ± 30.5 mg GAE/ 100 g DW.

In control samples with no grape powder addition, for three of the samples, it was seen that bread parts and dough possessed significantly different total phenolic contents than each other ($p < 0.05$), crumb being the poorest sample among others. This was in accordance with previous researchers who found that the crumb of bread baked using white hard wheat “Blanca Grande” flour having the lowest total phenolic content among all bread fractions (Lu et al., 2014). Lu et al. (2014) also

found similar results with present findings, as crust fraction having the highest amount of phenolics and this might be related to either the effect of Maillard reaction or intense heat application during baking to render the phenolic acids more physically accessible or chemically more susceptible to the hydrolysis process. Wheat protein substitution increased the total phenolic content for crust, crumb and dough parts significantly ($p < 0.05$). Furthermore, increase in substitution level from 6.5% to 13% made more increase in total phenolic content however it was not found statistically significant ($p > 0.05$). This might be related with the specific property of Folin-Ciocalteu reagent that can detect all phenolic groups found in extracts, including those found in the extractable proteins such as wheat protein isolate (Shahidi and Naczek, 1995).

In Concord grape powder extract (CGEP) substituted samples, phenolic content of dough samples were significantly higher than that of crumb and crust parts ($p > 0.05$) however, it is different than the results found for no-Concord grape extract powder added samples. Total phenolics in Concord grape extract substituted samples changed between 1449.0 and 2032.7 mg GAE/100 g DW. Other researches on different grape fractions gave different results. In bread samples substituted with 10% grape pomace quite lower results were gathered and total phenolic content was measured as 89.43 ± 0.02 mg (Hayta et al., 2014). The lower result might be related with the lower phenolic content of grape pomace (1550 mg GAE/100 g DW) in comparison to Concord grape extract powder used in this current study (34623.8 mg GAE/100 g DW). Different phenolic contents found for freeze-dried extract powders and fresh extracts were similar to other previous research which detected the ellagic acid content of the wet sediment of blackberry juices as 0.05 g/100 g, whereas for freeze-dried sediment of blackberry juice as 7.41 g/100 g in (Siriwoharn et al., 2004). Increase in wheat protein isolate level seemed to make no significant ($p < 0.05$) contribution on the measured phenolic content in Concord grape extract powder substituted samples.

Some studies reported decrease or no change in phenolic content through baking (Gelinas and McKinnon, 2006) which might be related with heat induced reactions of grape phenolics with components, such as proteins or starch, producing large molecules that could not be extracted (Peng et al., 2010). The comparison for changes between flour samples before and after baking into bread were given in

Table 5.4. According to the results, the total phenolic content of control hard wheat bread flour (721.8 ± 6.5 mg GAE/100 g DW) was lost at 71%, 84% and 60% levels in crust, crumb and dough parts of bread samples, respectively. The minimum loss was found for dough samples. This finding was in agreement with previous literature (Lu et al., 2014) involving two dough samples obtained before and after fermentation and slightly lower phenolic content than their flour was observed. However, substitution with wheat protein isolate made a decrease in the phenolic contents, losses down to 45%, 61% and 44% for crust, crumb and dough, respectively. On the other hand, loss of phenolics were lower in grape substituted samples; average values of 35%, 36% and 18% were measured for crust, crumb and dough, respectively and wheat protein isolate substitution made no more increase to the phenolic content.

Table 5.4 : Total antioxidant activity and total phenolic contents of WPI and CGEP substituted bread and dough samples.

	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough
	TAA-DPPH (mg TEAC/100 g DW)			TAA-ABTS (mg TEAC/100 g DW)			TP (mg GAE/ 100 g DW)		
P1	105.0± 9.7 dx	28.0± 5.4 dy	39.9± 7.0 cy	287.8± 7.9 dx	84.9± 0.5 by	312.0± 8.1 cx	199.0± 3.0 dy	108.7± 3.3 cz	277.7± 6.8 cx
P2	104.0± 19.3 dx	38.7± 2.1 dy	55.6± 4.5 cy	476.5± 11.5 cdx	169.2± 1.3 bz	317.0± 7.3 cy	378.7± 5.5 cx	271.3± 5.4 bx	385.7± 75.6 bcx
P3	92.2± 13.0 dy	121.5± 0.7 cxy	128.3± 0.0 cx	524.4± 6.0 cx	196.1± 11.6 bz	396.5± 16.2 cy	387.6± 13.8 cy	298.1± 10.4 bz	557.5± 0.4 bx
GP1	1166.5± 40.0 ax	1133.9± 17.0 ax	1310± 77.8 ax	2171± 139.1 bx	2147± 59.5 ax	2368± 203.1 bx	1571± 9.5 aby	1480± 40.3 ay	1893± 53.9 ax
GP2	1111.8± 2.6 by	1130.7± 53.6 ay	1289± 9.1 ax	2288± 8.6 aby	2228± 236.0 ay	2802± 12.3 ax	1449± 61.2 by	1504± 59.6 ay	1922± 110.9 ax
GP3	1049.7± 43.4 cx	1058.0± 4.9 bx	1147± 88.7 bx	2395± 49.7 ay	2189± 1.2 az	2724± 50.2 ax	1599± 85.6 ay	1602± 54.7 ay	2032± 30.5 ax

Data given is the mean value± standard deviation of triplicate analyses. Values with different letters (a–d) within the same column and different letters (x–z) within the same row differ significantly ($p < 0.05$)

TAA: Total antioxidant activity; TP: Total phenolics; DW: Dry weight; TEAC: Trolox Equivalent Antioxidant Capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,20-azinobis-3-ethybenzothiazoline-6-sulphonic acid diammonium salt; GAE: gallic acid equivalence.

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

Table 5.5: The % loss of total phenolics and antioxidant activity of WPI and CGEP substituted bread and dough samples.

	Crust				Crumb				Dough			
Loss (%)												
	TP	ABTS	DPPH	TF	TP	ABTS	DPPH	TF	TP	ABTS	DPPH	TF
P1	71	20	-84	n.d.	84	77	51	n.d.	60	14	30	n.d.
P2	45	-32	-83	n.d.	61	53	32	n.d.	44	12	2	n.d.
P3	44	-45	-62	n.d.	57	46	-114	n.d.	20	-10	-125	n.d.
GP1	34	37	22	-61	38	38	24	-62	20	31	12	14
GP2	39	33	25	-47	37	35	24	-34	19	18	14	2
GP3	33	30	30	-12	33	36	29	20	14	21	23	-11

TP: Total phenolics; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,20-azinobis-3-ethybenzothiazoline-6-sulphonic acid diammonium salt; TF: Total flavonoids.

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

5.3.2.2 Total antioxidant capacity

Antioxidant activity in different parts of bread samples and dough were measured using two radical scavenging methods and results were given in Table 5.4. DPPH radical scavenging activity measurements revealed that the antioxidant activity of the bread samples changed between 28.0 ± 5.4 and 1310.1 ± 77.8 mg TEAC/100 g DW. In samples without grape powder, increase in wheat protein isolate substitution level made no significant increase except for the crumb and also for higher wheat protein substitution (13%). When the antioxidant activity using in different parts of bread samples and dough were evaluated by DPPH method, crust was found to have richest antioxidant activity as much as 2-3 folds in comparison to crumb and dough, however at 13% wheat protein isolate substitution difference between samples became statistically insignificant ($p > 0.05$).

After grape extract powder substitution, antioxidant activity by DPPH radical scavenging method increased at least 10-folds in all samples. Different from the no-grape powder added samples, dough fraction of the bread samples displayed the highest antioxidant activity. Wheat protein isolate addition seemed to lower the antioxidant activity, particularly being significant at 13% substitution level ($p < 0.05$). It was claimed that presence of flavonoids and proteins together in samples might have been an effective in masking part of the antioxidant activity in flavonoid-rich samples (Arts et al., 2002).

According to the study by Peng et al. (2010) who added grape seed extract, into bread formulations at three different levels (300 mg, 600 mg and 1 g), thermal processing decreased the antioxidant activity in grape seed extract added breads about 30-40% in comparison to standard solutions of grape seed extract with the same concentrations. The researchers reported that this decrease might be related with the effect of heat on proanthocyanidins of grape in addition to the interactions of protein and starch macromolecules of bread samples. Another possibility they suggested might have been the thermal degradation of proanthocyanidins (Peng et al., 2010).

The DPPH radical scavenging activity results measured were generally lower than measurements made by ABTS radical scavenging assay. Presence of anthocyanins was proposed as a factor to affect the performance of DPPH radical scavenging activity. DPPH is purple and it changes color from purple to yellow as the antioxidant scavenges its proton and its maximum absorbance is at around 515 nm, however since anthocyanins are red in color and also absorb at 520 nm, the absorption spectra of DPPH and anthocyanins may overlap at 515 nm. Therefore, the purple/red color of grape extract samples might affect the absorbance of DPPH and cause an overexpressing in the absorbance of DPPH (Munoz-Espada et. al., 2004).

The antioxidant activity was found to be lost about 20, 77 and 14% in the crust, crumb and dough which were baked using control hard wheat bread flour without making any ingredient substitution. Wheat protein substitution (6.5%) seemed to decrease the extent of antioxidant activity in all parts of the bread and dough. Moreover, the extent of protection increased with the increase in WPI level substituted. This effect might also be related with the intrinsic phenolic content of WPI and their antioxidant activity added (Labat et al., 2000).

ABTS radical scavenging activity measurements for CGEP and wheat protein isolate substituted bread samples were given in Table 5.4. The results revealed that the antioxidant activity of the bread samples ranged between 84.9 ± 0.5 and 2802.7 ± 12.3 mg TEAC/100 g DW.

In samples without CGEP, crumb samples had the lowest antioxidant activity and their difference from the crust and dough was statistically significant ($p < 0.05$) in all cases. In bread samples baked using hard wheat bread flour without any ingredient

substitution, dough was slightly higher in antioxidant activity than crust, however wheat protein isolate significantly increased the antioxidant activity of the crust samples while making no change in other fractions. The increase in substitution level also increased the antioxidant activity of the crust.

In samples with CGEP, antioxidant activity was at least 7-fold more than the samples without CGEP and without wheat protein isolate substitutions and the distribution of the measured ABTS radical scavenging activity was similar in all samples. And different from the samples without CGEP, a significant increase in antioxidant activity was detected in only dough samples.

It was reported previously that the presence of protein sources might have a masking role in decreasing the total amount of measured antioxidant activity (using ABTS radical scavenging method) significantly depending on both the type of polyphenol and the type of protein (Arts et al., 2002).

According to the measured antioxidant activity of the samples without CGEP before and after baking, lesser amounts of losses were measured with the presence of wheat protein isolate in all fractions. The crust results were found to be about 30 to 45% more than that of flour. In samples with CGEP no significant changes occurred in the extent of losses in the antioxidant activity.

5.3.2.3 Total flavonoids

Total flavonoids in Concord grape extract powder and wheat protein isolate substituted bread samples were given in Table 5.6. Total flavonoid contents for samples ranged between 1399.8 ± 75.5 mg QE/ 100 g DW and 2907.0 ± 474.7 mg QE/ 100 g DW. Although distribution of flavonoids is balanced in all parts of bread and dough and statistically similar in only grape powder substituted breads ($p > 0.05$), wheat protein isolate seemed to lower the measured amount of flavonoids in the dough at 6.5% substitution level to lower levels than crust and crumb. At 13% substitution level, further significant losses were detected and also in the crumb as well. Increasing levels of wheat protein isolate substitution decreased the total flavonoids in crumb and dough significantly ($p < 0.05$), while the decline in the crust was insignificant ($p > 0.05$).

Total flavonoid content seemed to increase in the CGEP added crust and crumb samples, while dough samples lost 14% of its total flavonoids. With wheat protein isolate substitution, measured flavonoid content showed a significant decreasing trend but it was still greater than the flavonoid content before bread baking. Only opposite effect was evident in the dough samples and the amount of flavonoid loss decreased with the increase in wheat protein isolate substitution level.

Table 5.6: Total anthocyanin and total flavonoid content of WPI and CGEP substituted bread and dough samples.

	Crust	Crumb	Dough	Crust	Crumb	Dough
	TAC- (mg Cy-3-glc/100 g DW)			TF(mg QE/ 100 g DW)		
CGEP, No amylose, No WPI, (GP1)	12.2± 2.9ax	15.5± 2.3ax	18.4± 4.1ax	2806.7± 48.6ax	2830.9± 34.8ax	2907.0± 474.7ax
CGEP, 6.5% WPI, (GP2)	11.7± 0.0ax	15.8± 2.8ax	17.5± 4.4ax	2561.3± 138.1abx	2346.9± 177.6bx	1455.5± 60.9by
CGEP, 13% WPI (GP3)	14.0± 1.4ax	9.8± 2.2bx	15.6± 0.0ax	1956.8± 348.5bx	1399.8± 75.5cy	1442.3± 79.6by

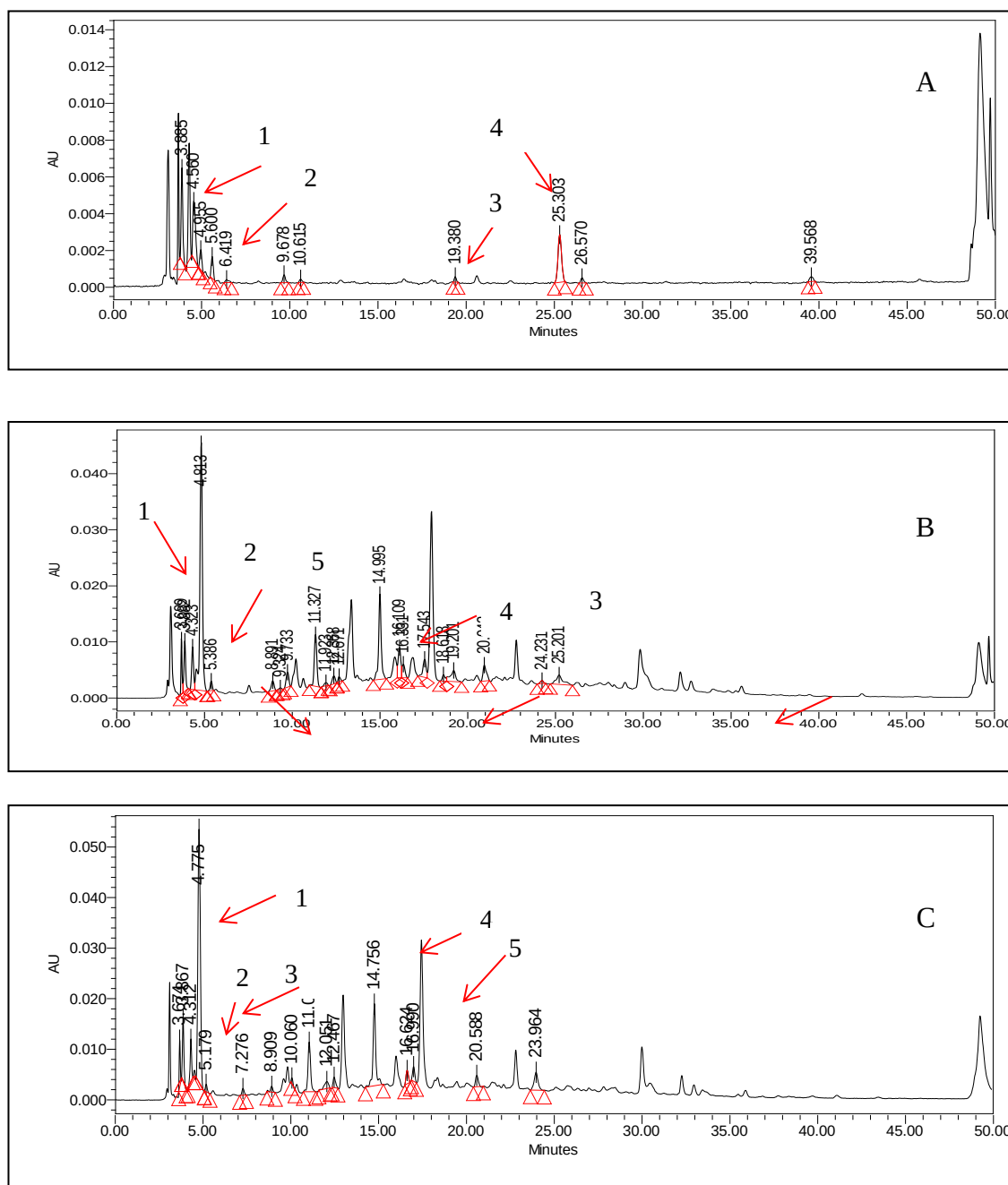
Data given is the mean value± standard deviation of triplicate analyses. Values with different letters (a–d) within the same column and different letters (x–z) within the same row differ significantly ($p < 0.05$). TF: Total flavonoids; TAC: Total anthocyanin content; DW: Dry weight; QE: quercetin equivalence; Cy-3-glc: cyanidin-3-glucoside

5.3.2.4 Total anthocyanins

Total anthocyanins in Concord grape extract powder and wheat protein isolate substituted bread samples were given in Table 5.6. Anthocyanin content of samples changed between 9.8 ± 2.2 and 18.4 ± 4.1 mg Cy-3-glc/100 g DW. These compounds are known for their very low stability against process and/environmental changes such as changes in pH, the presence of ascorbic acid, sulfite, light, oxygen, and enzymatic and non-enzymatic browning reactions (Wesche-Ebeling and Argaiiz-Jamet, 2002). Differences in anthocyanin contents either in different parts of the bread samples and dough or after increasing amount of wheat protein isolate substitution were found as statistically insignificant ($p > 0.05$). Although monomeric anthocyanin detection method is considered as a common approach for measuring the anthocyanin contents of the cereal samples and correlated well with HPLC results in some studies (Hosseinian et al., 2008), it has also been reported many times as a method that detected lower anthocyanin contents than HPLC detections (Sánchez-Moreno et al., 2003; Wu et al., 2004; Wu et al., 2006; Lee and Finn, 2007).

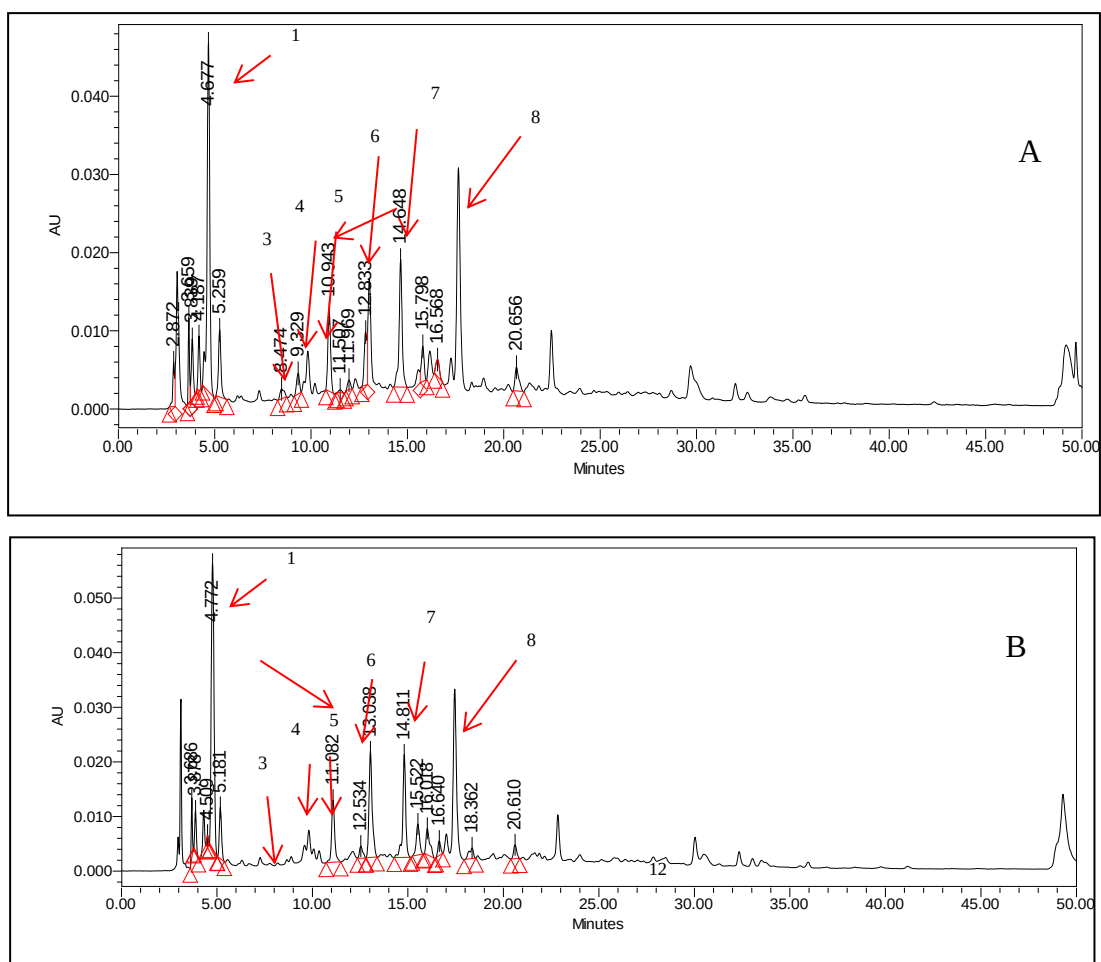
5.3.2.5 Phenolic and anthocyanin profiles and composition of CGEP and WPI substituted bread samples

Phenolic compound profiles and anthocyanin profiles of CGEP and wheat protein isolate substituted bread samples were given in Figures 5.18-5.26 and Tables 5.7 and 5.8. 280 nm, dough, crust and crumb samples of no CGEP or no WPI added samples (P1) were found to have similar amounts of gallic acid.



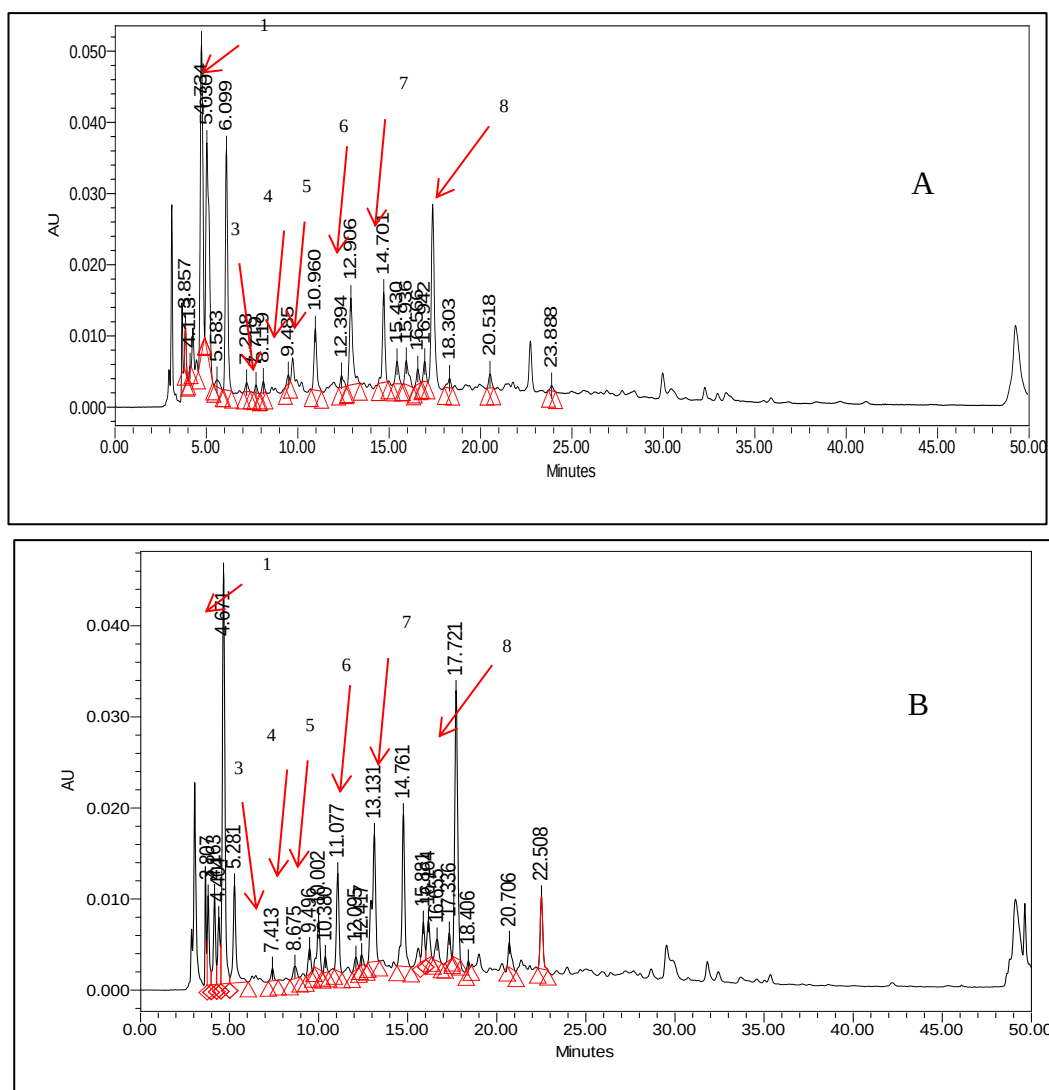
(1: Gallic acid, 2: Protocatechuic acid, 3: Vanillic acid, 4: Ferulic acid; 5: Sinapic acid)

Figure 5.18 : Representative HPLC-PDA chromatograms extracted at 280 nm for A: P1 bread dough; B: GP1 bread dough C: GP3 bread dough.



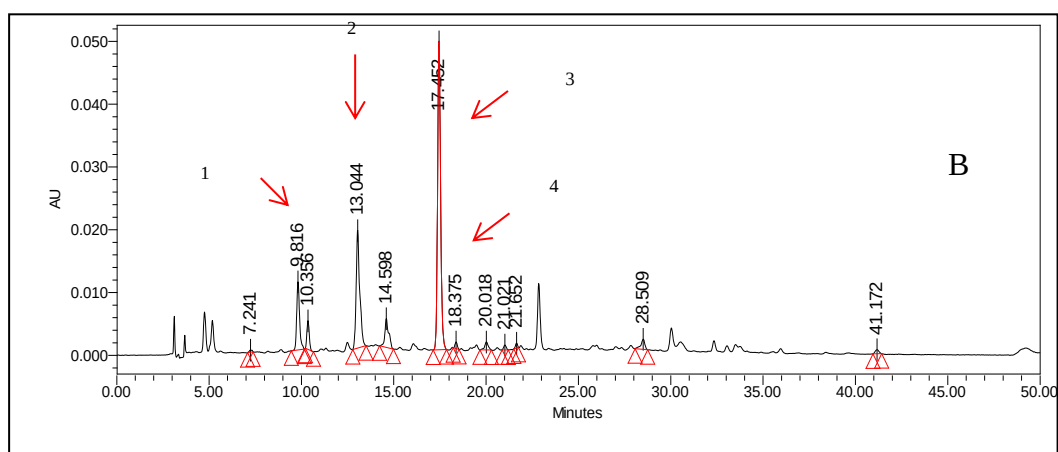
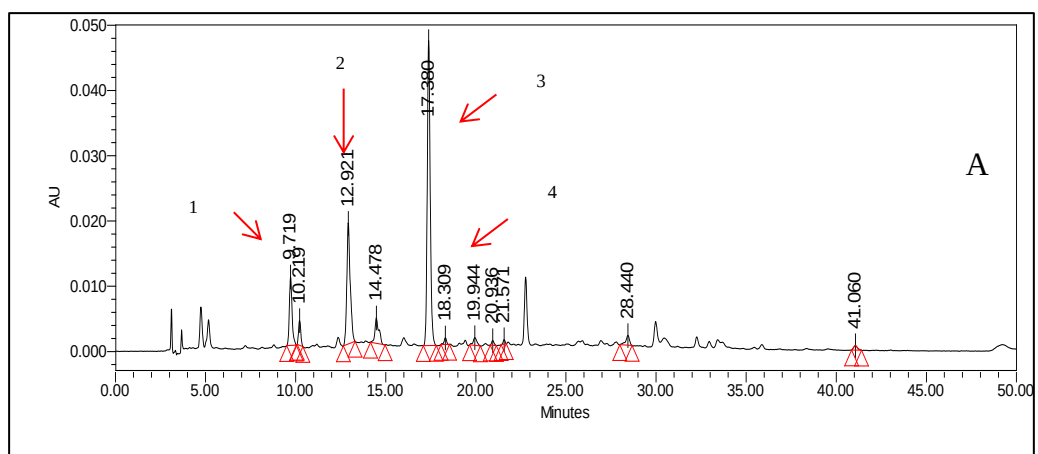
(1: Gallic acid, 2: Protocatechuic acid, 3: Epigallocatechin; 4: Catechin hydrate; 5: Epicatechin; 6: Syringic acid, 7: Vanillic acid, 8: n.d)

Figure 5.19 : Representative HPLC-PDA chromatograms extracted at 280 nm for A: GP1 Crumb; B: GP3 Crumb.



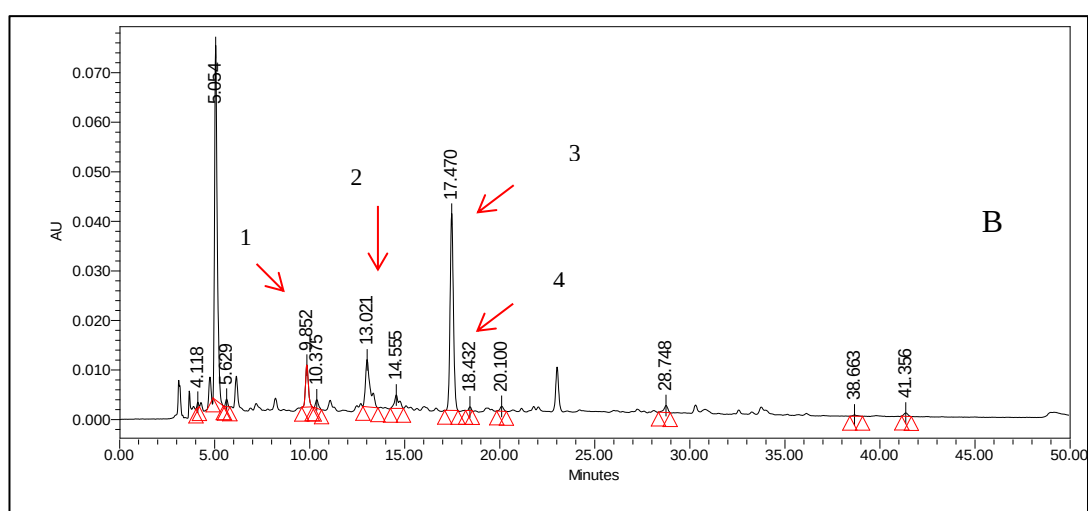
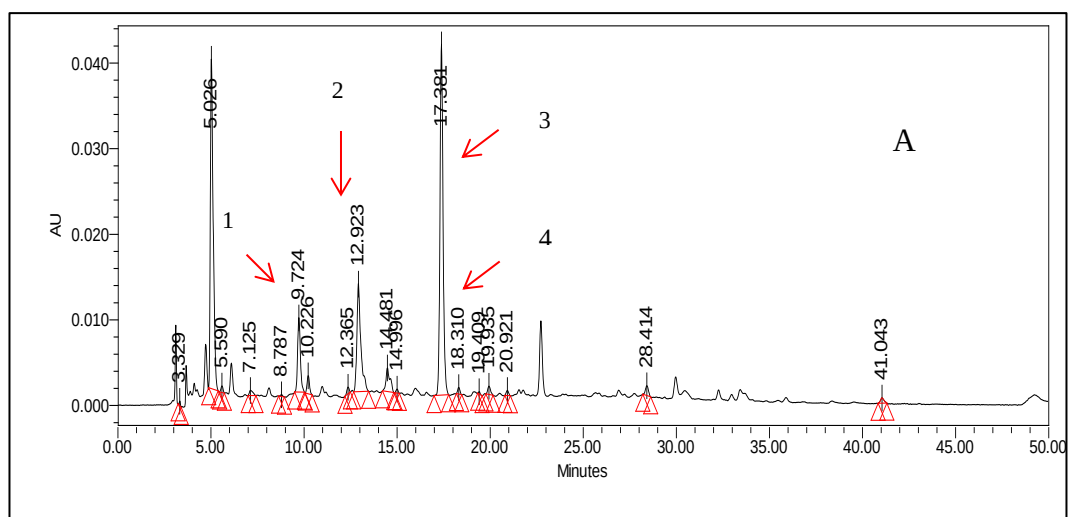
(1: Gallic acid, 2: Protocatechuic acid, 3: Epigallocatechin; 4: Catechin hydrate; 5: Epicatechin; 6: Syringic acid, 7: Vanillic acid, 8: n.d)

Figure 5.20 : Representative HPLC-PDA chromatograms extracted at 280 nm for A: GP1 Crust; B: GP3 Crust.



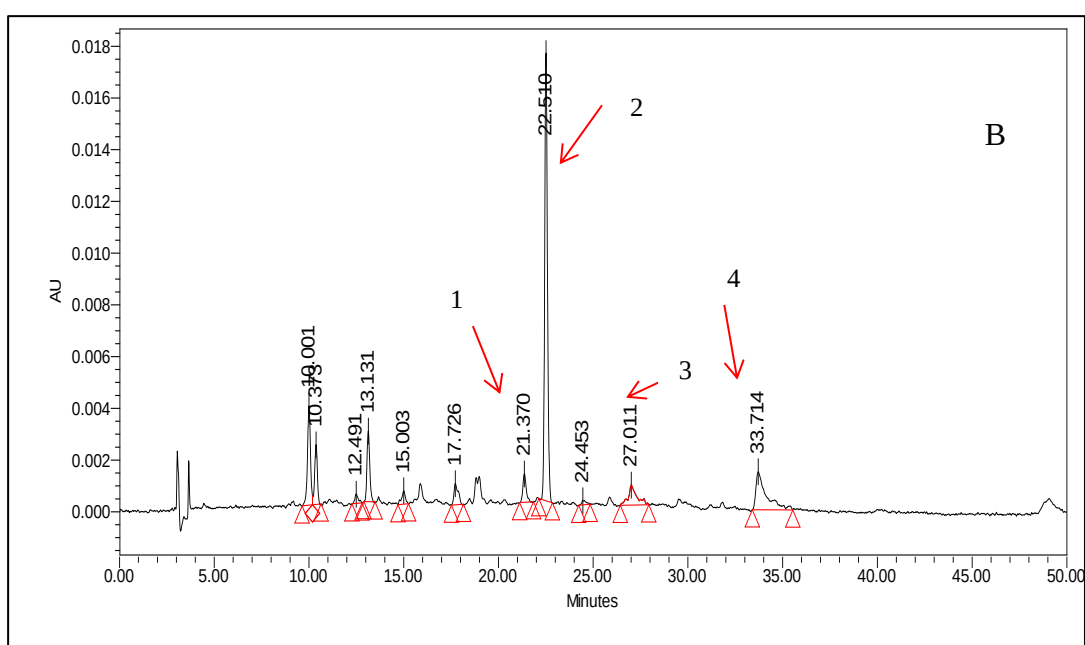
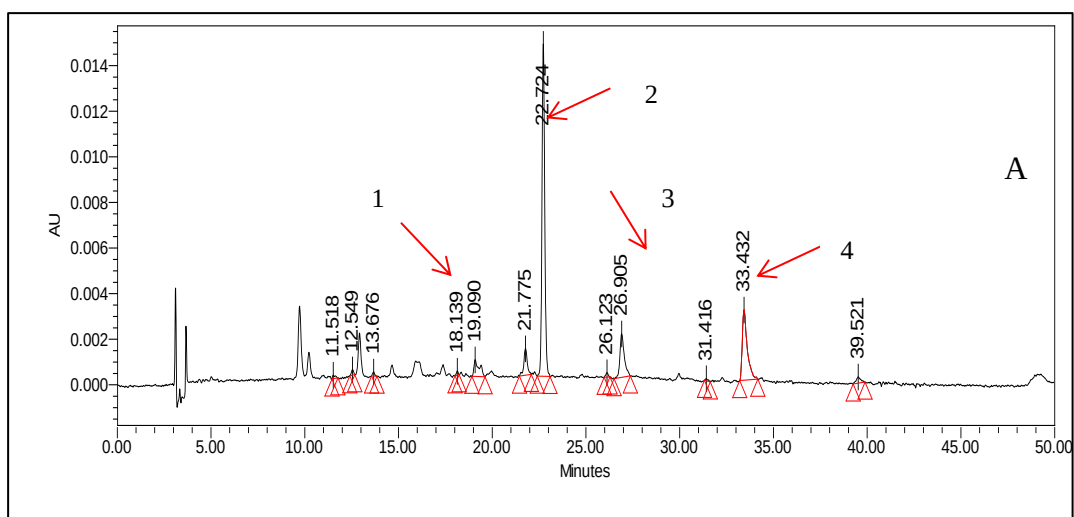
(1: Chlorogenic acid; 2: Caffeic acid, 3: P-coumaric acid, 4: Ferulic acid)

Figure 5.21 : Representative HPLC-PDA chromatograms extracted at 312 nm for A: GP1 Crumb, B: GP3 Crumb.



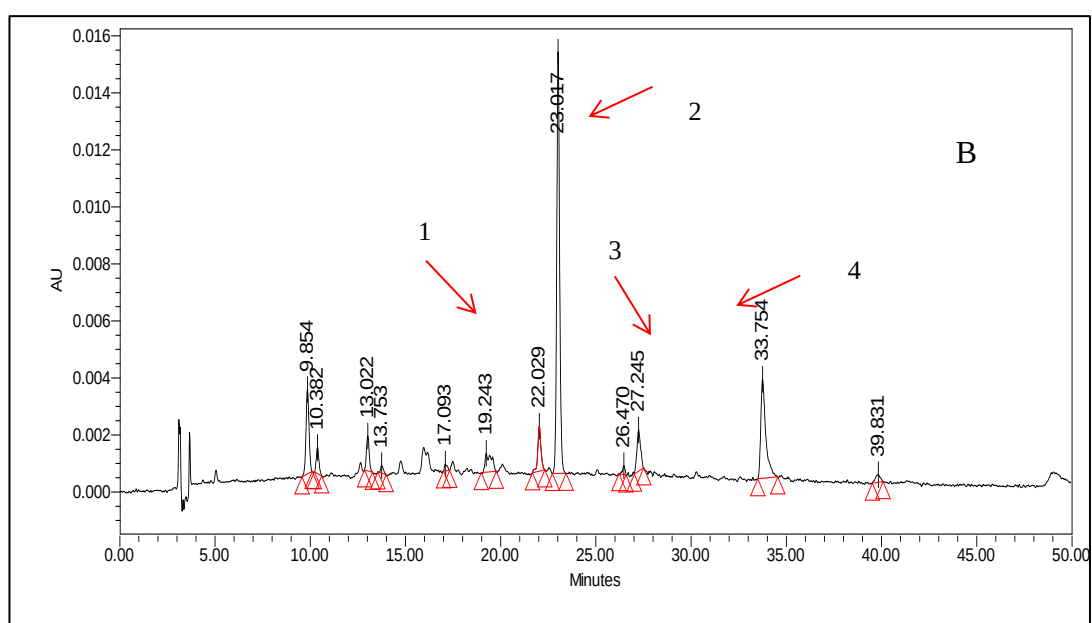
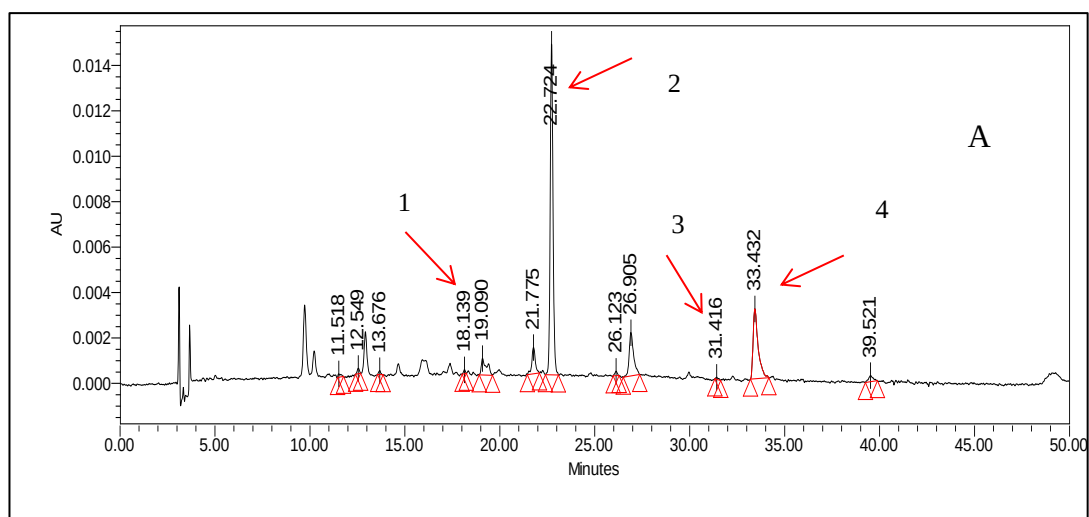
(1: Chlorogenic acid; 2: Caffeic acid, 3: P-coumaric acid, 4: Ferulic acid)

Figure 5.22 : Representative HPLC-PDA chromatograms extracted at 312 nm for A: GP1 Crust, B: GP3 Crust.



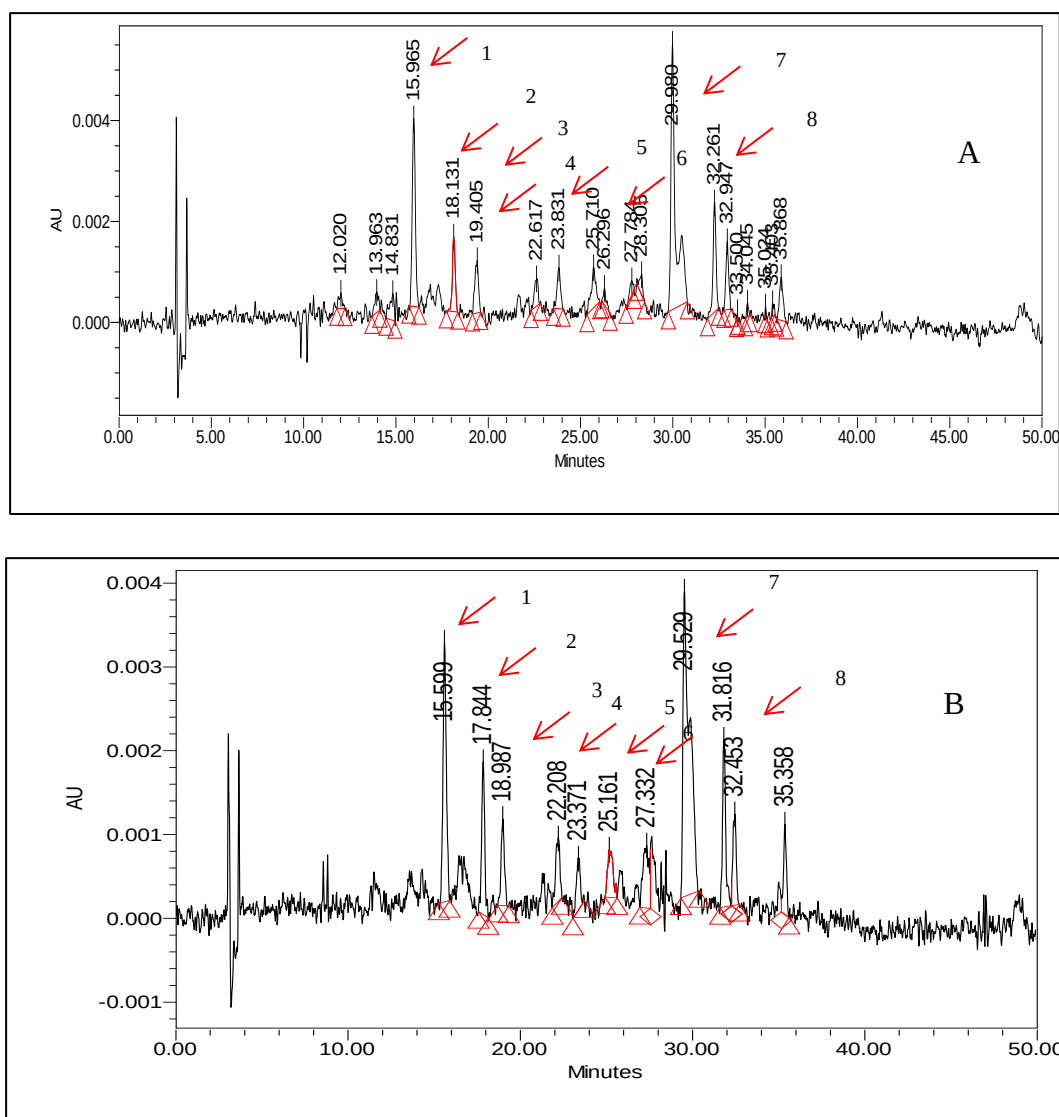
(1: Quercetin-3-beta-glucoside; 2: Myricetin 3: Rutin, 4: Quercetin)

Figure 5.23 : Representative HPLC-PDA chromatograms extracted at 360 nm for A: GP1 Crumb, B: GP3 Crumb.



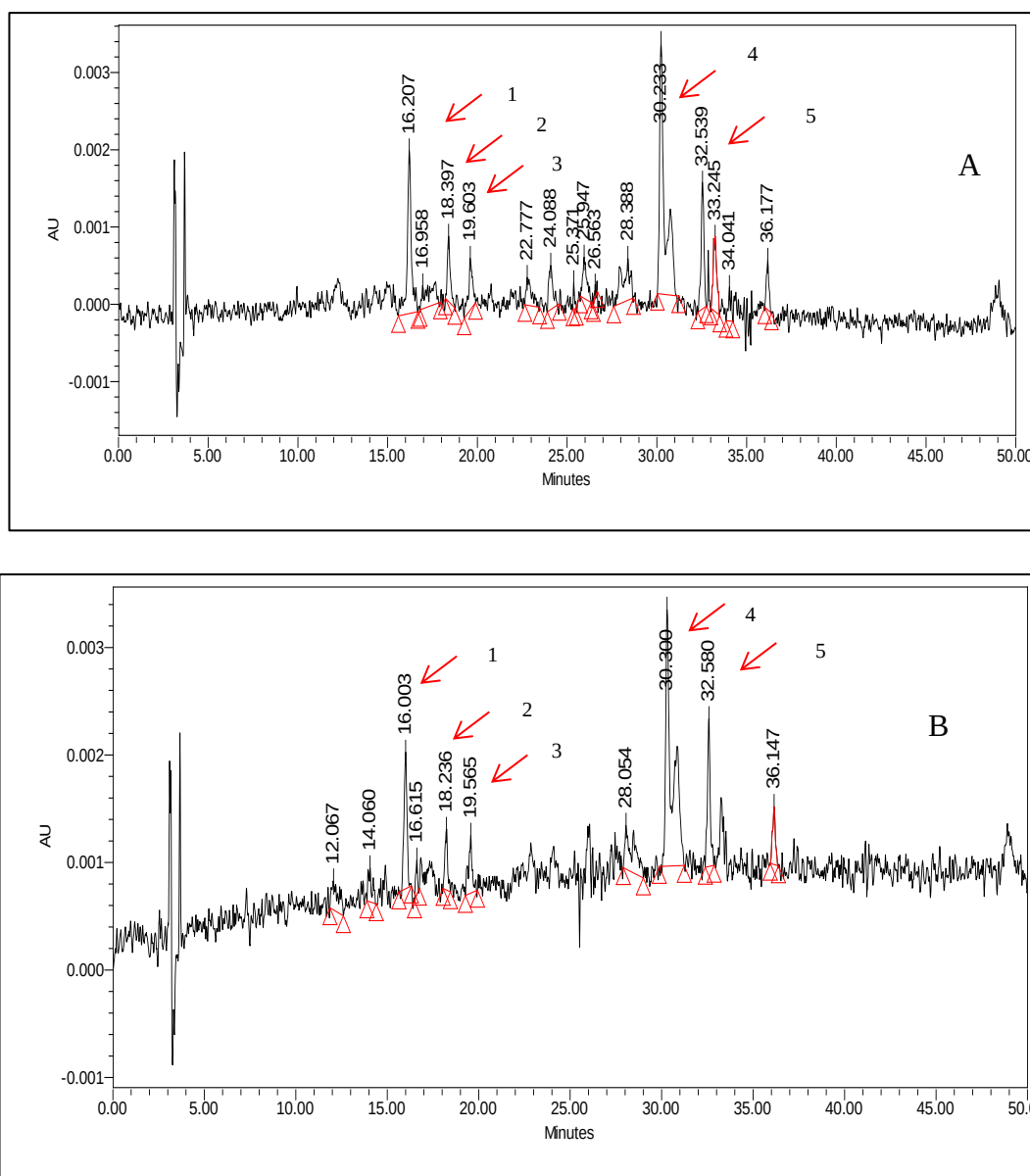
(1: Quercetin-3-beta-glucoside; 2: Myricetin 3: Rutin, 4: Quercetin)

Figure 5.24 : Representative HPLC-PDA chromatograms extracted at 360 nm for A: GP1 Crust, B: GP3 Crust.



(1: Delphinidin-3-glucoside; 2:Cyanidin-3-o-glucoside; 3: Cyanidin-3,5-d,glucoside; 4: delphinidin-3,5-diglucoside; 5: delphinidin; 6:cyanidin; 7:malvidin; 8: malvidin-3-o-glucoside)

Figure 5.25 : Representative HPLC-PDA chromatograms extracted at 520 nm for A: GP1 Crumb, B: GP3 Crumb.



(1: Delphinidin-3-glucoside; 2: Cyanidin-3-o-glucoside; 3: Cyanidin-3,5-d,glucoside; 4: malvidin; 5: malvidin-3-o-glucoside)

Figure 5.26 : Representative HPLC-PDA chromatograms extracted at 520 nm for A: GP1 Crust, B: GP3 Crust.

Research revealed that behaviors of phenolic compounds during baking were different. Therefore to make any generalization about their behavior was hard to manage as suggested by Sivam et al. (2010).

In control samples baked without CGEP, amount of gallic acid was - around 6 - 18 $\mu\text{g}/100 \text{ g DW}$. Crust parts of bread had significantly lower amount of gallic acid in comparison to crumb and dough, generally.

Protocatechuic acid, gallic acid, sinapic acid, vanillic acid, p-coumaric and ferulic acid were the major phenolic compounds detected in no-CGEP added bread samples and generally parts of bread and dough differed significantly ($p > 0.05$) for all detected compounds. Similarly, vanillic, caffeic, syringic, p-coumaric, ferulic, and sinapic acids were the identified phenolics in hard wheat bread samples by Lu et al. (2014).

Before baking, the amount of syringic acid was around $2.5 \mu\text{g}/100 \text{ g DW}$, vanillic and sinapic acids were around $2 \mu\text{g}/100 \text{ g DW}$, and gallic acid was found as around $6.5 \mu\text{g}/100 \text{ g DW}$ in control flour (A1). Gallic acid and vanillic acid were found to increase significantly (around 2-fold) after baking. Increases from flour to bread parts in certain phenolic acids such as vanillic acid and sinapic acid were also evident in previous research carried out on bread samples baked using refined hard white wheat variety “Blanca Grande” (Lu et al., 2014). In the present study generally fermented dough and crumb part of bread had higher amount of phenolics in contrast with the findings of Lu et al. (2014). Increasing effect of fermentation on the amounts of certain phenolic acids such as ferulic acid was previously mentioned by other researchers (Moore et al., 2009). Highest level of vanillic, caffeic and sinapic acid, ferulic acid and total phenolics in the upper crust of bread baked using refined hard white wheat variety “Blanca Grande” was evident. According to their findings, flour of the same wheat had lowest levels of vanillic, caffeic and p-coumaric acids. Therefore, researchers suggested possible changes in the hydrolysis or chemistry of the phenolics acids during bread baking as the reason for the increase in these phenolics (Lu et al., 2014). In a previous study, the loss in catechins was suggested to be related with the combined effect of oxidation, isomerization/epimerization, and degradation during bread making, the reduction of phenolic content could possibly be associated with the interactions between phenolics and wheat proteins via hydrogen bonding during dough preparation (Wang and Zhou 2004).

Although wheat gluten was reported to contain ferulic acid ($4.9\text{-}17.7 \mu\text{g}/100 \text{ mg}$), also sinapic acid ($1.4\text{-}3.5 \mu\text{g}/100 \text{ mg}$), and traces of p-coumaric acid and vanillic acid were detected after saponification (Labat et al., 2000), present findings did not reveal significant amounts of these phenolics in only WPI substituted flour samples (P2). Therefore, increase in WPI substitution level did not make any certain effects on the phenolic compounds found in crust, crumb and dough of no-CGEP added samples. However protocatechuic acid was higher in WPI substituted samples.

Gallic acid was also dominant phenolic acid in CGEP substituted bread samples (changed between 45 and 53 $\mu\text{g}/100\text{ g DW}$). The gallic acid content of only CGEP substituted wheat flour (GA1) was calculated as 65.5 $\mu\text{g}/100\text{ g DW}$ before baking, so a considerable amount (app. 20%) of loss was detected in CGEP added samples. Syringic acid, p-coumaric acid, sinapic acid and epigallocatechin were also present in CGEP added bread samples but the content of these compounds were much lower (around 10-15 $\mu\text{g}/100\text{ g DW}$).

Effect of increase in WPI substitution level in CGEP-substituted bread samples was slightly different, since 6.5% substitution made slight increases in various fractions of bread samples when the protocatechuic acid, gallic acid, sinapic acid, epigallocatechin, catechin and epicatechin were considered. However, 13% substitution decreased the measured amount of these compounds back to previous levels. This might be related with the effect of decrease in flour level and level of flour phenolics after certain level. or with the decrease in protein content during bread fermentation.

In CGEP-substituted bread samples different bread parts and dough generally had significant difference in their individual phenolics.

Rutin was the most abundant flavonol in CGEP added bread samples, although it was lost as much as 25 % during baking. Heat susceptibility of anthocyanins were observed for CGEP- substituted bread samples, since the dough fraction had significantly higher level of individual anthocyanins for almost all samples ($p < 0.05$). For rutin and quercetin-3- β -glucoside, increase in WPI level had a preserving effect.

Table 5.6: Phenolic acids and flavan-3-ols, and resveratrol contents of WPI and CGEP substituted bread samples.

	P1			P2			P3			GP1			GP2			GP3		
	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough
Protocath. acid	0.98 dy	3.19 bx	2.85 cdx	1.67 cdy	1.31 cy	3.77 bcx	2.80 cx	3.52 bx	4.30 bx	6.80 abx	6.74 ax	0.28 ey	7.36 ax	6.38 axy	6.21 ay	5.76 bx	6.97 ax	1.96 dey
Gallic acid	nd	14.19 bx	18.15 bx	7.20 by	15.39 bx	17.43 bx	6.14 bz	11.78 by	15.34 bx	45.49 ax	49.56 ax	49.83 ax	45.26 ax	51.46 ax	53.73 ax	43.97 ax	51.27 ax	53.87 ax
Sinapic acid	nd	6.58 bcx	nd	nd	9.09 bcx	7.84 cy	nd	8.87 bcx	9.65 bx	9.23 by	10.53 aby	19.80 ax	11.78 ax	11.12 abxy	9.33 by	10.35 by	14.51 ax	9.53 by
Epigallo-catechin	nd	nd	nd	nd	nd	nd	nd	nd	nd	9.53 ay	11.05 ax	9.52 by	9.82 ay	12.28 ax	10.03 by	8.58 ay	12.95 ax	11.29 axy
Catechin Hydrate	nd	nd	nd	nd	nd	nd	nd	nd	nd	6.54 ax	8.21 ax	6.82 bx	4.49 ay	5.42 by	10.86 ax	7.27 ax	6.33 ax	9.62 ax
Epicatechin	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.64 bz	5.32 cy	7.34 ax	4.26 ay	6.68 ax	5.73 bx	3.58 by	6.86 ax	7.00 ax
Syringic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	13.26 ay	13.10 ay	15.85 ax	3.23 by	7.25 bx	2.93 by	2.38 cx	5.80 bx	3.58 bx
Epicatechin gallate	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.76 bx	0.98 abx	1.29 ax	0.60 bx	0.91 abx	1.13 ax	1.01 ax	1.02 ax	0.85 ax
Vanilic acid	12.39 bx	6.98 bcy	3.69 abz	14.39 ax	3.61 cy	7.30 axy	12.57 bx	5.40 bcx	7.12 ax	5.64 dy	13.21 abx	3.76 aby	6.15 dx	12.45 abx	5.05 ax	7.02 cy	16.15 ax	nd
Chlorogenic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.78 cy	1.73 by	3.43 bx	8.88 ay	11.74 ax	10.84 ax	9.87 ay	12.58 ax	11.25 axy
Caffeic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.59 cy	0.58 by	1.14 bx	2.96 ay	3.91 ax	3.61 ax	3.29 ay	4.19 ax	3.75 axy
p-coumaric acid	nd	0.02 dx	nd	nd	0.02 dx	0.04 cx	nd	0.07 dx	0.06 cx	13.05 abx	12.45 cx	13.81 abx	12.17 by	13.77 bx	13.47 bx	12.64 ay	14.74 ax	14.21 axy
Ferulic acid	nd	0.33 cx	0.42 bx	nd	0.38 cx	0.43 bx	0.28 dy	0.34 cxy	0.37 bx	0.51 bx	0.53 bx	0.60 abx	0.47 cy	0.77 ax	0.94 ax	0.64 ax	0.84 ax	0.64 abx
t-resveratrol	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.41 abx	0.39 cx	nd	0.33 by	0.53 bx	nd	0.52 ax	0.71 ax	nd

Results are given as µg/100 g DW. Data given is the mean value of triplicate analyses. Values with different letters (a–e) of different bread samples at each fraction and different letters (x–z) within the bread sample among crust, crumb or dough differ significantly ($p < 0.05$). nd: not detected.

Table 5.7: Flavonols and anthocyanin contents of WPI and CGEP substituted bread samples.

	GP1			GP2			GP3		
	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough
Quercetin-3- β -glucoside	1.59 ax	1.15 ay	1.08 cy	1.64 ax	1.09 ay	1.82 bx	1.81 axy	1.56 ay	2.05 ax
Rutin	52.58 bx	57.23 ax	57.27 ax	52.49 by	59.73 ax	57.66 axy	54.49 ay	62.91 ax	58.44 axy
Myricetin	3.35 ax	2.65 ax	3.05 ax	2.43 bx	2.51 ax	3.07 ax	2.10 bx	2.58 ax	3.10 ax
Quercetin	4.04 ax	4.12 ax	3.88 ax	4.15 ax	3.46 ax	3.32 ax	4.10 ax	3.85 ax	3.57 ax
Delphinidin-3-o-glucoside	1.60 az	2.33 ay	3.08 ax	0.84 bz	2.47 ay	3.22 ax	0.87 bz	2.27 ay	3.36 ax
Cyanidin-3-o-glucoside	0.58 ax	0.95 ax	1.14 ax	0.38 ay	1.03 ax	1.01 ax	0.39 ay	1.09 ax	1.22 ax
Cyanidin-3,5-diglucoside	1.03 az	1.65 ay	2.27 ax	0.48 cy	1.55 axy	2.27 ax	0.78 by	1.83 ax	1.93 ax
Delphinidin-3,5-diglucoside	0.33 ay	0.41 bxy	0.63 ax	0.22 ay	0.57 ax	0.66 ax	0.33 ay	0.72 ax	0.62 ax
Delphinidin	0.61 ay	0.80 axy	1.18 ax	0.17 by	0.79 axy	1.25 ax	0.25 bz	0.65 ay	1.01 ax
Cyanidin	0.58 ay	0.94 axy	1.20 ax	0.68 ay	0.91 ay	1.38 ax	0.33 by	0.78 ax	1.16 ax
Malvidin	0.12 ay	0.18 bxy	0.47 ax	nd	0.67 ax	nd	nd	0.62 ax	0.31 ax
Malvidin-3-o-glucoside	5.12 ay	7.07 ay	11.86 ax	3.88 az	7.34 ay	9.37 ax	4.18 az	7.07 ay	12.85 ax

Results are given as $\mu\text{g}/100\text{ g DW}$. Data given is the mean value $\pm$ standard deviation of triplicate analyses. Values with different letters (a–e) of different bread samples at each fraction and different letters (x–z) within the bread sample among crust, crumb or dough differ significantly ($p < 0.05$). nd: not detected

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

On the other hand, significant decreases in delphinidin-3-o-glucoside, delphinidin, cyanidin-3-o-glucoside, and cyanidin-3,5-diglucoside in particularly crust of bread samples were evident.

No change in cyanidin was detected. The study about the processing steps of Concord juice revealed that anthocyanins of grapes displayed different stability behaviors against processing conditions and specifically heat treatments. According to the study, although peonidin and peonidin-3-O-glucoside were the major phenolic components of Concord grape juice before heat treatment; malvidin and dimethoxyflavylium were formed after pasteurization. Therefore methylated anthocyanins showed higher stability to oxidative and thermal conditions than glucosylated and highly hydroxylated anthocyanins (Gollücke et al., 2009).

5.3.3 Interaction of CGEP with amylose

5.3.3.1 Total phenolics

Total phenolic content in bread samples were given in Table 5.7. Phenolic contents of different bread samples ranged on a wide scale of 101.8 ± 2.2 to 1893.9 ± 53.9 mg GAE/100 g DW.

All control samples containing only HACS had significantly lower levels of phenolics than samples with CGEP and HACS ($p < 0.05$), although level of HACS substitution made no significant effects on measured phenolic content of these samples ($p > 0.05$). In addition, crusts of those samples have a significantly higher level of phenolic content ($p < 0.05$) than crumb. This might be because of thermal processing that caused complex physical and chemical reactions of phenolic compounds, including leaching of water soluble phenolics, liberation of free phenolics from their bound forms, degradation of polyphenols, breakdown and transformation of phenolics and most probably formation of Maillard reaction products, such as formation of complex products from phenolics and proteins (Xu et al, 2008). Similarly, previous research has also revealed that crust parts of the white bread with hard wheat flour had more phenolic compounds than crumb ($p < 0.05$) (Gelinas and McKinnon, 2006; Lu et al., 2014) because of Maillard reaction products, whereas no significant difference detected in different parts of wholegrain bread (Gelinas and McKinnon, 2006). In a previous study, the total phenolic content of bread slices (without the addition of grape pomace powder) had a value of 35.29 mg GAE/100 g (Hayta et al., 2014) which was lower than the current findings ranging from 101.8 ± 2.2 to 108.7 ± 3.3 mg GAE/100 g DW in crumb and from to 175.4 ± 2.5 to 277.7 ± 6.8 mg GAE/100 g DW in the dough. Dough is the richest part of phenolics in bread samples without Concord grape extract powder, therefore baking lowered the total phenolics in those bread samples.

In samples with CGEP, amylose substitution generally caused no significant decline ($p > 0.05$) in measured phenolic content of crumb and crust, while making a significant decrease in the dough ($p < 0.05$). However, this decreasing trend was not linear in 20% substitution and increased in fermented dough. For only crust, 20% amylose substitution made a slight decline in total measured phenolics. Crumb for

Concord grape extract powder added breads were independent from the amylose substitution level.

When compared with total phenolic content of unprocessed control hard wheat flour (721.8 ± 6.5 mg GAE/100 g DW), an average of 74%, 85% and 70% loss of phenolics in crust, crumb and dough were found respectively. The amount of loss in no-CGEP added samples was significantly higher than CGEP substituted samples ($p < 0.05$). In CGEP substituted samples 36%, 40% and 23% of total phenolics in crust, crumb and dough samples, respectively were lost in average, comparison to only CGEP added flour (2374.2 ± 89.2 mg GAE/100 g DW). Similar to the present results, bread containing eriocitrin (30%), a lemon flavonoid, reported to retain only sixty-five percent of the eriocitrin and 78% of the antioxidative activity after baking the bread (Kanae et al., 2008). In accordance with the present findings for CGEP substituted samples, the amount of phenolic acids that were added individually were recovered at a ratio of 74–80% after baking (Han and Koh, 2010). Another study revealed that tea catechins were preserved to a higher extent since only 16% of the total tea catechins were lost after baking as well as during its shelf life (Wang and Zhou, 2004). In the current study, high amylose corn starch addition did not have any significant role on the remaining phenolic levels for all of the samples.

5.3.3.2 Total antioxidant activity

DPPH radical scavenging activity in bread samples were given in Table 5.7. Antioxidant activity measured using DPPH radical, ranged on a wide scale from 28.0 ± 5.4 to 1426.0 ± 39.7 mg GAE/100 g DW. Samples containing no CGEP had significantly lower levels of antioxidant activity than CGEP added ones ($p < 0.05$), although level of high amylose starch substitution made no significant effect on the antioxidant activity of those samples ($p > 0.05$). In addition, crusts of those samples have significantly higher level of antioxidant activity ($p < 0.05$) than crumb and dough. Current findings ranged from 28.0 ± 5.4 to 43.1 ± 2.4 mg TEAC/100 g DW for crumb and from 30.7 ± 9.1 to 41.8 ± 3.5 mg TEAC/100 g DW for the dough. The antioxidant activity of wheat products depends on the nature of antioxidant species, wheat variety, extraction method, and type of antioxidant activity assay (Fardet et al., 2008).

For samples with CGEP, amylose substitution (from 0% to 10%) caused significant decline ($p < 0.05$) in the measured DPPH radical scavenging activity of crumb and dough, while making no significant changes in phenolic content of crust. However, this decreasing trend was not linear in further (20 %) substitution and caused increases in the crumb and fermented dough. Antioxidant activity of white bread crust was reported to be twice as much high in comparison to flour or crust-free bread in previous studies. This difference was explained by the reductive intermediates formed during Maillard reaction during baking (Miller et al., 2002).

Table 5.8 : Total phenolic content and antioxidant activity results of HACS and CGEP substituted bread samples and dough.

	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough
	TAA-DPPH (mg TEAC/100 g DW)			TAA-ABTS (mg TEAC/100 g DW)			TP (mg GAE/ 100 g DW)		
A1	105.0± 9.7bx	28.0± 5.4cy	39.9± 7.0dy	287.8± 7.9bx	84.9± 0.5by	312.0± 8.1cx	199.0± 3.0cy	108.7± 3.3bz	277.7± 6.8cx
A2	100.2± 12.8bx	43.1± 2.4cy	30.7± 9.1dy	306.1± 7.0bx	81.4± 0.8by	317.0± 7.3cx	178.2± 8.7cx	101.8± 2.2by	179.5± 2.3cdx
A3	74.0± 10.8bx	34.4± 4.0cy	41.8± 3.5dy	213.9± 2.2bx	74.3± 4.3by	278.7± 4.4cy	161.2± 1.1cy	103.8± 1.4bz	175.4± 2.5dx
GA1	1166.5± 40.0ax	1133.9± 17.0ax	1310.1± 77.8bx	2171.5± 139.1ax	2147.0± 59.5ax	2368.8± 203.1bx	1571.2± 9.5ay	1480.5± 40.3ay	1893.9± 53.9ax
GA2	1080.5± 4.6az	1103.0± 7.5by	1193.1± 2.9cx	2286.9± 95.6ax	2109.8± 109.5ax	2480.4± 15.8bx	1573.8± 53.3ay	1411.7± 20.2az	1745.2± 6.4bx
GA3	1031.7± 18.2ay	1111.9± 18.3aby	1426.0± 39.7ax	2416.0± 113.9axy	2151.5± 102.2ay	3072.0± 202.7ax	1451.6± 61.9by	1428.± 22.2ay	1826.1± 44.3abx

Data given is the mean value± standard deviation of triplicate analyses. Values with different letters (a–d) within the same column and different letters (x–z) within the same row differ significantly ($p < 0.05$). A1: bread flour control; A2: bread flour substituted with 10% HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

TAA: Total antioxidant activity; TP: Total phenolics; DW: Dry weight; TEAC: Trolox Equivalent Antioxidant Capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,20-azinobis-3-ethylbenzo-thiazoline-6-sulphonic acid diammonium salt; GAE: gallic acid equivalence.

Results of total antioxidant activity determined by ABTS radical scavenging activity method were given in Table 5.7. According to the results, antioxidant activity of the samples changed between 74.3 ± 4.3 mg TEAC/100 g DW and 3072.0 ± 202.7 mg TEAC/100 g DW. In samples with no CGEP substitutions, crumb had significantly lower antioxidant activity in comparison to crust and dough ($p < 0.05$). Amylose substitution level was not significant in any of those samples ($p > 0.05$).

In samples with CGEP substitutions, ABTS radical scavenging results were at least 7-fold higher than no-grape powder added samples. The highest results were measured for bread dough samples and were found as significantly higher than crust

and crumb of bread ($p < 0.05$). Amylose substitution level generally made no significant effect on ABTS radical scavenging activity of the samples. Loss of ABTS radical scavenging activity after baking was also evident as seen from Table 5.8. Percent loss was highest in the crumb of no-CGEP added samples (77%). Generally HACS addition had no effect on the loss (%) of antioxidant activity analyzed by ABTS method, only exceptions were for dough and crust of 20% HACS substituted bread samples, in which the % loss significantly increased from around 15 and 12% to 40 and 78% for the crust and dough, respectively.

Table 5.9 : Phenolic and antioxidant activity losses (%) for HACS and CGEP substituted bread samples and dough.

	Crust				Crumb				Dough			
	TP	ABTS	DPPH	TF	TP	ABTS	DPPH	TF	TP	ABTS	DPPH	TF
Losses (%)												
A1	71	20	-84	n.d	84	77	51	n.d	60	14	30	n.d
A2	74	15	-76	n.d	85	77	24	n.d	74	12	46	n.d
A3	77	41	-30	n.d	85	79	40	n.d	75	78	27	n.d
GA1	34	37	22	-61	38	38	24	-62	20	31	12	-66
GA2	34	33	28	3	41	39	26	-11	26	28	20	-63
GA3	39	30	31	-1	40	37	25	15	23	11	4	-55

A1: bread flour control; A2: bread flour substituted with 10% HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

5.3.3.3 Total flavonoids

Total flavonoids in CGEP and high amylose corn starch (HACS) substituted bread samples were given in Table 5.8. Total flavonoid content of bread samples changed between 1936.5 ± 41.3 and 2907.0 ± 474.4 mg QE/100 g DW. Before HACS substitution, bread sample with only CGEP seemed to have an insignificant difference in flavonoid content between parts of bread samples and dough ($p > 0.05$). However, HACS seemed to decrease the measured amount of flavonoids in the crust and crumb. Flavonoid levels in 20% amylose substituted samples were found significantly different ($p < 0.05$) in different parts of bread and dough. Therefore, amylose substitution decreased flavonoids significantly ($p < 0.05$) in crust and crumb but made no effect for the dough. Increase in substitution level made only slight changes.

In comparison to flavonoid contents of flour samples, different parts of bread samples were found to have significant difference in the remaining % flavonoids. As shown in Table 5.8., flavonoid contents of the crust and crumb decreased with the increase in HACS level, whereas for dough no change was seen.

Table 5.10 : Total anthocyanin and total flavonoid contents of HACS and CGEP substituted bread and dough samples.

	Crust	Crumb	Dough	Crust	Crumb	Dough
	TAC- (mg Cy-3-glc/100 g DW)			TF(mg QE/ 100 g DW)		
GA1	12.2±	15.5±	18.4±	2806.7±	2830.9±	2907.0±
	2.9ax	2.3ax	4.1bx	48.6ax	34.8ax	474.4ax
GA2	17.6±	21.4±	32.0±	1704.0±	1936.5±	2847.4±
	1.3ay	3.6ay	0.6ax	187.8bx	41.3bx	398.0ax
GA3	14.4±	18.4±	31.2±	1769.9±	1493.4±	2701.4±
	1.4ay	2.8axy	4.0ax	79.1by	28.5cz	0.0ax

Data given is the mean value± standard deviation of triplicate analyses. Values with different letters (a–d) within the same column and different letters (x–z) within the same row differ significantly ($p < 0.05$).

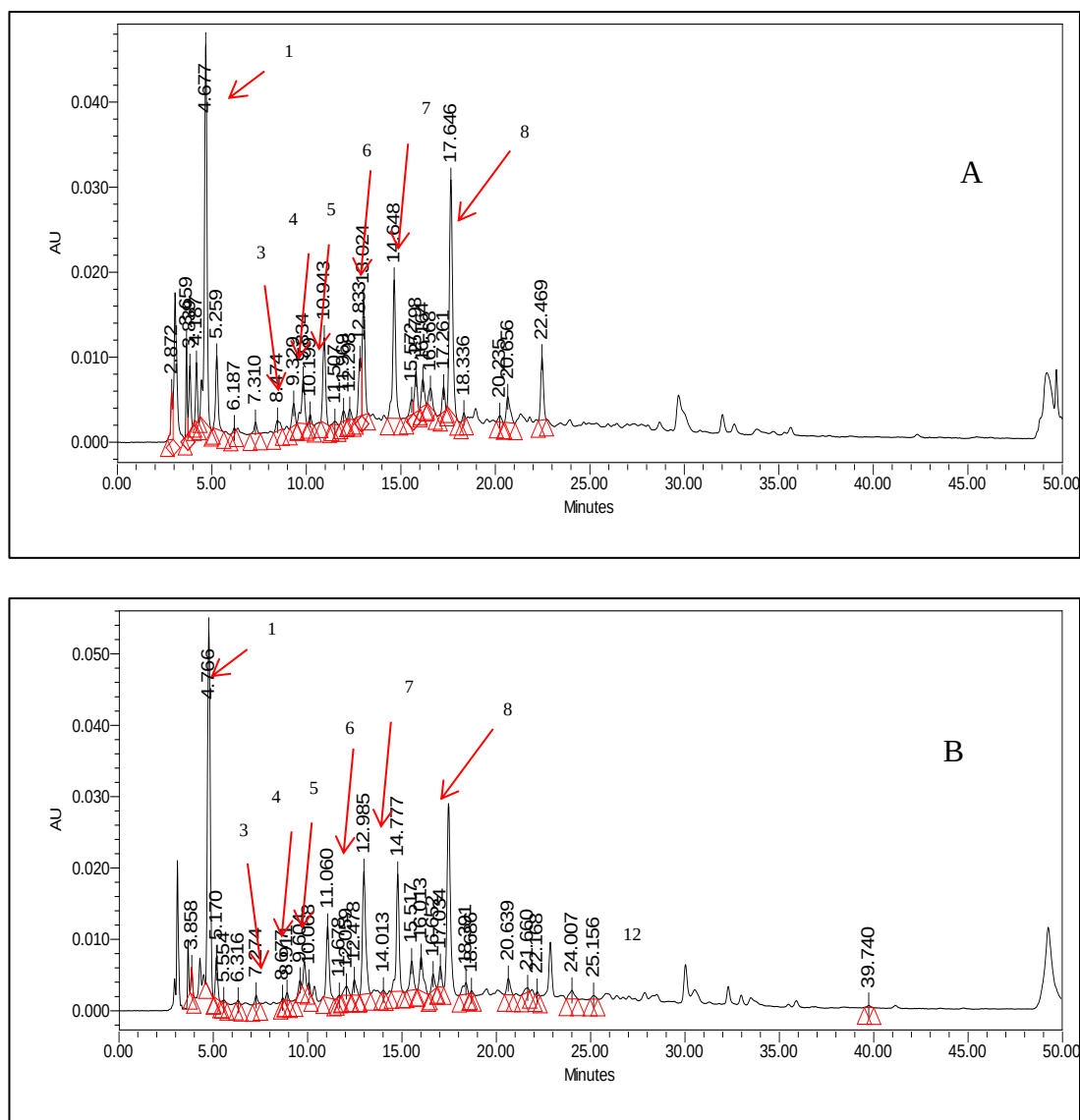
TF: Total flavonoids; TAC: Total anthocyanin content; DW: Dry weight; QE: quercetin equivalence; Cy-3-glc: cyanidin-3-glucoside

5.3.3.4 Total anthocyanins

Total anthocyanin contents in parts of bread samples and dough were given in Table 5.9. Amount of anthocyanins ranged between 12.2±2.9 and 32.0±2.9 mg Cy-3-glc/100 g DW. Although the anthocyanin content of bread samples with only CGEP substitutions were statistically similar ($p > 0.05$), dough had significantly higher total anthocyanins than crust and crumb of bread after HACS addition. Change in the anthocyanin contents of crumb and crust of breads with increase in HACS level was not found as statistically significant ($p > 0.05$). pH of Concord grape extract powder was found around as 2.5 – 3.5. High acidity might have influenced the monomeric anthocyanin detection method. Previous findings revealed that after high-temperature treatment (70°C, 30 min) of several grape cultivars for the production grape juice, only 12–32% of the total anthocyanins present in the grapes were measured (Lee et al., 2004).

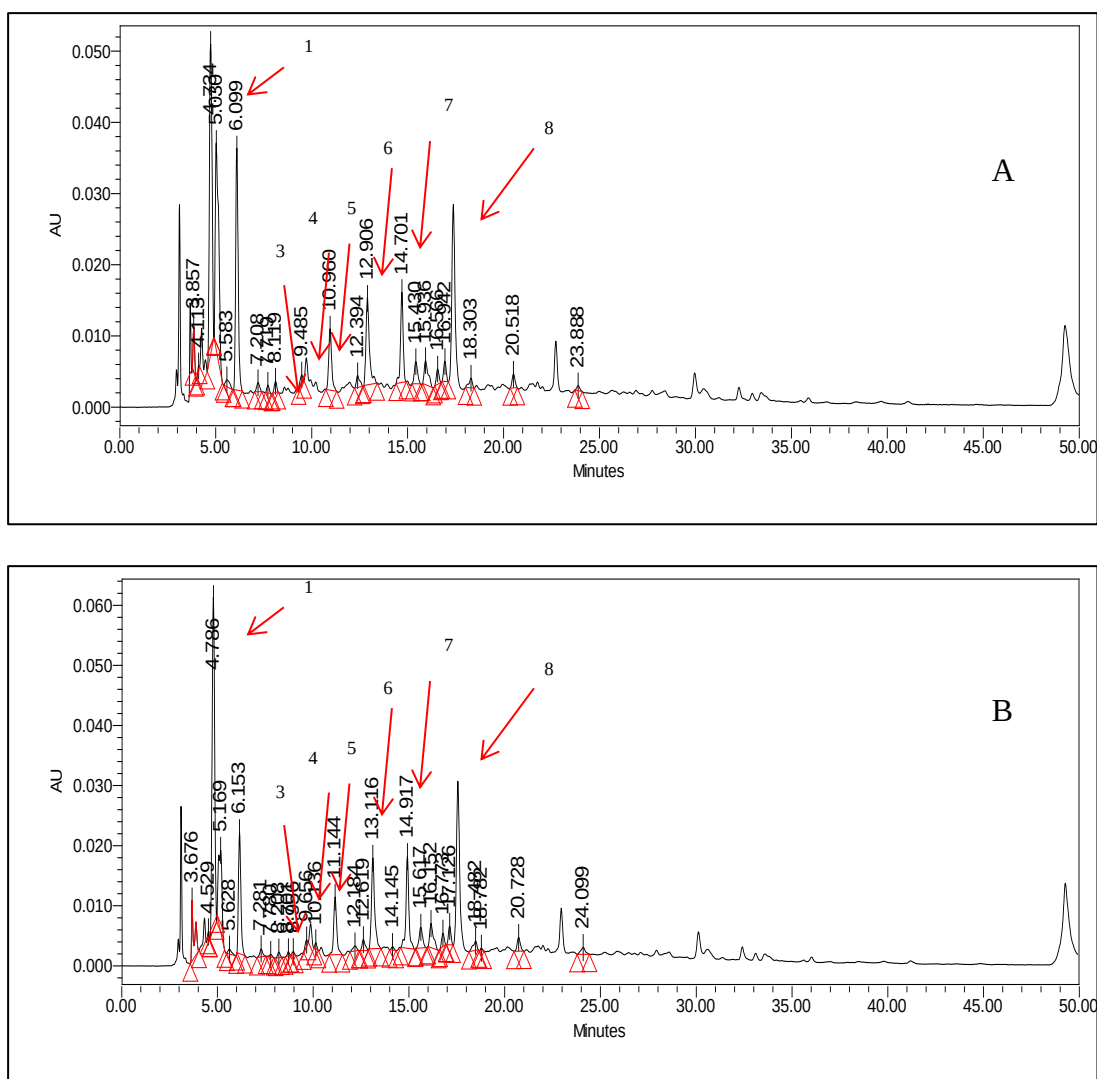
5.3.3.5 Phenolic and anthocyanin profiles and composition of CGEP and HACS substituted bread samples

Phenolic compound profiles and anthocyanin profiles of CGEP and HACS substituted bread samples were given in Figures 5.27-5.34 and Tables 5.11 and 5.12.



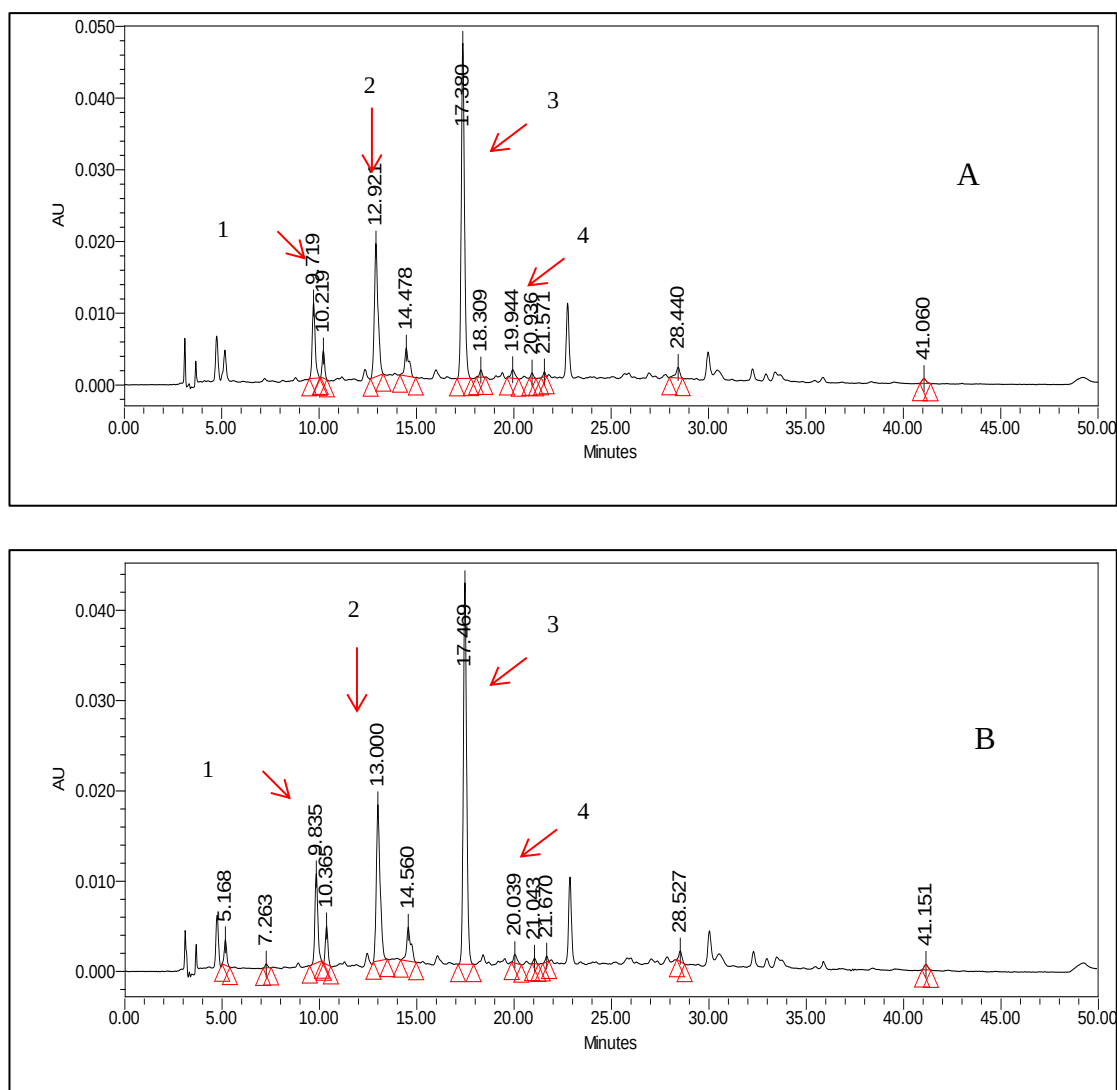
(1: Gallic acid, 2: Protocatechuic acid, 3: Epigallocatechin; 4: Catechin hydrate; 5: Epicatechin; 6: Syringic acid, 7: Vanilic acid, 8: n.d)

Figure 5.27: Representative HPLC-PDA chromatograms extracted at 280 nm for A: GA1 Crumb; B: GA3 Crumb.



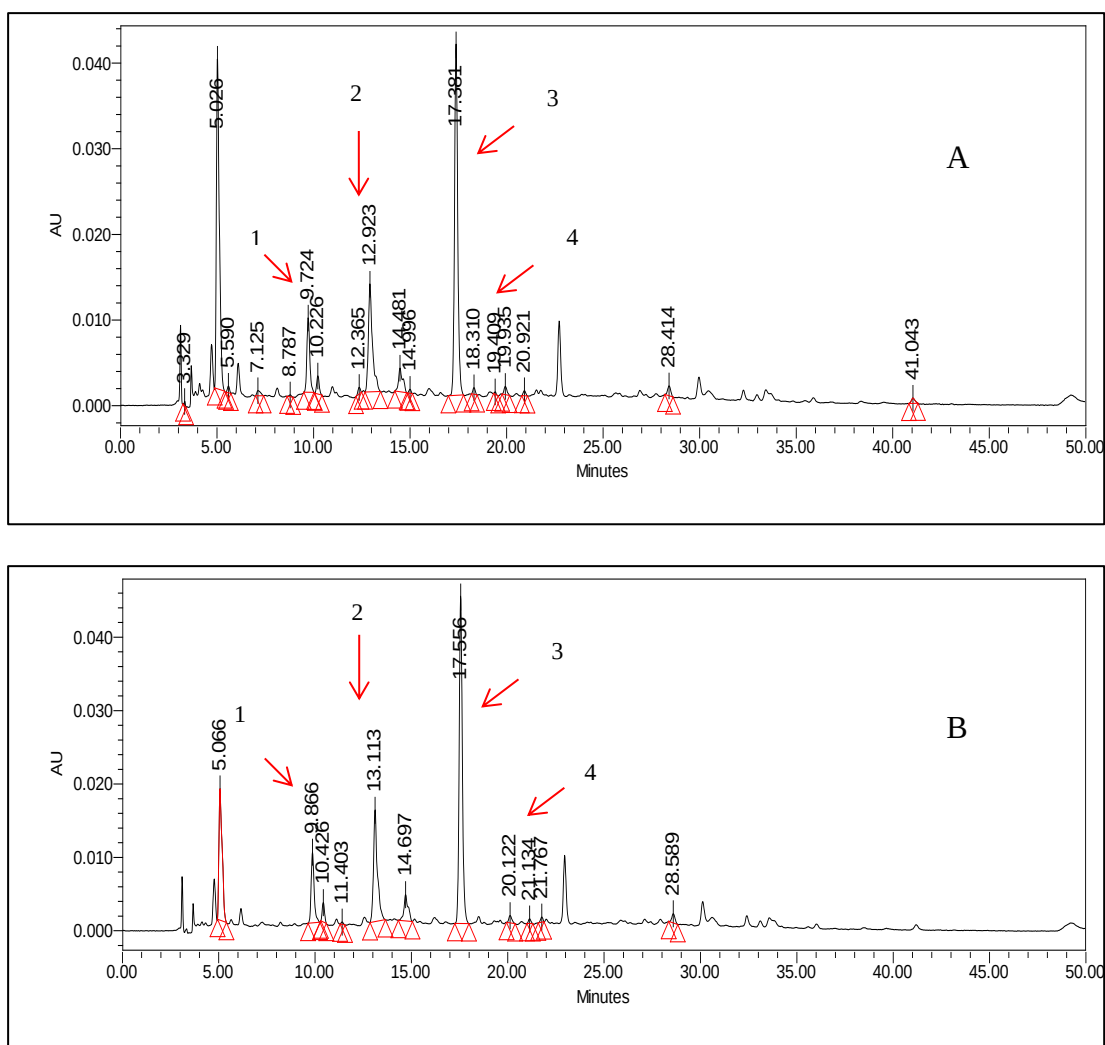
(1: Gallic acid, 2: Protocatechuic acid, 3: Epigallocatechin; 4: Catechin hydrate; 5: Epicatechin; 6: Syringic acid, 7: Vanilic acid, 8: n.d)

Figure 5.28: Representative HPLC-PDA chromatograms extracted at 280 nm for A: GA1 Crust; B: GA3 Crust.



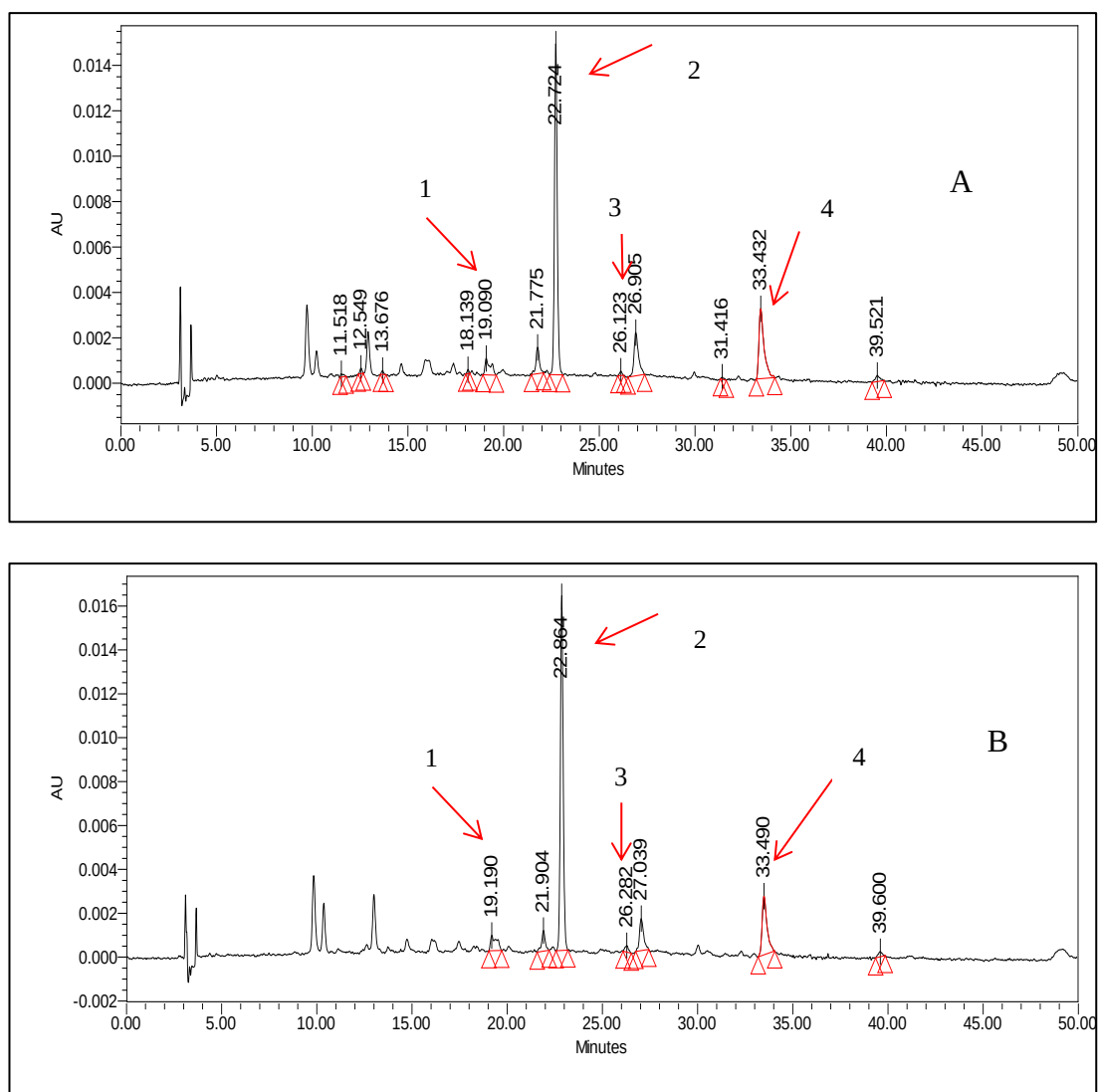
(1: Chlorogenic acid; 2: Caffeic acid; 3: P-coumaric acid; 4: Ferulic acid)

Figure 5.29: Representative HPLC-PDA chromatograms extracted at 312 nm for A: GA1 Crumb; B: GA3 Crumb.



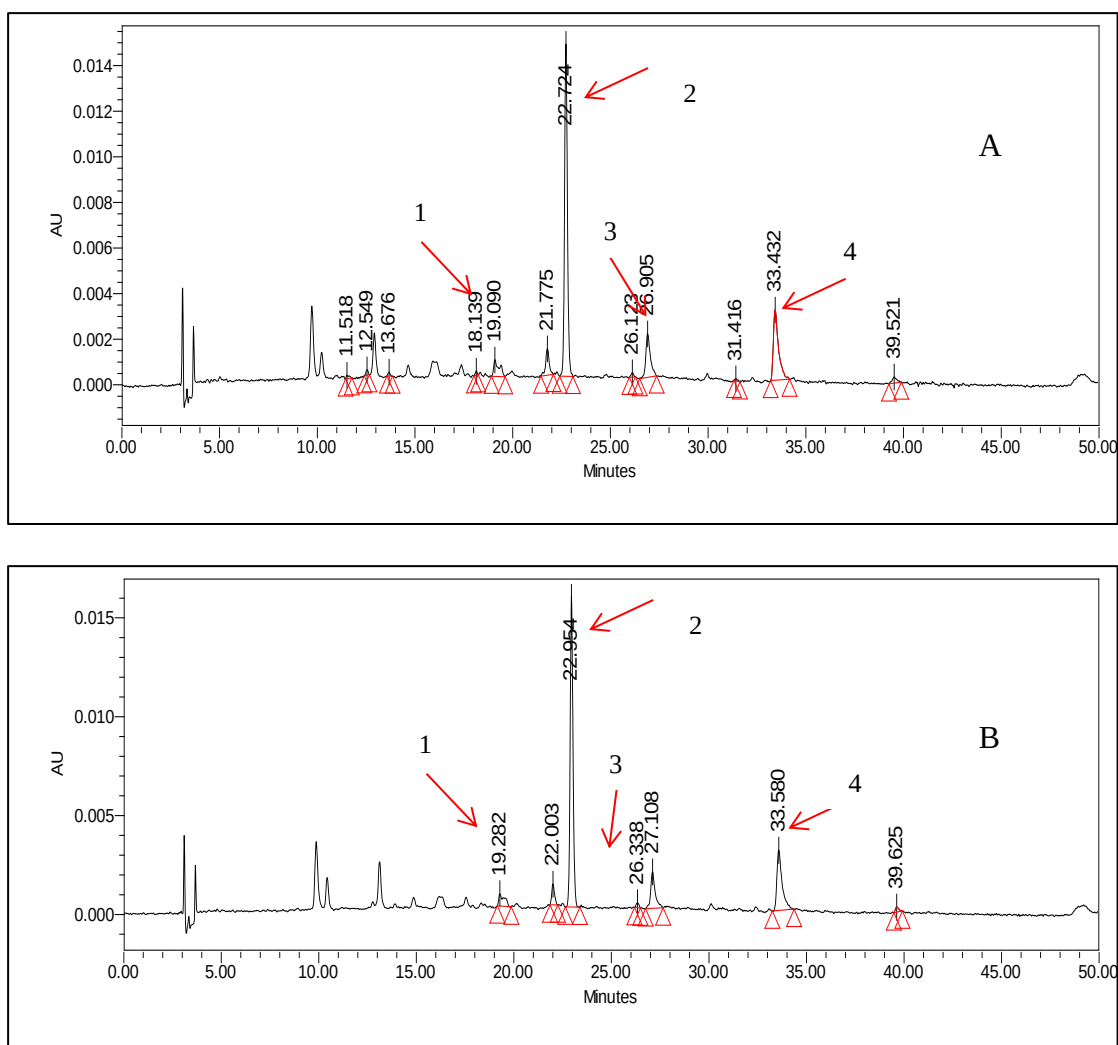
(1: Chlorogenic acid; 2: Caffeic acid; 3: P-coumaric acid; 4: Ferulic acid)

Figure 5.30: Representative HPLC-PDA chromatograms extracted at 312 nm for A: GA1 Crust; B: GA3 Crust.



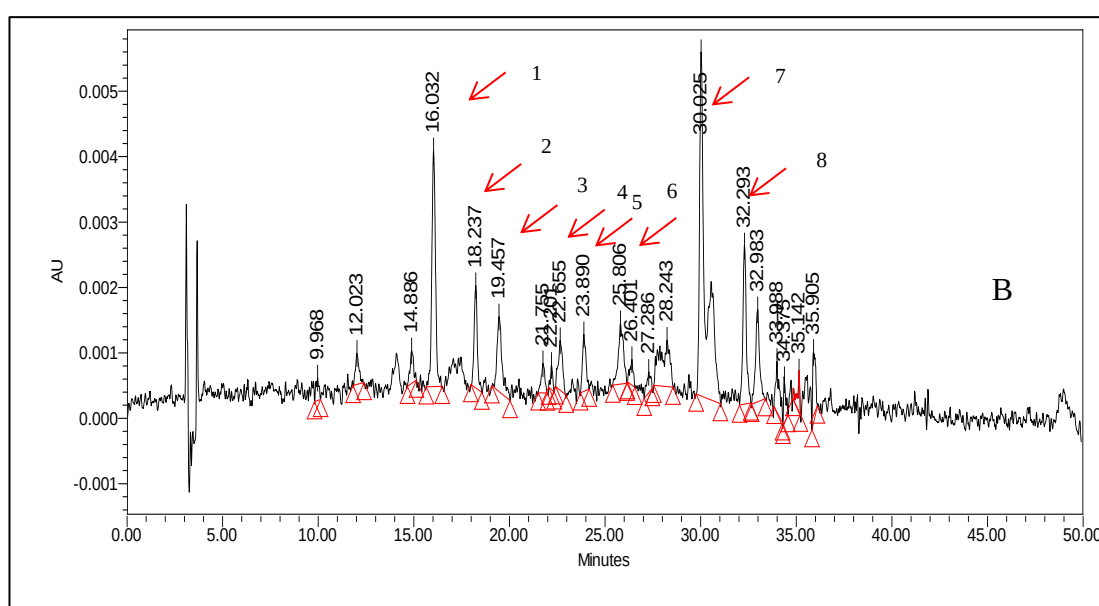
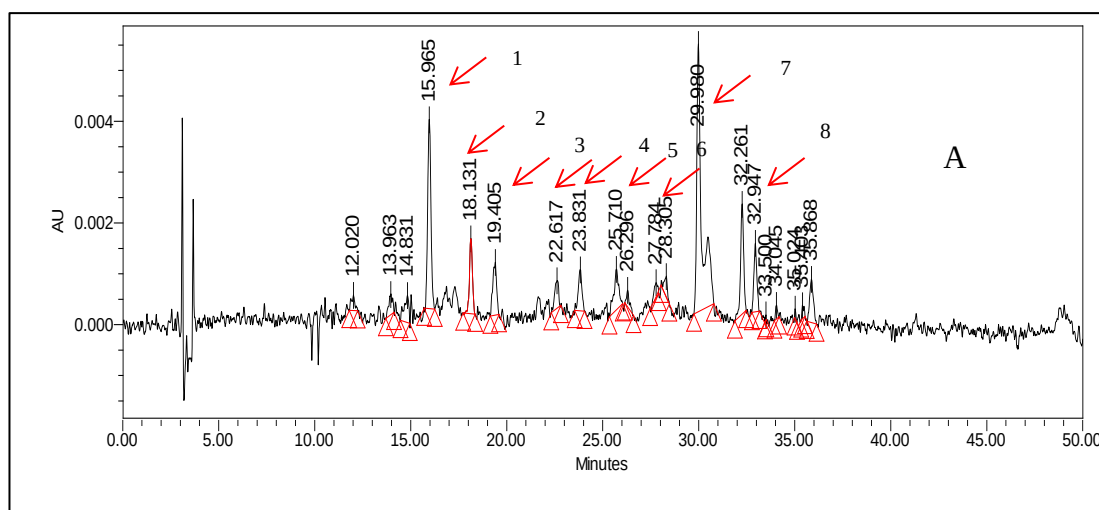
(1: Quercetin-3-beta-glucoside; 2: Myricetin 3: Rutin, 4: Quercetin)

Figure 5.31: Representative HPLC-PDA chromatograms extracted at 360 nm for A: GA1 Crumb; B: GA3 Crumb.



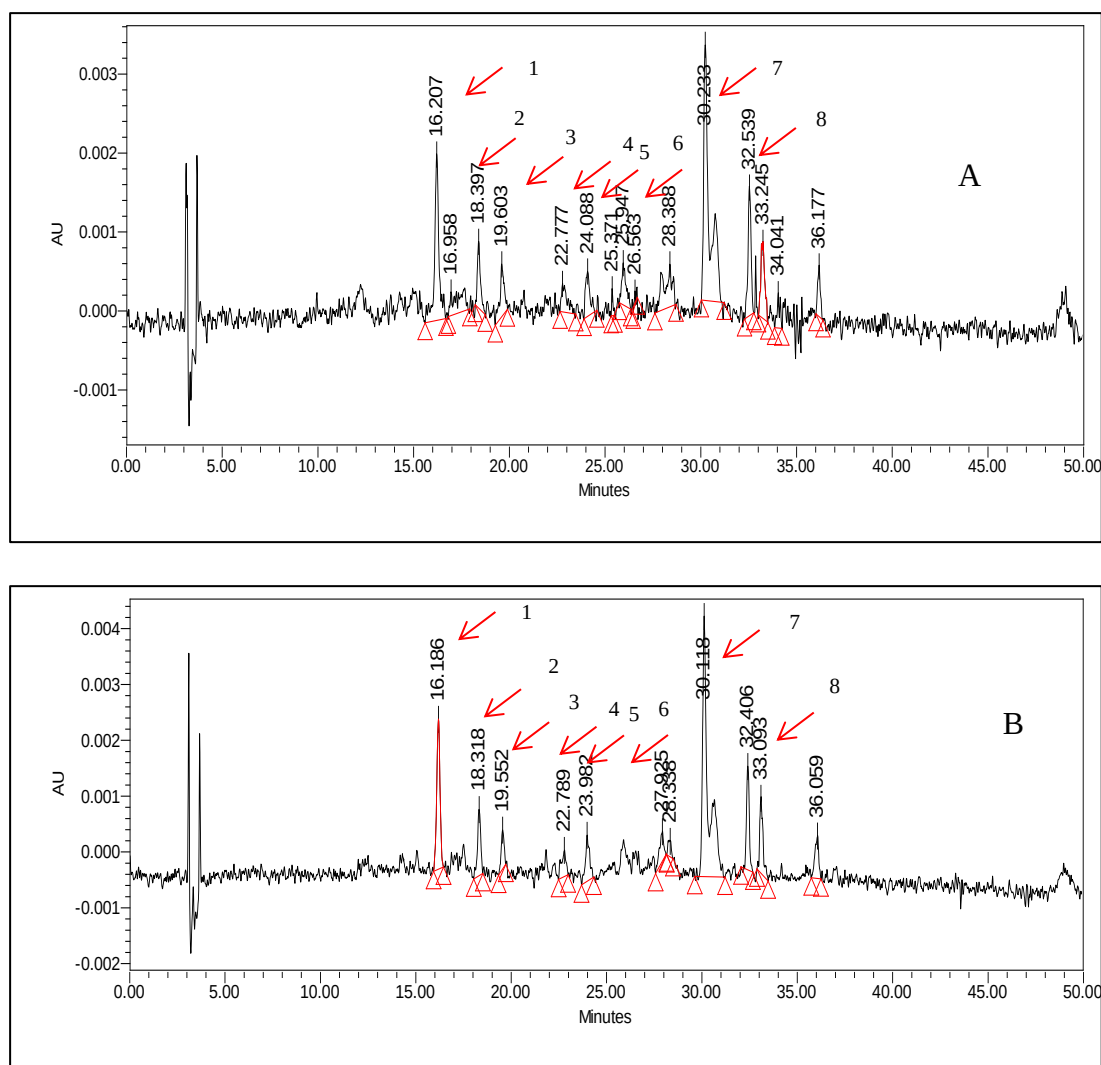
(1: Quercetin-3-beta-glucoside; 2: Myricetin 3: Rutin, 4: Quercetin)

Figure 5.32: Representative HPLC-PDA chromatograms extracted at 360 nm for A: GA1 Crust; B: GA3 Crust.



(1: Delphinidin-3-glucoside; 2: Cyanidin-3-o-glucoside; 3: Cyanidin-3,5-d,glucoside; 4: delphinidin-3,5-diglucoside; 5: delphinidin; 6: cyanidin; 7: malvidin; 8: malvidin-3-o-glucoside)

Figure 5.33: Representative HPLC-PDA chromatograms extracted at 520 nm for A: GA1 Crumb; B: GA3 Crumb.



(1: Delphinidin-3-glucoside; 2: Cyanidin-3-o-glucoside; 3: Cyanidin-3,5-d,glucoside; 4: delphinidin-3,5-diglucoside; 5: delphinidin; 6: cyanidin; 7: malvidin; 8: malvidin-3-o-glucoside)

Figure 5.34: Representative HPLC-PDA chromatograms extracted at 520 nm for A: GA1 Crust; B: GA3 Crust.

Ferulic acid is reported by other researchers as the main free phenolic acid of wheat flour (Hatcher and Kruger, 1997). Although that is the case, the total ferulic acid content of 11 wheat varieties has been reported to possess significantly different (Adom et al., 2003) levels even as high as even two-fold. However according to present findings; ferulic acid level was lower (around 0.15 $\mu\text{g}/100 \text{ g DW}$) in control flour (A1) before baking, in comparison to syringic acid (2.5 $\mu\text{g}/100 \text{ g DW}$), vanillic and sinapic acids (2 $\mu\text{g}/100 \text{ g DW}$), and gallic acid (6.5 $\mu\text{g}/100 \text{ g DW}$).

In control bread samples, without CGEP, the highest concentrations of phenolic acids were found as gallic acid (2.4 to 18 $\mu\text{g}/100 \text{ g DW}$) and vanillic acid (3.38 to 12.39 $\mu\text{g}/100 \text{ g DW}$).

Han and Koh (2010) revealed that among four phenolic acids, caffeic acid, ferulic acid, syringic acid and gallic acid, which were mixed with wheat flour at a concentration of 4.44 $\mu\text{mol/g}$ of flour, gallic acid increased in concentration after fermentation with respect to other added phenolic acids, indicating that fermentation liberated gallic acid by means of breaking bonds between gallic acid and other molecules (Han and Koh, 2010). Some phenolics such as ferulic, syringic, vanillic, and p-coumaric acids might increase in concentration by heat stress, or some bound phenolics might be released by the effect of heat (Cheng et al., 2006). Lu et al. (2014) detected ferulic acid as the predominant phenolic acid in white bread samples (Lu et al., 2014). Similarly, Sun-Waterhouse et al. (2011) detected only ferulic acid and also 3-O-p-coumaroylquinic acid as intrinsically occurring in their “control bread” without the addition of any fruit source.

According to present findings, no significant decrease between the phenolic concentrations of fermented dough samples and crumb samples was observed and this was in accordance with previous findings (Han and Koh, 2010). For some phenolic compounds; such as procatechuic acid, epigallocatechin, catechin hydrate, vanillic and p-coumaric acids, crumb had higher concentrations than dough. Significant differences in phenolic concentrations were generally observed in bread crusts which was not in agreement with some previous researchers (Lu et al., 2014).

HACS substitution made no significant change in the measured phenolics of no-CGEP added breads.

In CGEP substituted bread samples, individual phenolic concentration was highest for gallic acid (45.49-53.52 $\mu\text{g}/100\text{ g DW}$) and rutin (53.16-60.89 $\mu\text{g}/100\text{ g DW}$), followed by sinapic acid, syringic acid, and p-coumaric acid (around 10 $\mu\text{g}/100\text{ g DW}$). In the study of Mildner-Szkudlarz et al., sourdough mixed rye bread was prepared as control bread besides breads with four different levels of grape pomace (4%, 6%, 8% and 10%). In control bread, mainly gallic acid was detected at 280 nm, while phenolic profiles in supplemented mixed rye breads were dominantly consisting of also catechin and epicatechin. At 360 nm, caffeic acid and myricetin were detected as two dominant phenolic acids, in addition to quercetin and quercetin-3- β -D-glucoside (Mildner-Szkudlarz et al., 2011).

Recoveries of phenolics after baking vary among different phenolic compounds. For example after baking; changes in epigallocatechin (50% loss), gallic acid (no change), catechin hydrate (50% loss), epicatechin (2-3 fold increase) and syringic acid (20% loss) were significantly different from each other. Similar differences were also found in different studies; epicatechin was spiked into bread samples in a previous study and could only be recovered after baking at around 7-8%. The low extractability has been related with the polyphenol complexing with polysaccharides and/or proteins/ enzymes (Le Bourvellec et al., 2007). In another study, recovery rate of phenolic acids after baking was found to range from 73.8 to 80.9% (Han and Hoh, 2010).

It was reported that thermal degradation during processing is a factor to change the ratio between phenolic compounds. For example, vanillin and vanillic acid were possibly produced by thermal decomposition of ferulic acid (Fiddler et al., 1967; Pisarnitskii et al., 1979; Peleg et al., 1992), whereas p-hydroxybenzaldehyde from p-coumaric acid (Pisarnitskii et al., 1979). Caffeic acid, ferulic and p-coumaric acids are either heat labile or susceptible to thermal breakdown (Steinke and Paulson, 1964; Pisarnitskii et al., 1979; Huang and Zayas, 1991). On the other hand, some phenolics such as ferulic, syringic, vanillic, and p-coumaric acids increase in concentration either by heat stress, or by the release of some bound phenolics by the effect of heat (Cheng et al., 2006).

HACS substitution increased some phenolics measured; such as catechin hydrate, epicatechin gallate, chlorogenic acid and caffeic acid contents particularly at 20% substitution level and in crumb.

Quercetin-3- β -glucoside, delphinidin 3-O-glucoside, cyanidin 3-O-glucoside and their diglucosides, also anthocyanidins such as; delphinidin, cyanidin, malvidin and their glucosides were detected in CGEP added breads. Flavonols detected displayed no significant differences among crumb, crust and dough ($p>0.05$). However significant differences were observed between the anthocyanin profiles of crust, crumb and dough of bread samples. Generally crusts had lower amounts of anthocyanin compounds, as temperature during baking was the highest one among different parts. In another study carried on black currant extract and high methoxy pectin bread high concentrations of highly polar polyphenols such as gallic acid and protocatechuic acid, in addition to high concentrations of the anthocyanins with

medium polarity: delphinidin 3-O-glucoside, delphinidin 3-O-rutinoside, cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside were detected. They suggested that oxidation of myricetin or quercetin occurred to some extent during baking, generating oxidation products with higher polarity. Peaks increasing in the baked bread matrix suggested that the galactoside form of myricetin and quercetin (especially myricetin) might be less stable than the glucoside and rutinoside forms in the bread matrix, and the cleavage of glycoside residue from quercetin might be possible (Sun-Waterhouse, et al., 2011). However for further identification HPLC-MS/MS (High performance liquid chromatography-mass spectrometry) analysis would possibly give a better profiling.

On the other hand; HACS substitution seemed to have no effect on the remaining flavonols and anthocyanins. Only exception was malvidin, since the malvidin content increased with the increase in HACS substitution. Some researchers previously claimed that starch had a protecting role on anthocyanins (White et al., 2010).

Table 5.11: Phenolic acids, flavan-3-ols, and resveratrol contents of HACS and CGEP substituted bread samples.

	A1			A2			A3			GA1			GA2			GA3		
	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough
Protocat. acid	0.98 dy	3.19 cx	2.85 bx	2.07 cy	2.81 cxy	3.46 bx	2.22 cy	2.86 cx	2.78 bx	6.80 ax	6.74 ax	0.82 cy	1.65 dy	0.83 dz	6.49 ax	6.12 bx	6.15 bx	6.64 ax
Gallic acid	nd	14.19 cx	18.15 bx	2.41 cy	13.77 cx	12.24 cx	13.13 bxy	15.18 cx	10.42 cy	45.49 ax	49.56 bx	49.83 ax	48.48 ax	53.29 ax	51.08 ax	48.28 ax	53.52 ax	49.88 ax
Sinapic acid	nd	6.29 bx	nd	nd	12.13 ax	nd	nd	7.34 bx	nd	9.23 cy	10.53 aby	19.80 ax	10.00 ay	8.54 abz	22.08 ax	9.51 bx	9.33 abx	9.45 bx
Epigallo-catech.	nd	nd	nd	nd	nd	nd	nd	nd	nd	9.53 ay	11.05 ax	9.52 by	9.93 ax	11.10 ax	10.11 abx	10.03 ax	11.33± ax	11.12 ax
Catechin hydrate	nd	nd	nd	nd	nd	nd	nd	nd	nd	6.54 bx	8.21 bx	6.82 ax	3.24 by	10.01 ax	10.28 ax	22.80 ax	9.60 ay	7.52 ay
Epicatechin	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.64 bz	5.32 by	7.34 ax	0.78 bz	7.28 ax	5.20 by	5.47 ax	5.60 bx	5.74 bx
Syringic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	13.26 ay	13.10 by	15.85 ax	12.93 ay	18.86 ax	13.42 by	1.11 by	5.89 cx	2.04 cy
Epicatechin galleate	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.76 abx	0.98 bx	1.29 ax	0.77 abx	0.97 bx	0.99 ax	1.23 ax	1.61 ax	1.64 ax
Vanillic acid	12.39 ax	6.94 by	3.69 az	11.20 bx	6.25 bcy	3.38 az	7.20 cx	4.43 dx	4.82 ax	5.64 dy	5.61 cx	3.76 ay	nd	9.69 ax	5.75 ay	nd	4.50 dx	4.70 ax
Chlorogenic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.78 bcy	1.78 by	3.43 ax	2.20 aby	3.70 ax	3.34 ax	2.34 abz	4.15 ax	3.10 ay
Caffeic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.59 ybcd	0.59 by	1.14 ax	0.80 aby	1.23 ax	1.11 ax	0.78 abcz	1.38 ax	1.03 ay
p-coumaric acid	nd	0.02 cx	nd	nd	0.15 cx	0.15 bx	0.05 cy	0.09 cx	0.06 by	12.45 bx	12.45 bx	13.81 ax	12.82 abx	13.79 ax	13.64 ax	13.07 ax	13.61 ax	14.37 ax
Ferulic acid	nd	0.33 bcdx	0.42 ax	0.22 by	0.22 dy	0.36 ax	0.26 by	0.30 cdxy	0.33 ax	0.53 ax	0.53 abx	0.60 ax	0.59 ax	0.50 abcx	0.61 ax	0.58 ax	0.67 ax	0.75 ax
t-resveratrol	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.41 ax	0.39 bx	0.52 ax	0.39 ax	0.58 ax	0.52 ax	0.41 ax	0.33 bx	0.49 ax

Results are given as µg/100 g DW. Data given is the mean value of triplicate analyses. Values with different letters (a–e) of different bread samples at each fraction and different letters (x–z) within the bread sample among crust, crumb or dough differ significantly ($p < 0.05$). nd: not detected.

Table 5.12: Flavonols and anthocyanin contents of HACS and CGEP substituted bread samples.

	GA1			GA2			GA3		
	Crust	Crumb	Dough	Crust	Crumb	Dough	Crust	Crumb	Dough
Quercetin-3-β-glucoside	1.59 ax	1.15 ay	1.08 ay	1.30 ax	1.06 ax	1.10 ax	1.41 ax	1.13 ax	0.89 ax
Rutin	52.58 ax	57.23 ax	57.27 ax	53.16 ax	60.89 ax	57.10 ax	54.33 ay	60.47 ax	57.06 axy
Myricetin	3.35 ax	2.65 ax	3.05 ax	3.00 ax	3.15 ax	3.08 ax	2.40 ax	1.99 ax	3.14 ax
Quercetin	4.04 ax	4.12 ax	3.88 ax	4.05 ax	3.62 ax	3.78 ax	3.78 ax	3.59 ax	3.20 ax
Delphinidin-3-o-glucoside	1.60 az	2.33 ay	3.08 ax	1.52 az	2.50 ay	3.54 ax	1.81 az	2.52 ay	3.65 ax
Cyanidin-3-o-glucoside	0.58 ax	0.95 ax	1.14 ax	0.74 ay	1.03 axy	1.19 ax	0.69 ay	1.02 ax	1.04 ax
Cyanidin-3,5-diglucoside	1.03 az	1.65 ay	2.27 ax	1.22 az	1.71 ay	2.53 ax	0.90 ay	1.98 ax	2.25 ax
Delphinidin-3,5-diglucoside	0.33 ay	0.41 axy	0.63 ax	0.34 ax	0.53 ax	0.80 ax	0.40 ay	0.55 ay	0.91 ax
Delphinidin	0.61 ay	0.80 axy	1.18 ax	0.78 ay	0.76 ay	1.36 ax	0.87 ay	0.73 ay	1.38 ax
Cyanidin	0.58 ay	0.94 axy	1.20 ax	nd	nd	1.52 ax	0.55 ay	1.04 ax	1.96 ax
Malvidin	0.12 cy	0.18 bxy	0.47 abx	0.69 bx	0.80 ax	0.36 by	0.94 ax	0.68 ax	1.30 ax
Malvidin-3-o-glucoside	5.12 ay	7.07 ay	11.86 ax	4.15 ay	5.83 ay	12.64 ax	6.81 az	7.84 ay	13.34 ax

Results are given as $\mu\text{g}/100 \text{ g DW}$. Data given is the mean value $\pm$ standard deviation of triplicate analyses. Values with different letters (a–e) of different bread samples at each fraction and different letters (x–z) within the bread sample among crust, crumb or dough differ significantly ($p < 0.05$). nd: not detected.

A1: bread flour control; A2: bread flour substituted with 10% HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

6. RESULTS AND DISCUSSION ON CGEP ADDED EXTRUDATES

6.1 Effect of Protein on CGEP Added Extrudate Quality

6.1.1 Introduction

Extrusion has become a widely used operation for processing increasing numbers of food products over the years. However, it is still considered a complicated procedure with “multiple input-output” variables. During the process, protein and starch interact with each other under high temperature and pressure conditions within the barrel. Protein and starch available in cereal products are thermo-mechanically transformed into new food products in the extruder barrel with a number of textural advantages, such as increased expansion and crispiness, in addition to extrusion’s economic advantages of high productivity, low operating costs, and energy and time savings (Brennan et al., 2011).

In recent years, increased consumer interest in functional foods, especially ones rich in antioxidants, has driven extrusion research in how to more effectively incorporate functional ingredients into extrudate formulations. Fruits are rich in antioxidants, most of which are present in fruits as phenolic compounds. Grapes are good sources of these compounds, particularly anthocyanins.

Fruits have been added into extrudate formulations as fresh, pomace or powder. Application of fruit powders in extrusion feed is an easy way of incorporating fruit into extrusion processing, though only a small number of reports have focused on powder substitution (Camire et al., 2007; Potter et al., 2013). Fruit powder substitution could provide potential health benefits in terms of antioxidant activity and anthocyanin content in extruded samples, and substitution level could be increased to yield more significant health benefits (Camire et al., 2007). Published studies have usually been limited to either determination of phenolic compounds and antioxidant activity or evaluation of basic physical quality parameters of extrudates (Camire et al., 2007; Stojceska et al., 2009; White et al., 2010). Some studies have

mainly focused on the effects of extrusion conditions on the properties and quality of functional extrudates (Altan et al., 2009; Karkle et al., 2012; Yağcı and Göğüş, 2009; Yağcı and Göğüş, 2008). These studies investigated considerable increases in substitution levels of health-promoting substances; however the combined effects of adding phenolic compounds/ingredients and basic cereal components, particularly proteins, were not evaluated.

Behaviors of cereal proteins under various extrusion conditions warrants further research as generally proteins are highly affected by processing conditions. Temperature may be considered one of the most important factors as high temperature has irreversible effects on proteins, including protein unfolding and loss of functionality, as a result of aggregation and/or covalent bond breaking (Leon et al., 2003). In contrast, the effect of high pressure on protein denaturation is different as it leaves some parts of the molecule unchanged (Knorr et al., 2006). However, as proteins are exposed simultaneously to multiple extreme conditions, such as high temperature, high pressure, and mechanical shear during extrusion, changes occur in proteins, including conformational disassembling and reassembling through different interactions of disulfide, hydrogen and non-covalent bonds. This results in extrusion products that are completely different in comparison to their raw materials. Protein based sources (legumes and soy products as blends with cereals, being the most common) were generally added into extruded products to help nutritional balance (Bhattacharya, 2012).

Starch is another cereal macro molecule having a significant role during extrusion. Moreover, amylose is known to have a role as a protective barrier in terms of retention of fruit anthocyanins during extrusion, as suggested in a previous study (White et al., 2010). Also the former study by our group (Tacer Caba et al., 2014) investigated the effect of amylose on the quality of extrudate pertaining CGEP as the anthocyanin source. However, for the effect of proteins no such information is available.

Therefore, detailed investigation of interactions is required during extrusion. Proteins are known for their ability to react with other food components, including polyphenols, which lead to changes in structural, functional and nutritional properties both of the food components. The interactions between proteins and polyphenols during extrusion are mostly governed by temperature which can affect hydrogen

bonding and cause the formation of hydrophobic interactions (Ozdal et al., 2013). between health-promoting substances from fruit-based ingredients and basic cereal components, such as protein, and more importantly exploring combined effects on the quality of the extruded products.

The objective of the present study is to investigate the single and combined effects of wheat protein isolate and Concord grape extract powder (CGEP) substitutions on the quality parameters of wheat flour products extruded at various extrusion temperatures.

6.1.2 Specific mechanical energy (SME)

In the present study, SME values measured for all samples ranged between 0.170 and 0.288kW.h/kg (Table 6.1). For samples without CGEP, the decreases in SME due to a rise in temperature were not significant ($p>0.05$). However, in samples with CGEP, SME values were found to be decreasing significantly ($p<0.05$) with increasing extrusion temperature from 90 to 120 °C die temperature.

Specific mechanical energy is a common measure of the work input from the extruder to the extrusion material and is an indicator of the effect of molecular breakdown or degradation and enables a good characterization of the final product (Guerrero et al., 2012). It is considered to be highly related to the extrusion process parameters of screw speed, barrel temperature, feed moisture, and feed composition, all of which affect the viscosity and flow of the extrusion melt (Schaich and Rebello 1999).

The general decreasing trend of SME values with increasing extrusion temperature was similar to that noted in previous studies (Fischer, 2004). When samples at each extrusion temperature were evaluated, it was noticed that among samples without CGEP, SME values were significantly different only for 150°C extrudates. A significant decreasing trend detected for samples up to a certain level of WPI substitution (from 0% to 6.5%) was similar to that of Zhu et al. (2010) who found that change in the level of protein addition from 0% to 10% resulted in a sharp decrease in extrusion SME by means of viscosity decrease, although further increases in protein made almost no more significant changes in SME in their study. For the samples with CGEP, SME values were significantly different only among 120°C and 150°C samples. Other researchers have reported a decreasing trend or

even no differences in SME values (Camire et al., 2007) for corn flour extrudates substituted with fruit powder (Potter et al., 2013) or apple pomace (Karkle et al., 2012), similar to the results in the present study obtained for 150°C samples (P1 vs. GP1). The significant decrease in SME values between P1 and GP1 samples extruded at 150°C could be related to the lubricative roles played by sugar and fiber present in the fruit ingredients (Potter et al., 2013) which is CGEP in the present study. Another reason for this significant change in SME values among samples extruded at 150°C with CGEP could be related to WPI substitution in the formulations, since the decreasing effect of CGEP (observed in samples P1 vs. GP1) which was not statistically significant ($p>0.05$) was lost by the addition of WPI (P2 vs. GP2 and P3 vs. GP3) and the differences in SME values between P2 vs. GP2 and between P3 vs. GP3 were not found to be statistically significant ($p>0.05$).

Table 6.1 : Specific mechanical energy of samples extruded at three different temperatures.

Sample	SME (kWh/kg)		
	90°C	120°C	150°C
P1	0.200±0.001ax	0.205±0.000bcx	0.204±0.001ax
P2	0.208±0.007ax	0.188±0.008cx	0.190±0.001bcx
P3	0.226±0.021ax	0.196±0.006bcx	0.182±0.004cx
GP1	0.270±0.001ax	0.245±0.000ay	0.170±0.002dz
GP2	0.288±0.063ax	0.214±0.012by	0.196±0.006aby
GP3	0.223±0.003ax	0.193±0.003bcy	0.188±0.003bcy

Data given is the mean value± standard deviation of duplicate analyses. Values with different letters (a–d) within the same column and different letters (x–z) within the same row differ significantly ($p < 0.05$). P1: bread flour control; P2: bread flour substituted with 6.5% wheat protein isolate (WPI); P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% concord grape extract powder (CGEP); GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

6.1.3 Diametric expansion

Expansion indices increased gradually with increases in exit die temperature however these changes were only significant for WPI-substituted samples (P2, P3, GP2, and GP3) (Table 6.2). Among samples without WPI (P1 and GP1), extrusion temperatures had no significant effects on the extent of extrudate expansion ($p>0.05$). CGEP had also no effect on the expansion ratio; extruded samples with CGEP and their counterparts without CGEP were similar in their expansion characteristics.

Table 6.2 : Physical properties of extrudates produced at three different temperatures.

Sample	Diametric Expansion Ratio			Bulk Density (kg/m ³)			Hardness (N)			Water Absorption (ml water/g sample)		
	T (°C)											
	90	120	150	90	120	150	90	120	150	90	120	150
P1	1.2	1.8	2.4	1282.2	966.5	397.3	0.70	0.91	0.44	1.50	3.79	5.30
	±	±	±	±	±	±	±	±	±	±	±	±
	0.0	0.3	0.1	4.7	196.9	12.8	0.01	0.08	0.12	0.02	0.69	0.00
	ay	axy	abx	ax	ax	bcy	abxy	ax	ay	by	ax	abx
P2	1.2	1.4	2.3	1267.3	1239.4	469.4	0.66	0.94	0.54	1.58	2.71	5.51
	±	±	±	±	±	±	±	±	±	±	±	±
	0.0	0.0	0.0	5.2	4.1	27.6	0.01	0.03	0.07	0.10	0.12	0.51
	az	ay	bcx	ax	ax	ay	aby	ax	ay	bz	bcy	ax
P3	1.3	1.4	2.1	1184.9	1158.8	478.3	0.52	0.71	0.50	1.48	3.47	4.71
	±	±	±	±	±	±	±	±	±	±	±	±
	0.0	0.0	0.1	40.1	27.2	19.8	0.06	0.06	0.09	0.11	0.17	0.11
	ay	ay	dx	bx	ax	ay	bx	bx	ax	bz	aby	abx
GP1	1.2	1.7	2.5	1286.1	1033.4	333.0	0.75	0.99	0.50	2.11	3.50	5.62
	±	±	±	±	±	±	±	±	±	±	±	±
	0.0	0.3	0.0	0.7	279.7	16.6	0.07	0.02	0.04	0.22	0.31	0.35
	ay	axy	ax	ax	axy	cy	ay	ax	az	az	aby	ax
GP2	1.2	1.4	2.4	1249.4	1215.2	387.7	0.61	0.94	0.44	1.65	2.30	4.43
	±	±	±	±	±	±	±	±	±	±	±	±
	0.3	0.0	0.0	11.4	7.4	11.5	0.04	0.01	0.01	0.22	0.04	0.03
	az	ay	bx	ax	ax	cy	aby	ax	az	bz	cy	bx
GP3	1.2	1.3	2.2	1242.2	1225.2	456.8	0.59	0.91	0.44	1.36	2.42	5.43
	±	±	±	±	±	±	±	±	±	±	±	±
	0.0	0.0	0.0	0.2	4.6	2.3	0.07	0.01	0.04	0.04	0.6	0.14
	az	ay	cdx	ax	ax	aby	aby	ax	ay	bz	cy	ax

Data given is the mean value± standard deviation of duplicate analyses. Values with different letters (a–d) within the same column and different letters (x–z) within the same row differ significantly ($p < 0.05$).

P1: bread flour control; P2: bread flour substituted with 6.5% wheat WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

Diametric expansion indices of the samples increased gradually with increases in exit die temperature. This trend was in consistent with previous literature findings (Chang and Ng, 2011). Extrudate expansion was expected to decrease with the increase in protein levels in feed materials (Devi et al., 2013; Ding et al., 2006; Linko et al., 1981), however in this study, this decrease was observed only in samples extruded at 150°C die temperature. Decreases were generally related with the drop in the starch content in feed materials after protein levels were increased. On the other hand, although being statistically insignificant, a reduction at 6.5% WPI substitution followed by an increase at 13% WPI substitution was more remarkable. Similar trend was also detected by Faubion and Hoseney (1982) who noted a decrease in diametric expansion as gluten in extrudates was increased to 11%, followed by an increase in expansion as the protein content increased to 16% (Faubion and Hoseney, 1982).

6.1.4 Bulk density

Die temperature was one of the main factors affecting bulk density of extrudates (Table 6.2). For each formulation, bulk density decreased with increasing die temperature, showing a statistically significant drop at 150°C. Neither WPI- nor CGEP-substitution levels had significant effects on bulk density results, with the only exception for samples with CGEP which are extruded at 150°C, for which increase in protein levels significantly increased the extrudate bulk density ($p < 0.05$).

The decrease in bulk density with increasing die temperature was statistically significant at 150°C. This condition was due to the fact that during extrusion, by the increase in temperature the degree of superheated water increases, which leads to more bubble formation, decreased melt viscosity, thereby lower bulk density (Ding et al., 2006; Fletcher et al., 1985;). Neither WPI- nor CGEP-substitution levels had significant effects on bulk density results, with the only exception for samples with CGEP which are extruded at 150°C, for which increase in protein levels significantly increased the extrudate bulk density ($p < 0.05$). Increase in protein and/or fiber added to the starchy products was reported to cause the rupture of cell walls, thus related with the prevention of air bubbles from expanding and decrease in the bulk density previously (Bisharat et al., 2013).

6.1.5 Textural properties

Statistically significant changes in hardness were detected for samples with CGEP extruded at different die temperatures ($p < 0.05$). When the die temperature increased from 90°C to 120°C, extrudate hardness values for all samples increased; however from 120°C to 150°C hardness values decreased (Table 6.2). All extrudates at 150°C were less hard than their respective counterpart samples produced at lower extrusion die temperatures ($p < 0.05$).

Temperature was the main parameter to affect the hardness of the extrudates. Similar to the findings by Ding et al. (2006), the decrease in sample hardness with increase in extrusion temperature from 120°C to 150°C might be related to the lower melt viscosity that gave rise to bubble growth, increase in expansion ratio ($r = -0.512$, $p < 0.01$) and decrease in bulk density ($r = -0.961$, $p < 0.01$) yielding softer extruded samples.

In general, increases in protein content of samples were associated with decreases in hardness values of the extrudates in the present study. Increase in protein content is considered as a weakening factor for the mechanical properties of extruded corn products (Chanvrier et al., 2007). Previous studies linked this weakening and increased brittleness effect to changes in the microstructures of the extrudate, probably resulting from shear effects of the extrusion process on the protein network; as protein content is increased in the formulation, extrudate texture is much more affected by such shearing (Chanvrier et al., 2007; Chaunier et al., 2007). Addition of CGEP seemed to prevent the softening effect exerted by 13% WPI at 120 °C and at 150°C, as seen from the results of GP3 sample in the present study (Table 6.2). This might be related to the previously determined extrudate-hardening effect of fruit-based ingredients which are rich in fiber and sugar (Potter et al., 2013).

6.1.6 Water absorption

Water absorption values of extrudates were highly affected by temperature and increased by the increase in extrusion temperature for each sample tested (Table 6.2). These findings are in agreement with previous findings in the literature (Yağcı and Göğüş 2008). Water absorption of extrudates decreased with the increasing WPI substitution level, especially from 0% to 6.5% WPI substitution, only in samples with CGEP.

Water absorption of extrudates is mostly governed by the starch. Therefore it is widely used as an indirect measurement of starch gelatinization (Anderson et al., 1969). Only for samples with CGEP, water absorption of extrudates generally decreased with the increasing WPI substitution level, especially from 0% to 6.5% WPI substitution. Increase in the formation of gluten network by means of higher WPI substitutions can be related to lower water absorption values. Previous studies revealed that gluten formation restricted starch gelatinization and by this means decreased the level of water absorption since they are concurrently occurring changes during extrusion (Ding et al., 2006). However, from 6.5% to the 13% WPI substitution level, similar trend was not observed neither among the samples without CGEP nor among the samples with CGEP. Similarly, non-specific trends were reported by previous researchers, when comparing the effect of adding 0% to 20%

levels of soy protein concentrate to high amylose corn starch during extrusion (Zhu et al., 2010).

6.1.7 Color

Color parameters such as darkness/lightness (L^* values), redness (a^* values), and yellowness (b^* values) were compared among the formulations within each extrusion barrel temperature and among the barrel temperatures within each formulation (Table 6.3). Samples with CGEP were found to be significantly different from samples without CGEP, in all three color parameters measured ($p < 0.05$). When each parameter was evaluated individually for samples without CGEP produced at each temperature, it was observed that changes in color parameters among different formulations were generally not significant among samples ($p > 0.05$), indicating that protein substitution level did not seem to affect color parameter. However, for samples with CGEP, yellowness (b^* values) was found to decrease, whereas darkness (lower L^* values) and redness (a^* values) increased significantly ($p < 0.05$) when compared to values for the counterpart samples without CGEP. On the other hand, for samples with CGEP, increases in protein substitution levels increased lightness (higher L^* value) of extrudates while not effecting a^* and b^* values (except for a values for samples extruded at 90°C).

When the effects of different extrusion temperatures are evaluated, samples without CGEP and extruded at 150°C were found to have higher darkness, redness and yellowness than all other extrudates without CGEP obtained at lower temperatures ($p < 0.05$). However, for samples with CGEP and extruded at 150°C , only yellowness was higher ($p < 0.05$) whereas darkness and redness were not different than values of all other CGEP-containing samples extruded at other temperatures ($p > 0.05$).

Camire et al. (2007) substituted white corn meal with 1% dehydrated Concord grape powder to make extrusion at 163°C , and reported lower darkness and redness values than those found in the present study, possibly because the CGEP inclusion in their study was lower (1% w/w) than in the present study. Their yellowness values were found to be similar to those found for samples without CGEP in the present study, likely because of lower amount of CGEP substitution.

Table 6.3 : Hunter L*a*b* color measurements of flour samples extruded at three different temperatures.

	L*			a*			b*		
	Extrusion Temperature								
	90°C	120°C	150°C	90°C	120°C	150°C	90°C	120°C	150°C
P1	84.58± 0.26ax	84.35± 0.78xax	81.70± 0.04ay	-0.05± 0.01dz	0.22± 0.01by	0.62± 0.07bx	11.3± 0.12ay	11.48± 0.29 axy	12.4± 0.25 ax
	85.55± 0.07ax	82.09± 1.37axy	81.16± 0.00ay	-0.05± 0.01dy	0.46± 0.13bx	0.52± 0.21bx	10.1± 0.18bx	10.97± 0.68ax	11.9± 0.05 ax
	85.35± 0.16ax	83.23± 0.06ay	81.54± 0.45az	-0.03± 0.00dy	0.27± 0.03bx	0.41± 0.09bx	10.5± 0.07by	11.48± 0.40 axy	12.3± 0.57 ax
GP1	45.44± 2.09cx	46.23± 1.35cx	45.34± 1.54cx	5.94± 0.13axy	5.62± 0.00ay	6.48± 0.29ax	0.45± 0.05cy	0.57± 0.01by	1.82± 0.20 bx
	49.13± 0.56bx	48.33± 0.52bcx	48.50± 2.56bx	5.50± 0.03bx	5.62± 0.30ax	6.32± 0.78ax	0.32± 0.15cy	0.75± 0.12 bxy	2.05± 0.99 bx
	51.09± 0.27bx	49.47± 0.18bx	49.67± 1.40bx	5.12± 0.10cx	5.55± 0.03ax	5.85± 0.52ax	0.14± 0.00cy	0.80± 0.06by	1.68± 0.25 bx

Data given is the mean value± standard deviation of duplicate analyses. Among each of L*, a*, and b*, values with different letters (a–d) within the same column and different letters (x–z) within the same row differ significantly (p < 0.05).

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

6.1.8 Scanning electron microscope (SEM) images

Micrographs of some ingredients and extruded samples are presented in Figure 6.1. When SEM images of ingredients are evaluated, dense protein matrix was seen in WPI, whereas for CGEP more ordered crystal-like structure was observed. In flour blends; starch granules, protein matrix and protein adhesion to starch granules were noticed. For flour samples containing WPI (P3 and GP3), a more dense protein matrix in which all other components were embedded was a characteristic micro-structure.

In the microstructure of extruded samples evaluated by SEM, larger particles observed in the images might be related to swelling and gelatinization of starch granules during extrusion. For samples extruded at the highest die temperature (150°C), the voids observed between particles might be the reason for lower hardness and lower bulk density of those extruded samples. For extruded samples with CGEP, CGEP structure was still detectable after extrusion, indicating that the applied extrusion conditions did not completely alter the CGEP structure.

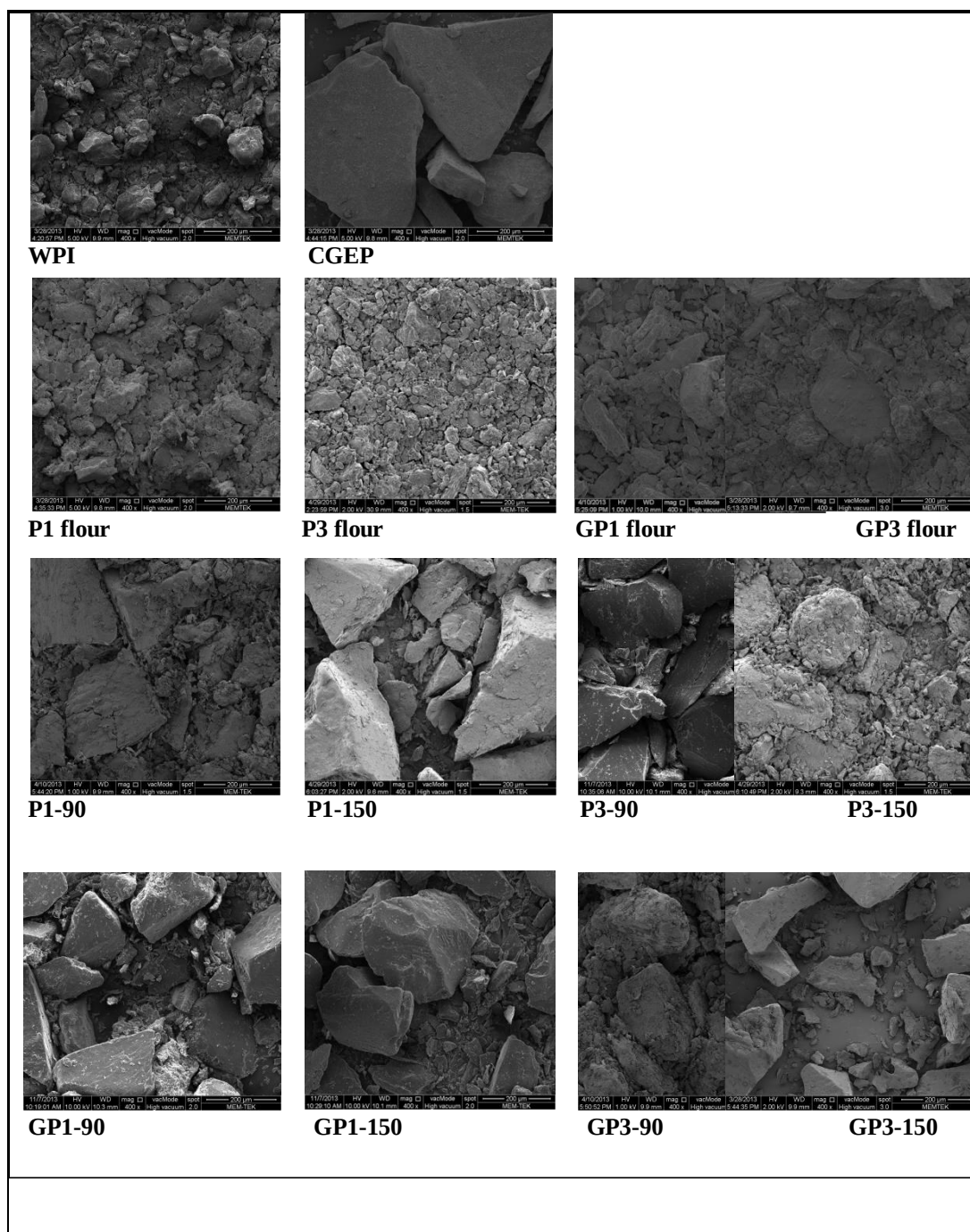


Figure 6.1 : SEM images of extruded samples with WPI and CGEP substitutions.

6.1.9 Pasting properties

Main pasting parameters of both non-extruded and extruded samples were evaluated (Table 6.4). The peak viscosity and final viscosity values of non-extruded samples were generally at least two-fold of the values for respective counterpart extruded samples. Peak viscosity and final viscosity values of non-extruded samples both of with CGEP (GP1, GP2, and GP3) and without CGEP (P1, P2, and P3) were found to

decrease significantly as the level of protein substitution increased. The lowest peak and final viscosity values among all were measured by samples extruded at 150°C. They were significantly lower than the values obtained for samples extruded at the lower die temperatures 120°C and 90°C ($p < 0.05$). The level of protein substitution had a significant effect on final viscosity values of samples without CGEP ($p < 0.05$). For all samples, except for GP1, pasting temperatures were significantly lower for samples extruded at 150°C (around 50°C difference) with respect to those extruded at 90°C or 120°C (around 80-90°C difference). Within each extrusion temperature studied, neither the level of protein substitution nor the CGEP substitution made significant differences ($p > 0.05$) among extruded samples (except for GP1 at 120°C).

According to the pasting parameters, the peak viscosity and final viscosity values of non-extruded native flour samples were generally at least two-fold of the values for respective counterpart extruded samples. This finding was consistent with the previous findings and might be related to the shear forces applied during extrusion which changed the structure of samples. Zaidul et al. (2007) reported that the lower final viscosity values of extruded samples were caused by the disruption of starch granules during extrusion.

At the extrusion temperature of 150°C, peak viscosities of all extruded samples were significantly lower than those of samples extruded at the other two die temperatures (120 and 90°C). This is probably because the extent of disruption of starch granules was greater, with rapid swelling at 150°C relative to lower temperatures (Chaunier et al., 2007). When the effect of CGEP presence was evaluated; peak viscosities of samples with CGEP were significantly lower (about 30%) than their counterpart samples without CGEP at each temperature studied. This might be both related to the weakening of protein network and retarding of paste formation in samples (Chaunier et al., 2007; Núñez et al., 2009) which may be attributed to the hygroscopic behavior of CGEP in formulations. However, the level of protein substitution for samples with CGEP did not have a significant reducing effect as it had for samples without CGEP.

Similar to the present results, gradual increase in protein levels in the formulations is considered as a weakening factor for the gel (Joshi et al., 2014). Each sample without CGEP was significantly higher than its counterpart sample with CGEP, both in peak and final viscosity measurements. This was expected since the starch proportion was lower in samples with CGEP.

Final viscosity values for all samples extruded at 150°C were significantly lower than the values obtained for samples extruded at the lower die temperatures 120°C and 90°C ($p < 0.05$). Moreover, for samples with CGEP, final viscosity values were about 35% lower than those of the counterpart samples without CGEP. The extent of reduction might be related to both the decreased amount of starch in samples with CGEP and also to the existence of starch-phenol complexes inhibiting the regular alignment of polymer chains which can affect pasting properties (Beta and Corke, 2004).

Decrease in final viscosity values with the increase in WPI levels might be associated with the formation of protein and starch surface complexes (Zaidul et al., 2007). In contrast, final viscosity values of samples with CGEP were not affected significantly by changes in WPI substitution level ($p > 0.05$).

6.1.10 Thermal properties

The gelatinization onset temperatures (T_o), peak temperatures (T_p) and enthalpies (ΔH) for extruded samples were found to be in the ranges of 60.74-69.36°C, 65.56-74.28°C and 0.02-6.09 (J/g), respectively (Table 6.5). T_o values for gelatinization increased significantly ($p < 0.05$) both in samples with and without CGEP as the die temperature was raised from 90 to 120°C. For samples extruded at 150°C, no gelatinization and retrogradation peaks were observed, indicating that all samples were fully gelatinized during the extrusion process at that temperature. Samples with 13% WPI (both with and without CGEP) extruded at 120°C were also found to be fully gelatinized. No significant changes were observed in T_p values of gelatinization with an increase in extrusion temperature from 90°C to 120°C for the wheat flour only (P1) and wheat flour with CGEP (GP1) samples. However, for extrudates containing 6.5% WPI, both with and without CGEP, T_p values increased significantly for the same die temperatures mentioned.

Table 6.4 : RVA pasting parameters of flour samples and their extrudates produced a three different temperatures.

Sample	Non-Extruded Samples						Extruded Samples					
				<u>Peak Viscosity</u>			<u>Final Viscosity</u>			<u>Pasting Temperature (°C)</u>		
	<u>Peak Viscosity</u>	<u>Final Viscosity</u>	<u>Pasting T(°C)</u>	<u>90°C</u>	<u>120°C</u>	<u>150°C</u>	<u>90°C</u>	<u>120°C</u>	<u>150°C</u>	<u>90°C</u>	<u>120°C</u>	<u>150°C</u>
P1	2558.8	2923.5	68.0	1378.8	1275.5	1108.2	1991.3	1560.0	888.2	91.34	88.5	50.14
	±55.7	±53.5	±0.2	±3.2	±66.5	±40.8	±42.8	±93.0	±9.7	±0.81	±0.35	±0.09
	a	a	b	ax	ax	ay	ax	ay	az	ax	ay	az
P2	2201.8	2481.0	68.8	1111.2	1278.0	932.3	1639.1	1654.6	766.0	90.95	89.65	50.28
	±74.8	±55.0	±0.6	±29.3	±60.5	±26.3	±27.0	±3.0	±11.5	±0.77	±1.6	±0.0
	b	b	b	bxy	ax	by	bx	ax	by	ax	ax	ay
P3	1835.0	2092.0	68.6	1084.6	1059.7	653.52	1548.7	1347.2	572.3	90.94	90.06	50.18
	±19.0	±15.0	±0.0	±9.5	±50.3	±11.5	±17.3	±5.8	±64.3	±0.01	±0.01	±0.08
	d	c	b	bx	bx	cy	bx	by	cz	ax	ax	ay
GP1	2014.0	2216.0	76.7	890.3	1088.5	516.0±	1227.0	1103.0	343.8	80.76	62.86	50.13
	±22.5	±12.0	±7.3	±95.3	±99.0	50.0	±54.5	±107.5	±14.3	±9.29	±12.66	±0.03
	c	c	ab	cxy	abx	dy	cx	cdx	dy	ax	bx	ax
GP2	1550.3	1676.5	84.6	789.5	955.8	603.2	1009.8	1266.0	430.8	89.46	91.74	50.15
	±26.8	±34.5	±1.8	±33.5	±50.2	±48.7	±36.3	±49.0	±48.3	±0.99	±0.41	±0.0
	e	d	a	cxy	bcx	cdy	dy	bcx	dz	ax	ax	ay
GP3	1403.7	1544.3	84.4	796.0	787.0	478.6	1049.0	1019.7	344.7	91.46	91.94	50.21
	±22.2	±18.8	±0.4	±23.0	±8.0	±18.5	±18.0	±2.7	±10.3	±0.96	±0.56	±0.14
	e	e	a	cx	cx	dy	dx	dx	dy	ax	ax	ay

Data given is the mean value± standard deviation of triplicate analyses. Values with different letters (a–e) within the same column and different letters (x–z) within the same row differ significantly (p < 0.05).

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

The presence of CGEP in flour (GP1) increased the onset (T_o) and peak temperatures of gelatinization (T_p) of extrudates in comparison to the counterpart extruded samples without CGEP (P1). This effect with CGEP substitution was evident at both die temperatures of 90°C and 120°C. Samples with WPI, on the other hand, behaved differently at the two different die temperatures. At 90°C, there were no significant differences ($p < 0.05$) among the T_o values or the T_p values of samples without CGEP (with WPI, P2 and P3) and the P1 samples (no WPI, no CGEP). Moreover, the shifting effect of CGEP to higher temperatures, detected in GP1 vs. P1 samples, became less pronounced by WPI substitution at samples with CGEP and WPI (GP2 and GP3).

For each formulation studied, the increase in extrusion die temperature from 90° to 120°C significantly decreased the measured enthalpy values ($p < 0.05$). Samples with CGEP had significantly lower enthalpy values than their counterpart samples without CGEP for extrudates produced at 90°C.

Onset and peak temperatures of retrogradation, for all samples produced at 90°C and 120°C, were lower than all T_o and T_p values of gelatinization (Table 6.5). There were no significant changes in T_o or T_p of retrogradation for any of the extrudates with the increase in die temperature from 90°C to 120°C, except for the T_o value of GP1.

According to the thermal measurements made with samples it was obvious that WPI substitution had no role on the onset of gelatinization for samples without CGEP which are extruded at 90°C. There are some contradictory findings in the literature on the effect of proteins on starch gelatinization. The present findings at 90°C were not in agreement with the general increasing effect of protein on gelatinization T_o and T_p , as reported previously (Eliasson, 1983; Mohamed and Duarte, 2003), but were in accordance with the findings of a study that found no effect of gluten on gelatinization (Erdogdu et al., 1995). However, on samples with CGEP, it displayed a normalizing effect, by preventing the retarding effect of CGEP. For samples extruded at 120°C, on the other hand, the increasing effect of WPI on T_o and T_p values was significant ($p < 0.05$). This difference was believed to be a result of much decreased levels of available water at the 120°C when compared to 90°C in the presence of WPI or a result of migration of water from starch to proteins, similar to

the findings of previous studies (Eliasson and Hegg, 1980; Mohamed and Rayas-Duarte, 2003).

Table 6.5 : Thermal properties of extrudates determined by DSC.

	Extrusion Temperatures					
	90°C	120°C	90°C	120°C	90°C	120°C
	Thermal Properties of Gelatinization					
	T _o (°C)		T _p (°C)		ΔH (J/g)	
P1	61.61±0.52 bcy	63.40±0.02 cx	65.75±0.36 bx	66.48±0.04 bx	3.52±0.08 bx	0.02±0.00 cy
P2	62.07±0.98 bcy	69.00±0.09 abx	65.56±0.47 by	72.59±0.56 ax	4.06±0.59 abx	0.08±0.02 cy
P3	60.74±0.71 c	n.d.	65.96±0.16 b	n.d.	6.09±1.44 a	n.d.
GP1	66.89±1.52 ay	69.36±0.43 ax	74.28±3.70 ax	72.92±0.82 ax	1.05±0.41 cx	0.43±0.09 by
GP2	63.59±0.74 by	68.49±0.26 abx	67.00±0.67 by	72.41±1.11 ax	2.58±0.64 bcx	0.90±0.06 ay
GP3	62.41±0.20 bc	n.d.	66.54±0.09 b	n.d.	3.33±1.15 b	n.d.
	Extrusion Temperatures					
	90°C	120°C	90°C	120°C	90°C	120°C
	Thermal Properties of Retrogradation					
	T _o (°C)		T _p (°C)		ΔH (J/g)	
P1	43.01±0.05 cx	43.12±0.37 bx	51.22±0.08 by	53.07±0.09 aby	0.38±0.01 bcx	0.19±0.01 by
P2	47.00±0.05 bx	43.11±0.31 bx	54.82±0.05 abx	55.75±1.40 abx	0.10±0.01 dy	0.53±0.00 ax
P3	47.26±2.08 ab	n.d.	55.08±1.10 ab	n.d.	0.49±0.01 b	n.d.
GP1	47.42±0.76 abx	41.78±0.85 by	53.70±0.30 abx	50.43±0.23 bx	0.25±0.08 cdy	0.45±0.02 abx
GP2	51.22±1.27 ax	43.37±0.86 ax	57.65±0.53 ax	56.09±0.7 ax	0.27±0.13 cdx	0.36±0.22 ax
GP3	42.88±1.85 c	n.d.	56.22±1.31 a	n.d.	0.81±0.07 a	n.d.

Data given is the mean value± standard deviation of triplicate analyses. Values with different letters (a–d) within the same column and different letters (x–y) within the same row differ significantly ($p < 0.05$). To: onset temperature, Tp: peak temperature, ΔH: enthalpy of gelatinization, n.d.: not detected.

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

The findings on the enthalpy decreases ($p < 0.05$) with increasing temperatures and presence of CGEP were similar to the findings of Zhu et al. (2009), who related phenolic compounds with the smaller enthalpies of gelatinization in wheat starches. However, for samples extruded at 120°C, the effect of CGEP was opposite to that at 90°C, and gelatinization enthalpies increased significantly ($p < 0.05$) in the presence of CGEP. Increase in gelatinization enthalpies for WPI-substituted samples (both in samples with and without CGEP) was found insignificant ($p > 0.05$) in present findings. It was previously shown that WPI had a decreasing effect on starch

gelatinization enthalpies (Yağcı and Göğüş, 2008). Moreover, for all parameters of retrogradation T_o , T_p and enthalpy, neither the change in WPI level nor the presence of CGEP had distinct effects ($p>0.05$). Mildner-Szkudlarz Ottenhof and Farhat (2004) revealed that gluten addition (9%) to freshly extruded wheat starch (with 34% water content) did not have any influence on the retrogradation enthalpy of the original sample. But in the present study, 13% WPI added sample with CGEP (GP3) had a significantly higher retrogradation enthalpy than its counterpart sample without CGEP (P3) extruded at 90°C (0.81 vs. 0.49 J/g, respectively). It was reported previously that polyphenols were effective in preventing the retrogradation of starch (Tacer Caba et al., 2014; Wu et al., 2009). However, this effect was not observed for CGEP in the present study. CGEP and CGEP with WPI retarded retrogradation to higher temperatures up to high levels (13%) in comparison to samples without CGEP. Different types of polyphenols were reported to have different effects on thermal properties as declared by other researchers (Zhu et al., 2009).

6.2 Effect of Amylose on CGEP Added Extrudate Quality

6.2.1 Introduction

The aim of this chapter is to investigate any effects related to anthocyanins, amylose and their combinations, on the quality parameters of extrudates produced at various extrusion temperatures.

6.2.2 Specific mechanical energy (SME) and die pressure

SME is the mechanical energy required to rotate the extruder screws together with the viscous melt (Colonna et al., 1989). It is an important indicator of the mechanical properties of extrusion products since the expansion, density and water absorption properties of the extrudates are related to SME.

The average SME was measured as 0.235 kWh/kg for all treatments. Generally SME had a decreasing trend as the extrusion temperature increased for GA-coded samples. This decline was observed by other researches as well (Ryu and Ng, 2001; Chanvrier et al., 2007). It was assumed that the decrease was due to the lower friction of the extrusion melt at high temperatures (Colonna et al., 1989). Generally, the SME required to extrude anthocyanin-substituted GA samples (at 90°C and 120°C) were

higher when compared to the counterpart A samples. This finding is in contrast with some previous studies that found lower SME when apple pomace was incorporated into corn flour (Karkle et al., 2012). However, as reported by Chanvrier et al. (2007), the lower SME findings might be related to the lubricative role of the higher amounts of proteins in A samples. Since the substitution in A samples included only high amylose starch, but not CGEP, the relative amount of protein in those samples was higher than in GA samples.

Extrusion temperature is the main factor affecting extruder die pressure. In this study, samples with CGEP substitution generally displayed a decrease in die pressure compared with no-CGEP-added C samples. This decrease became more evident as the extrusion temperature increased and became nearly two-fold for A1 and A2 when compared with their counterpart GA1 and GA2 samples. However changes in amylose substitution levels did not result in considerable changes in die pressures of samples.

As shown in Table 6.6, the correlation between SME and die pressure was found to be low ($r=0.203$). It has been documented by Ryu and Ng (2001) that the viscosity of the melt is high at low extrusion temperatures. Moreover, low level of starch gelatinization at low temperatures was proposed as another reason for the increase in melt viscosity and subsequent increase in die pressure (Karkle et al., 2012).

Table 6.6 : Pearson's correlation matrix for extrudate parameters.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1Water Absorption	1.000																		
2Diametric Expansion	0.896**	1.000																	
3Bulk Density	-0.867**	-0.958**	1.000																
4Hardness	-0.410*	-0.473**	0.653**	1.000															
5Diametric Pressure	-0.578**	-0.663**	0.731**	0.627**	1.000														
6SME	-0.245	-0.082	0.263	0.616**	0.203	1.000													
7Peak Viscosity	-0.315	-0.466**	0.330*	0.009	0.432**	-0.427**	1.000												
8Final Viscosity	-0.637**	-0.728**	0.604**	0.108	0.565**	-0.351*	0.875**	1.000											
9Setback Viscosity	-0.681**	-0.767**	0.658**	0.139	0.581**	-0.270	0.857**	0.976**	1.000										
10Peak time	-0.704**	-0.843**	0.844**	0.473**	0.735**	0.064	0.603**	0.745**	0.779**	1.000									
11Pasting Temp	-0.758**	-0.761**	0.728**	0.262	0.631**	-0.038	0.365*	0.685**	0.697**	0.731**	1.000								
12To Gel	-0.758**	-0.886**	0.922**	0.697**	0.732**	0.266	0.428**	0.599**	0.656**	0.868**	0.708**	1.000							
13Tp Gel	-0.750**	-0.882**	0.918**	0.698**	0.737**	0.262	0.428**	0.594**	0.648**	0.869**	0.712**	0.998**	1.000						
14 ΔH Gel	-0.624**	-0.613**	0.561**	0.133	0.289	-0.122	0.256	0.460**	0.461**	0.550**	0.470**	0.427**	0.426**	1.000					
15Moisture Content	-0.564**	-0.640**	0.761**	0.707**	0.523**	0.540**	0.026	0.182	0.290	0.562**	0.351*	0.766**	0.756**	0.169	1.000				
16Resistant Starch	-0.030	0.100	-0.040	0.159	-0.173	0.391*	-0.575**	-0.491**	-0.510**	-0.238	-0.129	-0.113	-0.107	0.306	-0.066	1.000			
17To Retro	-0.027	0.105	-0.130	-0.136	-0.175	0.116	-0.045	-0.071	-0.040	-0.057	-0.116	-0.221	-0.217	0.200	-0.132	0.080	1.000		
18Tp Retro	-0.014	0.100	-0.095	-0.088	-0.180	0.135	-0.089	-0.125	-0.075	-0.045	-0.220	-0.204	-0.205	0.274	-0.073	0.158	0.891**	1.000	
19 ΔH Retro	-0.135	-0.121	0.150	0.071	-0.045	0.122	-0.215	-0.173	-0.118	0.071	-0.138	0.094	0.085	0.509**	0.118	0.503**	-0.170	0.176	1.000

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

Table 6.7 : Some physical properties of the extrudates.

	Hardness (N)			Water Absorption (ml water/g sample)			Diametric Expansion Ratios			Bulk Density (kg/m ³)		
	90°	120°	150°	90°C	120°C	150°C	90°C	120°C	150°C	90°C	120°C	150°C
A1	0.696±0.005 dx	0.892±0.005 bx	0.447±0.085 ay	1.50±0.02 dy	3.79±0.69 ax	5.30±0.00 ax	1.16±0.01 by	1.78±0.27 bxy	2.45±0.06 ax	1282.0±4.5 abx	769.6±52.6 ay	397.3±12.8 az
A2	0.792±0.016 cdxy	0.897±0.098 bx	0.570±0.027 ay	1.59±0.05 dy	4.54±0.63 ax	5.35±0.14 ax	1.15±0.01 bz	1.93±0.18 aby	2.49±0.07 ax	1275.1±21.8 abx	926.4±68.6 ay	362.9±11.9 abz
A3	0.924±0.093 bcy	1.238±0.010 ax	0.476±0.015 az	1.77±0.02 cdz	4.01±0.10 ay	4.97±0.23 abx	1.42±0.27 abx	1.86±0.27 abx	2.53±0.19 ax	1266.7±11.8 abx	1053.2±105.3 ay	270.8±1.4 dz
GA1	0.746±0.051 dy	0.946±0.018 bx	0.463±0.015 az	2.11±0.22 bcz	3.50±0.31 ay	5.62±0.35 ax	1.25±0.05 aby	1.74±0.30 bxy	2.52±0.01 ax	1286.1±0.7 ax	745.2±51.2 ay	333.0±16.6 bcz
GA2	1.013±0.048 bx	0.906±0.045 bx	0.454±0.094 ay	2.30±0.04 by	4.46±0.12 ax	5.06±0.28 abx	1.43±0.02 aby	2.57±0.12 abx	2.50±0.02 ax	1336.5±14.9 ax	445.7±18.4 by	290.4±7.3 cdz
GA3	1.327±0.010 ax	1.04±0.0450 by	0.393±0.071 az	3.42±0.16 ay	4.13±0.11 ax	4.43±0.10 bx	1.70±0.15 ay	2.64±0.16 ax	2.57±0.01 ax	1165.0±75.0 bx	376.3±35.2 by	212.0±23.0 ey
Ave.	0.916±0.213	0.987±0.123	0.467±0.053	2.12±0.65	4.07±0.36	5.12±0.37	1.35±0.19	2.09±0.37	2.51±0.04	1268.6±51.4	723.6±242.4	311.1±61.2

Different letters (a–d) within the same column, and different letters (x–z) within the same row differ significantly ($p < 0.05$). Data given is the average of duplicate analysis $\pm$ standard deviations.

A1: bread flour control; A2: bread flour substituted with 10% HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

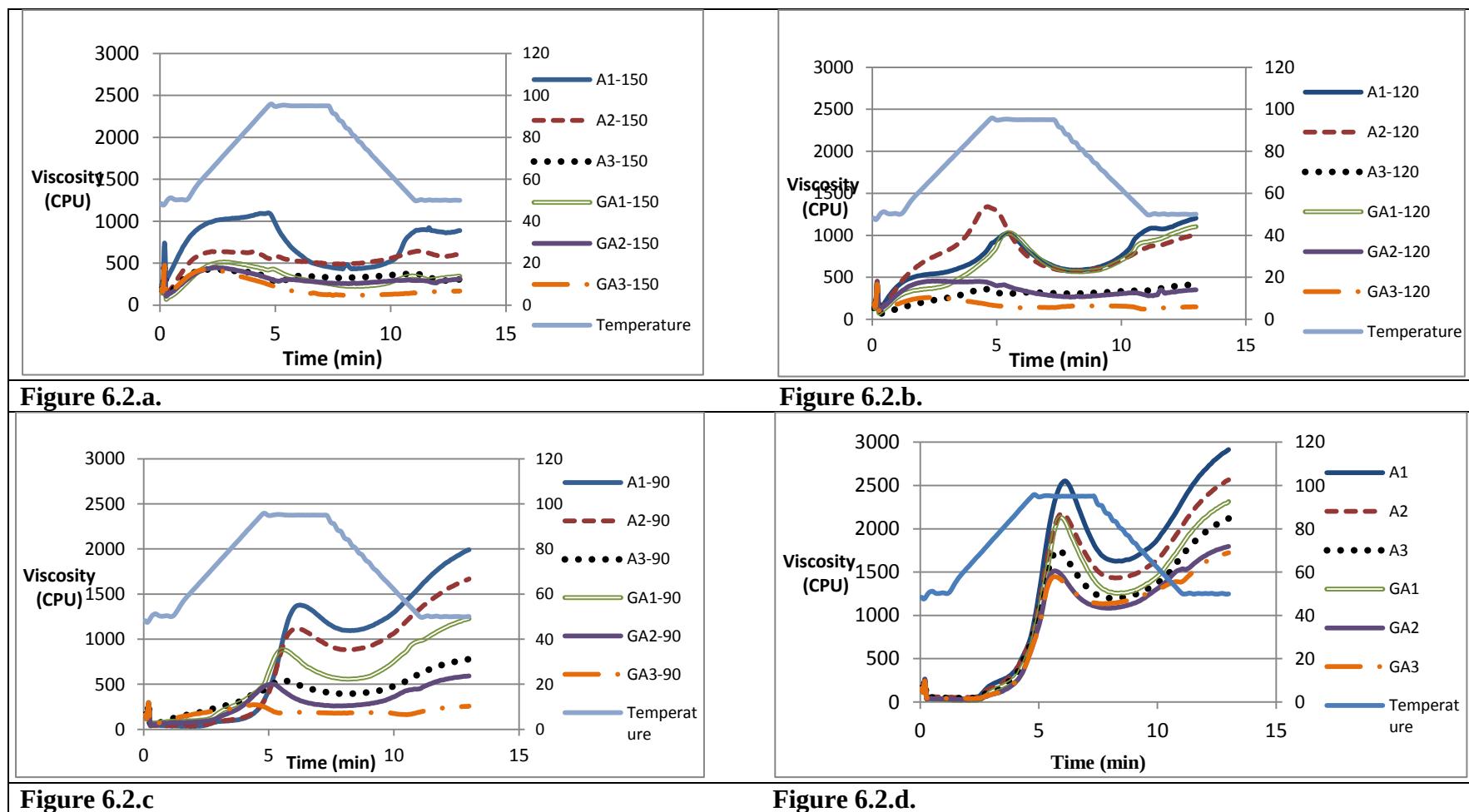


Figure 6.2 : Pasting profiles of HACS and CGEP substituted extrudates and samples before extrusion

6.2.3 Pasting properties of extrudates

Pasting curves of extrudates according to the RVA data are depicted in Figure 6.2 (a, b, c). Peak viscosity of the extrudates differed significantly as the die temperature changed. In particular, samples extruded at 150°C had significantly lower peak viscosity values than the other two temperature treatments ($p < 0.05$). Previous studies revealed that high temperature may be considered as a factor contributing to lower peak viscosity measurements of the extrudates, by accelerating the degradation of starch granules (Mason and Hoskeney, 1986; Ryu and Ng, 2001). This behavior was common in starches from other sources as well. The peak viscosities of extrudate pastes from rice flour were found to decrease with increases in extrusion temperature (Guha et al., 1998).

Moreover, except for samples extruded at 90°C, shapes of the pasting curves were unlike the characteristic shape of the pasting curves of non-extruded samples.

Incorporation of CGEP was found to decrease the peak viscosity of the extrudates significantly. However, the impact of CGEP was not as pronounced as that of temperature. At 90°C, peak viscosities drastically decreased by almost 50%, from 114.9, 93.88 and 53.33 RVU for A1, A2 and A3, respectively, to 74.19, 42.13 and 23 RVU for their counterparts GA1, GA2 and GA3, respectively. This trend was found to be statistically significant ($p < 0.05$). According to Núñez, Sandoval, Müller, Valle and Lourdin. (2009), a drop in peak viscosity might be related to the presence of hygroscopic substances as they decrease the amount of available water. However this effect was less significant at higher extrusion temperatures, possibly because effect of temperature on decreased viscosity was more evident.

Peak viscosity also declined as the level of high amylose starch substitution increased (Figures 6.2 a., 6.2.b., 6.2.c. and 6.2.d.). The peak is hardly observed for GA2 and GA3 samples at 120°C and for none of samples except A1 at 150°C. High amylose content at high barrel temperature is the main cause for this particular case relationship but it can also be related to the possible interactions of amylose with CGEP, since peaks get smaller with CGEP incorporation into the formula. Another reason for lower or even undetectable peaks might be linked to the higher gelatinization temperatures ($>100^{\circ}\text{C}$) of high-amylose starches in the A samples: that

these starches did not gelatinize entirely under the RVA conditions (Jane et al., 1999), since peak viscosity is an indicator for degree of starch gelatinization.

In the present study, amylose-substituted extrudates also displayed lower values for breakdown viscosity. The inverse relationship between amylose content and breakdown viscosity was previously revealed by other researchers as well (Noda et al., 2004). Moreover, as temperature decreases as a part of the RVA temperature regimen, the gel structure will be formed mainly due to amylose and lead to an increase in final viscosity. This part is defined as the set-back region and is attributed to the retrogradation or reordering of amylose (Lei, Ji-Chun, Cai-Ling and Chun 2008). Some researchers reported that the presence of high levels of amylopectin was found to be a factor on lower pasting temperature and set-back viscosity with respect to normal starch (Jane et al., 1999). However, according to the current results obtained, the determining and consequently lowering factor for pasting temperatures was not only the amylose content in the extrudates but, more dominantly, the CGEP addition. Findings of the current study are also opposed to their findings (Jane et al., 1999) that the lower set-back viscosity is mainly due to the low amount of amylose since it is effecting the gel network formation. In the current study, it was found that the set-back viscosity decreased with increases in amylose content. It may be linked to the unique properties of the extrusion process.

6.2.4 Hardness values for extrudates

Generally, hardness results for the samples extruded at 150°C were significantly lower ($p < 0.05$) than those from other extrusion temperature samples (Table 6.7). For all samples, the extrudates became more brittle at higher extrusion temperatures. CGEP substitution at 90°C produced harder extrudates (A samples) when compared to their counterpart C samples. However, no similar effect was detected at higher extrusion temperatures. A negative correlation was detected between the hardness versus diametric expansion and water absorption measurements ($r = -0.473$ and -0.410 , respectively). Other researchers (Robin et al., 2012) found relationships between hardness and some other parameters as well, such as porosity, cell size and cell wall thickness and the density of the final product. Similarly, hardness was significantly correlated with bulk density ($r = 0.653$). Extrusion parameters such as

SME and diametric pressure were also found to be significant factors ($p < 0.01$) affecting hardness ($r = 0.616$ and 0.627).

Increase in amylose content may also become an important factor for the extrudate structure. The combination of a high amount of protein and lower starch content in extrudate formulation may be considered a weakening factor in the cell wall materials which leads to an increase in brittle extrudate structure (Chaunier et al., 2007). However, such weakness was not found to be significant in the present study.

6.2.5 Water absorption values for extrudates

For all samples, water absorption increased significantly with increasing extrusion temperatures (Table 6.7). This is in agreement with a previous report by Yağcı and Göğüş, (2008). Generally, the amount of water absorption was significantly lower ($p < 0.05$) at extrusion temperature of 90°C when compared with the other two temperatures. It was detected that the difference between the degrees of water absorption was more distinct when wheat flour was substituted only with CGEP (GA1). The amylose and CGEP substitution, on the other hand, together gave more similar products particularly at 120 and 150°C . Therefore, amylose had a compensating role for water absorption. Water absorption is an indicator for measuring the volume of water that is absorbed after swelling of starch in excess water. Therefore the degree of starch gelatinization and fragmentation during extrusion may be considered among the main factors affecting the degree of water absorption (Rayas-Duarte et al., 1998). Since the degree of starch damage is greater at higher temperatures, water absorption increases at higher temperatures. It is therefore, considered as an indirect index for porosity of the extrudates (Colonna et al., 1989).

6.2.6 Diametric expansion ratio for extrudates

As given in Table 6.7, each increase in barrel temperature generally had a significant increasing effect on diametric expansion ratio of the extrudates ($p < 0.05$). However, substitution of wheat flour with only high amylose starch (C samples) made no significant change in samples produced. Extrudates substituted with both CGEP and amylose (GA2 and GA3) displayed different characteristics, with highest diametric expansion ratios obtained at 120°C . The A samples expanded more at 90°C when

compared to C samples. The physicochemical aspects of plasticized starch matrix during extrusion, the effects of process conditions and porosity of the material are among the main parameters that determine the expansion mechanism of extruded cereal products (Robin et al., 2011; Robin et al., 2012).

The negative correlation detected between expansion ratio and paste peak viscosity values of the extrudates were considerably high and statistically significant (-0.466, $p < 0.01$), similar to the findings reported by some other researchers (Alvarez-Martinez et al., 1988; Della Valle et al., 1997).

6.2.7 Bulk density of extrudates

Bulk density is an important parameter to evaluate the degree of puffing of extrudates and it is highly related with the expansion ratio (Asare et al., 2004). Temperature was found to be the main factor significantly affecting the bulk density of the extrudates, as given in Table 6.7 ($p < 0.05$). Bulk density was found to decrease as the extrusion temperature increased for all treatments. This effect might be related to the lowering effect of high extrusion temperatures on shear viscosity and also the increase in steam pressure temperature which resulted in further expansion (Chanvrier et al., 2007). Bulk density measurements correlated well with peak viscosity ($r = 0.330$ at $p < 0.05$). CGEP substitution alone (GA1) did not significantly affect the bulk density values at extrusion temperatures of 90 and 120°C; significance was only detected at 150°C. However, interaction of CGEP with amylose further decreased bulk density values of the extrudates to below those of C samples.

6.2.8 Total resistant starch content of extrudates

Resistant starch (RS) is defined as the portion of starch that is not digested in the small intestine but reaches the large intestine intact (Englyst et al., 1992). In extruded samples, resistant starch levels ranged between 0.76 - 4.73% (dry matter basis, Table 6.8). Levels of amylose substitution were the main determining factor for variation in resistant starch measurements. Starch digestibility is lowered by higher amylose content, thus amylose content and resistant starch formation are positively correlated (Singh et al., 2010). Extrusion temperature had no effect on RS contents within each studied sample (Table 4). Previous studies revealed that phenolic and anthocyanin fractions in fruits are important for the digestibility of starch and resistant starch

contents by pertinent inhibitory effects on α -glucosidase and α -amylase enzyme activities (McDougall et al., 2005). The results of the present study revealed an increasing effect on resistant starch formation in the CGEP-substituted samples, with the trend more evident as extrusion temperature was increased.

Table 6.8 : Resistant starch contents of the samples.

Sample	Resistant Starch %, dry matter		
	90	120	150
A1	1.13±0.04dx	0.87±0.07cxy	0.76±0.10ey
A2	3.30±0.16bx	2.70±0.28bxy	2.24±0.15cy
A3	4.73±0.36ax	3.81±0.67ax	3.46±0.39ax
GA1	0.95±0.17dx	0.90±0.09cx	1.11±0.07dx
GA2	2.21±0.19cx	2.24±0.23bx	2.77±0.16bx
GA3	3.54±0.34bx	3.94±0.22ax	3.76±0.18ax

Different letters (a–d) within the same column, and different letters (x–y) within the same row differ significantly ($p < 0.05$). Data given is the average of duplicate analysis $\pm$ standard deviations.

A1: bread flour control; A2: bread flour substituted with HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

At low temperatures, formation of resistant starch was probably more significantly influenced by an increase in amylose level than CGEP substitution level, which was presumably not high enough to inhibit enzymatic activity. However, at 150°C extrusion temperature lower moisture content of the samples may be the reason for a more packed structure of starch, with limited starch available for digestion (Mulinacci et al., 2008). This structure may provide possible inhibitory action of CGEP on digestion enzymes to increase total resistant content. Significant negative correlations for resistant starch formation were obtained for peak viscosity (-0.575, $p < 0.01$), final viscosity (-0.491, $p < 0.01$) and setback viscosity measurements of the samples (-0.510, $p < 0.01$).

6.2.9 Thermal properties of extrudates

Onset temperatures (T_o), peak temperatures (T_p) and endothermic enthalpies (ΔH) for the gelatinization and retrogradation transitions of extrudates by using DSC are given in Table 6.9. At 90°C extrusion temperature, a gelatinization peak was observed for each of the samples. Both T_o and T_p values were significantly different from each other and ranged between 66.89-74.28°C and 51.51-73.20°C, for CGEP-substituted samples and for C samples, respectively. Due to interactions between CGEP and

high amylose starch, a statistically significant shift to higher temperatures was detected in A-coded samples. This alteration might be related with the effect of the hydroxyl groups of CGEP that interact with side chains of amylopectin found in high amylose starch. This interaction possibly changed the coupling forces between the crystallites and amorphous matrix through binding to the amorphous region of starch granules and thus affected the T_o and T_p values (Zhu et al., 2009). Without CGEP addition, their C-coded counterparts had significantly lower T_o and T_p temperatures.

Similar increasing effects on onset and peak temperatures were detected in red colored varieties of potatoes in comparison to white potatoes by other researchers (Mulinacci et al., 2008). The interaction between functional groups such as phenolic acids and amylose and amylopectin in the system through hydrogen bonding and van der Waals forces was proposed as the reason for differences by other researchers (Zhu et al., 2008). Lower thermal energy measured in CGEP-substituted samples could be related to the interaction of hydrophilic OH groups in phenolic compounds in CGEP with amylopectin side-chains which helped the hydration of starch granules by decreasing the coupling forces (Zhu et al., 2009; Wu et al., 2009).

Absence of a peak temperature in some of the samples (represented by “n.d.”, or not shown in Table 6.9) indicated that the starch in the sample was fully gelatinized during extrusion and was in an amorphous state (Robin et al., 2012).

All extruded and stored samples had lower retrogradation temperatures in comparison to extruded samples before storage (Table 6.9). C samples were significantly affected by the increase in amylose level, probably because it was an indirect measurement for the decrease in amylopectin level in the samples. Relative decrease in amylopectin from A1 to A3 delayed the retrogradation to higher temperatures and significantly decreased the retrogradation enthalpies. At higher extrusion temperatures, this effect became less significant and was completely lost at 150°C.

Wu et al. (2009) emphasized the effect of tea polyphenols on preventing the retrogradation of rice starch. Phenolics hydrate through hydroxyl groups, and change the water content of starch gels. Moreover the interaction of hydroxyl groups of

Table 6.9 : Thermal properties of extrudates determined by DSC.

	Thermal Properties of Gelatinization					
	T _o (°C)		T _p (°C)		ΔH (J/g)	
	Barrel Temperatures					
	90°C	120°C	90°C	120°C	90°C	120°C
A1	61.61±0.52by	63.4±0.02bx	65.75±0.36by	66.48±0.04cx	3.52±0.08ax	0.02±0.00cy
A2	61.27±0.09bx	51.51±0.12cy	65.63±0.02bx	58.55±0.13dy	4.19±0.06ax	0.12±0.02cy
A3	59.98±2.62bx	n.d.	64.74±3.08by	n.d	5.76±1.74ax	n.d
GA1	66.89±1.52ay	69.36±0.43ax	74.28±3.70ax	72.92±0.82bx	1.05±0.41bx	0.43±0.09by
GA2	68.30±0.38ax	n.d.	73.25±0.18ax	n.d.	0.34±0.13bx	n.d.
GA3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Thermal Properties of Retrogradation					
	T _o (°C)		T _p (°C)		ΔH (J/g)	
	Barrel Temperatures					
	90°C	120°C	90°C	120°C	90°C	120°C
A1	43.01±0.05cx	45.26±1.46by	51.22±0.08cy	53.07±0.09cx	0.38±0.01ax	0.19±0.01cy
A2	47.32±0.22bx	n.d	55.69±0.12bx	n.d	0.24±0.00bx	n.d
A3	52.56±1.66ax	n.d.	59.50±1.21ax	n.d.	0.25±0.03bx	n.d.
GA1	46.77±0.76bx	41.78±0.85cy	53.70±0.30bcx	50.43±0.23dy	0.25±0.08bx	0.45±0.02bx
GA2	49.27±1.06bx	n.d.	56.06±1.25bx	n.d.	0.21±0.01bx	n.d.
GA3	n.d	n.d.	n.d.	nd	n.d.	nd

Different letters (a–d) within the same column and different letters (x–y) within the same row differ significantly ($p < 0.05$). T_o is the onset temperature, T_p is the peak temperature, ΔH is the enthalpy of gelatinization, n.d.: not detectable. Data given is the average of triplicate analysis $\pm$ standard deviations.

phenolic acids with amylose/amylopectin might affect the amylopectin retrogradation by reorganizing the starch polymer chains (Beta and Corke, 2004; Zhu et al., 2008; Wu et al., 2009). At 90°C, presence of CGEP significantly delayed retrogradation compared to that of the A1 sample. In addition, the retrogradation peak detected was significantly smaller ($p < 0.05$). However, at higher extrusion temperatures this preventive effect was not present, probably because of the degradation effect of higher temperatures on phenolics. Retrogradation started at higher temperatures with the increase in the amylose substitution level. However, this trend became less significant after interaction with CGEP.

6.3 Evaluation of CGEP Added Extrudate As a Functional Food

6.3.1 Introduction

This chapter aimed to investigate the changes in functional properties of the extruded products, focusing on the effect of incorporating either wheat protein isolate or high amylose corn starch, distinctly. Therefore, a detailed discussion on the changes in total phenolic contents, total antioxidant activity, total flavonoid contents, total anthocyanin contents, in addition to phenolic/anthocyanin profiles of extrudates obtained from HPLC chromatograms have been made in this chapter.

6.3.2 Interaction of CGEP with protein

6.3.2.1 Total phenolics

Total phenolic contents of wheat protein isolate and grape powder extract substituted and extruded samples were given in Table 6.10.

Total phenolic content of the extrudates ranged between 129.5 ± 0.2 and 2095.7 ± 1.0 mg GAE/100 g DW. In no Concord grape powder extract substituted samples, phenolic contents of the samples significantly decreased with the increase in extrusion temperature ($p < 0.05$). Measured amount of phenolics increased significantly with the increase in wheat protein isolate (WPI) substitution. Only exception was for 150°C at which the amount of phenolics were at same levels for all three wheat protein isolate substitutions. That was probably because of the denaturation of protein moieties at 150°C. It is known that Folin-Ciocalteu reagent can detect all phenolic groups in extracts but also those in extractable proteins,

therefore proteins found in wheat protein isolate might have contribution to total phenolic content as well (Shahidi and Naczsk, 1995).

Use of Concord grape extract powder in extruded samples increased the total phenolic content about 8-13 fold, in comparison to samples without Concord grape powder (CGEP). Generally, increase in extrusion temperature was significantly more detrimental to phenolic content at 150°C, significant decreases were detected especially when the temperature changed from 120 to 150°C. Use of CGEP for substitution of flour was not that much affected as in the no CGEP added samples. Therefore no statistically significant increases detected in the measured phenolics generally for CGEP added samples.

The WPI substitution was found as a significant factor to decrease the % loss of phenolics after extrusion in samples without CGEP at 90°C and 120°C. On the other hand, the same effect was not evident in CGEP substituted samples and WPI seemed to have only an insignificant role on the loss of total phenolics in samples with CGEP. The loss in total phenolics was around 15, 20 and 36% for 90, 120 and 150°C, respectively. Higher losses in total phenol content were reported in previous studies. Total phenolics in the tomato pulp added extrudates decreased from 15.37 ± 0.09 to 7.49 ± 0.11 mg gallic acid equivalent/g dry weight after extrusion process (Caltinoglu et al., 2014).

Different vital wheat gluten samples were reported to contain phenolic acids such as ferulic acid (4.9-17.7 µg/100 mg), sinapic acid (1.4-3.5 µg/100 mg), and also traces of *p*-coumaric acid and vanillic acid (Labat et al., 2000). Therefore, they might have affected the amount of total phenolics, being more significant at samples with lower phenolic content (e.g. samples with no Concord grape extract powder).

6.3.2.2 Total antioxidant activity

Total antioxidant activity measurements by DPPH radical scavenging method in Concord grape extract powder and wheat protein isolate substituted extrudates were given in Table 6.10. DPPH radical scavenging activity of the extrudates changed between 26.0 ± 2.6 mg TEAC/100 g DW and 1395.1 ± 30.9 mg TEAC/100 g DW.

Extrusion temperature made no significant effect on the measured total antioxidant activity by DPPH radical in samples with no CGEP ($p < 0.05$). Similarly, DPPH

Table 6.10 : Total antioxidant activity and total phenolic contents of WPI and CGEP substituted samples extruded at different temperatures.

	T(°C)								
	90	120	150	90	120	150	90	120	150
	TAA-DPPH (mg TEAC/100 g DW)			TAA-ABTS (mg TEAC/100 g DW)			TP (mg GAE/ 100 g DW)		
P1	40.9± 7.3dx	31.5± 7.8 cx	26.0± 2.6cx	87.8± 2.8dx	77.8± 8.2dx	73.4± 2.2dx	239.6± 4.6cx	133.5± 6.2ey	129.5± 0.2by
P2	121.6± 5.1cx	113.2± 3.1cx	109.8± 3.3cx	255.9± 16.1dx	73.2± 1.3dy	61.6± 1.0dy	645.8± 47.5bx	370.3± 15.5cy	132.9± 1.2bz
P3	165.3± 29.2cx	110.9± 4.8cx	113.4± 0.8cx	276.2± 16.2dx	115.6± 4.0dy	60.7± 0.1dz	656.6± 71.0bx	225.6± 5.1dy	156.6± 0.9by
GP1	1308.8± 9.4bx	1164.3± 38.3abx	965.7± 57.3by	1850.2± 64.8cx	1752.4± 5.1cx	1341.6± 44.4by	1850.2± 64.8ax	1752.4± 5.1ax	1492.3± 59.6ay
GP2	1395.1± 30.9ax	1348.6± 14.6ax	1171.5± 8.9ay	3274.4± 60.0ax	3122.0± 91.1ax	2632.8± 75.3ay	2019.7± 78.9ax	1940.7± 19.0ax	1529.9± 19.4ay
GP3	1256.4± 31.9bx	1074.8± 181.2bx	970.3± 114.9bx	2696.3± 151.4bx	2457.1± 51.4bxy	2126.6± 119.6by	2095.7± 1.0ax	1774.2± 39.5by	1483.1± 18.3az

Different letters (a–d) within the same column, and different letters (x–y) within the same row differ significantly ($p < 0.05$). Data given is the average of triplicate analysis $\pm$ standard deviations.

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

radical scavenging activities of buckwheat flour extrudates were not affected significantly during extrusion at 170°C (Sensoy et al, 2006). Substitution with WPI in those samples, increased the total antioxidant activity about 3 fold, although the differences between substitution levels were not statistically significant in general ($p < 0.05$).

CGEP substituted samples increased the total antioxidant activity of samples about 32-27 fold and changed between 965.7 and 1395.1 mg TEAC/100 g DW. Extrusion temperature made a significant decrease in the antioxidant activity only at 150°C ($p < 0.05$). WPI addition made a different effect than the counterpart no-Concord grape added samples. The lower level of substitution (6.5%) resulted in a significantly higher antioxidant activity, while higher substitution levels (13%) lowered the activity back to original levels at which no wheat protein isolate addition was made.

According to the measurements made before and after extrusion, the loss of total antioxidant activity by DPPH method was around 30%, 45% and 55% for 90, 120 and 150°C, respectively in no CGEP added samples. In contrast, WPI substitutions to these samples seemed to increase the antioxidant activity at least around 2-fold in all of these samples. No such recovery effect for WPI was detected in the CGEP added

samples and the loss of DPPH radical scavenging activity was around 10, 20 and 30 % for 90, 120 and 150°C extrusions, respectively being independent from the WPI level. Antioxidant activity of tomato pulp added extrudates decreased from 16.71 ± 0.42 to 12.84 ± 0.36 $\mu\text{mol Trolox equivalent/g dry weight}$ after extrusion, therefore the % loss was around 20 % (Caltinoglu et al., 2014).

Total antioxidant activity measurements by ABTS radical scavenging activity method in CGEP and WPI substituted extrudates were given in Table 6.10. ABTS radical scavenging activities of the extruded samples were between 60.7 ± 0.1 and 3274.4 ± 60.0 mg TEAC/100 g DW. Antioxidant activity showed no significant change ($p > 0.05$) in samples with no CGEP and no WPI and extruded at different extrusion temperatures. However, after WPI addition, the ABTS radical scavenging activity of the samples extruded at 90°C increased significantly ($p < 0.05$) and became significantly higher than the measurements made at two other temperatures. Therefore, substitutions with WPI made a significant effect only in samples extruded at 90°C.

In samples with CGEP, antioxidant activity was significantly lower ($p < 0.05$) at 150°C than the other extrusion temperatures of 90 and 120°C. This trend did not change after WPI substitution, as well. Although antioxidant activity results were lowest at 150°C for all samples, WPI made a significant increasing effect on the ABTS radical scavenging activity at all extrusion temperatures ($p < 0.05$).

Decrease in antioxidant activity after extrusion has been reported to be related with decarboxylation of phenolic compounds depending on the high barrel temperature and polymerization of phenols and tannins due to high moisture content during extrusion (Repo-Carrasco-Valencia et al., 2009; Dlamini et al., 2007).

Table 6.11 : % Loss of phenolics, antioxidant activity and flavonoids in WPI and CGEP substituted extruded samples.

	Extrusion Temperature (T°C)											
	90				120				150			
Loss (%)												
	TP	ABTS	DPPH	TF	TP	ABTS	DPPH	TF	TP	ABTS	DPPH	TF
P1	65	76	28	n.d	81	78	45	n.d	81	80	54	n.d
P2	7	29	-114	n.d	47	80	-99	n.d	81	83	-93	n.d
P3	5	24	-191	n.d	67	68	-95	n.d	77	83	-99	n.d
GP1	18	46	12	-13	24	49	22	-5	37	61	35	14
GP2	15	5	6	-21	18	9	10	-9	36	23	21	2
GP3	12	22	16	-29	25	28	28	-22	38	38	35	-11

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

After extrusion, ABTS radical scavenging activity of the no CGEP added samples were lost around 76, 78, and 80% for 90, 120 and 150°C extrusion temperatures, respectively (Table 6.11). However, WPI substitution significantly decreased the % loss of antioxidant activity to around 30% at 90°C. Such a decrease in the loss of antioxidant activity was not evident at other extrusion temperatures. In CGEP substituted samples, the effect of WPI substitution was not consistent and firstly made a decrease from 46% to 5 % (at 6.5% wheat protein isolate substitution level) and then slightly increased back to 22%. Similar effect was evident at 120°C, as well.

6.3.2.3 Total flavonoids

Total flavonoids in CGEP and wheat protein isolate (WPI) substituted extrudates were given in Table 6.12. Total flavonoid contents of samples changed between 1499.8 ± 26.6 and 2262.3 ± 33.1 mg QE/ 100 g DW. Substitutions of WPI and gradual increase of substitution level seemed to increase the total flavonoid content in all different extrusion temperatures, although the differences were not statistically significant ($p > 0.05$). In samples with no wheat protein isolate substitution, the flavonoid content had a sharper decrease from 120°C to 150°C, and standard deviations among different batches gave higher differences, on the other hand, with the increase in wheat protein levels the measured flavonoid contents became higher. This effect was more evident at 150°C.

In CGEP substituted samples, increases in extrusion temperatures significantly decreased the flavonoid contents ($p < 0.05$). With WPI substitutions (and specifically at 13% substitution level) the effect of temperature on the measured flavonoid was less evident since the differences between samples' flavonoid contents at 90, 120 and 150°C were 2262.3, 2126.4 and 1936.3 mg QE/ 100 g DW, respectively, being insignificant ($p > 0.05$). Therefore WPI might be regarded as a barrier to heat damage of CGEP flavonoids.

When the unprocessed flour was compared with extrudate samples, it was evident that total flavonoid contents were higher in 90 and 120°C extrusion temperatures. Moreover, the gradual increase in the remaining flavonoid contents with increase in WPI levels were evident at all extrusion conditions. In accordance with the present findings, thermal processes of steaming and boiling were also found to increase the total flavonoid contents around 60-90% in yellow and black soybeans (Durge et al., 2011) and suggested that it might be related with the release of phenolic or phenolic analogue substances having reactivity toward phenolic content measuring reagents such as Folin-Ciocalteu reagent) from polymerized structural substances (such as lignin) in cell walls upon thermal processing (Xu and Chang, 2008).

Table 6.12 : Total flavonoid and anthocyanin contents of WPI and CGEP substituted samples extruded at different temperatures.

	T(°C)					
	90	120	150	90	120	150
Samples	TAC (mg Cy-3-glc/100 g DW)			TF (mg QE/ 100 g DW)		
GP1	25.1± 3.7ax	24.5± 8.4ax	22.5± 1.6ax	1970.6± 198.0ax	1836.3± 120.0ax	1499.8± 26.6bx
GP2	23.4± 0.6ax	23.7± 6.0ax	17.9± 1.4bx	2123.2± 15.2ax	1897.4± 0.4ay	1714.6± 27.5abz
GP3	18.2± 5.6ax	14.2± 0.3ax	16.2± 2.1bx	2262.3± 33.1ax	2126.4± 95.1ax	1936.3± 113.3ax

Different letters (a–d) within the same column, and different letters (x–y) within the same row differ significantly ($p < 0.05$). Data given is the average of triplicate analysis $\pm$ standard deviations.

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

6.3.2.4 Total anthocyanins

Total anthocyanin contents of CGEP and wheat protein isolate substituted extrudates ranged between 14.2 and 25.1 mg Cy-3-glc/100 g DW and were presented in Table 6.12. Total anthocyanin contents of extrudates displayed no significant difference

between 90 and 120°C ($p>0.05$). However significant decreases were detected when samples were substituted with WPI and extruded at 150°C ($p<0.05$). Similarly, although extrusion was considered as a good method for increasing procyanidin monomer and dimer contents in grape seed and pomace samples, it was found to reduce the total anthocyanins in grape pomace extrudates in the range of 18% to 53% (Khanal et al., 2010). Similarly in a different study, although anthocyanin retention was around 57% at 140°C die temperature, when extrusion temperature raised to 180 °C, retention decreased to about 40 % (Durge et al., 2011) confirming the sensitivity of anthocyanin to temperature.

WPI substitutions presented no effects on the retained anthocyanin levels, but instead generally they decreased the measured anthocyanin contents. However their effect was not found to be statistically significant ($p>0.05$).

6.3.2.5 Phenolic and anthocyanin profiles and composition

Phenolic and anthocyanin profiles of CGEP and wheat protein isolate substituted extrudate samples were given in Figures 6.3-6.10 and Tables 6.13 and 6.14.

Gallic acid was the most abundant phenolic acid for all samples, having a concentration of 0.23 to 20.30 µg/100 g DW in no-CGEP added extruded samples and 40.63 to 62.45 µg/100 g DW in samples with CGEP. Sinapic acid was also detected as another major compound in no-CGEP added samples. On the other hand, protocatechuic acid, sinapic acid, epigallocatechin, catechin hydrate, syringic acid, vanillic acid, chlorogenic acid, and *p*-coumaric acid might be regarded as other major compounds in CGEP substituted samples at about 5-30 µg/100 g DW concentrations. These findings were in consistent with other researcher's findings (Pastrana-Bonilla et al., 2011; Fraige et al., 2014; Xia et al., 2010).

Increase in extrusion temperature had a detrimental effect on phenolics and flavan-3-ols, generally. However gradual increase in WPI substitution levels made differences in the effect of extrusion temperature for some of the compounds. The most profound effect was observed for CGEP substituted samples, gallic acid increased significantly with the WPI level. For sinapic acid (in samples without CGEP), and epigallocatechin, catechin hydrate, epicatechin, syringic acid, epicatechin gallate and vanillic acid (for samples with CGEP) similar linear trends were detected with WPI, although the effects were changing in statistical significance.

However, phenolic compounds are known to behave differently at various extrusion temperatures and selected compounds might not be detected in extruded samples, although they were present in raw samples (Zielinski et al., 2010). For instance, the very high content of gallic acid in CGEP added and extruded sample has been related to excision of the gallate group attached to the C-ring of the flavonoids or to the hydrolysis of gallotannins present in grape pomace (Brennan et al., 2011).

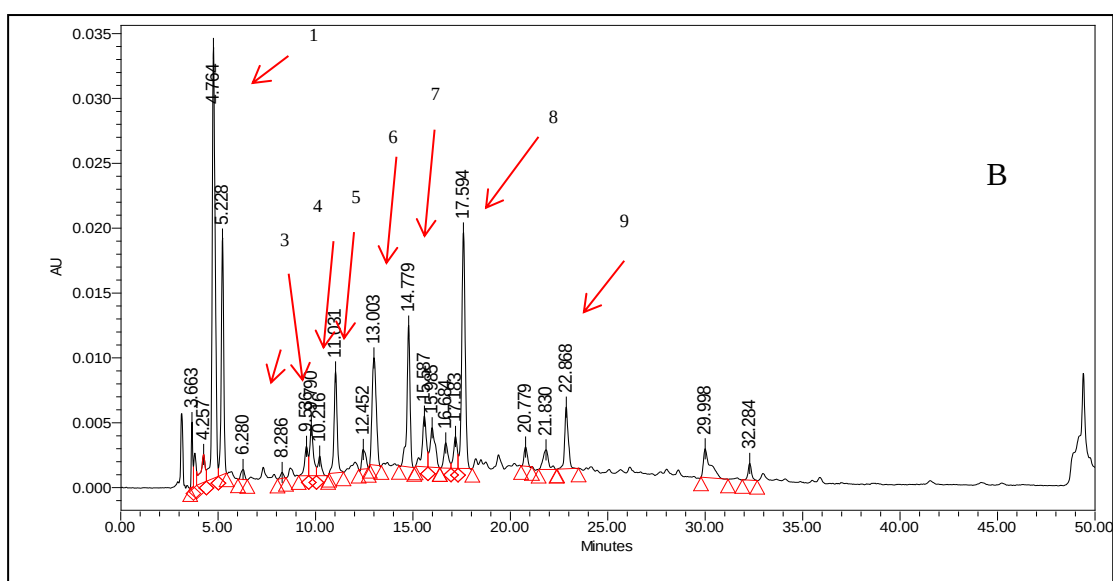
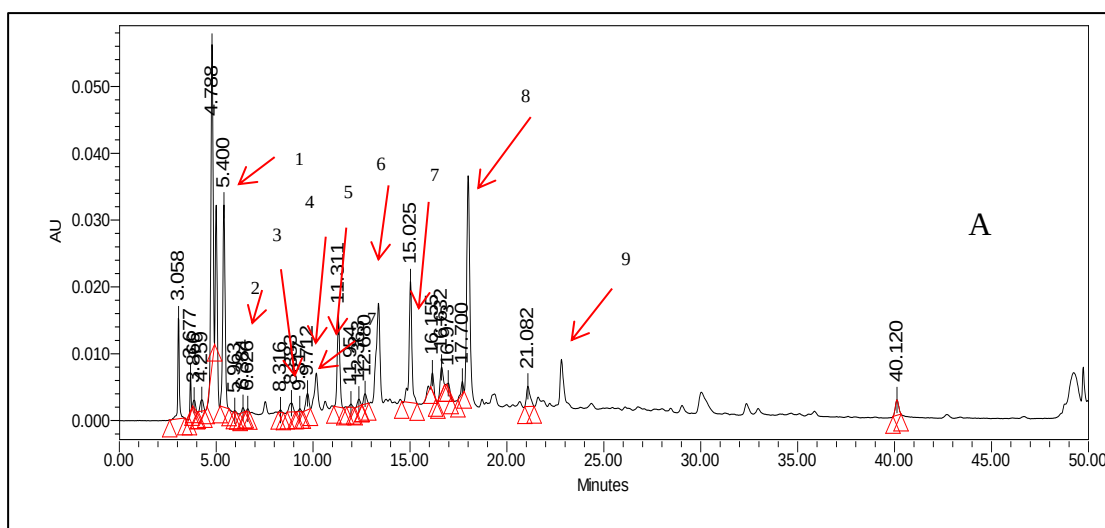
Rutin was the most abundant flavonol in extrudate samples (38.7-85.66 µg/100 g DW). Among anthocyanins and anthocyanidins the most significant compound was malvidin-3-o-glucoside (5.63-12.30 µg/100 g DW). The rest of the components were measured at around 5 µg/100 g DW or less. Malvidin-3-o-glucosides and its derivatives such as *p*-coumaryl were also reported as the major compounds in different red grape varieties (Guerrero et al., 2009).

WPI substitutions had no effect on 150°C, however at 120°C and 90°C extrusion temperatures the effect of WPI substitutions on samples was generally negative, however not being statistically significant for all samples ($p > 0.05$).

For anthocyanin and anthocyanidins before WPI substitution, thermal degradation was more significant at 150°C, instead of 90 and 120 °C extrusion temperatures.

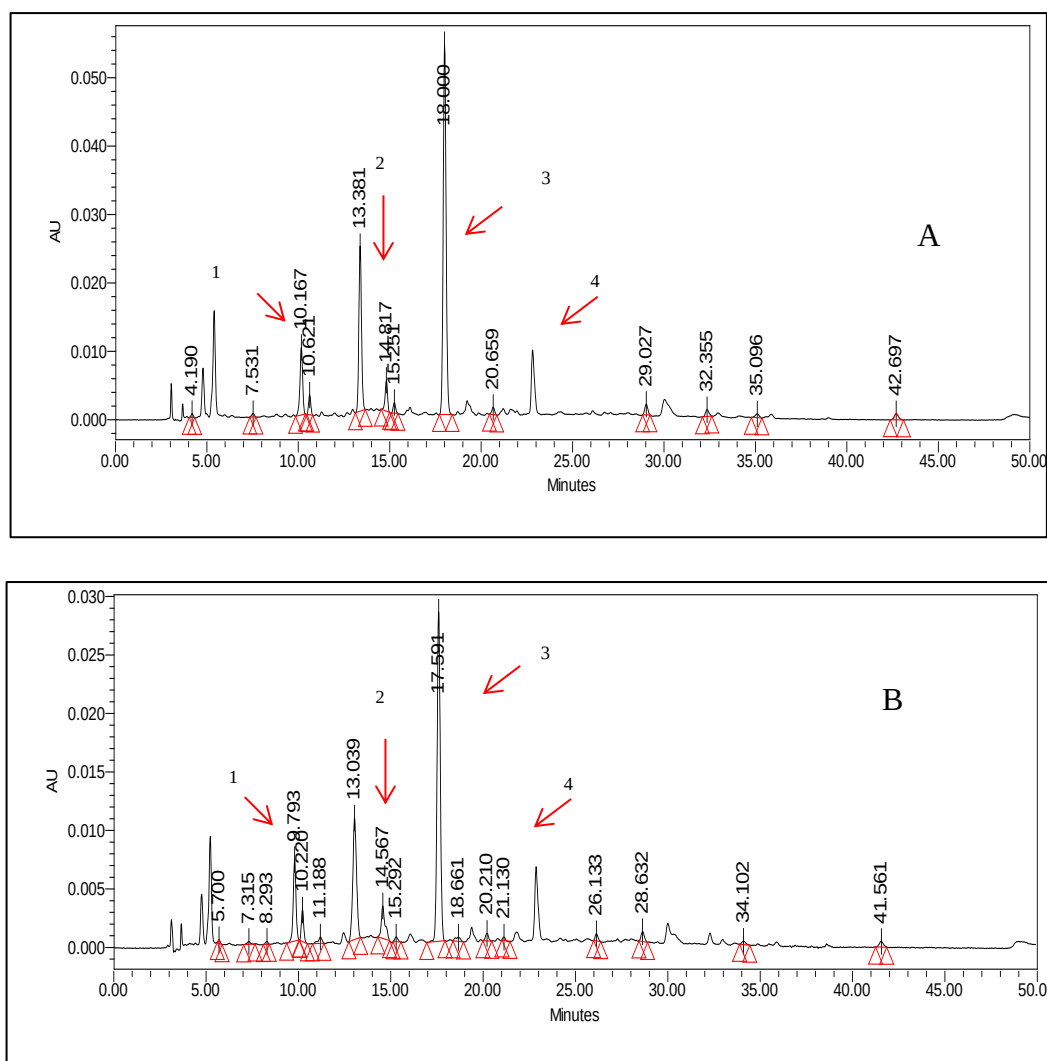
Khanal et al (2010) revealed that the decrease in procyanidins for the forced air oven dried grape pomace was significant when they were heated at 60°C, but when their temperature was further raised from 105 to 120°C, no more significant losses were measured (Khanal et al., 2010). Anthocyanins were reported to be more sensitive to the effect of heat above 70°C (Markakis et al., 1957).

Thermal processing of steaming and boiling caused significant ($p < 0.05$) decreases in cyanidin-3-glucoside and peonidin-3-glucoside levels in black soybeans (Xu and Chang, 2008). They also revealed that pressure steaming treatments caused significant ($p < 0.05$) increases in gallic acid and 2,3,4-trihydroxybenzoic acid, whereas all treatments caused significant ($p < 0.05$) decreases both in two predominant phenolic acids (chlorogenic acid and trans-cinnamic acid), and in total phenolic acids of yellow and black soybeans.



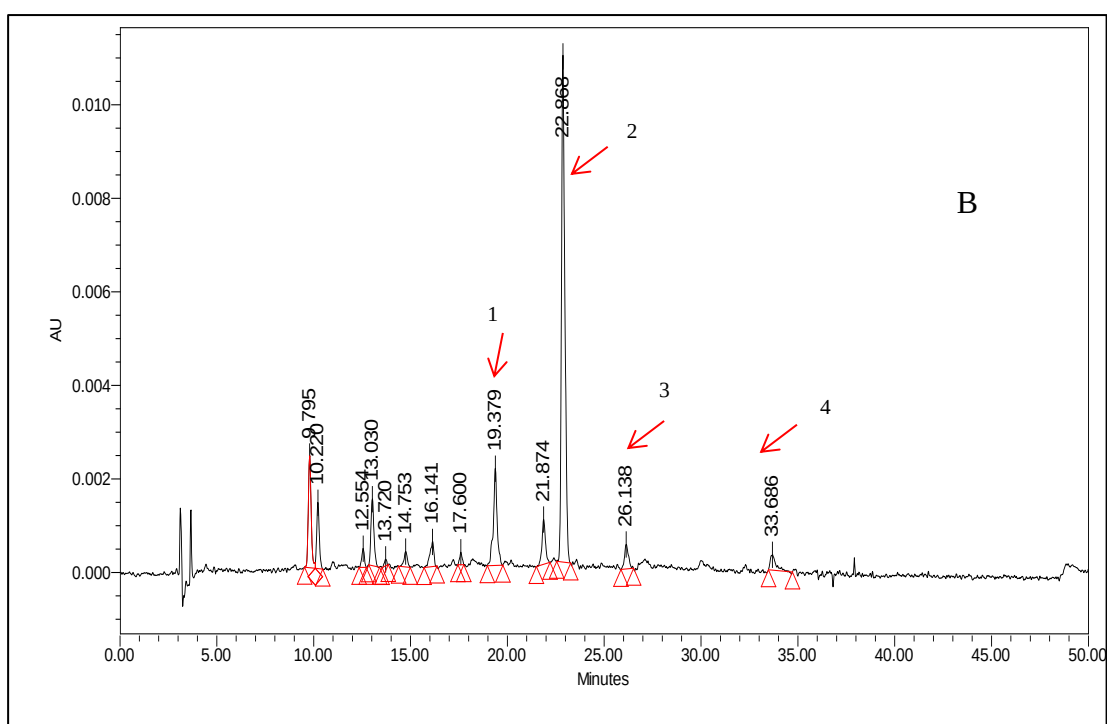
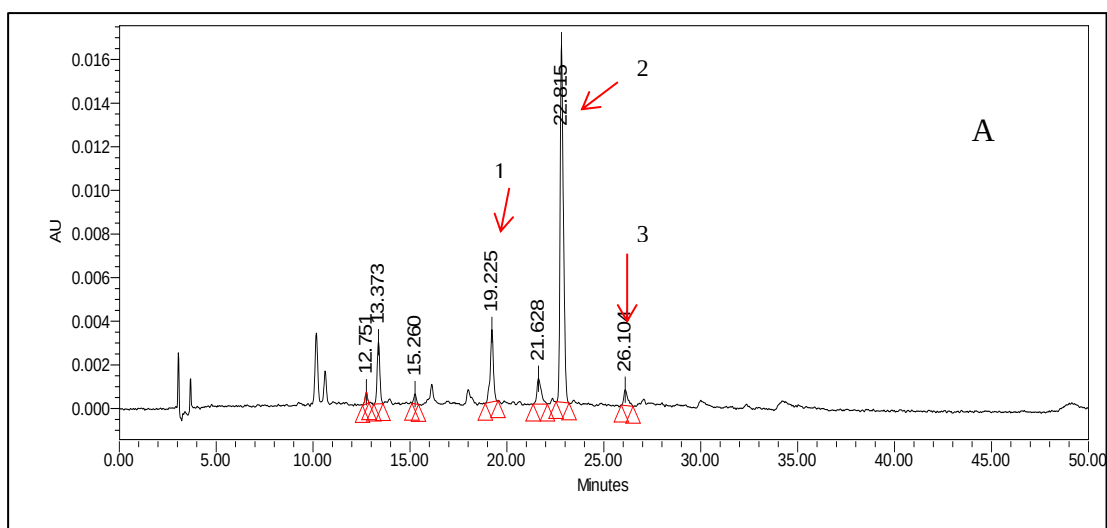
(1: Gallic acid, 2: Protocatechuic acid, 3: Epigallocatechin; 4: Catechin hydrate; 5: Epicatechin; 6: Syringic acid, 7: Vanilic acid, 8: n.d)

Figure 6.3 : Representative HPLC-PDA chromatograms extracted at 280 nm for A: GP1 extruded at 150°C; B: GP3 extruded at 150°C.



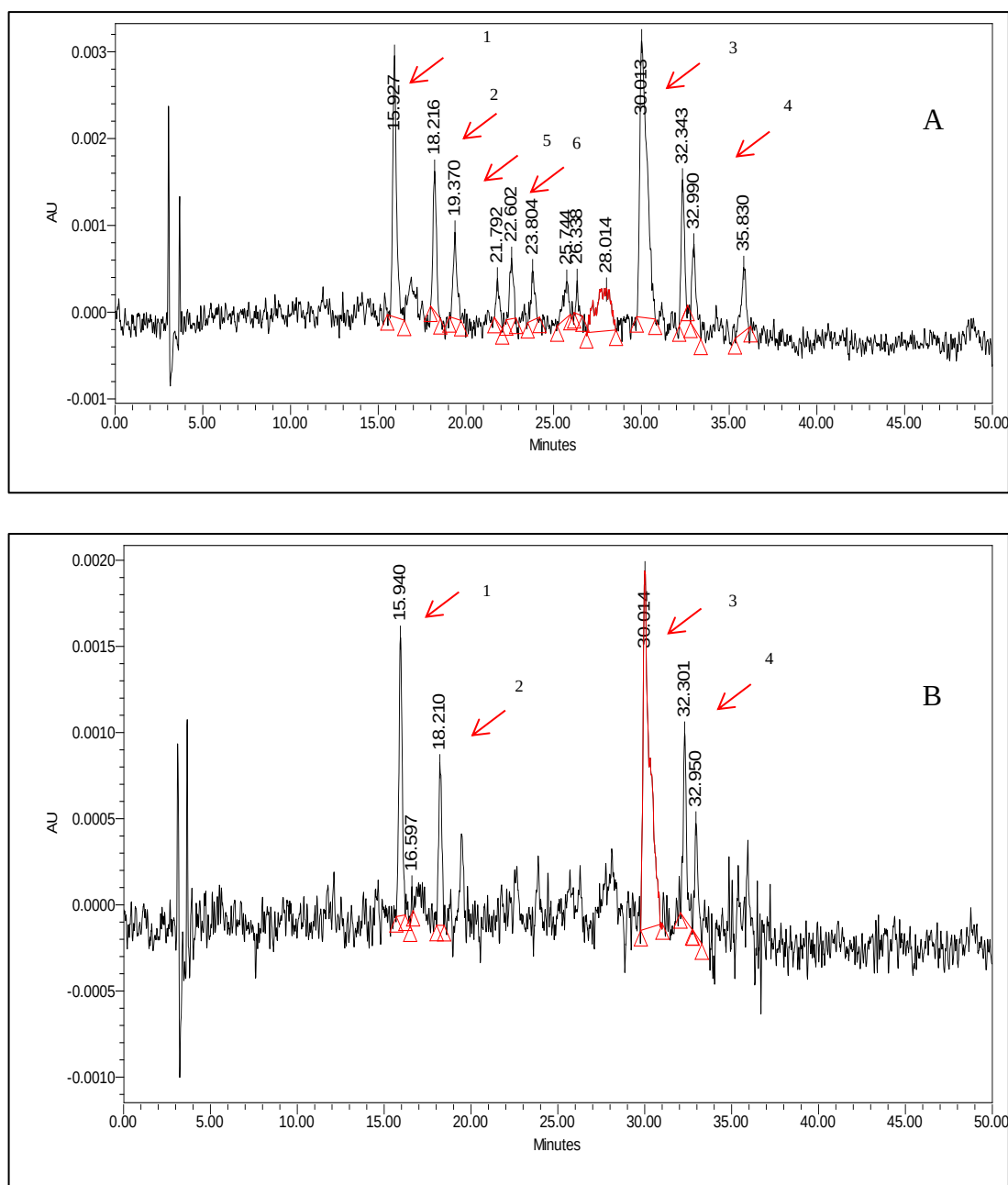
(1: Chlorogenic acid, 2: Caffeic acid, 3: P-coumaric acid, 4: Ferulic acid)

Figure 6.4 : Representative HPLC-PDA chromatograms extracted at 312 nm for A: GP1 extruded at 150°C; B: GP3 extruded at 150°C.



(1:Quercetin-3-beta-glucoside; 2: Myricetin; 3: Rutin, 4: Quercetin)

Figure 6.5 : Representative HPLC-PDA chromatograms extracted at 360 nm for A: GP1 extruded at 150°C; B: GP3 extruded at 150°C.



(1: Delphinidin-3-glucoside; 2: Cyanidin-3-o-glucoside; 3: malvidin; 4: malvidin-3-o-glucoside; 5: Cyanidin-3,5-diglucoside; 6: Delphinidin-3,5-diglucoside)

Figure 6.6 : Representative HPLC-PDA chromatograms extracted at 520 nm for A: GP1 extruded at 150°C; B: GP3 extruded at 150°C.

Table 6.13 : Phenolic acids and flavan-3-ols of WPI and CGEP substituted extrudate samples.

	P1			P2			P3			GP1			GP2			GP3		
	150	120	90	150	120	90	150	120	90	150	120	90	150	120	90	150	120	90
Protocat. acid	0.66 dx	0.48 by	nd	1.00 cx	nd	nd	0.36 ex	nd	nd	7.15 ax	5.47 ay	6.02 ay	7.01 ax	0.55 bz	5.52 ay	3.07 bx	nd	2.74 bx
Gallic acid	20.30 bx	12.68 by	2.41 dz	16.23 bx	17.71 bx	29.20 ac	0.23 cx	nd	nd	40.63 ay	43.34 ay	50.85 bx	48.94 ax	43.06 ay	50.37 bx	48.25 ay	50.75 ay	62.45 ax
Sinapic acid	4.77 dx	5.23 dx	nd	6.67 dy	12.83 bx	2.91 ez	8.56 cx	6.26 cy	6.67 cy	18.99 ay	20.46 ax	17.31 az	8.66 cx	nd	10.46 bx	11.76 bx	4.02 dy	nd
Epigallo-catechin	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.64 bx	11.40 ax	12.64 ax	13.23 ax	11.30 ax	12.59 ax	9.16 cx	12.47 ax	2.70 by
Catechin hydrate	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.52 bx	8.47 aby	7.18 ay	11.10 bx	10.48 ax	6.23 ay	36.65 ax	8.51 aby	5.82 ay
Epicatechin	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.35 cy	0.36 y	1.01 bx	0.62 by	0.81 aby	6.43 ax	0.78 ax	1.55 ax	0.80 bx
Syringic acid	nd	nd	nd	7.28 bx	nd	nd	7.30 bx	nd	nd	1.59 cy	21.46 ax	22.16 ax	1.60 cz	17.74 bx	10.99 by	15.73 ax	21.39 ax	1.55 cy
Epicatechin galleate	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.55 aby	4.44 ax	1.30 ay	1.89 ax	1.73 bx	1.05 ax	0.82 bx	1.54 bx	0.67 bx
Vanilic acid	7.04 cx	4.32 by	3.83 by	nd	5.15 bx	4.53 bx	2.87 dy	nd	3.91 bx	4.98 cdx	3.64 ax	4.43 bx	10.84 by	16.96 ax	16.36 ax	31.97 ax	22.18 ay	6.00 bz
Chlorogenic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	10.45 ay	12.97 ax	13.25 ax	3.07 ax	3.16 cx	3.52 bx	2.78 axy	4.72 bx	0.95 cy
Caffeic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.48 ay	4.32 ax	4.42 ax	1.02 bx	1.05 bx	1.17 bx	0.93 by	1.57 bx	0.32 cz
p-coumaric acid	0.13 bx	0.06 cy	0.10 cx	0.09 bx	0.03 cx	0.03 cx	0.15 bx	0.06 cy	0.03 cy	15.46 ax	15.66 ax	15.41 ax	15.54 ax	12.03 by	14.60 ax	12.26 axy	15.79 ax	6.96 by
Ferulic acid	0.16 bx	nd	0.16 ax	0.16 bx	nd	0.26 ax	0.18 by	nd	0.44 ax	0.41 ay	0.28 az	0.62 ax	0.48 ax	0.61 ax	0.73 ax	0.40 ay	0.92 ax	0.88 ax
t-resveratrol	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.57 ay	0.96 ax	0.43 ay	0.61 ax	0.33 ay	0.62 ax	0.36 by	0.81 ax	0.25 ay

Results are given as µg/100 g DW. Data given is the mean value of triplicate analyses. Values with different letters (a–e) of different extruded samples at each formulation and different letters (x–z) within the extruded sample among different temperatures differ significantly ($p < 0.05$). nd: not detected.

Table 6.14 : Flavonols and anthocyanins of WPI and CGEP substituted extrudate samples.

	GP1			GP2			GP3		
	150	120	90	150	120	90	150	120	90
Quercetin-3- β -glucoside	1.46 ax	2.20 ax	0.98 ax	1.88 ax	1.03 bx	1.05 ax	1.67 ax	1.24 bxy	0.44 by
Rutin	67.86 ax	81.96 ax	85.66 ax	73.58 ay	65.86 az	81.52 ax	58.80 axy	38.73 by	85.57 ax
Myricetin	1.09 ax	0.55 ax	0.85 ax	0.35 ax	1.06 ax	0.65 ax	0.88 ax	0.60 ay	0.41 ay
Quercetin	nd	nd	0.93 ax	nd	0.32 ay	0.72 ax	nd	0.82 ax	0.84 ax
Delphinidin-3-o-glucoside	2.06 ay	4.50 ax	4.53 ax	1.82 az	3.14 ay	4.53 ax	1.65 ay	5.45 ax	2.46 by
Cyanidin-3-o-glucoside	1.09 ay	2.94 ax	2.16 ax	0.88 ay	1.46 ay	1.98 ax	0.84 ax	2.71 ax	1.03 bx
Cyanidin-3,5-diglucoside	1.34 ay	2.93 ax	2.71 ax	1.34 ay	2.55 ax	2.51 ax	1.33 ay	2.30 ax	1.38 by
Delphinidin-3,5-diglucoside	0.63 ax	1.37 ax	0.89 ax	0.44 ay	nd	1.06 ax	nd	1.16 ax	0.54 ay
Delphinidin	0.52 ay	2.02 ax	1.30 ax	0.66 ay	0.82 ay	1.32 ax	nd	1.25 ax	0.99 ax
Cyanidin	0.64 ax	1.22 ax	0.99 ax	0.66 ay	0.80 axy	1.05 ax	0.68 ax	0.76 ax	nd
Malvidin	0.15 ay	0.61 ax	0.35 ax	0.17 ax	0.55 ax	0.22 ax	nd	0.48 ax	0.25 ay
Malvidin-3-o-glucoside	5.63 ay	17.06 ax	11.84 ax	5.88 az	8.61 ay	12.30 ax	5.39 ay	11.76 ax	5.73 by

Results are given as $\mu\text{g}/100 \text{ g DW}$. Data given is the mean value $\pm$ standard deviation of triplicate analyses. Values with different letters (a–e) of different extruded samples at each formulation and different letters (x–z) within the extruded sample among different temperatures differ significantly ($p < 0.05$). nd: not detected

P1: bread flour control; P2: bread flour substituted with 6.5% WPI; P3: bread flour substituted with 13% WPI; GP1: bread flour substituted with 7% CGEP; GP2: bread flour substituted with 6.5% WPI and 7% CGEP; GP3: bread flour substituted with 13% WPI and 7% CGEP.

6.3.3 Interaction of CGEP with amylose

6.3.3.1 Total phenolics

Total phenolic contents of extrudates with CGEP and HACS extruded at different temperatures were given in Table 6.13. Amount of total phenolics changed between 108.8 ± 0.7 and $1947.5 \pm 39.4 \text{ mg GAE}/100 \text{ mg DW}$. In samples with no CGEP substitution, from 90°C to 120°C significant decreases in total phenolic content were evident at all HACS substitution levels ($p < 0.05$). When the effect of increase in HACS substitution levels were evaluated, HACS level seemed to have no effect on the phenolic content all of the samples extruded at different die temperatures.

CGEP substitution significantly increased the total phenolic content in all samples; minimum about 6 fold. The phenolic contents of the CGEP-substituted samples changed between 1408.7 and 1947.5 mg GAE/100 mg DW.

Total phenolic contents in only CGEP substituted samples presented a decrease only when the die temperature was at 150°C. On the other hand, after HACS substitution significant decreases were evident at lower die temperatures of 120°C. At each distinct extrusion temperatures, phenolics contents differed by means of changing the HACS substitution levels. Therefore it is hard to talk about a linear relationship with total phenolic contents and HACS substitution levels. Changes in extractability of polyphenols were proposed to be related with the complex formation between polyphenolics and wheat proteins or polysaccharides via H bonding and/or hydrophobic interactions (Almajano et al., 2007; Shahidi and Naczk, 2005).

Losses in total phenolics for no CGEP-added samples after extrusion were around 67, 80 and 81% for 90, 120 and 150°C, respectively. HACS substitution had no significant effect on the % loss of phenolics. In previous studies, phenolic content of barley extrudates were found to decrease around 46–60% in comparison to the unprocessed barley flour (Altan et al, 2009). On the other hand, losses were around 25%, 30% and 37% for extrusion temperatures of 90, 120 and 150 °C, respectively. HACS substitution slightly increased the % loss of phenolics and the most significant change was evident for sample extruded at 120°C.

6.3.3.2 Total antioxidant activity

Total antioxidant activity measured by DPPH radical scavenging activity method for extrudates with CGEP and HACS were given in Table 6.13. DPPH radical scavenging activity of samples ranged between 19.9 and 1251.9 mg TEAC/100 g DW.

Increase in extrusion temperature had no significant effect on the antioxidant activities of no-CGEP added extrudate samples. Similarly, total antioxidant activity was not affected from the differences in barrel temperature (100-160°C), extruder screw speed (180-720 l/min) and moisture content (18-28% wet base). Hirth et al. (2014) worked on the effects of extrusion cooking on the retention of the phenolics, antioxidants and anthocyanins of bilberry extract added (2%) maize starch extrudates and for explaining no change in the phenolic content and antioxidant activity they referred to the short processing time in the extrusion process(Hirth et al., 2014).

%Losses in DPPH radical scavenging activities for no-CGEP added extruded samples were around 50, 55 and 56% for the 90, 120 and 150°C die temperatures,

respectively. HACS addition increased the % loss at all three extrusion temperatures. On the other hand, in CGEP added samples 25, 32 and 40% losses were detected respectively as the extrusion die temperature increased from 90 to 150°C. HACS also increased the % loss at all three extrusion temperatures, mostly at the 20% substitution levels (GA3). Similarly, in barley extrudates antioxidant activity of the samples was found to decline about 60-68% in comparison with the unprocessed barley flour (Altan et al, 2009). Antioxidant activity and total phenols also decreased after extrusion in the blends of corn starch with navy or small red beans (Anton, et al., 2009).

In CGEP substituted samples, amount of antioxidant activity measured by DPPH radical scavenging activity method was about 40-fold of that for no-CGEP added samples. The decreasing effect of die temperature was only evident at 150°C for only CGEP added samples (GA1). With the HACS substitution the drop in antioxidant activity became significant at extrusion temperature of 120°C.

ABTS radical scavenging activity of no-CGEP added samples changed between 68.4 and 87.8 mg TEAC/100 g DW. Antioxidant activity measured by ABTS method was not affected from die temperatures. HACS substitution also made no changes in antioxidant activities of the samples.

CGEP addition raised the antioxidant activity measured by ABTS method at about 20 fold. The range of antioxidant activities by ABTS radical scavenging method was between 1341.6 and 1850.2 mg TEAC/100 g DW. Significant decreases in ABTS radical scavenging activities were detected with increases in extrusion temperatures. HACS substitutions also decreased the antioxidant activity significantly ($p < 0.05$). A generalization was made that those conditions that promote extrudate expansion (e.g., increased screw speed or reduced moisture, or amylose as in present case) generally resulted in reduced antioxidant activity in the product (Ozer et al. 2006).

%Losses in ABTS radical scavenging activities for no-CGEP added extruded samples were constant around 78-80% for all three die temperatures. HACS substitutions made no effect on the ABTS radical scavenging activities of these samples. In CGEP added samples, on the other hand, losses were around 49%, 55 and 59% for 90, 120 and 150°C die temperatures, respectively. Findings about the decrease in antioxidant activity after extrusion was in agreement with the previous studies as well (Özer et al., 2006; Potter et al., 2013). A possible explanation was given as Maillard reaction

may have been inhibited during the extrusion process by fruit powders, thus the production of Maillard reaction products with antioxidant capacity was inhibited (Özer et al., 2006). In contrast, small but significant increases in both total antioxidant capacity and also total phenols were reported for extruded blends of red cabbage with corn starch or with wheat flour (Stojceska et al. 2009).

Table 6.15 : Total antioxidant activity and total phenolic contents of HACS and CGEP substituted samples extruded at different temperatures.

	T(°C)								
	90	120	150	90	120	150	90	120	150
	TAA-DPPH (mg TEAC/100 g DW)			TAA-ABTS (mg TEAC/100 g DW)			TP (mg GAE/ 100 g DW)		
A1	40.9± 7.3cx	31.5± 7.8cx	26.0± 2.6cx	87.8± 2.8dx	77.8± 8.2cx	73.4± 2.2cx	239.6± 4.6cx	133.5± 6.2cy	129.5± 0.2cy
A2	20.4± 1.9cx	25.1± 2.9cx	26.1± 0.6cx	73.4± 5.9dx	69.9± 8.6cx	74.9± 0.4cx	227.4± 1.4cx	137.5± 14.5cy	117.8± 3.2cy
A3	24.2± 3.4cx	19.9± 1.9cx	22.7± 1.2cx	76.1± 8.6dx	68.4± 2.9cx	71.1± 3.2cx	219.3± 2.5cx	121.7± 11.1cy	108.8± 0.7cy
GA1	1308.8± 9.4ax	1164.3± 38.3ax	965.7± 57.3ay	1850.2± 64.8ax	1752.4± 5.1ax	1341.6± 44.4by	1947.5± 39.4ax	1813.3± 10.8ax	1492.3± 59.6aby
GA2	1251.9± 42.6ax	979.3± 68.9by	960.8± 45.5ay	1744.6± 33.0bx	1457.3± 59.9by	1437.3± 0.1ay	1848.7± 87.5abx	1476.3± 13.2by	1408.7± 6.6by
GA3	1054.4± 12.4bx	885.7± 83.9bxy	751.6± 19.5by	1633.1± 29.5cx	1434.9± 101.5bxy	1354.1± 7.3by	1764.3± 58.2bx	1509.4± 49.2by	1556.7± 14.6ay

Different letters (a–d) within the same column, and different letters (x–y) within the same row differ significantly ($p < 0.05$). Data given is the average of duplicate analysis $\pm$ standard deviations.

A1: bread flour control; A2: bread flour substituted with HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

6.3.3.3 Total flavonoids

Total flavonoids in CGEP and HACS added extrudates were given in Table 6.15. According to the results; flavonoid contents of the samples were between 1492.6 ± 134.7 and 1970.6 ± 198.0 mg QE/ 100 g DW. Increase in die temperatures displayed a decrease in measured flavonoid contents, although they did not seem statistically significant except for GA2 samples extruded at 120°C.

HACS substitution levels did not make any significant change on the measured flavonoid content of the extrudates ($p > 0.05$).

When the flavonoid contents of extrudates were compared with the unprocessed flour samples, it was observed that extrusion at 90°C increased the total amount of flavonoids, in an average level of 12%. Such a significant increase (30-34%) in total flavonols in extruded cranberry pomace/corn starch products compared to control was also observed by White et al. (2010).

6.3.3.4 Total anthocyanins

Total anthocyanin contents of Concord grape powder extract and high amylose starch substituted extrudates were given in Table 6.15. Total anthocyanins changed between 21.6 ± 1.4 and 25.4 ± 5.2 mg Cy-3-glc/100 g DW. Neither the effect of HACS levels, nor the extrusion temperature was found significant on the anthocyanin level measured. Camire et al. (2007) worked with fruit powders during extrusion of corn flour and observed that sufficient amount of colorants remained and produced a purple color corn meal extruded product (Camire et al., 2007). However the extent of anthocyanin retention in their study was around 10% and was very low indeed, as another study by White et al. (2010) used dried cranberry pomace (formulated with 30,40 and 50% of corn starch) and observed higher anthocyanin retentions. Particularly at higher corn starch content (50%), the anthocyanins were lost around only 50% and were suggested to be protected by the presence of starch present in the extrudate mix (White et al., 2010). According to Hirth et al (2014) extent of anthocyanin retention was different for different extrusion conditions comprising bilberry extract added (2%) maize starch extrudates. They found that highest amount of anthocyanins was measured at lowest barrel temperature (100°C), highest moisture content (28%), lowest screw speed and the highest flow rate (Hirt et al., 2014).

Table 6.16 : Total flavonoid and total anthocyanin contents of HACS and CGEP substituted samples extruded at different temperatures.

	T($^{\circ}\text{C}$)					
	90	120	150	90	120	150
	TAC- (mg Cy-3-glc/100 g DW)			TF (mg QE/ 100 g DW)		
GA1	25.1 $\pm$ 3.7ax	24.5 $\pm$ 8.4ax	22.5 $\pm$ 1.6ax	1970.6 $\pm$ 198.0ax	1836.3 $\pm$ 120.0ax	1499.8 $\pm$ 26.6ax
GA2	25.4 $\pm$ 5.2ax	22.1 $\pm$ 3.7ax	22.3 $\pm$ 3.7ax	1958.5 $\pm$ 22.3ax	1560.4 $\pm$ 52.0ay	1492.6 $\pm$ 134.7ay
GA3	22.1 $\pm$ 4.0ax	21.6 $\pm$ 1.4ax	25.4 $\pm$ 2.5ax	1945.5 $\pm$ 28.8ax	1890.5 $\pm$ 103.9ax	1686.9 $\pm$ 53.9ax

Different letters (a–d) within the same column, and different letters (x–y) within the same row differ significantly ($p < 0.05$). Data given is the average of duplicate analysis $\pm$ standard deviations.

A1: bread flour control; A2: bread flour substituted with HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

Moreover, heat sensitivity of Concord grapes was mentioned previously when pigment losses in Concord grape anthocyanins were reported to be increased from 32% to 82%, as their process temperature has raised from 77 to 121°C (Sastry and

Tischer, 1952). Khanal et al (2009) observed about 120% increase in monomer contents of grape pomace at die temperature of 170°C and screw speed of 200 rpm, even though they observed a significant decrease (18-53%) in total anthocyanins in pomace during extrusion.

Table 6.17 : % Loss of phenolics, antioxidant activity and flavonoids in HACS and CGEP substituted extruded samples.

	Extrusion Temperature (T°C)											
	90				120				150			
Loss (%)												
	TP	ABTS	DPPH	TF	TP	ABTS	DPPH	TF	TP	ABTS	DPPH	TF
A1	65	76	28	n.d	81	78	45	n.d	81	80	54	n.d
A2	67	80	64	n.d	80	81	56	n.d	83	79	54	n.d
A3	68	79	57	n.d	82	81	65	n.d	84	80	60	n.d
GA1	18	46	12	-13	24	49	22	-5	37	61	35	14
GA2	22	49	16	-12	38	58	34	11	41	58	36	15
GA3	26	52	29	-11	36	58	41	-8	34	61	50	4

A1: bread flour control; A2: bread flour substituted with 10% HACS; A3: bread flour substituted with 20% HACS; GA1: bread flour substituted with 7% CGEP; GA2: bread flour substituted with 10% HACS and 7% CGEP; GA3: bread flour substituted with 20% HACS and 7% CGEP.

% Losses in total phenolic contents of CGEP added extruded samples were around 22, 33 and 37 % for 90, 120 and 150°C die temperatures, respectively. HACS addition seemed to affect the phenolic losses at 120 and 150°C extrusion temperatures, while having no effect at 90°C.

6.3.3.5 Total phenolics and anthocyanin profiles of CGEP and HACS substituted extrudates

It was observed that the extrusion temperature has significant effect on the phenolic and anthocyanin profile of extrudates.

According to the results given in Table 6.18, gallic acid and vanillic acid were the major phenolic acids found in extruded samples without CGEP.

For samples with CGEP, gallic acid, in addition to sinapic, syringic, chlorogenic and *p*-coumaric acids were found at concentrations more than 10 µg/100 g DW. Epigallocatechin and catechin hydrate were also compounds found in moderate levels.

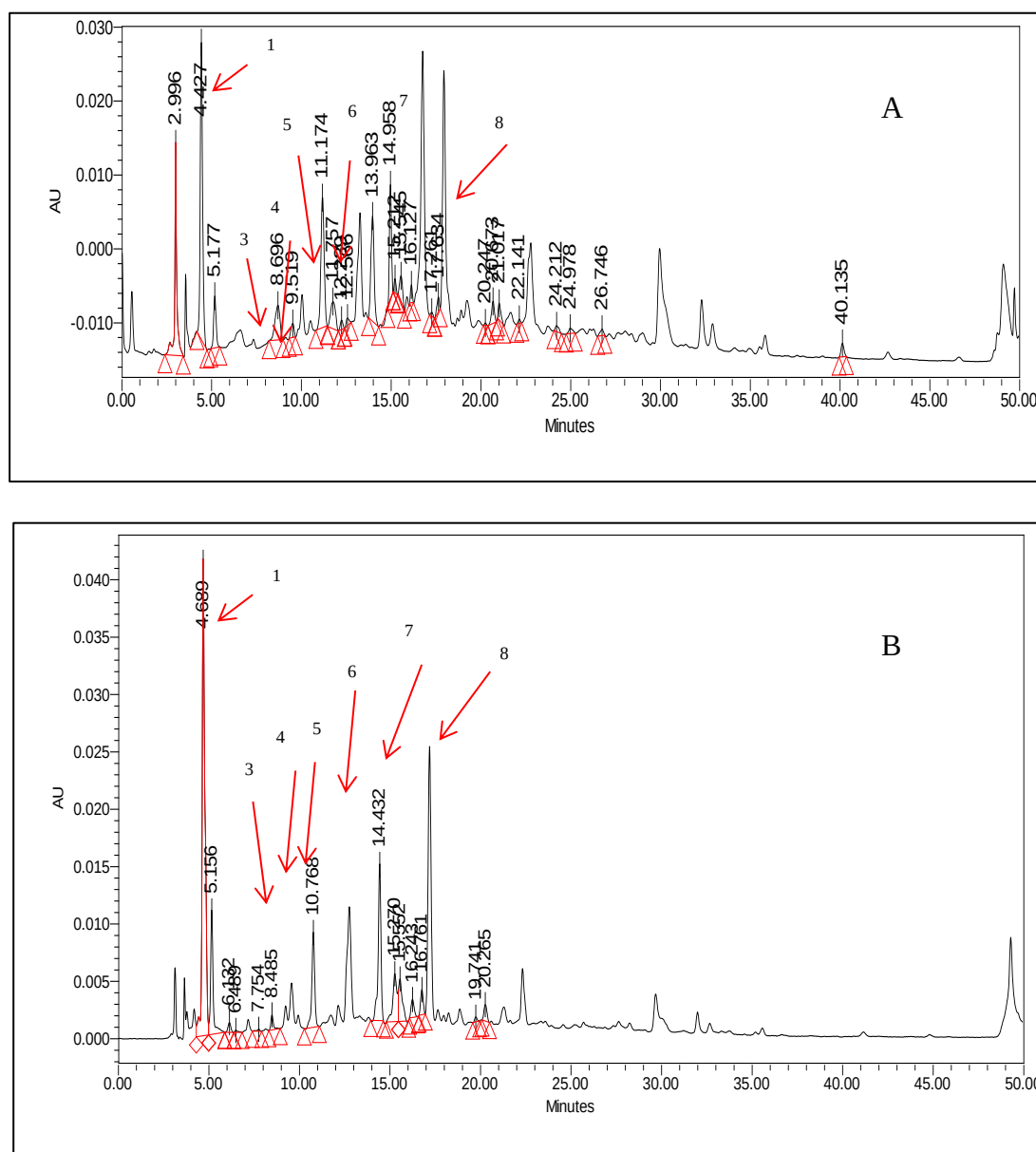
Gallic acid content of the unprocessed flour with CGEP (GA1) was 48.75 µg/100 g DW. During extrusion of samples at 150°C and 120°C with HACS substitutions, gallic acid content increased. In consistent with the present finding, increase in gallic acid content was also observed when grape seed flour extract was heated at 120 °C for 90 minutes (Ross et al., 2011). Moreover, Chamorro et al (2009) also measured an increase in gallic acid (71%), gallocatechin (100%) and epicatechin gallate (129%) in grape pomace samples after autoclaving at 100° for 15, 30 and 60 minutes. Release of gallic acid was related with the excision of the gallate group attached to the C-ring of the flavonoids or from the hydrolysis of gallotannins present in grape pomace (Chamorro et al., 2009). Similar release of gallic acid in tea by the effect of heat and oxygen was also detected (Wang et al., 2000). However, no significant increases in concentrations of epigallocatechin, catechin hydrate, epicatechin and epicatechin gallate (before extrusion 12.23, 11.35, 19.61 and 1.99 µg/100 g DW, respectively) were detected after extrusion as proposed in another study conducted with grape pomace (Chamorro et al., 2009).

In no-CGEP added samples, sinapic acid (at 150°C), gallic acid (120 and 90°C), and vanilic acid (90°C) increased with HACS substitutions, whereas other components generally exerted either no change or some losses. In samples with CGEP on the other hand, gallic acid content revealed significant increases by HACS substitution, at 150°C and 120°C while no effect was observed at extrusion temperature of 90°C.

Sensoy et al. (2005) revealed that extrusion has changed only the polar compounds, whereas roasting changed the structure of both polar and non-polar compounds. Mechanical energy applied by the extruder has been proposed as a factor on the formation of some complexes between flour components while also a factor on the degradation of larger molecules such as starch (Sensoy et al., 2005).

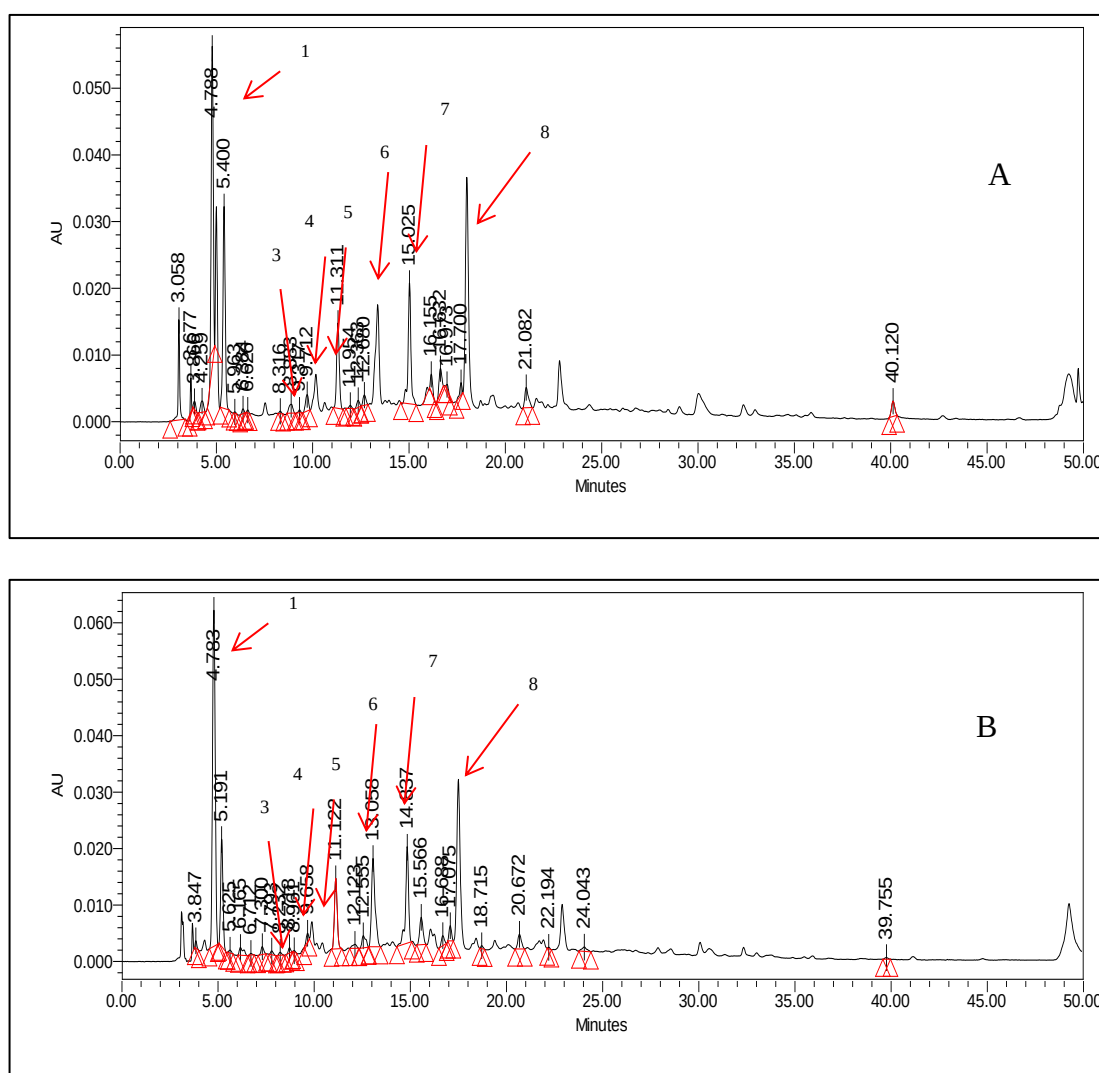
The role of HACS in this respect, might be similar with the binding of large molecular weight procyanidins to polysaccharides through hydrogen bonding and/or hydrophobic interactions as has been revealed for apple pomace procyanidins by other researchers (Le Bourvellec et al., 2004; Renard et al., 2001). At higher extrusion temperatures, the starch degradation mentioned by Sensoy et al. (2005) might have occurred.

Changes in anthocyanin and flavonols of extruded products with CGEP and HACS substitutions were given in Table 6.19. Among different samples, rise in extrusion temperature generally decreased the anthocyanin contents of samples.



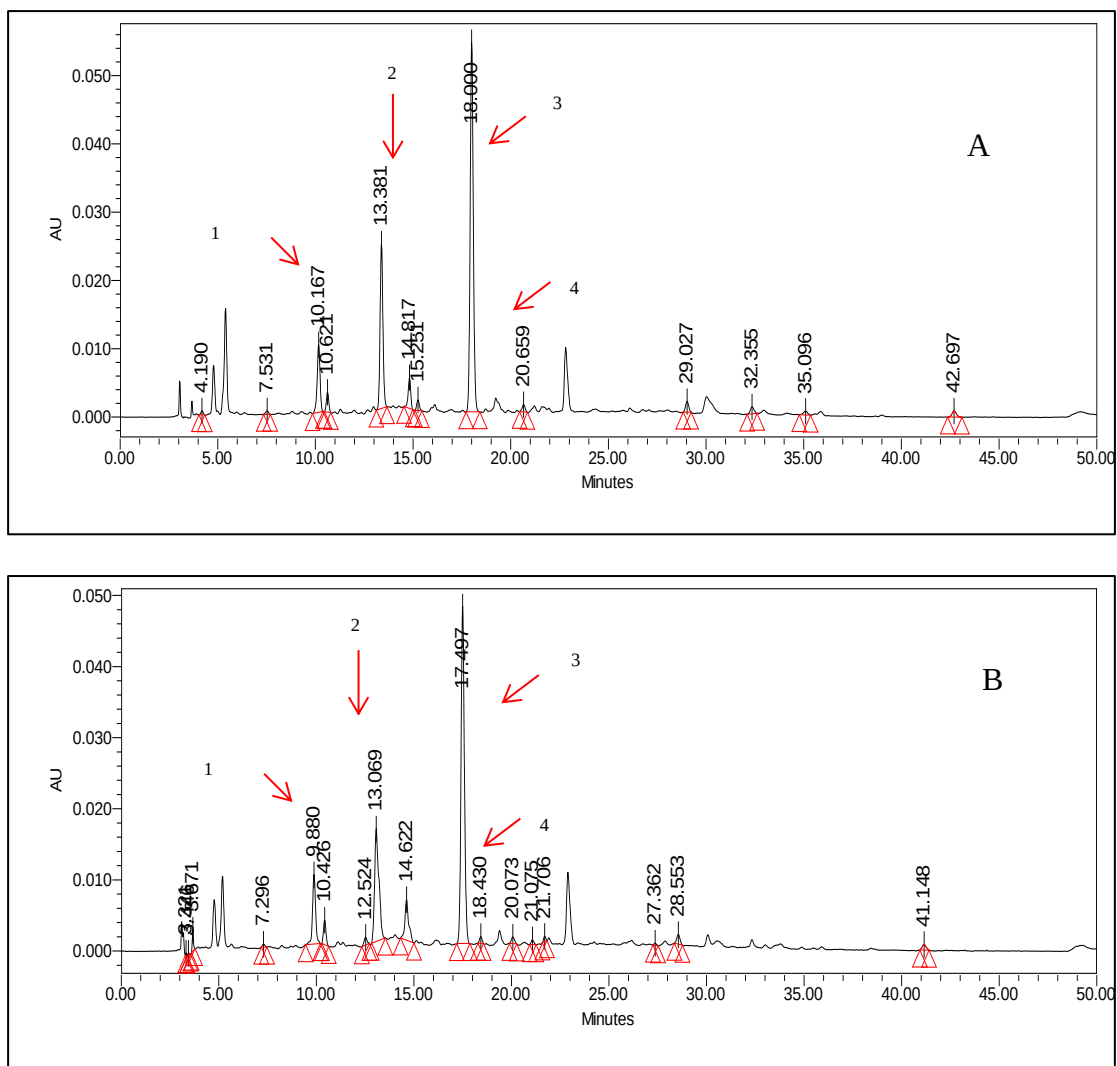
(1: Gallic acid, 2: Protocatechuic acid, 3: Epigallocatechin; 4: Catechin hydrate; 5: Epicatechin; 6: Syringic acid, 7: Vanillic acid, 8: n.d)

Figure 6.7: Representative HPLC-PDA chromatograms extracted at 280 nm for A: GA1 extruded at 120°C; B: GA3 extruded at 120°C.



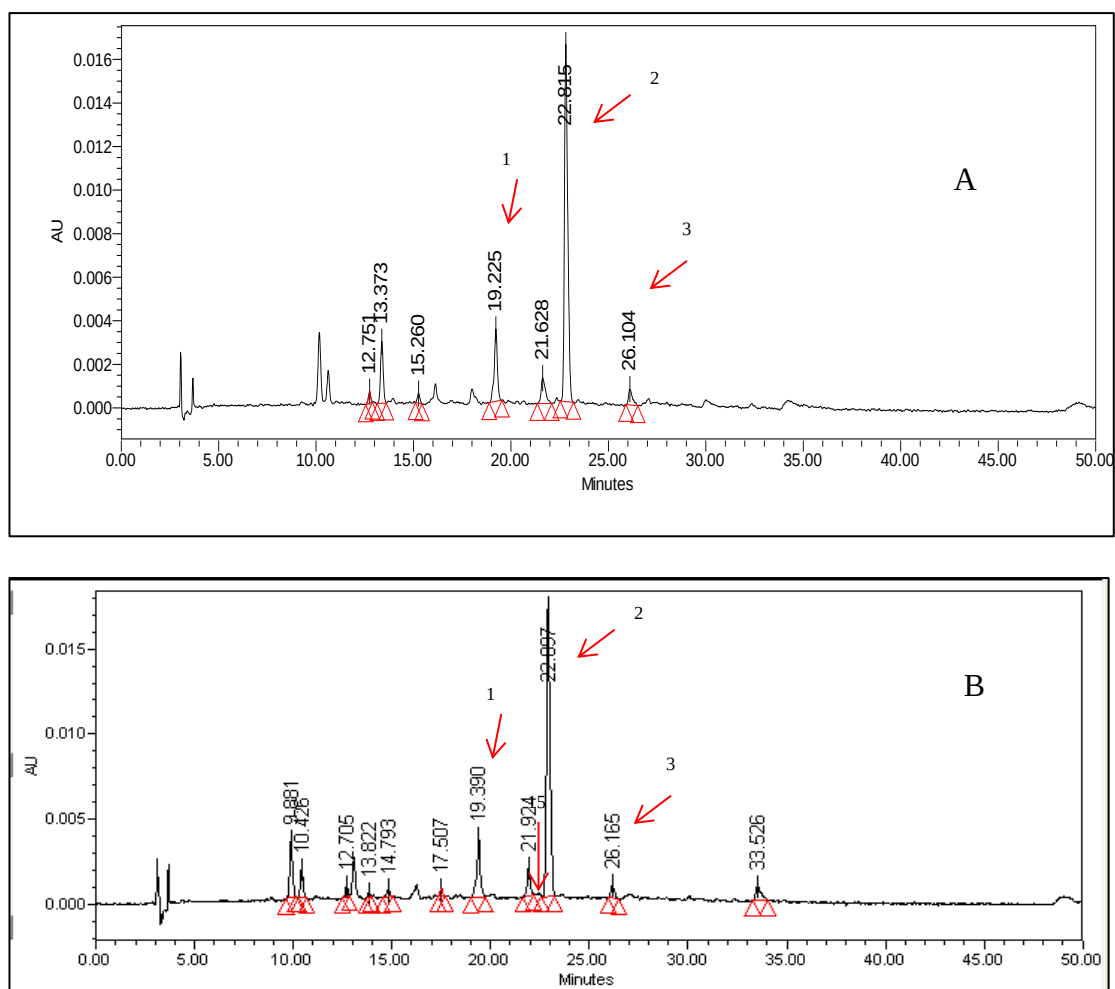
(1: Gallic acid, 2: Protocatechuic acid, 3: Epigallocatechin; 4: Catechin hydrate; 5: Epicatechin; 6: Syringic acid, 7: Vanilic acid, 8: n.d)

Figure 6.8: Representative HPLC-PDA chromatograms extracted at 280 nm for A: GA1 extruded at 150°C; B: GA3 extruded at 150°C.



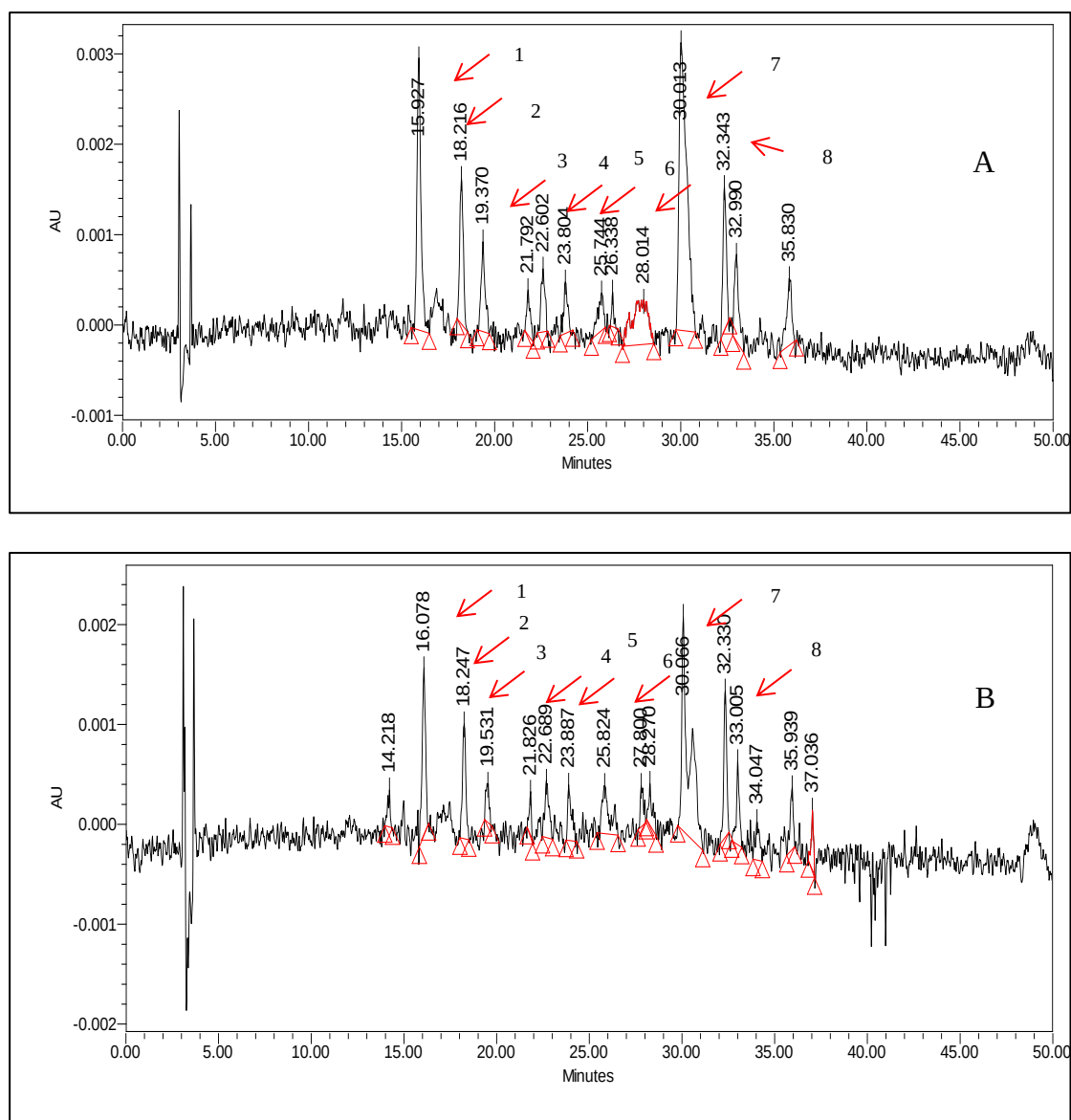
(1: Chlorogenic acid, 2: Caffeic acid, 3: P-coumaric acid, 4: Ferulic acid)

Figure 6.9: Representative HPLC-PDA chromatograms extracted at 312 nm for A: GA1 extruded at 150°C; B: GA3 extruded at 150°C.



(1:Quercetin-3-beta-glucoside; 2: Myricetin; 3: Rutin)

Figure 6.10: Representative HPLC-PDA chromatograms extracted at 360 nm for A: GA1 extruded at 150°C; B: GA3 extruded at 150°C.



(1: Delphinidin-3-glucoside; 2: Cyanidin-3-o-glucoside; 3: Cyanidin-3,5-d,glucoside; 4: delphinidin-3,5-diglucoside; 5: delphinidin; 6: cyanidin; 7: malvidin; 8: malvidin-3-o-glucoside)

Figure 6.11: Representative HPLC-PDA chromatograms extracted at 520 nm for A: GA1 extruded at 150°C; B: GA3 extruded at 150°C.

HACS caused various effects at different process conditions in samples with CGEP. At 90°C barrel temperature, the amount of anthocyanin were found to be independent from HACS and no particular decreasing or increasing effect on anthocyanin contents was evident. For extrusion temperature of 120°C, on the other hand, almost all anthocyanin compounds displayed the same behavior and cyanidin-3,5-diglucoside, delphinidin-3,5-diglucoside, quercetin-3- β -glucoside and aglycones delphinidin, cyanidin, malvidin and malvidin-3-o-glucoside contents decreased significantly as the HACS substitution level increased.

At 150 °C, although anthocyanidins seemed unaffected, increase in HACS substitution level significantly decreased the concentrations of anthocyanin mono glucosides (delphinidin-3-o-glucoside, cyanidin-3-o-glucoside, and malvidin-3-o-glucoside) found in extruded samples significantly. On the other hand, in diglucosides of cyanidin-3,5-diglucoside and delphinidin-3,5-diglucoside no such effect was notified. Previous findings by stated that the anthocyanin loss during extrusion was lower when the ratio of corn starch to pomace in an extruder mix was higher. Therefore, they suggested that starch might be acting like a barrier to preserve anthocyanins (White et al., 2010). Therefore similarly, HACS might have formed some kind of a barrier like structure with anthocyanins at 150°C.

In extruded products anthocyanin degradation has been attributed to the breaking of structures at high temperatures. Possibly high temperature could be opening either the pyrlum ring of anthocyanins and forming chalcone (Sadilova et al., 2007) or hydrolyzing the glycosidic moiety and forming aglycon (Sadilova et al.,2006) so that producing degradation products such as quercetin, phloroglucinaldehyde, and protocatechuic acid.

In unprocessed flour with CGEP (GA1) 0.73 μ g/100 g DW quercetin-3- β -glucoside, 73.5 μ g/100 g DW rutin, 1.05 μ g/100 g DW myricetin and 0.72 μ g/100 g DW quercetin were quantified. Only quercetin-3- β -glucoside content of the extruded samples was higher than that of unprocessed flour. Similarly, White et al. (2010) found that extruded cranberry pomace and corn samples contained 30-34% more total flavonols (including aglycones and glucosides myricetin and quercetin) than unprocessed flour. The enhanced flavonol compounds after extrusion was explained by the disruption of pomace matrix during extrusion to release the flavonols bound to cell walls (Hutzler et al., 1998).

Table 6.18 : Phenolic acids, flavan-3-ols, and resveratrol contents of HACS and CGEP substituted extrudate samples.

	A1			A2			A3			GA1			GA2			GA3		
	150	120	90	150	120	90	150	120	90	150	120	90	150	120	90	150	120	90
Protocat. acid	0.66 cx	0.48 cy	nd	nd	nd	0.37 cx	nd	nd	0.45 cx	7.15 bx	5.47 by	6.02 ay	7.64 ax	6.29 ay	5.95 ay	7.22 abx	6.32 ay	5.50 by
Gallic acid	20.30 bx	12.68 by	2.41 cz	nd	nd	22.78 bx	nd	16.81 by	24.34 bx	40.63 bx	43.34 ax	50.85 ax	50.05 bx	52.21 ax	43.44 abx	61.56 ax	47.46 ay	43.63 aby
Sinapic acid	4.77 cx	5.23 bcx	nd	6.21 cx	3.54 cy	nd	5.18 cx	6.07 bcx	5.81 bx	18.99 ay	20.46 ax	17.31 az	18.28 ax	20.76 ax	nd	13.78 bxy	11.10 ay	16.11 ax
Epigallo catechin	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.64 ax	11.40 ax	12.64 ax	13.65 ax	11.55 ax	12.60 ax	13.06 ax	10.66 ax	11.83 bx
Catechin hydrate	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.52 ax	8.47 ay	7.18 ay	8.07 bx	5.64 bx	10.20 ax	4.44 ax	2.20 cxy	1.70 by
Epicatechin	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.35 ay	0.36 ay	1.01 ax	0.37 axy	0.30 ay	0.52 bx	0.45 ax	0.39 ax	0.44 bx
Syringic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	18.59 az	21.46 ay	22.16 ax	20.27 ax	18.32 ax	21.16 bx	19.87 ax	17.76 ax	20.34 cx
Epicatechin galleate	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.55 by	4.44 ax	1.30 cy	2.58 ax	1.87 cx	2.11 bx	1.54 bx	3.43 bx	3.62 ax
Vanilic acid	7.04 bx	4.32 ay	3.83 ay	4.70 bx	4.10 aby	3.94 ay	2.81 by	3.33 by	5.16 ax	4.98 bx	3.64 bx	4.43 ax	5.03 bx	3.72 bx	4.56 ax	21.31 ax	4.05 aby	5.21 ay
Chlorogenic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	10.45 by	12.97 ax	13.25 ax	11.56 ax	9.48 by	13.31 ax	11.55 ax	9.38 bx	11.28 ax
Caffeic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.48 by	4.32 ax	4.42 ax	3.85 bx	3.16 by	4.44 ax	3.85 ax	3.13 bx	2.38 ax
p-coumaric acid	0.12 bx	0.06 bx	0.10 bx	0.12 bx	0.08 by	nd	0.05 bx	0.03 bx	0.07 bx	15.46 ax	15.66 ax	15.41 ax	15.74 ax	14.83 ax	15.40 ax	15.05 ax	13.39 ax	15.32 ax
Ferulic acid	0.16 cx	0.15 bx	0.16 dx	0.18 cx	0.15 bx	0.20 dx	nd	0.14 bx	0.08 ex	0.41 bcx	0.28 by	0.40 ax	0.78 ax	0.57 abx	0.48 bx	0.51 bx	1.07 ax	0.55 ax
t-resveratrol	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.57 ax	0.96 ax	0.43 bx	0.59 ax	0.58 abx	0.69 ax	0.56 ax	0.22 by	0.56 abx

Results are given as µg/100 g DW. Data given is the mean value of triplicate analyses. Values with different letters (a–e) of different extruded samples at each formulation and different letters (x–z) within the extruded sample among different temperatures differ significantly ($p < 0.05$). nd: not detected.

Table 6.19 : Flavonols and anthocyanins of HACS and CGEP substituted extrudate samples.

	GA1			GA2			GA3		
	150	120	90	150	120	90	150	120	90
Quercetin-3- β -glucoside	1.46 ax	2.20 ax	0.98 ax	1.90 ax	1.21 ay	1.15 ay	1.89 ax	1.12 axy	1.09 ay
Rutin	67.86 ax	81.96 ax	85.66 ax	71.02 axy	61.29 cy	82.89 ax	72.72 ax	71.25 bx	76.78 bx
Myricetin	1.09 ax	0.55 ax	0.85 ax	0.63 ax	0.64 ax	0.53 ax	0.82 ax	1.09 ax	1.14 ax
Quercetin	nd	nd	0.93 ax	nd	nd	0.73 ax	nd	nd	1.15 ax
Delphinidin-3-o-glucoside	2.06 by	4.50 ax	4.53 ax	3.26 ax	3.00 bx	4.44 ax	0.85 cy	3.05 bxy	3.95 ax
Cyanidin-3-o-glucoside	1.09 by	2.94 ax	2.16 ax	1.36 ay	1.34 by	2.15 ax	0.79 cx	1.48 bx	2.01 bx
Cyanidin-3,5-diglucoside	1.34 aby	2.93 ax	2.71 ax	1.57 ay	1.79 by	3.00 ax	0.71 by	1.84 bxy	2.86 ax
Delphinidin-3,5-diglucoside	0.63 ax	1.37 ax	0.89 ax	0.48 az	0.77 by	1.04 ax	0.48 ay	0.70 bx	1.03 ax
Delphinidin	0.52 ay	2.02 ax	1.30 ax	0.50 ay	0.94 bxy	1.33 ax	0.56 ay	1.07 bx	1.30 ax
Cyanidin	0.64 ax	1.22 ax	0.99 ax	0.71 ax	0.58 bx	0.64 cx	0.96 ax	nd	0.80 by
Malvidin	0.15 ay	0.61 ax	0.35 bx	0.21 ay	0.40 ax	0.43 bx	0.29 ay	0.54 ay	2.14 ax
Malvidin-3-o-glucoside	5.63 aby	17.06 ax	11.84 ax	6.46 ay	7.24 b	12.19 ax	3.40 by	7.41 bxy	10.47 bx

Results are given as $\mu\text{g}/100\text{ g DW}$. Data given is the mean value of triplicate analyses. Values with different letters (a–e) of different extruded samples at each formulation and different letters (x–z) within the extruded sample among different temperatures differ significantly ($p < 0.05$). nd: not detected.

7. COMPARISON OF FUNCTIONAL PROPERTIES OF CGEP ADDED BREAD AND EXTRUDATE SAMPLES

For making comparisons bread crumb samples of GA1 (with only 7% CGEP substitution), GA3 (with 7% CGEP and 20% HACS substitution) and GP3 (with 7% CGEP and 13% WPI substitution) representing the highest WPI and HACS substitutions were chosen. These three samples were compared with extruded samples (at 150°C) of GA1 (with only 7% CGEP substitution), GA3 (with 7% CGEP and 20% HACS substitution) and GP3 (with 7% CGEP and 13% WPI substitution). Extruded samples were also chosen as representing the highest WPI and HACS substitutions.

7.1 Total Phenolics, Antioxidant Activity, Flavonoids and Anthocyanins

Total phenolic contents of HACS and WPI substituted bread crumb samples and extruded products were not found to be significantly different ($p>0.05$). However, when antioxidant activity by DPPH and ABTS radical scavenging methods were evaluated, bread samples were found to be higher than their counterpart extruded samples, although WPI substitution in extrudate samples caused a significant increase ($p<0.05$).

Total flavonoid contents for GA1 crumb sample was significantly higher than all other samples ($p<0.05$). After HACS or WPI substitutions flavonoid content of bread crumbs decreased about 50%. For samples extruded at 150°C on the other hand, flavonoid contents were better retained as about 20 % increase was observed.

WPI substitutions had detrimental effects on total anthocyanin content, whereas HACS act as an improver for the measured anthocyanin contents both for bread crumb and extruded product samples.

Table 7.1: Comparison of total phenolics, antioxidant activity, flavonoids and anthocyanins and phenolic profiles of selected bread and extruded samples.

	Bread Crumb Samples			Extrudate samples		
	GA1 (Only 7% CGEP)	GA3 (7% CGEP, 20% HACS)	GP3 (7% CGEP, 13% WPI)	GA1 (Only 7% CGEP)	GA3 (7% CGEP, 20% HACS)	GP3 (7% CGEP, 13% WPI)
TPC (mg GAE/ 100 g DW)	1480.5a	1428.4a	1602.3a	1492.3a	1556.7a	1483.1a
DPPH (mg TEAC/ 100 g DW)	1133.9a	1111.9a	1058.0a	965.7a	751.6b	970.3a
ABTS (mg TEAC/ 100 g DW)	2147.0a	2151.5a	2189.2a	1341.6b	1354.1b	2126.6a
TFC (mg QE/ 100 g DW)	2830.9a	1493.4bc	1399.8c	1499.8b c	1890.5b	1936.3b
TAC (mg Cy-3-glc/ 100 gDW)	15.5c	18.4bc	9.8d	22.5ab	25.4a	16.2c
Protocat. acid	6.74ab	6.15ab	6.97ab	7.15a	7.22a	3.07b
Gallic acid	49.56ab	53.52ab	51.27ab	40.63b	61.56a	48.25ab
Sinapic	10.53bc	9.33c	14.51ab	18.99a	13.78abc	11.76bc
Epigallocatech	11.05b	11.33b	12.95a	12.64a	13.06a	9.16c
Catechin hyd.	8.21bc	9.60bc	6.33cd	11.52b	4.44d	36.65a
Epicatechin	5.32a	5.60a	6.86a	0.35b	0.45b	0.78b
Syringic acid	13.10a	5.89b	5.80b	1.59b	19.87a	15.73a
Epic. gallate	0.98a	1.61a	1.02a	1.55a	1.54a	0.82a
Vanillic	5.61c	4.50c	16.15b	4.98c	21.31b	31.97a
Chloro. acid	1.78d	4.15c	12.58a	10.45b	11.55ab	2.78cd
Caffeic acid	0.59d	1.38c	4.19a	3.48b	3.85ab	0.93cd
<i>p</i> -coum. acid	12.45a	13.61a	14.74a	15.46a	15.05a	12.26a
Ferulic acid	0.53ab	0.67ab	0.84a	0.41b	0.51ab	0.40b
<i>t</i> -resveratrol	0.39b	0.33b	0.71a	0.57ab	0.56ab	0.36b
Q3G	1.15a	1.13a	1.56a	1.46a	1.89a	1.67a
Rutin	57.23a	60.47a	62.91a	67.86a	72.72a	58.80a
Myricetin	2.65a	1.99ab	2.58a	1.09bc	0.82c	0.88c
Quercetin	4.12a	3.59a	3.85a	nd	nd	nd
D3G	2.33a	2.52a	2.27a	2.06a	0.85b	1.65b
Cy3G	0.95a	1.02a	1.09a	1.09a	0.79a	0.84a
Cy3,5Di	1.65a	1.98a	1.83a	1.34ab	0.71b	1.33ab
D3,5Di	0.41b	0.55ab	0.72a	0.63a	0.48ab	nd
Delphinidin	0.80a	0.73ab	0.65ab	0.52b	0.56b	nd
Cyanidin	0.94a	1.04a	0.78ab	0.64b	0.96a	0.68b
Malvidin	0.18a	0.68a	0.62a	0.15a	0.29a	nd
M3G	7.07a	7.84a	7.07a	5.63ab	3.40b	5.39ab

Catechin hydrate: Catechin hyd.; Epicatechin gallate: Epic. gallate; *p*-coumaric acid: *p*-coum. acid; Chlorogenic acid: Chloro. acid; Quercetin-3- β -glucoside: Q3G; Delphinidin-3-*o*-glucoside: D3G; Cyanidin-3-*o*-glucoside: Cy3G; Cyanidin-3,5-diglucoside: Cy3,5Di; Delphinidin-3,5-diglucoside: D3,5Di; Malvidin-3-*o*-glucoside: M3G

TP: Total phenolics; DW: Dry weight; TEAC: Trolox Equivalent Antioxidant Capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,20-azinobis-3-ethylbenzo-thiazoline-6-sulphonic acid diammonium salt; GAE: gallic acid equivalence. TF: Total flavonoids; TAC: Total anthocyanin content; QE: quercetin equivalence; Cy-3-glc: cyanidin-3-glucoside

7.2 Comparison of Individual Phenolic Compounds Phenolic Compounds

For most of the phenolic compounds except for gallic acid, syringic acid, ferulic acid, flavan-3-ol epicatechin and flavonols, extruded sample (GA1) without any HACS or WPI substitution was richer than its counterpart bread crumb sample GA1. GA1 was also significantly higher in sinapic acid, epigallocatechin, chlorogenic acid, and caffeic acid when compared to the bread crumb sample with the same formulation. Anthocyanin compounds were better retained in bread samples. Variable results were obtained for phenolic compounds which might be related with their structural differences behaving differently under certain process conditions as reported previously (Brennan et al., 2010).

HACS substitution during extrusion increased gallic acid, syringic acid and vanillic acid significantly in comparison to the extrudate samples with no HACS or WPI. Whereas in the same sample of bread it was not a very good alternative as syringic acid and caffeic acid contents were found as significantly lower.

On the other hand use of WPI substitution generally decreased the phenolic acids and anthocyanins in extrudates, while giving better results with bread samples.

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8. CONCLUSIONS AND RECOMMENDATIONS

Adding a rich bioactive source; Concord grape extract powder alone and together with amylose and protein to see their interactions and effects on the quality and functional properties of two common cereal products, bread and extruded snack gave significant results.

Concord grape extract powder substitution has been highly detrimental on the bread quality, specifically in terms of bread quality parameters of loaf volume, loaf specific volume and crumb firmness. Use of HACS with CGEP increased the quality losses in bread system, since the damage of gluten structure was higher in those samples. On the other hand, pertaining the quality of CGEP added bread samples was possible by WPI. Since the protein supplementation had positive effect on gluten network which was beneficial for the bread quality parameters. Generally no change in quality of the extrudates was detected by CGEP substitution. Those products' quality properties were highly affected by extrusion die temperatures. Specifically extrusion at 150°C caused some losses in product quality parameters.

The results on functional properties of raw ingredients revealed that hard wheat bread flour became a very rich raw material after CGEP substitution, since all functional parameters (total phenolics, antioxidant activity and flavonoids) displayed an increase at least around 330% with respect to hard wheat flour. According to the results on the functional properties of crust, crumb and dough fractions of bread sample with CGEP and WPI, all functional properties increased significantly by means of CGEP substitution. Loss of phenolics after baking was lower in CGEP substituted samples in comparison to control samples without CGEP. WPI substitution decreased the extent of phenolic loss in samples without CGEP; however WPI substitution made no significant changes in CGEP substituted samples. Increasing levels of WPI substitution decreased the total flavonoids in crumb and dough significantly ($p < 0.05$), while the decline in the crust was insignificant ($p > 0.05$). Gallic acid and vanillic acid were found to increase significantly (around 2-

fold) after baking. WPI substitution level did not make any certain effects on the phenolic compounds found in crust, crumb and dough of no-CGEP added samples. Gallic acid was also dominant phenolic acid in CGEP substituted bread samples but after baking, a considerable amount (app. 20%) of loss was detected in CGEP added samples. For rutin and quercetin-3- β -glucoside, increase in WPI level had a preserving effect. On the other hand, significant decreases in anthocyanidins and mono glucoside anthocyanins of delphinidin-3-o-glucoside, delphinidin, cyanidin-3-o-glucoside, and cyanidin-3,5-diglucoside in particularly crust of bread samples were evident, although the measured levels were higher generally, in comparison to extrudate samples.

In samples with HACS and CGEP, HACS substitution generally caused no significant changes ($p>0.05$) in measured phenolic content of crumb and crust, while making a significant decrease in the dough ($p<0.05$). The amount of loss of phenolics after baking in no-CGEP added samples was significantly higher than CGEP substituted samples ($p<0.05$). HACS addition did not have any significant role on the remaining phenolic levels for all of the samples. HACS substitution made no significant change in the measured phenolics of no-CGEP added breads. Generally crusts had lower amounts of anthocyanin compounds, as temperature during baking was the highest one among different parts. On the other hand; HACS substitution seemed to have no effect on the remaining flavonols and anthocyanins. Only exception was malvidin, since the malvidin content increased with the increase in HACS substitution.

As a result of this study, addition of CGEP increased the total phenolic content of extrudates about 10-fold, whereas for bread it provided 10-14 fold increase with respect to no-CGEP added products. So it can be said that addition of CGEP increased the functionality of the cereal products in a significant manner. However WPI substitution besides CGEP gave better quality in bread samples whereas for extrudates CGEP addition had not so much detrimental effect on the quality. WPI level of 6.5% seemed to preserve the bioactive compounds in a better way with respect to other levels of substitution. Whereas for HACS substitutions no specific was evident for different substitution levels. For extrusion temperatures studied we suggest 120°C which preserved CGEP and related functional properties besides maintaining the extrudate quality.

Use of CGEP is suggested for its functional properties and providing acceptable quality in both baked and extruded bakery products. We recommend the use of only CGEP in extrudates whereas for bread it should be used with WPI or other protein ingredients to have better product quality. The mechanisms behind the interaction of bioactive compound with basic cereal components need further evaluation both in model systems as well as in different bakery products.

REFERENCES

- AACC International.** (2000). *Approved methods of the AACC, 10th ed.* St. Paul, MN: American Association of Cereal Chemists International.
- Abdellatif Mohamed, A., Xu, J. & Singh, M.** (2010). Yeast leavened banana-bread: Formulation, processing, colour and texture analysis. *Food Chemistry*, 118, 620–626.
- Altan, A., McCarthy, K. L. & Maskan, M.** (2009). Effect of extrusion cooking on functional properties and in vitro starch digestibility of barley-based extrudates from fruit and vegetable by-products. *Journal of Food Science*, 74(2), E77-86.
- Alvarez-Martinez, L., Kondury, K. P. & Harper, J. M.** (1988). A general model for expansion of extruded products. *Journal of Food Science*, 53(2), 609–615.
- Amanor-Boadu, V., Boland, M., Barton, D., Anderson, B. & Henahan, B.** (2013). National Grape Cooperative Association. Retrieved: http://www.agmrc.org/media/cms/nationalgrapecooperative_F58D9D7E0352B.pdf . 17.12.2014.
- Anon.** (2011). The Concord Grape Association. The history of Concord grapes, <http://www.concordgrape.org/>. Retrieved 21 November 2014.
- Asare, E. K., Sefa-Dedeh, S., Sakyi-Dawson, E. & Afoakwa, E. O.** (2004). Application of response surface methodology for studying the product characteristics of extruded rice-cowpea-groundnut blends. *Internal Journal of Food Sciences and Nutrition*, 55, 431–439
- Ashwell M.** (2002). *Concepts of functional foods: A proposal for a scientific base for claims.* Brussels, Belgium: ILSI Press Europe.
- Beta, T. & Corke, H.** (2004). Effect of ferulic acid and catechin on sorghum and maize starch pasting properties. *Cereal Chemistry*, 81, 418–422.
- Blanche, S. & Sun, Z.** (2004). Physical characterization of starch extrudates as a function of melting transitions and extrusion conditions. *Advances in Polymer Technology*, 23(4), 277–290.
- Bourne M. C.** (1982). *Food texture and viscosity.* New York: Academic Press, pp. 44–117.
- Borkowski, T., Szymusiak, H., Gliszczyńska-Swigło, A. & Tyrakowska, B.** (2005). The effect of 3-O- β -glucosylation on structural transformations of anthocyanidins. *Food Research International*, 38, 1031–1037.

- Borrelli RC, Mennella C, Barba F, Russo M, Russo GL, Krome K, Erbersdobler HF, Faist V. & Fogliano V.** (2003). Characterization of coloured compounds obtained by enzymatic extraction of bakery products. *Food Chemistry and Toxicology*, 41, 1367–74.
- Brennan, C., Brennan, M., Derbyshire, E. & Tiwari, B.K.** (2011). Effects of extrusion on the polyphenols, vitamins and antioxidant activity of foods. *Trends in Food Science and Technology*, 22, 570-575.
- Burin, V. M., Ferreira-Lima, N. E., Panceri, C. P. & Bordignon-Luiz, M. T.** (2014). Bioactive compounds and antioxidant activity of *Vitis vinifera* and *Vitis labrusca* grapes: Evaluation of different extraction methods. *Microchemical Journal*, 114, 155–163.
- Caltinoglu, C., Tonyali, B. & Sensoy, I.** (2014). Effects of tomato pulp addition on the extrudate quality parameters and effects of extrusion on the functional parameters of the extrudates. *International Journal of Food Science and Technology*, (49)2, 587–594.
- Camire, M. E., Dougherty, M. P. & Briggs, J. L.** (2007). Functionality of fruit powders in extruded corn breakfast cereals. *Food Chemistry*, 101, 765–770.
- Careri, M., Corradini, C., Elviri, L., Nicoletti, I. & Zagnoni, I.** (2003). Direct HPLC analysis of quercetin and trans-resveratrol in red wine, grape, and winemaking byproducts. *Journal of Agricultural and Food Chemistry*, 51, 5226-5231.
- Castellarin, S. D., Bavaresco, L., Falginella, L., Gonçalves, M. I. V. Z. & Di Gaspero, G.** (2012). Phenolics in grape berry and key antioxidants. In: S. Delrot, M. Chaves & H. Gerós (Eds.), *The biochemistry of the grape berry* (pp. 89-110). Bussum, The Netherlands: Bentham Science Publishers
- Celik, H., Kose, B. & Cangı, R.** (2008). Determination of Fox grape genotypes (*Vitis labrusca* L.) grown in Northeastern Anatolia. *Horticultural Sciences (Prague)*, 35(4), 162–170.
- Chang, Y. H. & Ng, P. K. W.** (2009). Effects of extrusion process variables on extractable ginsenosides in wheat-ginseng extrudates. *Journal of Agricultural and Food Chemistry*, 57, 2356–2362.
- Chanvrier, H., Appelqvist, I. A. M., Bird, A. R., Gilbert, E., Htoon, A. & Li, Z.** (2007). Processing of novel elevated amylose wheats: functional properties and starch digestibility of extruded products. *Journal of Agricultural and Food Chemistry*, 55, 10248–10257
- Chaunier, L., Della Valle, G. & Lourdin, D.** (2007). Relationships between texture, mechanical properties and structure of cornflakes. *Food Research International*, 40, 493–503.
- Colonna, P., Tayeb, J. & Mercier, C.** (1989). Extrusion cooking of starch and starchy products. In P. L. Mercier & J. M. Harper (Eds.), *Extrusion cooking* (pp. 247-319). St. Paul, MN: American Association of Cereal Chemists.

- Day, L., Augustin, M. A., Batey, I. L. & Wrigley, C. W.** (2006). Wheat-gluten uses and industry needs. *Trends in Food Science & Technology*, 17, 82–90.
- Dell Agli, M., Galli, G.V., Vrhovsek, U., Mattivi, F. & Bosisio, E.** (2005). In vitro inhibition of human cGMP-specific phosphodiesterase-5 by polyphenols from red grapes. *Journal of Agricultural and Food Chemistry*, 53, 1960–1965.
- Della Valle, G., Vergnes, B., Colonna, P. & Patria, A.** (1997). Relations between rheological properties of molten starches and their expansion behavior in extrusion. *Journal of Food Engineering*, 31, 277-296.
- Dlamini, N. R., Taylor, J. R. N. & Rooney, L. W.** (2007). The effect of sorghumtype and processing on the antioxidant properties of African sorghum-based foods. *Food Chemistry*, 105(4), 1412-1419.
- Eliasson, A. & Gudmundsson, M.** (2006). Starch: Physicochemical and functional aspects. In A. Eliasson (Ed.), *Carbohydrates in food, 2nd Edition*, (pp. 391–469). Baco Raton: CRC Press..
- Englyst, H. N., Kingman, S. M. & Cummings, J. H.** (1992). Classification and measurement of nutritionally important strach fractions. *European Journal of Clinical Nutrition*, 46, S33–S50.
- Falchi, M., Bertelli, A., Scalzo, R. L., Morassut, M., Morelli, R., Das, S., Cui, J. H. & Das, D. K.** (2006). Comparison of cardioprotective abilities between the flesh and skin of grapes. *Journal of Agricultural and Food Chemistry*, 54, 6613–6622
- FAOSTAT** (Food and Agriculture Organizaton of the United States, Statistics Division),(2014.),<http://faostat.fao.org/site/567/DesktopDefault.aspxPa geID =567 #ancor>. Son erişim tarihi: 17.12.2014.
- Faubion, J. M. & Hoseney, R. C.** (1989). The viscoelastic properties of wheat flour doughs. In H. Faridi & J. M. Faubion (Eds.), *Dough Rheology and Baked Product Texture*. New York: AVI.
- Fraige, K., Pereira-Filho, E. R. & Carrilho, E.** (2014). Fingerprinting of anthocyanins from grapes produced in Brazil using HPLC-DAD-MS and explanatory analysis principal component. *Food Chemistry*, 145, 395-403.
- Fremont, L., Belguendou, L. & Delpal, S.** (1999). Antioxidant activity of resveratrol and alcohol-free wine polyphenols realted to LDL oxidation and polyunsaturated fatty acids. *Life Sciences*, 64, 2511-2521.
- Flamini, R., Mattivi, F., De Rosso, M., Arapitsas, P. & Bavaresco, L.** (2013). Advanced knowledge of three important classes of grape phenolics: Anthocyanins, stilbenes and flavonols. *International Journal of Moleculaer Sciences*, 14, 19651-19669.
- Goesaert, H., Brijs, K., Veraverbeke, W. S., Courtin, C. M., Gebruers, K. & Delcour, J. A.** (2005). Wheat flour constituents: how they impact bread quality, and how to impact their functionality. *Trends in Food Science and Technology*, 16, 12–30.

- Gollücke, A. P. B., Catharino, R. R., Souza, J. C., Eberlin, M. N. & Tavares, D. Q. (2009).** Evolution of major phenolic components and radical scavenging activity of grape juices through concentration process and storage. *Food Chemistry*, 112, 868–873.
- Guha, M., Ali, S. Z. & Bhattacharya, S. (1998).** Effect of barrel temperature and screw speed on rapid viscoanalyser pasting behaviour of rice extrudate. *International Journal of Food Science and Technology*, 33, 259–266.
- Hathorn, C. S., Biswas, M. A., Gichuhi, P. N. & Bovell-Benjamin, A. C. (2008).** Comparison of chemical, physical, micro-structural, and microbial properties of breads supplemented with sweetpotato flour and high-gluten dough enhancers. *LWT- Food Science and Technology*, 41, 803–815.
- Hayta, M., Özüğür, G., Etgü, H. & Şeker, İ. T. (2014).** Effect of grape (*vitis vinifera* L.) Pomace on the quality, total phenolic content and anti-radical activity of bread. *Journal of Food Processing and Preservation*, 38, 980–986.
- Hayta, M. & Özüğür, G. (2011).** Phytochemical fortification of flour and bread. In R. Preedy, R. R. Watson & V. B. Patel (Eds.), *Flour and breads and their fortification in health and disease prevention* (pp. 293-299). V. New York: Academic Press.
- Hirth, M., Leiter, A., Beck, S. M. & Scuchmann, H. P. (2014).** Effect of extrusion cooking process parameters on the retention of bilberry anthocyanins in starch based foods. *Journal of Food Engineering*, 125, 139-146.
- Hosseinian, F. S., Li, W. & Beta, T. (2008).** Measurement of anthocyanins and other phytochemicals in purple wheat. *Food Chemistry*, 109, 916–924
- Hung, P. V., Maeda, T. & Morita, N. (2006).** Waxy and high-amylose wheat starches and flours—characteristics, functionality and application. *Trends in Food Science and Technology*, 17, 448–456.
- Hu, L., Hsieh, F. & Huff, H. E. (1993).** Corn meal extrusion with emulsifier and soybean fibre. *LWT – Food Science and Technology*, 26, 544–551.
- Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S. & Radosavljevic, M. (1999).** Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry*, 76(5), 629-637.
- Kanae, O., Tomoko, K. & Yoshiaki, M. (2008).** Effect of lemon flavonoid on the properties of bread. *Japan Society of Cookery Science*, 41, 297-303.
- Karkle, E. L., Keller, L., Dogan, H. & Alavi, S. (2012).** Matrix transformation in fiber-added extruded products: Impact of different hydration regimens on texture, microstructure and digestibility. *Journal of Food Engineering*, 108, 171–182.
- Kim, J. H., Tanhehco, E. J. & Ng, P. K. W. (2006).** Effect of extrusion conditions on resistant starch formation from pastry wheat flour. *Food Chemistry*, 99, 718–723.

- Knorr, D., Heinz, V. & Buckow, R.** (2006). High pressure application for food biopolymers. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, 1764, 619–631.
- Labat, E., Morel, M. & Rouau, X.** (2000). Wheat gluten phenolic acids: occurrence and fate upon mixing. *Journal of Agricultural and Food Chemistry*, 48, 6280-6283.
- Lee, J. & Finn, C. E.** (2007). Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. *Journal of the Science of Food and Agriculture*, 87, 2665–2675.
- Lee, J. H. & Talcott, S. T.** (2004). Fruit maturity and juice extraction influences ellagic acid derivatives and other antioxidant polyphenolics in Muscadine grapes, *Journal of Agricultural and Food Chemistry*, 52, 188–195.
- Lei, F., Ji-Chun, T., Cai-Ling, S. & Chun, L.** (2008). RVA and farinograph properties study on blends of resistant starch and wheat flour. *Agricultural Sciences in China*, 7(7), 812-822.
- Manach, C., Scalbert, A., Morand, C. Rémésy, C. & Jimenez, L.** (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition*, 79, 727-747.
- Maskan, M. & Altan, A.** (2011). *Advances in food extrusion technology*. Boca Raton, Florida, USA: CRC Press. 396 pp.
- Mason, W. R. & Hoseney, R. C.** (1986). Factors affecting the viscosity of extrusion- cooked wheat starch. *Cereal Chemistry*, 63(5), 436-441.
- Mayer, A. S., Heinonen, M. & Frankel, E. N.** (1998). Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation. *Food Chemistry*, 61, 71-75.
- McDougall, G. J., Shpiro, F., Dobson, P., Smith, P., Blake, A. & Stewart, D.** (2005). Different polyphenolic components of soft fruits inhibit α -amylase and α -glucosidase. *Journal of Agricultural and Food Chemistry*, 53, 2760-2766.
- Merike Vaher, M., Matso, K., Levandi, T., Helmja, K. & Kaljurand, M.** (2010). Phenolic compounds and the antioxidant activity of the bran, flour and whole grain of different wheat varieties. *Procedia Chemistry*, 2, 76–82.
- Mildner-Szkudlarz, S., Zawirska-Wojtasiak, R., Szwengiel, A. & Pacynski, M.** (2011). Use of grape by-product as a source of dietary fibre and phenolic compounds in sourdough mixed rye bread. *International Journal of Food Science and Technology*, 46, 1485–1493.
- Montgomery D. C. & Runger G. C.** (2007). *Applied statistics and probability for engineers*. (4th ed.) Hoboken, NJ: Wiley.
- Moore, J., Luther, M., Cheng, Z. & Yu, L.** (2009). Effects of baking conditions, dough fermentation, and bran particle size on antioxidant properties of whole-wheat pizza crusts. *Journal of Agricultural and Food Chemistry*, 57, 832–839.

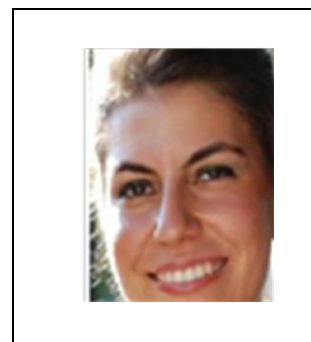
- Mulinacci, N., Ifri, F., Giaccherini, C., Innocenti, M., Andrenelli, L. & Canova, G.** (2008). Effect of cooking on the anthocyanins, phenolic acids, glycoalkaloids, and resistant starch content in two pigmented cultivars of *Solanum tuberosum* L. *Journal of Agricultural and Food Chemistry*, 56, 11830-11837.
- Mullen, W., Marks, S. C. & Crozier, A.** (2007). Evaluation of phenolic compounds in commercial fruit juices and fruit drinks. *Journal of Agricultural and Food Chemistry*, 55, 3148–3157.
- Munoz-Espada, A. C., Wood, K., Bordelon, V. B. & Watkins, B. A.** (2004). Anthocyanin quantification and radical scavenging capacity of Concord, Norton, and Marechal Foch grapes and wines. *Journal of Agricultural and Food Chemistry*, 52, 2200-2204.
- Noda, T., Tsuda, S., Mori, M., Takigawa, S., Matsuura-Endo, C. & Saito, K.** (2004). The effect of harvest dates on the starch properties of various potato cultivars. *Food Chemistry*, 86, 119–125.
- Núñez, M., Sandoval, A. J., Müller, A. J., Valle, G. D. & Lourdin, D.** (2009). In thermal characterization and phase behavior of a ready-to-eat breakfast cereal formulation and its starchy components. *Food Biophysics*, 4, 291–303.
- Pyler, E. J. & Gorton, L. A.** (2008). *Baking science and technology Volume 1: fundamentals and ingredients*. MO, USA: Sosland Publishing Co.
- Rayas-Duarte, P., Majewska, K. & Doetkott, C.** (1998). Effect of extrusion process parameters on the quality of buckwheat flour mixes. *Cereal Chemistry*, 75(3), 338–345.
- Robin, F., Dubois, C., Pineau, N., Labat, E., Théoduloz, C. & Curti, D.** (2012). Process, structure and texture of extruded whole wheat. *Journal of Cereal Science*, 56, 358-366.
- Robin, F., Théoduloz, C., Gianfrancesco, A., Pineau, N., Schuchmann, H.P. and Palzer, S.** (2011). Starch transformation in bran-enriched extruded wheat flour. *Carbohydrate Polymers*, 85, 65–74.
- Ryu, G. H. & Ng, P. K. W.** (2001). Effects of selected process parameters on expansion and mechanical properties of wheat flour and whole cornmeal extrudates. *Starch/Stärke*, 53, 147–154.
- Sidhu, J. S., Al-Saqer, J. & Al-Zenki, S.** (1997). Comparison of methods for the assessment of the extent of staling in bread. *Food Chemistry*, 58, 161–167.
- Singh, J., Dartois, A. & Kaur, L.** (2010). Starch digestibility in food matrix: a review. *Trends in Food Science & Technology*, 21, 168-180.
- Stojceska, V., Ainsworth, P., Plunkett, A. & İbanoğlu, Ş.** (2009). The effect of extrusion cooking using different water feed rates on the quality of ready-to-eat snacks made from food by-products. *Food Chemistry*, 114, 226–232.
- Swanson, R. B.** (2004). Bakery: Yeast-leavened breads. In J. S. Smith & Y. H. Hui (Eds.), *Food processing: principles and applications*. Iowa, USA: Blackwell Publishing.

- Tester, R. F. & Morrison, W. R.** (1990). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chemistry*, 67(6), 551-557.
- Van Soest, J. J. G. & Knooren, N.** (1998). Influence of glycerol and water content on the structure and properties of extruded starch plastic sheets during aging. *Journal of Applied Polymer Science*, 64(7), 1411-1422.
- Onyeneho, S. N. & Hettiarachchy, N. S.** (1992). Antioxidant activity of durum wheat bran. *Journal of Agricultural and Food Chemistry*, 40, 1496-1500.
- Pace-Asciak, C. R., Hahn, S. E., Diamandis, E. P., Soleas, G. & Goldberg, D. M.** (1995). The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. *Clinical Chemistry Acta*, 235, 207-219.
- Pastrana-Bonilla, E., Akoh, C.C., Sellappan, S. & Krewer, G.** (2003). Phenolic content and antioxidant capacity of Muscadine grapes. *Journal of Agricultural and Food Chemistry*, 51, 5497-5503
- Repo-Carrasco-Valencia, R., Pena, J., Kallio, H. & Salminen, S.** (2009). Dietary fiber and other functional components in two varieties of crude and extruded kiwicha (*Amaranthus caudatus*). *Journal of Cereal Science*, 49(2), 219-224.
- Rosell, C. M.** (2011). The science of doughs and bread quality. In: In V. R. Preedy, R. R. Watson & V. B. Patel (Eds.), *Flour and breads and their fortification in health and disease prevention* (pp. 3-13). New York NY: Academic Press.
- Sánchez-Moreno, C., Cao, G., Ou, B. & Prior, R. L.** (2003). Anthocyanin and proanthocyanidin content in selected white and red wines. Oxygen radical absorbance capacity comparison with nontraditional wines obtained from highbush blueberry. *Journal of Agricultural and Food Chemistry*, 51, 4889-4896.
- Sarawong, C., Schoenlechner R., Sekiguchi, K., Berghofer, E. & Ng, P.K.W.** (2014). Effect of extrusion cooking on the physicochemical properties, resistant starch, phenolic content and antioxidant capacities of green banana flour. *Food Chemistry*, 143, 33-39.
- Sarker, Md. Z. I., Yamauchi, H., Kim, S., Matsuura-Endo, C., Takigawai N. M. & Noda, N.** (2008). A farinograph study on dough characteristics of mixtures of wheat flour and potato starches from different cultivars. *Food Science and Technology Research*, 14(2), 211-216.
- Sensoy, I., Rosen, R. T., Ho, C. T. & Karwe, M. V.** (2005). Effect of processing on buckwheat phenolics and antioxidant activity. *Food Chemistry*, 99, 388-39.
- Shanmuganayagam, D., Warner, T. F., Krueger, C. G., Reed, J. D. & Folts, J. D.** (2007). Concord grape juice attenuates platelet aggregation, serum cholesterol and development of atheroma in hypercholesterolemic rabbits. *Atherosclerosis*, 190, 135-142.

- Shukitt-Hale, B., Carey, A., Simon, L., Mark, D. A. & Joseph, J. A.** (2006). Effects of Concord grape juice on cognitive and motor deficits in aging. *Nutrition*, 22, 295–302.
- Singletary, K. W., Stansbury, M. J., Giusti, M., Breemen, R. B. V., Wallig, M. & Rimando, A.** (2003). Inhibition of rat mammary tumorigenesis by concord grape juice constituents. *Journal of Agricultural and Food Chemistry*, 51, 7280–7286.
- Sluimer, P.** (2005). Principles of breadmaking: Functionality of raw materials and process steps. St. Paul, MN: American Association of Cereal Chemists.
- Stalmach, A., Edwards, C. A., Wightman, J. D. & Crozier, A.** (2011). Identification of (poly)phenolic compounds in Concord grape juice and their metabolites in human plasma and urine after juice consumption. *Journal of Agricultural and Food Chemistry*, 59, 9512–9522.
- Sun-Waterhouse, D., Sivam, A. S., Cooney, J., Zhou, J., Perera, C. O. & Waterhouse, G. I. N.** (2011). Effects of added fruit polyphenols and pectin on the properties of finished breads revealed by HPLC/LC-MS and Size-Exclusion HPLC. *Food Research International*, 44, 3047–3056.
- Vitrac, X., Bornet, A. L., Vanderlinde, R., Valls, J., Richard, T., Delaunay, J. C., Mérillon, J. M. & Teissédre, P. L.** (2005). Determination of stilbenes (δ -viniferin, trans-astringin, trans-piceid, cis- and trans-resveratrol, ϵ -viniferin) in Brazilian wines. *Journal of Agricultural and Food Chemistry*, 53, 5664–5669.
- Wang, R., Zhou, W. & Isabelle, M.** (2007). Comparison study of the effect of green tea extract (GTE) on the quality of bread by instrumental analysis and sensory valuation. *Food Research International*, 40, 470–479.
- Wang, R. & Zhou, W.** (2004). Stability of tea catechins in breadmaking process. *Journal of Agriculture and Food Chemistry*, 52(26), 8224–8229.
- Wesche-Ebeling, P. & Argaiiz-Jamet, A.** (2002). Stabilization mechanisms for anthocyanin: the case for copolymerization reactions. In J. Welti-Chanes, G. V. Barbosa-Canovasi & J. M. Aguilera (Eds.), *Engineering and food for the 21st century*. Roca Baton: CRC Press.
- White, B. L., Howard, L. R. & Prior, R. L.** (2010). Polyphenolic composition and antioxidant capacity of extruded cranberry pomace. *Journal of Agricultural and Food Chemistry*, 58, 4037–4042.
- Wu, Y., Chen, Z., Li, X. & Li, M.** (2009). Effect of tea polyphenols on the retrogradation of rice starch. *Food Reserach International*, 42, 221–225.
- Wu, X., Gu, L., Prior, R. L. & McKay, S.** (2004). Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 52, 7846–7856.

- Wu, X., Beecher, G. R., Holden, J. H., Haytowitz, D. B., Gebhardt, S. E. & Prior, R. L.** (2006). Concentration of anthocyanins in common foods in the United States and estimation of normal consumption. *Journal of Agricultural and Food Chemistry*, 54, 4069–4075.
- Xia, E., Deng, G., Guo, Y. & Li, H.** (2010). Biological activities of polyphenols from grapes. *International Journal of Molecular Sciences*, 11, 622–646.
- Xu B. & Chang, S. K. C.** (2008) Total phenolics, phenolic acids, isoflavones, and anthocyanins and antioxidant properties of yellow and black soybeans as affected by thermal processing. *Journal of Agricultural and Food Chemistry*, 56, 7165–7175.
- Yağcı, S. & Göğüş, F.** (2008). Response surface methodology for evaluation of physical and functional properties of extruded snack foods developed from food-by-products. *Journal of Food Engineering*, 86, 122–132.
- Zhu, F., Cai, Y., Sun, M. & Corke, H.** (2008). Effect of phenolic compounds on the pasting and textural properties of wheat starch. *Starch/Stärke*, 60, 609–616.
- Zhu, F., Cai, Y. Z., Sun, M. & Corke, H.** (2009). Effect of phytochemical extracts on the pasting, thermal, and gelling properties of wheat starch. *Food Chemistry*, 112, 919–923.
- Zhu, L., Shukri, R., Mesa-Stonestreet, N. J., Alavi, S., Dogan, H. & Shi, Y.** (2010). Mechanical and microstructural properties of soy protein – high amylose corn starch extrudates in relation to physiochemical changes of starch during extrusion. *Journal of Food Engineering*, 100, 232–238.

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PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

▪ **Tacer Caba, Z.**, Nilufer Erdil, D., Boyacıoğlu, M. H., and Ng, P.K.W., 2014: Evaluating the effects of amylose and grape extract powder substitution on physicochemical properties of extrudates produced at different temperatures. *Food Chemistry*, 157, 476–484.

▪ **Tacer Caba, Z.**, Nilufer Erdil, D., Boyacıoğlu, M.H., Ng, P.K.W. 2014. Evaluating the effects of protein addition to wheat flour extrudates enriched with

grape powder. ([Quality Assurance and Safety of Crops and Foods], accepted)

- **Tacer Caba, Z.**, Nilufer Erdil, D., Boyacioglu, M. H., Ng, P. K. W., 2014: Starch properties of wheat flour samples, with or without grape powder substitution, and their products. *Novel Approaches in Food Industry (NAFI) 2014*, May 26-29, 2014, İzmir, Turkey.
- **Tacer Caba, Z.**, Nilufer Erdil, D., Boyacioglu, M. H., Ng, P. K. W., 2013: Effects of amylose and hard wheat flour on measured phenolic content and antioxidant activity in bread substituted with Concord grape extract powder. *AACC International Annual Meeting*, September 29 - October 2, 2013, Albuquerque New Mexico, USA. *Cereal Foods World* 58:A71.
- **Tacer Caba, Z.**, Nilufer Erdil, D., Boyacioglu, M. H., Ng, P. K. W., 2013: Evaluating the changes in bread and dough substituted with concord grape extract powder (CGEP) and amylose. *IFT 2013 Annual Meeting & Food Expo*, July 13-16 2013, Chicago, Illinois, USA.
- **Tacer Caba, Z.**, Nilufer Erdil, D., Boyacioglu, M. H., Ng, P. K. W., 2013: Rheological and Textural Changes in Dough and Bread by Addition of Concord Grape Extract Powder (CGEP) and Wheat Protein Isolate (WPI). *EUROFOODCHEM VII*, May 7-10, 2013, İstanbul, Turkey.
- **Tacer Caba, Z.**, Nilufer Erdil, D., Boyacioglu, M. H., Ng, P. K. W., 2012: Antosiyaninlerin ekstrüde gıda matrisindeki diğer bileşenlerle etkileşimi ve ekstrüde ürünlerin fiziksel özelliklerine etkileri. *Türkiye 11. Gıda Kongresi*; October 10-12, 2012, Hatay, Turkey.
- **Tacer Caba, Z.**, Nilufer Erdil, D., Boyacioglu, M. H., Ng, P. K. W., 2012: Interactions between anthocyanins and cereal ingredients during extrusion. *AACC International Annual Meeting*, September 30 -October 3, 2012, Hollywood Florida, USA. *Cereal Foods World* 57:A72.

OTHER PUBLICATIONS, PRESENTATIONS AND PATENTS

- **Tacer Caba, Z.**, Nilufer Erdil, D., Ai, Yongfeng. 2015. Chemical Composition of Cereals and Their Products in Handbook of Food Chemistry; Ed. Peter Chi-Keung Cheung. Springer-Verlag Berlin Heidelberg. DOI 10.1007/978-3-642-41609-5_33-1.
- **Tacer Caba, Z.**, Nilufer Erdil, D., Ng, P. K. W., 2012: Determination of Gallic Acid in Model Systems Comprised of Either Gluten Protein or Corn Starch. *AACC International Annual Meeting*, September 30 -October 3, 2012, Hollywood, Florida, USA. *Cereal Foods World* 57:A72.
- **Tacer Caba, Z.**, Boyacıoğlu, D., Boyacioglu, M. H., 2012: Bioactive healthy components of bulgur. *International Journal of Food Sciences and Nutrition*, 63(2), 250–256.
- **Tacer, Z.**, Karaali, A., 2007: Şarapta coğrafi işaretlemeye temel oluşturabilecek otantisite analizleri. *Dünya Gıda Mart* 2007, 80 - 84.

- **Tacer Caba, Z.**, Nilüfer Erdil, D., Ng, P. K. W., 2012: Determination of Gallic Acid in Model Systems Comprised of Either Gluten Protein or Corn Starch. *AACC International Annual Meeting*, September 30 -October 3, 2012, Hollywood Florida, USA. *Cereal Foods World* 57:A72.
- **Tacer Caba, Z.**, Nilüfer Erdil, D., Boyacioglu, M. H., 2011: Current approaches on functional cereal products. *International Food Congress Novel Approaches In Food Industry*, May 26 – 29, 2011, Çeşme - İzmir, Turkey.
- Toydemir Otkun, G., **Tacer Caba, Z.**, Çapanoğlu Güven, E., Boyacıoğlu, D., 2011: A comparison of different antioxidant activity methods in fresh-cut rocket salad. *IFT 2011 Annual Meeting & Food Expo*, June 11-14, 2011, New Orleans, Louisiana, USA.
- **Tacer Caba, Z.**, Boyacioglu, M.H., Boyacioglu, D., 2010: Starch digestibility and glycemic index of bulgur. *IFT Annual Meeting & Food Expo.*, July, 17-20, 2010., Chicago, IL, USA.
- **Tacer Caba, Z.**, Boyacıoğlu, M. H., Boyacıoğlu, D., 2010: Glisemik indeks ölçümü için kullanılan in vitro yöntemler–Bir uygulama olarak bulgur. VII. *Uluslararası Beslenme ve Diyetetik Kongresi*, April, 14-18, 2010, İstanbul, Turkey
- **Tacer Caba, Z.**, Boyacıoğlu, M.H., Boyacıoğlu, D., 2010: Gelenekselden geleceğe sağlıklı bir gıda alternatifi olarak bulgur. *I.Uluslararası “Adriyatik'ten Kafkaslar'a Geleneksel Gıdalar” Sempozyumu*, April, 15-17, 2010, Tekirdağ, Turkey
- Toydemir-Otkun, G., **Tacer Caba, Z.**, Çapanoğlu-Güven, E., Boyacıoğlu, D., Demir, N., 2010: Effects of minimal processing and refrigerated storage on phenolic content and antioxidant capacity of rocket salad (*Eruca sativa*, *Brassicaceae*). Workshop on Antioxidant Measurement Assay Methods, April 21, 2010. Istanbul, Turkey.
- **Tacer, Z.**, Boyacioglu, M. H., Boyacioglu, D., 2009: Bioactive compounds of a healthy whole wheat product “Bulgur”. *IFT Annual Meeting & Food Expo.*, June, 6-9 2009., Anaheim, OC, California, USA.
- **Tacer, Z.**, Boyacioglu, M. H., Boyacioglu, D., 2008: Total phenols and antioxidant capacity of Bulgur. *AACC International Annual Meeting*, September 21 - 24, 2008, Hawaii, USA.
- **Tacer, Z.**, Şahin Yeşilçubuk, N., Karaali, A., 2007: Fenolik Bileşikler Yardımıyla Türk Şaraplarında Otantisitenin Belirlenmesi. *Gıda Mühendisliği 5. Kongresi*, November 8 - 10 2007, Ankara, Turkey.
- Arduzlar, D., **Tacer, Z.**, Boyacıoğlu, M. H., 2007: Dietary management of phenylketonuria patients (Developments of foods for phenylketonuria patients). *2nd International Congress on Food and Nutrition-Food for Future*, October 24-26 2007, Istanbul, Turkey.
- **Tacer, Z.**, Arduzlar, D., Boyacıoğlu M. H., 2007: A different point of view for the health benefits of wholegrains: phytochemicals. *2nd International Congress on Food and Nutrition-Food for Future*, October 24-26 2007, Istanbul, Turkey.
- **Tacer, Z.**, Boyacıoğlu, D., Boyacıoğlu, M. H., Çolak, H., Arduzlar, D., Nilüfer, D., 2007: From tradition to modernity: A new bulgur product with vegetables. *IFT Annual Meeting & Food Expo*, July 29 - August 1 2007, Chicago, USA.

- **Tacer, Z.**, Boyacioglu, D., Boyacioglu, M. H., Çolak, H., Arduzlar D., Nilufer, D., 2007: Evaluation of starch gelatinization during cooking and drying processes in bulgur production. 2007. 6th *European Young Cereal Scientists and Technologists Workshop*, April 30-May 2, 2007, Montpellier, La Grande Motte, France.