

CHANGES IN THE STRUCTURE AND DIGESTION FRACTIONS OF WHEAT
STARCH COOKED WITH AND WITHOUT FIBER

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WHEAT STARCH COOKED WITH AND WITHOUT FIBER**

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ABSTRACT

CHANGES IN THE STRUCTURE AND DIGESTION FRACTIONS OF WHEAT STARCH COOKED WITH AND WITHOUT FIBER

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Starch digestibility affects blood glucose levels. Dietary fibers had been shown to slow down starch digestibility. Therefore, the objective was to understand the effect of fiber type on *in vitro* digestion of wheat starch. Wheat starch with and without fiber was heated at 90°C for 10 minutes at three different water concentrations (50%, 65%, and 80%). The starch to fiber ratio was 1:1 (dry basis) for the fiber added samples. *In vitro* digestion analyses were conducted for wheat starch with or without psyllium and cellulose fibers, cooked at different water concentrations. Changes in the structures were determined with Differential Scanning Calorimeter (DSC), X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR) Spectroscopy and Scanning Electron Microscope (SEM) analysis.

The results showed that fiber addition and decreasing water concentration during cooking reduced the digestibility of starch. Digested starch fractions were affected by gelatinization, fiber type, and dilution due to the added fiber. The type of fiber or the formed matrix affected both the gelatinization and the accessibility to the digestive enzymes differently. The impact of psyllium fiber on keeping the long and

short-range order of wheat starch molecules was more pronounced than cellulose fiber.

Keywords: Gelatinization, crystallinity, *in vitro*, psyllium, cellulose



ÖZ

LİFLİ VE LİFSİZ PİŞİRİLEN BUĞDAY NİŞASTASININ YAPISINDA VE SİNDİRİM FRAKSİYONLARINDAKİ DEĞİŞİKLİKLER

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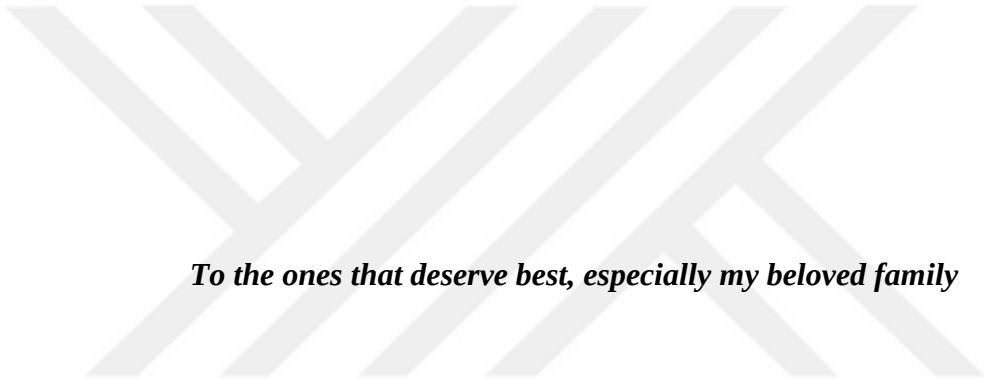
Nişastanın sindirilebilirliği, kan şekeri seviyesini etkileyen önemli bir faktördür. Diyet liflerinin nişasta sindirilebilirliğini yavaşlattığı gösterilmiştir. Bu doğrultuda çalışmanın amacı, lif türünün buğday nişastasının *in vitro* sindirimi üzerindeki etkisini araştırmaktır. Lifli ve lifsiz buğday nişastasını, üç farklı su konsantrasyonunda (% 50, % 65 ve % 80) 90° C'de 10 dakika ısıtma işlemi uygulanmıştır. Nişasta-lif oranı, lif eklenen numuneler için 1:1 (kuru baz) olarak belirlenmiştir. Farklı su konsantrasyonlarında pişirilmiş, pisilyum ve selüloz lifleri içeren ve içermeyen buğday nişastasını için *in vitro* sindirim analizleri yapılmıştır. Örneklerin yapısındaki değişiklikler; Diferansiyel Taramalı Kalorimetre (DSC), X-Işını Difraktometresi (XRD), Fourier Dönüşümlü Kızılötesi (FTIR) Spektroskopisi ve Taramalı Elektron Mikroskobu (SEM) analizi ile belirlenmiştir.

Sonuçlar, pişirme işlemi sırasında lif ilavesi ve su konsantrasyonunun azaltılmasının nişastanın sindirilebilirliğini düşürdüğünü göstermiştir. Sindirilmiş nişasta grupları jelatinizasyon, lif türü ve lif eklenmesine bağlı olan seyreltmeden etkilenmiştir. Lif

türü veya oluşan matris, hem jelatinizasyonu hem de sindirim enzimlerine erişilebilirliği farklı şekilde etkilemiştir. Pisilyum lifinin buğday nişastası moleküllerinin long-range ve short-range düzeni korumadaki etkisinin, selüloz lifine kıyasla daha belirgin olduğu gözlenmiştir.

Anahtar Kelimeler: Jelatinizasyon, Kristallik, *in vitro*, pisilyum, selüloz





To the ones that deserve best, especially my beloved family

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TABLE OF CONTENTS

ABSTRACT.....	v
ÖZ	vii
ACKNOWLEDGMENTS	x
LIST OF TABLES	xiv
LIST OF FIGURES	xx
LIST OF ABBREVIATIONS	xxii
CHAPTERS	
1 INTRODUCTION	1
1.1 Starch.....	1
1.2 Wheat Starch	5
1.3 Effects of Food Processing on Starch Granules	6
1.3.1 Mechanical Effect	6
1.3.2 Thermo-mechanical Effect.....	7
1.3.3 Enzymatic Degradation Effect	7
1.3.4 Thermal Effect	8
1.4 Dietary Fibers	9
1.5 Effects of Dietary Fibers on Human Health.....	11
1.6 Psyllium.....	13
1.7 Cellulose.....	14
1.8 Starch Digestion	17
1.8.1 Effects of Dietary Fibers on Starch Digestion	20
1.9 Methods Used in The Characterization Analysis	21

1.9.1	Differential Scanning Calorimetry (DSC) Analysis	21
1.9.2	X-Ray Diffraction (XRD) Analysis.....	22
1.9.3	Fourier Transform Infrared (FTIR) Spectroscopy Analysis.....	26
1.9.4	Scanning Electron Microscope (SEM) Analysis	27
1.10	Objectives	28
2	MATERIALS AND METHODS	29
2.1	Materials	29
2.2	Summary of Experimental Design.....	30
2.3	Method	30
2.3.1	Preparation of the Samples	30
2.3.2	Characterization.....	31
2.3.3	<i>In vitro</i> Digestion Analysis.....	32
2.3.4	Calculations of Starch Fractions.....	36
2.3.5	Statistical Analysis	37
3	RESULTS AND DISCUSSION.....	39
3.1	Physical Properties.....	39
3.1.1	Thermal Properties with Differential Scanning Calorimeter (DSC) .	39
3.1.2	Crystalline Properties with X-Ray Diffraction (XRD) Analysis.....	44
3.1.3	Short-range order with Fourier Transform Infrared (FTIR) Spectroscopy Analysis.....	50
3.1.4	Granular Morphology with Scanning Electron Microscope (SEM) Analysis	51
3.1.5	<i>In vitro</i> digestibility of wheat starch.....	55
4	CONCLUSIONS AND RECOMMENDATIONS	61

REFERENCES.....	63
APPENDICES	77
A. DIFFERENTIAL SCANNING CALORIMETER (DSC) THERMOGRAMS.....	77
B. FOURIER TRANSFORM INFRARED (FTIR) SPECTRA.....	80
C. STATISTICAL ANALYSES.....	84



LIST OF TABLES

TABLES

Table 2.1. Experimental design parameters.....	30
Table 2.2. Reagents of Glucose Assay Kit	36
Table 3.2 IR ratios of 1043/1016 cm^{-1} of raw materials (wheat starch, psyllium and cellulose fibers) and starch with and without psyllium and cellulose fibers cooked at different water concentrations.	51
Table 3.3. Digestion fractions of wheat starch with or without added psyllium and cellulose fibers cooked at different water concentrations. RDS (%): rapidly digestible starch SDS (%): slowly digestible starch and RS (%): remaining starch percentage per gram of starch in the samples.	59
Table C.1. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on fiber addition at 80% water concentration uncooked wheat starch	84
Table C.2. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on fiber addition at 80% water concentration uncooked wheat starch	85
Table C.3. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on fiber addition at 80% water concentration uncooked wheat starch	86
Table C.4. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on fiber addition at 80% water concentration uncooked wheat starch	87
Table C.5. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on fiber addition at 65% water concentration uncooked wheat starch	88
Table C.6. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on fiber addition at 65% water concentration uncooked wheat starch	89

Table C.7. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on fiber addition at 65% water concentration uncooked wheat starch.....	90
Table C.8. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on fiber addition at 65% water concentration uncooked wheat starch.....	91
Table C.9. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on fiber addition at 50% water concentration uncooked wheat starch.....	92
Table C.10. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on fiber addition at 50% water concentration uncooked wheat starch.....	93
Table C.11. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on fiber addition at 50% water concentration uncooked wheat starch.....	94
Table C.12. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on fiber addition at 50% water concentration uncooked wheat starch.....	95
Table C.13. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on different water concentrations for uncooked wheat starch	96
Table C.14. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on different water concentrations for uncooked wheat starch	97
Table C.15. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on different water concentrations for uncooked wheat starch	98
Table C.16. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on different water concentrations for uncooked wheat starch	99

Table C.17. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on different water concentrations for uncooked wheat starch-psyllium mixture (1:1)	100
Table C.18. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on different water concentrations for uncooked wheat starch-psyllium mixture (1:1)	101
Table C.19. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on different water concentrations for uncooked wheat starch-psyllium mixture (1:1)	102
Table C.20. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on different water concentrations for uncooked wheat starch-psyllium mixture (1:1)	103
Table C.21. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on different water concentrations for uncooked wheat starch-cellulose mixture (1:1)	104
Table C.22. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on different water concentrations for uncooked wheat starch-cellulose mixture (1:1)	105
Table C.23. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on different water concentrations for uncooked wheat starch-cellulose mixture (1:1)	106
Table C.24. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on different water concentrations for uncooked wheat starch-cellulose mixture (1:1)	107
Table C.25. One way ANOVA and Tukey's Comparison Test with 95% confidence level for 1043/1016 cm ⁻¹ depending on water concentration for wheat starch without fiber	108
Table C.26. One way ANOVA and Tukey's Comparison Test with 95% confidence level for 1043/1016 cm ⁻¹ depending on water concentration for cooked wheat starch-psyllium mixture (1:1)	109

Table C.27. One way ANOVA and Tukey's Comparison Test with 95% confidence level for 1043/1016 cm ⁻¹ depending on water concentration for cooked wheat starch-cellulose mixture (1:1)	110
Table C.28. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on fiber addition at 80% water concentration	111
Table C.29. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on fiber addition at 80% water concentration	112
Table C.30. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on fiber addition at 80% water concentration	113
Table C.31. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on fiber addition at 65% water concentration	114
Table C.32. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on fiber addition at 65% water concentration	115
Table C.33. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on fiber addition at 65% water concentration	116
Table C.34. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on fiber addition at 50% water concentration	117
Table C.35. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on fiber addition at 50% water concentration	118
Table C.36. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on fiber addition at 50% water concentration	119

Table C.37. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on different water concentrations for cooked wheat starch and raw starch.....	120
Table C.38. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on different water concentrations for cooked wheat starch and raw starch.....	121
Table C.39. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on different water concentrations for cooked wheat starch and raw starch	122
Table C.40. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on different water concentrations for cooked wheat starch-psyllium mixture and raw mixture (1:1)	123
Table C.41. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on different water concentrations for cooked wheat starch-psyllium mixture and raw mixture (1:1)	124
Table C.42. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on different water concentrations for cooked wheat starch-psyllium mixture and raw mixture (1:1).....	125
Table C.43. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on different water concentrations for cooked wheat starch-cellulose mixture and raw mixture (1:1)	126
Table C.44. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on different water concentrations for cooked wheat starch-cellulose mixture and raw mixture (1:1)	127
Table C.45. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on different water concentrations for cooked wheat starch-cellulose mixture and raw mixture (1:1).....	128
Table C.46. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on fiber addition for raw starch and mixtures (1:1)	129

Table C.47. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on fiber addition for raw starch and mixtures (1:1).....	130
Table C.48. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on fiber addition for raw starch and mixtures (1:1)	131



LIST OF FIGURES

FIGURES

Figure 1.1. Schematic diagram of (a) amylose; and (b) amylopectin with a branch point at the O-6 position. (BeMiller & Whistler, 2009)	4
Figure 1.2. The crystalline unit cell for native cellulose (cellulose I) (Benjamin Caballero, Paul Finglas, 2003).	15
Figure 1.3. The electromagnetic spectrum of radiations (Rethwisch & Callister, 2010).....	22
Figure 1.6. XRD patterns of different types of starch granules (Bul��on et al., 1998; Luckett, 2012).....	25
Figure 1.7. Diffraction patterns examples of (a) amorphous, (b) semicrystalline and (c) highly crystalline (Nagaraj, Subramani, Shivanna, & Siddaramaiah, 2016)	26
Figure 3.1. X-ray diffractograms of raw starch, cellulose, and psyllium. S: Starch, C: Cellulose, P: Psyllium.	45
Figure 3.2. X-ray diffractograms of raw and cooked wheat starch (80%, 65% and 50% water concentration). S: Starch.	46
Figure 3.3. X-ray diffractograms of raw and cooked wheat starch-psyllium mixtures (80%, 65% and 50% water concentration). S+P: Starch and Psyllium at 1:1 dry weight base.	46
Figure 3.4. X-ray diffractograms of raw and cooked wheat starch-cellulose mixtures (80%, 65%, and 50% water concentration). S+C: Starch and Cellulose at 1:1 dry weight base.	47
Figure 3.5. Scanning electron micrographs of raw and cooked wheat starch with and without fibers at 1500x magnification. a. Cooked 80%S, b. Cooked 80%S+C, c. Cooked 80%S+P, d. Cooked 65%S, e. Cooked 65%S+C, f. Cooked 65%S+P g. Cooked 50%S, h. Cooked 50%S+C, i. Cooked 50%S+P, j. Raw S, k. Raw S+C, l. Raw S+P	54
Figure A.1. DSC thermogram of cooked samples at 80% water concentration.....	77
Figure A.2. DSC thermogram of cooked samples at 65% water concentration.....	78

Figure A.3. DSC thermogram of cooked samples at 50% water concentration	79
Figure B.1. FTIR spectra of raw starch, cellulose and psyllium.....	80
Figure B.2. FTIR spectra of raw and cooked starch	81
Figure B.3. FTIR spectra of cooked wheat starch-psyllium mixtures at different water concentrations.	82
Figure B.4. FTIR spectra of cooked wheat starch-cellulose mixtures at different water concentrations	83



LIST OF ABBREVIATIONS

ABBREVIATIONS

AG : Available glucose

DSC : Differential scanning calorimeter

FSG : Free sugar glucose

FTIR : Fourier Transform Infrared

GI : Glycemic index

RAG : Rapidly available glucose

RDS : Rapidly digestible starch

RS : Remaining starch

SAG : Slowly available glucose

SDS : Slowly digestible starch

SEM : Scanning electron microscope

TG : Total glucose

XRD : X-ray diffraction

CHAPTER 1

INTRODUCTION

Today, people focus on nutritionally balanced food with low calorie and high dietary fiber content for their health. Excessive consumption of carbohydrates due to abundance and availability causes increased obesity rates in the population and is a public health concern. High consumption of carbohydrates is harmful due to their effect on postprandial digestion metabolism, the balance between nutrient storage, hunger, and satiety. The digestibility of starch is closely related to the distribution of this energy balance. The energy balance of the body can be regulated by consuming starch in the presence of non-starch polysaccharides, such as dietary fibers (Livesey, 1995).

1.1 Starch

Starch is the most crucial energy source for human consumption. Besides its nourishing effect, starch has an important impact on food products' quality with its effects on physical and sensory properties. Starch has been used in the food industry for a long time due to its technological and physiological properties.

Starch is the most abundant storage polysaccharide on Earth. The excess glucose is stored as starch in many plants, especially cereals, legumes, and most tubers. It is stored within the cells of plants as partially crystalline granules (Englyst, Hudson, & Englyst, 2006). Leaves, roots, shoots, fruits, grains, and stems of plants have starch granules storage. The endosperm is the main area of synthesis and accumulation of starch granules in the amyloplasts, generally found in vegetative plant tissues with duties to produce and store starch (Eliasson, 2004).

Starch granules can be found in different shapes, sizes, and compositions according to their botanical source. Wheat, barley, and rye have two types of granules; large and lenticular, small and spherical shaped. First of all, amyloplasts produce one large lenticular starch granule, and then small granules are formed and separated from amyloplast in those plants. The overall shape of the starch granules can be observed by an optical microscope. Under the polarised light, native starch granules show a characteristic Maltese cross-shape due to starch granule's birefringence (Whistler & BeMiller, 2012).

Physically, starch has a heterogeneous structure and contains crystal and amorphous phases altogether. Starch granule is arranged in amorphous areas and regions of high and low crystallinity, and the transition between them is gradual. The crystalline domain consists of linear fractions of amylopectin, while the branching points and amylose structure are defined as major components of amorphous domains (Ai & Jane, 2016; Oates, 1997). Crystalline characteristics of starch granules have been studied with the help of x-ray diffraction techniques. According to these studies, native starch granules have crystallinity ranging from 15 to 45 %. Starch granules can be characterized as A, B, and C type due to their x-ray diffraction (XRD) patterns. Cereal starches generally give A-type; tuber starches such as potatoes gives B-type pattern. Legume starches such as peas and beans show a C-type pattern, which is a combination of A and B-type XRD patterns. There is also a Vh-type pattern that is not found naturally. However, this pattern can be formed due to gelatinization of starch, treatment of starch with lipids, or similar components (Saldamlı, 2017).

The starch granule contains some minor components such as lipids and proteins and two major polymers, amylose and amylopectin. The assortment of these minor components is related to the botanical source of starch. Generally, cereal starches

contain approximately 0.25% protein and 1% lipid (Hu et al., 2018). Native starches also contain phosphorus and nitrogen in smaller amounts (Saldamlı, 2017).

Constitutively, starch is composed of D-glucose units linked by α -1,4 and α -1,6 glucosidic linkages. Amylose has a linear structure, while amylopectin has a branched structure (Eliasson, 2004). Amylose is predominantly linearly linked by α -1,4 glucosidic connections (Figure 1.1). The molecular weight of amylose ranges from 40 000 to 340 000, and the chains contain 250 to 2000 anhydroglucose units, which differs according to species and maturation of plants (Saldamlı, 2017).

Since amylose is an essentially linear compound with mainly α -D-1,4 glucosidic bonds, this kind of bonding causes amylose to form flexible random-coil helical conformations. In aqueous solutions, amylose becomes unstable and forms double-helices, which precipitate easily. These precipitates display the B-type x-ray diffraction polymorph, which has the double helices packed in a hexagonal unit cell. Single amylose molecules also can form helical complexes with other compounds such as alcohols and fatty acids, which consist of a hydrophobic part. The hydrophobic regions of these helical complexes are stationed inside the hydrophobic cavity of the helix structure. This helical conformation of amylose shows V- type pattern according to x-ray diffraction analysis. Many starch granules, especially cereals, reveal a characteristic rising peak at 2θ of 20° , which refers to the amylose-lipid complex in granule (Bertoft, 2017).

Generally, the amylose structure involves 20-30 % of the starch granule. When starch granules are heated above the gelatinization temperature, amylose leaks out from the starch granule and passes into the solution. With the increasing temperature, the linear structure of amylose turns into a branched structure with α -1,6 glycosidic bonds and higher molecular weight. However, the amylose molecule is still

considered as unbranched because of the long chains and rare branches in the structure (Saldamlı, 2017).

Amylopectin has the duty to produce the principal structure of starch granules. The amylopectin molecules are orientated radially in starch granules (Coultate, 2002). Besides α -D-1,4 glucosidic linkages, amylopectin consists of branching points at O-6 position (Figure 1.1). The molecular weight and size of the amylopectin structure are much larger than amylose. Amylopectin is considered to be composed of anhydroglucose chains with many α -1,6 branching points, and the molecular weight of amylopectin may reach as high as 80 000 000 (Bertoft, 2017).

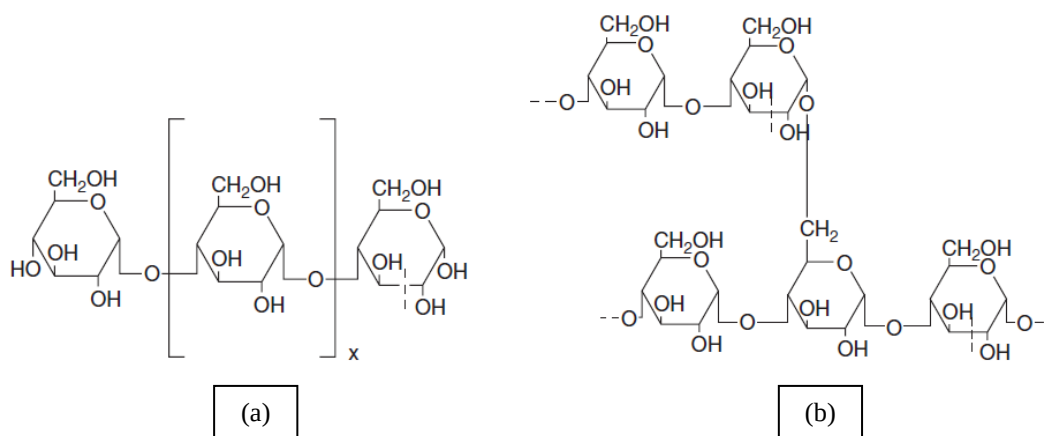


Figure 1.1. Schematic diagram of (a) amylose; and (b) amylopectin with a branch point at the O-6 position. (BeMiller & Whistler, 2009)

As a highly branched structure, branch chains of amylopectin can be divided into three groups. A-chains are generally short and consist of α -D-1,4 glucosidic bonds with no branch points. B-chains consist of D-glucose units linked by α -1,4 and α -1,6 glucosidic linkages, and they can have different chain lengths. C-chains which is unique in carrying the single reducing-end group of the molecule together with α -1,4 and α -1,6 glucosidic bonds. The branched-chain and length distribution of amylopectin depends on the botanical source of starch and affect the polymorphic

form of the crystalline structure, the gelatinization, pasting, and retrogradation properties of the starch (Ai & Jane, 2016).

The crystalline region of starch granules consists of double helices of amylopectin; meanwhile, amylose and branched chains of amylopectin structure constitute the amorphous region of the molecule. The crystallinity of starch is provided by packing amylopectin double helices into one unit cell. Wheat starch consists of an amylopectin structure with more short branch chains and displays A-type x-ray diffraction pattern. The crystalline portion of wheat starch consists of left-handed parallel-stranded double helices packed in a monoclinic unit cell in this type of pattern (Dome, Podgorbunskikh, Bychkov, & Lomovsky, 2020).

1.2 Wheat Starch

Wheat is one of the essential food grains grown globally, and it constitutes a large portion of daily caloric intake for humans (Li, Li, & Guo, 2020). Wheat is used in food products, animal feed, and other industrial applications. Paper, textile, and cosmetic industry can be examples for non-food applications. Wheat starch contains 67 % of the food industry's total consumption, while feed and seed have only 20 % and 7 %, respectively. Generally, wheat starch consists of 15-25 % amylose and 75-85 % amylopectin (Bao, Shen, Sun, & Corke, 2006). The granules of wheat starch have bimodal size distribution with large-lenticular and small-spherical shaped granules (Pokhrel, 2015). Approximately 70 % of wheat starch comprises large granules, and 30 % consists of small granules starch by volume. Large granules are about 25 micrometers, and small spherical ones are smaller than 10 micrometers in diameter (BeMiller & Whistler, 2009). Large granule starch is called A-type, and small granule starch is called B-type starch (Velicogna & Shea Miller, 2015). These two starch granule fractions differ in their chemical and physical properties. They vary in composition, molecular structure, swelling, gelatinization properties, pasting, and rheological behaviors (Geera, Nelson, Souza, & Huber, 2006).

Wheat starch has been used in the food industry for a long time due to its technological and physiological properties. During the cooking of pasta, there is a physical competition for protein coagulation. If protein coagulation occurs during the cooking of pasta, starch particles will be trapped in this protein network, and it will promote firmness in pasta, but if starch swelling and gelatinization prevail instead of the protein coagulation, then it will result in the soft and sticky pasta. Wheat starch has been used due to its water-binding properties to avoid firmness and obtain the desired structure in meat products. In custard type milk desserts, it is used to enhance thickening and gelling properties. In batters, breadings, breakfast cereals, ice creams, gravies, vitamin and flavor mixtures, wheat starch can be used to provide adhesion, structure, moisture control and can enhance gelling properties (Eliasson, 2004).

1.3 Effects of Food Processing on Starch Granules

Food processing causes changes in the starch structure due to mechanical and thermal effects.

1.3.1 Mechanical Effect

Milling is one of the mechanical processes and used in the large scale extraction of cereals. Milling processes cause substantial mechanical damage to the granules, which is visible and includes internal alterations. After the milling process, the water absorption and swelling properties of starch molecules increases. Gelatinization enthalpy can be changed by milling because double helices and the crystallinity are affected during milling. These changes occur due to the shear stress and compression caused by the process (Eliasson, 2004).

1.3.2 Thermo-mechanical Effect

Extrusion is a thermo-mechanical process widely used in the food industry. The extrusion method contains a high temperature with a large amount of mechanical energy input. By changing the extruder parameters, it is possible to obtain various forms of mechanical deterioration (Eliasson, 2004). Many studies have been shown the effect of the extrusion process on the characteristics of the final product and on the structure of starch. Many researchers indicated that the extrusion process results in complete gelatinization and disruption of starch granules. Therefore, starch becomes more susceptible to digestive enzymes depending on the process conditions (Svihus, Uhlen, & Harstad, 2005).

1.3.3 Enzymatic Degradation Effect

Enzymatic susceptibilities of starches differ depending on the granule type, ratio of amylose to amylopectin, crystallinity, polymorphic patterns, the existence of amylose-lipid complex, enzyme type, and hydrolysis conditions such as pH and temperature (Rocha, Carneiro, & Franco, 2010). Amylolysis is an enzymatic process that affects the structure of granules by the breakdown of the starch structure by α -amylase enzyme. This enzyme is naturally found in grains and human saliva. The amorphous rings, which are less organized, are more susceptible to enzymes than crystalline areas of granules, which have higher resistance (Oates, 1997). Crystallinity and gelatinization enthalpy can be affected as a result of enzymatic hydrolysis caused by the disruption of crystalline areas of starch granule (Hoover & Zhou, 2003). Mechanical processes such as milling may increase enzymatic degradation due to the increased surface area (Eliasson, 2004).

1.3.4 Thermal Effect

Starch granules are insoluble in water. The bonds between the chains in the crystalline regions are very strong; therefore, they form steric hindrance against water molecules. When starch granule absorbs water, granule starts to swell. Water is linked to the O-H groups in the granule structure by H-bonding. The swelling of the starch granule is a reversible change, and the volume of the granule increases by up to 30 % (Alcázar-Alay & Meireles, 2015).

When starch is heated in the presence of excess water, after a specific temperature, starch granules undergo a transition phase and lose their structure and birefringence property. This particular temperature is defined as the gelatinization temperature, which differs according to starch species. Gelatinization is an endothermic process that occurs when starch granule swells substantially with the absorption of water.

Starch gelatinization starts in the amorphous region of the granule and is preferred by the weak hydrogen bonds present in this region. Then, this endothermic process continues with the crystalline area of the granule. Thermal properties such as transition temperatures and gelatinization enthalpy are characteristic to the starch granule and give information about the gelatinization process and structure (Alcázar-Alay & Meireles, 2015). If the starch sample has high transition temperatures, this can indicate high degree crystallinity, high stability, and resistance to the gelatinization process (Tester, Karkalas, & Qi, 2004).

Gelatinization occurs in different temperature ranges for different starch species. As the heating continues, the starch granules continue to swell and partially dissolve. Starch granules become fully hydrated and distinguish themselves from the micellar network of the granule. After that, they pass into the aqueous medium. Starch

granules swell and gelatinized until available water runs out in the medium. As heating continues, the bonding forces in the granule become weaker, and the susceptibility of the granule to mechanical and thermal deterioration increases. Some of the dissolved starch that had previously spread into the surrounding aqueous region may pass through the swollen granules due to occupation of the entire volume by highly swollen granules.

When the cooling process is applied after cooking, the viscosity of the system increases with the formation of new H-bonds between the molecules, eventually forms a firm gel structure. The water in the gel is trapped and does not leak out of the network. However, if this gel is kept longer time, the starch chains interact with each other to form new H-bonds. This leads water to leak from the structure. This is called syneresis and can be observed only in systems with high water content. As the retention continues, the interaction between the starch chains increases over time. The starch chains begin to be rearranged in double helices, strengthened by hydrogen bonds, resulting in the crystal structure. This process is called retrogradation, which occurs after new intermolecular hydrogen bonding occurred (Tako, Tamaki, Teruya, & Takeda, 2014).

1.4 Dietary Fibers

Eben Hipsley, in 1953, was the first person who defined “dietary fiber” as non-digestible components that create the cell wall of the plants (Caprita, 2016). The Codex Alimentarius Commission’s Committee on Nutrition and Foods for Special Dietary Uses postulated a new dietary fiber definition included in the Guidelines for Nutrition and Health Claims. The committee described dietary fiber as; “carbohydrate polymers containing ten or more monomeric units which are not hydrolyzed by endogenous enzymes in the small intestine of humans” (CODEX, 2010).

Dietary fibers are obtained from the endosperm, shell, vascular tissue, epidermal tissue, and cotyledons of plants, including compounds of diverse chemical and morphological structure. Dietary fibers are resistant to human digestion enzymes but can be digested by the gut microflora (Caprita, 2016; Dülger & Şahan, 2011).

Dietary fibers are categorized according to their botanical source, solubility, fermentability, and physiological effects. Typing dietary fibers as water-soluble and insoluble is one of the simple classifications commonly used to differentiate. The structure of fibers affects their solubility. Polysaccharides have a strong affinity for water molecules due to the presence of multiple -OH groups. This situation leads to a strong interaction between polysaccharide molecules due to hydrogen bonding. The solubility of a polysaccharide can be recognized by the balance between molecule-molecule interaction and molecule-water interaction. For soluble polysaccharides, interactions between polysaccharide molecules and water molecules are energetically favorable. However, for insoluble polysaccharides, the intramolecular interactions between polymer segments dominate and eventually result in aggregation and precipitation (Guo, Hu, Wang, & Ai, 2017).

Celluloses, some hemicelluloses, lignin, and resistant starch are the primary examples of “insoluble” fiber, while oligosaccharides, pectins, gums, β -glucans, and psyllium are examples of “soluble” fiber. From the soluble fiber group, pectin is found in apple and quince; gums are found in resins; β -glucan is found in oats. From the insoluble dietary fiber group, cellulose is found in bran, and there is plenty of lignin in wheat.

Both soluble and insoluble dietary fibers have water and mineral cations binding capacity. They can impart some functional properties to food materials with their physical and chemical properties, such as increasing the water holding capacity and

gel formation properties. The water holding capacity of dietary fibers has significant effects on the intestinal tract, such as absorption of nutrients, postprandial response, and intestinal motility in the intestinal tract (Elleuch et al., 2011).

Dietary fibers can be incorporated into many food products such as bakery products, dairy products, jams, meat products, and soups to modify the textural properties, avoid syneresis, and stabilize high-fat food and emulsions and improve the shelf-life.

For healthy nutrition, both of these dietary fiber types should be consumed, and mixtures of these dietary fiber types can be more effective in competing with diseases such as diabetes, gastrointestinal and allergic diseases. According to the FDA recommended daily value of dietary fiber for healthy adults is 28 g per day. This value is based on the reference caloric intake of 2,000 calories for adults and children aged four years and older and pregnant women and lactating women (FDA, 2020).

1.5 Effects of Dietary Fibers on Human Health

Dietary fibers have many benefits to human health. They have an impact on the gastrointestinal system, serum lipids, metabolism of carbohydrates, and absorption of minerals. While food is usually broken down with the help of digestive enzymes, dietary fibers are not affected by digestive enzymes. Dietary fibers can be degraded by beneficial bacteria by fermentation in the colon. It has been reported that dietary fiber protects bowel health due to the increase in colonic fermentation. Approximately half of the total dietary fibers are fermented by the endogenous flora (Brownlee, 2011).

Dietary fiber increases the viscosity of the stomach contents due to the water-absorption properties and delays the emptying of the stomach. Since the stomach does not drain, the individual's desire to eat diminishes. They create a feeling of fullness. Dietary fibers are known to produce a feeling of fullness for a longer period when supplemented with abundant water (Thompson & Manore, 2012). Also, eating foods containing dietary fibers take a longer time to chew and swallow.

Water-insoluble fibers have positive effects on intestinal motility and the duration of intestinal transit. The increase in the intake of dietary fibers increase the fecal bulk and decrease the bowel transit time. The increase in the amount of stool is mainly due to the water-binding properties of the fibers that help to prevent constipation. It is also stated that dietary fibers are very useful in avoiding colon-rectal cancers. This effect occurs by altering the colon bacterial flora, reducing the formation of toxic metabolites and accelerating fecal excretion, and by shortening the contact times of toxic metabolites with the intestinal cells. Dietary fibers have a positive effect on probiotics in human gut (Konar, Toker, Oba, & Sagdic, 2016).

Consumption of dietary fibers has been shown to reduce the level of cholesterol in excess of 20 % (Dülger & Şahan, 2011). Therefore, dietary fiber consumption is of great importance in terms of reducing the risk of cardiovascular diseases. High dietary fiber intake is known to have benefits on diabetic individuals by reducing serum glucose and insulin requirements (Kahlon, Chow, Hoefer, & Betschart, 2001; Saldamlı, 2017).

The soluble fiber co-existing with the complex carbohydrates modifies the absorption of the blood sugar by the body and adjusts the sugar level in the blood by providing the glucose very slowly in the bloodstream. There are a lot of studies about the impacts of dietary fibers on diabetes. In a research, especially water-soluble

fibers had been shown to reduce the postprandial glucose and insulin concentrations in individuals with and without diabetes (Stevens et al., 2002).

1.6 Psyllium

Psyllium seeds and seed husks come from the plant genus *Plantago* including *P. ovata*, *P. psyllium*, and *P. Indica*. The European and Asiatic species of *Plantago*, which are cultivated in India, France, and Spain, are used for obtaining commercial psyllium (Whistler & BeMiller, 2012). Psyllium is composed of substituted acidic arabinoxylan. The xylan backbone has both β -1 $\rightarrow$ 4 and β -1 $\rightarrow$ 3 linkages linked with arabinose, rhamnose, and galacturonic acid units (Swaroop, Bagchi, Moriyama, & Bagchi, 2017).

Psyllium is soluble in water and forms gels; thus, classified as viscous soluble fiber (Figueroa, Dello, Ingeniería, Plapiqui, & Blanca, 2018). Psyllium is included in the complex dietary fiber group known as a functional and bioactive ingredient with a health claim approved by the Food and Drug Administration (FDA, 2015).

Studies have shown that psyllium fiber has benefits for body weight gain, satiety, diarrhea, cholesterol and triglyceride levels, fasting glycemia, and blood pressure. Psyllium has a potential role in treating metabolic syndrome, reducing the risk of cardiovascular diseases, irritable bowel syndrome, anti-inflammation, and colon cancer (Giacosa & Rondanelli, 2010; Yu, Xie, & Liu, 2012; Ziai et al., 2005).

Many studies have been done with different animal models and human clinical trials to understand the mechanism and the efficiency of psyllium on reducing plasma cholesterol concentration (Yu, Lutterodt, & Cheng, 2008; Chan & Heng, 2008).

In a research where several hamsters were used in animal models, it has been shown that psyllium fiber reduced the plasma cholesterol due to enhanced fecal bile acid excretion and hepatic bile acid synthesis. This effect was significant when compared with pectin and guar gum. Some cholesterol concentration lowering effect of psyllium was also seen in healthy human studies (Yu et al., 2012).

Several studies indicated that psyllium might improve the postprandial glycemic index and insulin sensitivity (Yu et al., 2008). These effects were related to limiting glucose diffusion to the small intestinal epithelium for absorption, delayed gastric emptying, and reducing carbohydrate digestion by delaying access to digestive enzymes. These properties can be attributed to psyllium's strong gel-forming ability and increasing fluid viscosity in intestinal and gastric systems.

It has been reported that psyllium may lower the speed of colon transit and provide the production of short-chain fatty acids due to fermentation by microflora in the colon. The usage of psyllium for the treatment of irritable bowel syndrome has also been mentioned in many studies (Yu et al., 2008; Marteau et al., 1994; Yu et al., 2012).

1.7 Cellulose

Cellulose is known as a substantial structural component of primary cell walls of plants, algae, vegetables, and some bacteria. It is composed of glucose monomers that are linked by β -(1 $\rightarrow$ 4) glycosidic linkages. The cellulose chain structure is unbranched. In the glucose ring, the hydroxyl groups are asymmetrically located, and this allows the linking of adjacent chains in one plane via means of hydrogen bonding. Intra- and intermolecular non-covalent hydrogen bonds of crystalline cellulose make it insoluble in water (Bin-fen, Zhen-hua, & Ju-fang, 2008; Puranen, Alapuranen, & Vehmaanperä, 2014). Cellulose becomes soluble in concentrated

acids such as phosphoric, sulfuric, and hydrochloric acids and in inorganic salt solutions. All of the cellulose hydrogen atoms are in the axial position, while all of the hydroxyl groups are equatorial. Equatorial hydroxyl groups allow cellulose to crystallize by bonding hydrogen with their closest neighbors.

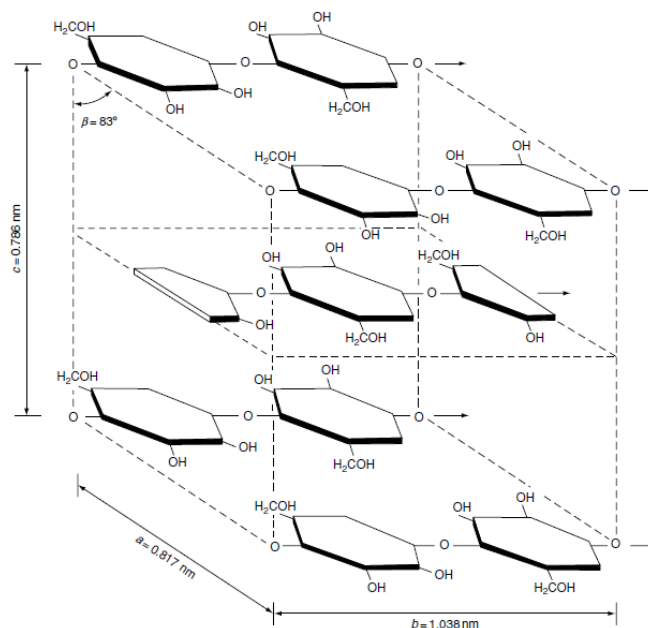


Figure 1.2. The crystalline unit cell for native cellulose (cellulose I) (Benjamin Caballero, Paul Finglas, 2003).

The structure of naturally occurring cellulose is known as Cellulose I, which is also divided into the crystalline structures as I α and I β . The cellulose microfibrils from the cell walls of the algae called *Cladophora*, *Halicystis*, and *Valonia* mainly have crystalline polymorphs of cellulose I α . On the other hand, cellulose microfibrils of cotton mostly have highly crystalline cellulose I β (Zugenmaier, 2008). Other crystalline forms of native cellulose (cellulose I) can be obtained by treating cellulose with sodium hydroxide, precipitating from solutions of alkali or salt. In addition, removing the added functional groups from cellulose derivatives can be used to obtain cellulose II as a different type of crystalline structure of native cellulose. There

are also other crystalline forms of cellulose, which are known as Cellulose III and Cellulose IV. These forms can be obtained by treating cellulose with cold and hot chemical liquids (anhydrous ammonia or glycerol).

Cellulose structure can stay stable up to 300 °C (Benjamin Caballero, Paul Finglas, 2003). For the same cellulose polymorph, the size and the shape of the crystallites of native cellulose diversify from species to species (Zugenmaier, 2008). Native cellulose involves crystalline and amorphous regions where the amorphous regions are more porous than the crystalline areas. The amorphous regions allow water penetration due to their porous structure and increase cellulose's enzymatic hydrolysis and reactivity to acid. Cellulose hydrolyzes to its glucose monomers via cellulase and cellobiase enzymes and resistant to digestive enzymes in the human small intestine. It may be fermented in the large intestine by colonic bacteria. (Benjamin Caballero, Paul Finglas, 2003).

Fermentability, solubility, and viscosity are the physicochemical characteristics of fibers that affect fermentation and therapeutical effects of consumption. Although bacteria poorly ferment cellulose in the human large intestine, it plays a crucial role in diet and overall health by increasing bowel transit rate and contributes to stool bulk (Holscher, 2017).

Some processed food products containing psyllium and cellulose are consumed as good sources of fiber. Both psyllium and cellulose are included in the fermentable fiber category. Fermentation products of the fibers in the human large intestine are short-chain fatty acids (SCFAs) such as butyrate, acetate, and propionate. These short-chain fatty acids (SCFAs) are energy sources for the intestine cells that may reduce the risk of cancer (Benjamin Caballero, Paul Finglas, 2003; Harris, Tasman-Jones, & Ferguson, 2000; Holscher, 2017).

The gastric emptying rate may be slowed down due to gel-forming properties and viscosity of polysaccharides. The gel-forming fibers such as psyllium and oat bran contribute to a feeling of repletion by maintaining a feeling of fullness for a more extended period after a meal. In contrast, fibers that do not have the gel-forming capability, such as cellulose and wheat bran, have little effect on the rate at which the meal exits from the stomach. Although studies have shown that some certain viscous fibers such as psyllium, guar gum, and pectin reduce serum cholesterol in humans and animals via its effect on glucose tolerance, cellulose does not lower the plasma cholesterol (Stipanuk & Caudill, 2013).

1.8 Starch Digestion

The oral digestion step is the first step of food digestion, and there are two actions. The first one is the mechanical grinding by chewing to break food into smaller particles, which generates a larger surface area for the digestive enzymes in subsequent sections of the gastrointestinal tract. The second one is enzymatic hydrolysis by the salivary amylase (α -amylase), which carry out the preliminary steps in the breakdown of carbohydrates. When the partially digested starch reaches the small intestine, it continues to be digested with the pancreatic α -amylase upon entry into the duodenum. The digestion of carbohydrates to absorbable monosaccharides was accomplished by α -glucosidases through the small intestine, where glucose units enter the bloodstream (Jackson & McLaughlin, 2009; Priebe, Eelderink, Wachters-Hagedoorn, & Vonk, 2018). Glucose is taken up by epithelial cells and enters through carrier proteins in the jejunum. Complex carbohydrates such as dietary fibers get to the colon because they cannot be digested by intestinal enzymes. Dikeman & Fahey (2006) claimed that the glucose absorption rate could be delayed with the presence of viscous fiber by means of hindering the transport of glucose molecules to the epithelial cells.

For a long time, it has been known that starch has been digested slowly but entirely in the small intestine, which results in modest glycemic responses, and starch has no other physiological role rather than being a source of energy. Now, this perspective has changed, and it is known that the metabolic fate and the physiological response to starch can vary significantly. The botanical source of starch, granular structure, food processing, and other components such as dietary fiber impact starch digestibility. The extent and rate of digestibility of starch in the human gut depend on the accessibility of starch to α -glucosidases. Other food ingredients such as polysaccharides may reduce the hydrolysis and disruption of starch molecules due to increasing the viscosity of the aqueous starch suspension. Englyst, Kingman, & Cummings (1992) claimed that adding guar gum to the suspension of potato starch caused a reduction in the enzymatic hydrolysis depending on viscosity. The presence of fibers can intervene in starch digestion by preventing contact between starch and digestive enzymes.

Raw starch enclosed within a particulate material or plant cell walls, and starch in the form of intact granules is digested slowly due to restriction of the physical access of amylolytic enzymes. Thermal processes increase starch digestibility because, with heat and water, bonding forces in the granule become weaker, and starch granules become susceptible to digestion enzymes.

The metabolism of the ingested starch in the human small intestine causes a wide variety of glycemic responses. Physiological measurements have been used to describe the effect of carbohydrates on postprandial blood glucose concentration via glycemic index values of foods (Aller et al., 2011; Englyst et al., 2006).

Glycaemic Index (GI) refers to the ability to raise blood glucose concentration after a certain period of time when a carbohydrate food is consumed. There are many *in*

vitro digestion studies that indicate that glycemic response and starch digestion are closely associated. Foods with high glycemic index values are rapidly digested and absorbed in the human body, cause significant fluctuations in the blood glucose level. However, foods with low glycemic index values are slowly digested and absorbed, resulting in a gradual rise in the blood glucose level. In the human diet, increased intake of low GI carbohydrates has been associated with several health benefits, such as protection against diabetes, coronary heart diseases, and obesity. Several researchers observed a significant correlation between the *in vitro* starch digestion rate and reported GI responses of products (Germaine et al., 2008; Oh, Bae, & Lee, 2014).

Starch digested entirely in the human intestine can be divided into two sub-groups: rapidly digestible starch (RDS) and slowly digestible starch (SDS). Rapidly digestible starch causes a rapid increase in the glucose concentration in the blood. Englyst et al. (2018) defined RDS fraction as the amount of starch digested and absorbed in the duodenum and proximal regions of the small intestine at the first 20 minutes, which can be harmful to individuals with diabetes. This rapid increase in blood glucose concentration can also cause tissue and organ damages. SDS fraction is defined as the starch amount digested after RDS before 120 minutes during the incubation with amylolytic enzymes. Compared with RDS, SDS is digested slowly throughout the small intestine to provide sustained and slow glucose release with a low initial glycemia. Thus, foods with a higher SDS ratio provide stable glucose metabolism and help individuals manage their diabetes and satiety (Aller et al., 2011; Dona, Pages, Gilbert, & Kuchel, 2010). Resistant starch fraction represents the remaining starch, which escapes digestion in the small intestine and is fermented by the large intestine microflora (Aller et al., 2011; Englyst et al., 2018). The short-chain fatty acids produced by the RS fraction's fermentation were associated with reducing cardiovascular diseases, including heart attacks. They may stabilize blood glucose concentration, cholesterol synthesis in the liver and reduce the risk of cancer.

This impact of resistant starch is similar to the dietary fibers, which can resist the digestion enzymes (Dona et al., 2010; Zhou et al., 2020).

RDS and SDS fractions represent the entire fraction of the available starch linked with the available glucose (AG). The term rapidly available glucose (RAG) combines RDS and free sugar glucose (FSG) that provide the glucose load that will be delivered quickly and will probably significantly impact the glycemic response. The SDS fraction is defined as the source of slowly available glucose (SAG) directly related to the concept of slow-release carbohydrates (Englyst et al., 2018). Creating starch-hydrocolloid mixtures can slow down starch digestion by covering the starch granule surface or increasing the medium viscosity. Altering the starch digestion rate depends on the hydrocolloid type (Zhou et al., 2020).

1.8.1 Effects of Dietary Fibers on Starch Digestion

Potentially, starch can be digested entirely into glucose monomers by pancreatic amylase and brush border glucoamylase in the small intestine. The degree of hydrolysis can be significantly increased by the cooking process (due to gelatinization), which takes place under the influence of water and heat. However, restricted water and other components that compete for available water may cause limited internal rearrangement of starch polymers in the granule during processing. Therefore, the fraction of starch that resists amylolysis can increase.

Bai et al. (2017) examined the effect of pectin on digestibility and evolution of starch molecular structure during digestion. They observed that the digestion rate of pure starch was higher than the starch-pectin mixture, and the viscosity of the starch-pectin mixture was higher than the pure starch. They claimed that pectin slowed the starch digestion by changing the conformation of the enzyme (amyloglucosidase) or hinder the access of enzyme to the starch.

Many studies have indicated that oat and barley β -glucan fiber positively influenced blood glucose response after eating a meal containing carbohydrates. This positive effect was explained by the ability of fibers to develop a high viscosity in the gut (Ekström, Henningsson Bok, Sjöö, & Östman, 2017; Zhang, Luo, & Zhang, 2017).

Repin, Cui, & Goff (2018) have used four dietary fiber types, which are yellow mustard mucilage, soluble flaxseed gum, fenugreek gum, and oat gum at different concentrations to observe the changes in digesta sugar content and apparent viscosity. They have found that even if each fiber has a different concentration in the samples, both digesta sugar content and apparent viscosity decreased similarly.

Hardacre, Yap, Lentle, & Monro (2015) aimed to observe the effects of different fiber types on the digestion rate of gelatinized starch by using *in vitro* digestion model. They stated that the starch digestion rate was altered little by the smaller cellulosic wheat fiber particles but reduced significantly when wood fibers were added.

1.9 Methods Used in The Characterization Analysis

1.9.1 Differential Scanning Calorimetry (DSC) Analysis

Differential scanning calorimetry (DSC) is a commonly used thermoanalytical technique that monitors changes in the physical or chemical properties of foods and their components. In a typical DSC thermogram of an aqueous suspension of starch, temperature values, T_o , T_p , and T_c and transition enthalpies associated with gelatinization are observed. The enthalpy of transition (ΔH) refers to the amount of crystal order that can also be defined as a double-helical structure in the suspensions of starch which is disrupted with heating process.

1.9.2 X-Ray Diffraction (XRD) Analysis

X-rays were discovered by German scientist Wilhelm Conrad Roentgen in 1895 while he was a Professor at Wuerzburg University in Germany. X-rays are invisible, unlike ordinary light. The most significant difference between X-rays and ordinary light is penetration depth. X-rays can easily penetrate opaque objects like the human body, wood, quite thick pieces of materials, and food because of their shorter wavelength (Cullity & Stock, 2014; Manickavasagan & Jayasuriya, 2014). The wavelength region of X-rays is between 10^{-8} m and 10^{-12} m (Figure 1.3), between ultraviolet and gamma rays (Cullity & Stock, 2014; Rethwisch & Callister, 2010).

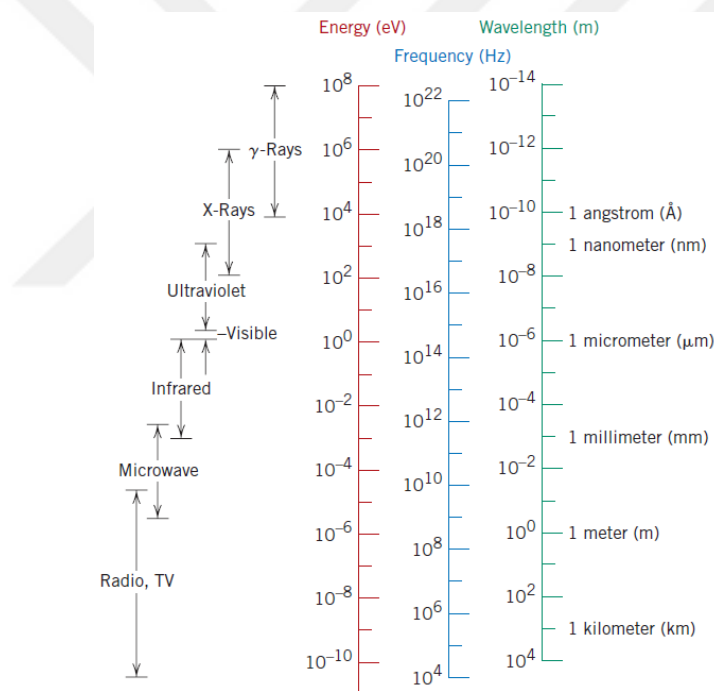


Figure 1.3. The electromagnetic spectrum of radiations (Rethwisch & Callister, 2010)

Since x-rays are electromagnetic radiation, they have both wave and particle motion behavior. They have two different spectrums; continuous due to wave motion and

characteristics due to particle motion. X-rays are the product of the kinetic energy of electrically charged particles like electrons. When some electrons decelerate rapidly by hitting the target, the continuous spectrum is obtained. During continuous radiation, the tube wavelength of radiation increases rapidly, then decreases slowly. On the other hand, when the x-ray tube's voltage increases above the particular critical value, sharp intensity will occur at specific wavelengths, and these wavelengths are called characteristic radiations. Furthermore, these radiations show different characteristics for different target materials because each material has a different atomic structure.

A diffractometer is an instrument (Figure 1.4) used to obtain diffraction angles and intensities for studying crystalline and non-crystalline materials. The most common use of x-ray diffractometers is the determination of the crystal structure of materials. Modern automated diffractometers have the capacity to collect the angular data on the order of 0.01° . In Figure 1.4, S represents the specimen put on a rotating table. O is an axis perpendicular to the plane of the drawing, and T is the monochromatic x-ray source, which is parallel to the diffractometer axis O. C is the detector that collects the intensities of diffracted beams. The counter detector C is supported on the carriage rotating about the axis O. While the detector is moving at a constant angular velocity, the recorder starts to plot the diffracted beam intensities as a function of 2θ , called as diffraction angle, with the help of a computer. The diffraction pattern is obtained after the collection of data. The high-intensity peaks show the crystallinity and crystal structure of the specimen (Cullity & Stock, 2014; Rethwisch & Callister, 2010).

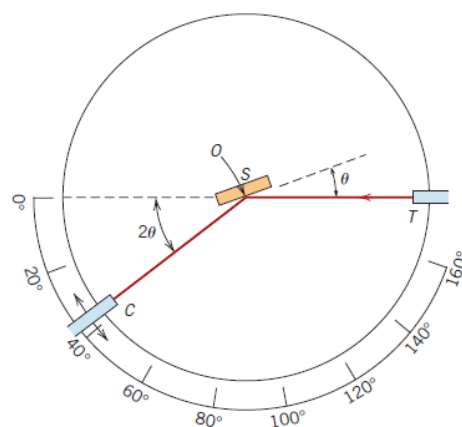


Figure 1.4. Schematic diagram of x-ray diffractometer (Rethwisch & Callister, 2010)

Starch granules are generally in semi-crystalline structure and show both long- and short-range order. Starches can have different types of crystalline structures; A-type, B-type, A- and B-type, which is called C-type, and Vh-type (Figure 1.5). A-type pattern for cereal starches, like corn, can generally have two sharp peaks at 2θ angles of around 15° and 23° , and two small peaks at 17° and 18° . Tuber and amylose-rich starches, like potato, show B-type diffraction pattern, which consists of a sharp peak at 17° and a few small peaks at about 5° , 20° , 22° and 23° (Buléon, Colonna, Planchot, & Ball, 1998; Luckett, 2012; Xie et al., 2006). Another difference between A- and B-type is the length and percentage of the branch chains. B-type starches have longer amylopectin branch chains and a higher percentage of long chains than A-type starches. In addition to that, short-chain fractions in A-type starches are in a larger amount than of B-type starches (Hanashiro, Abe, & Hizukuri, 1996; Hizukuri, 1985). C-type is the mixture of A- and B-type patterns and characteristics of legume starches. Peaks of C-type appear at 5° belongs to B-type; 18° and 23° of A-type pattern. Vh-type can be seen in amylose complexed with organic molecules like linear alcohols, fatty acids, iodine, and monoglycerides. 2θ angles of Vh-type pattern are at around 7° , 13° , 20° , and 23° (Buléon et al., 1998; Luckett, 2012; Xie et al., 2006).

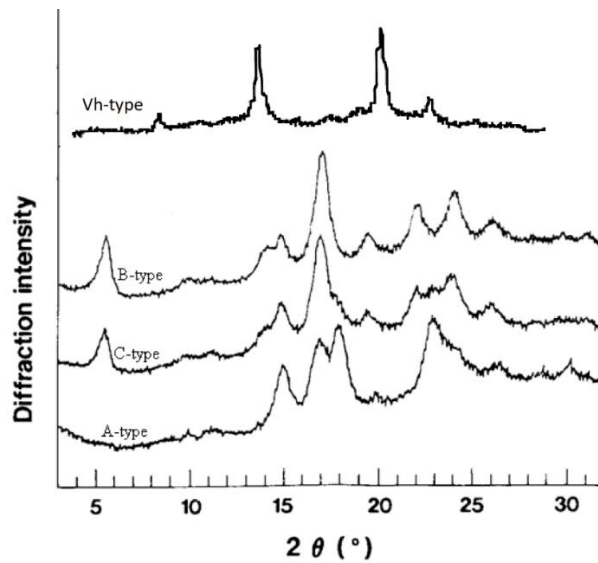


Figure 1.5. XRD patterns of different types of starch granules (Buléon et al., 1998; Luckett, 2012)

1.9.2.1 Long-Range & Short-Range Order Structure

In long-range order, atoms are arranged over large atomic distances in a three-dimensional structure observed in crystalline structures. XRD analysis detects long-range sequential structures, including regular and repeating double helix arrangement, and reflects the three-dimensional order of starch crystallinity (Wang, Li, Copeland, Niu, & Wang, 2015). If long-range order is broken and absent, this is called a non-crystalline or amorphous structure. When the distribution of atoms is less repeating array over short atomic distances, short-range order exists, which is generally observed in semi-crystalline or partially crystalline structures (Cullity & Stock, 2014; Rethwisch & Callister, 2010). While high sharp peaks occur in the diffraction pattern of crystalline structures, the diffraction pattern shows one or two broad peaks for amorphous structures, as shown in Figure 1.6. Moreover, the diffraction pattern of the short-range order includes less low, broad intense peaks at small angles (Figure 1.6.b) (Cullity & Stock, 2014).

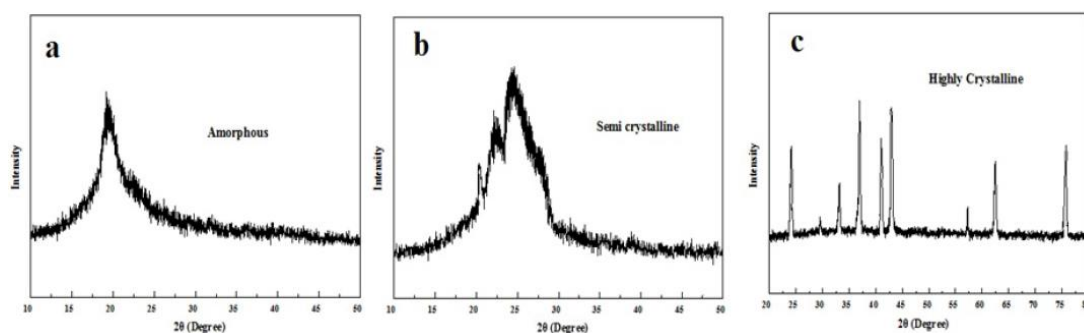


Figure 1.6. Diffraction patterns examples of (a) amorphous, (b) semicrystalline and (c) highly crystalline (Nagaraj, Subramani, Shivanna, & Siddaramaiah, 2016)

Although XRD is the only method that can measure long-range crystalline order, there are several methods available to measure short-range double-helical order in starch granules. FTIR has been suggested as a method to measure short-range order in starch (Capron, Robert, Colonna, Brogly, & Planchot, 2007; Jeroen J.G. Van Soest & Vliegenthart, 1997; Warren, Gidley, & Flanagan, 2016). FTIR has some notable advantages over other commonly used methods for measuring starch structure. FTIR is simple to use and maintain, offers a highly flexible sample presentation.

1.9.3 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FTIR has been using for many years to analyze the molecular structure and chemical bonding of organic compounds such as carbohydrates, proteins, enzymes and lipids (Gerwert & Kötting, 2010).

FTIR spectroscopy provides information on functional groups as infrared signatures or wavelengths in terms of chemical composition and physical state. The peaks produced by functional groups are determined by their atomic vibrations (Berthomieu & Hienerwadel, 2009).

Electromagnetic radiation that interacts with the sample is absorbed, reflected or transmitted, thereby providing information about the structure of the molecule and the energy transition level. Functional groups and chemical bonds in a molecule are determined according to the intensity of the peak absorbance (Munajad et al., 2018).

A source, sample compartment, amplifier, interferometer, computer, analog to digital (A/D) converter, and a detector are the parts of standard FTIR spectrophotometers. The radiation that generated from the source passes the sample through the interferometer and reaches the detector which results with the spectral information that appears on the monitor. On the FTIR graph, the infrared spectrum is placed in the x-axis or horizontal axis, where the frequency or absorbance takes place on the y-axis or vertical axis.

The infrared spectra of organic compounds are specific and include two regions which are defined as the functional group region ($4000\text{--}1500\text{cm}^{-1}$) and the fingerprint region ($1500\text{--}400\text{cm}^{-1}$). The functional group region peaks are used to determine if there is a specific functional group according to characteristic bonds of the sample and the fingerprint region peaks represent the molecular deformation due to the multiple bond deformation. The vibrations at frequency between $900\text{--}1200\text{ cm}^{-1}$ and $700\text{ to }900\text{ cm}^{-1}$ are defined as C–O stretching vibrations and the vibrations between $3000\text{ and }3600\text{ cm}^{-1}$ defined as the O–H group stretching which mainly observed in carbohydrates (Kizil, Irudayaraj, & Seetharaman, 2002).

1.9.4 Scanning Electron Microscope (SEM) Analysis

Scanning Electron Microscope has a resolution range (ultimate values below 3 nm), magnification capacity (between 10,000x and 150,000x), and depth of focus higher than an optical or light microscope. Capacities of SEM stay between the optical

microscope and transmission electron microscope (ASM International, 2004; Pomeranz, 1976).

The electrons in the electron beam hit the specimen and are emitted from the surface of the specimen. These emitted electrons, which have a smaller amount of current than the current of the primary electron beam, are collected by an electron detector, and then an amplifier is used to modify the contrast and brightness of the beam in a display tube. This tube is used to display the visual image and includes a cathode ray tube like the second tube, but this second tube is used to recording the image with the help of a camera (ASM International, 2004).

1.10 Objectives

Nowadays, people focus on nutritionally balanced food with low calorie and high dietary fiber content for a healthy diet. There is a need to regulate caloric intake by consciously designing new starchy foods. Dietary fibers are also functional nutrients that are highly crucial to human health and widely used in food applications.

Therefore, the objective was to understand the effect of fiber type (soluble or insoluble) on *in vitro* digestion of starch. *In vitro* digestion analyses were conducted for wheat starch with or without fiber (psyllium and cellulose) cooked at different water concentrations. Changes in thermal, structural, and morphological characteristics of samples were determined with DSC, FTIR, XRD, and SEM analysis.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Wheat starch was from Smart Chemistry (Izmir, Turkey). The wheat starch consisted of 28% amylose and 72% amylopectin. The average size was 10 μm . Psyllium and Cellulose fibers were from Tunçkaya Chemical (Istanbul, Turkey). Psyllium has 95% purity and passed through 40 mesh sieve. Cellulose has 99 % purity, with an average size of 32 μm . Glucoamylase (EC 3.2.1.3) 10,000 U/ml, from *Aspergillus niger*, Guar gum powder, Pancreatin 8 x USP from porcine pancreas, calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), and sodium acetate ($\text{C}_2\text{H}_3\text{NaO}_2$) were from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Invertase (EC 3.2.1.26) 11,600 U/ml, from *Saccharomyces cerevisiae*, was from Fisher Scientific (Massachusetts, USA). Ethanol ($\text{C}_2\text{H}_5\text{OH}$), methanol (CH_3OH), potassium hydroxide (KOH), hydrochloric acid (HCl), benzoic acid ($\text{C}_7\text{H}_6\text{O}_2$), acetic acid (CH_3COOH) were from Isolab (Wertheim, Germany).

2.2 Summary of Experimental Design

Table 2.1. Experimental design parameters

Analysis	Factors	Levels
1. Differential Scanning Calorimeter (DSC)	Fiber Type	Psyllium (Soluble) Cellulose (Insoluble)
2. X-Ray Diffraction (XRD)	Fiber Ratio (Starch:Fiber)	1:0
3. Fourier Transform Infrared (FTIR) Spectroscopy		1:1
4. Scanning Electron Microscope (SEM)	Moisture Concentration	50 %
5. <i>In vitro</i> Digestion		65 % 80 %

2.3 Method

2.3.1 Preparation of the Samples

Moisture contents of all the raw samples and cooked samples were measured before the analyzes. Wheat starch, psyllium, and cellulose had moisture contents of 11.50 ± 0.41 %, 8.01 ± 0.76 %, and 6.03 ± 0.22 %, respectively. Wheat starch with and without added fiber (psyllium or cellulose) was mixed with the predetermined three different water content (50, 65, and 80 %). The starch to fiber ratio was 1:1 (dry weight basis) for fiber added samples. Samples were weighed into beakers, and determined amounts of distilled water were added to obtain water contents of 50, 65, and 80% (wet basis) except the control (no added water) samples. Ingredients were mixed with a magnetic stirrer (Jeio Tech, MS-52M, Korea). Samples were transferred into polypropylene bags in amounts of 40 g for the cooking process, where the samples that would not need cooking were used as they were in the analyzes. The bags were sealed and allowed to stand at room temperature for 2 hours

before heating at 90 °C for 10 min in a water bath. After the heating process, samples were kept at room temperature over a period of 10 min. Then, the samples were frozen at -80°C for 24 h and freeze-dried (Martin Christ, Germany). Before the analysis, all the cooked samples were grounded into powder with a mill for seven seconds and passed through a 425 µm sieve.

2.3.2 Characterization

2.3.2.1 Differential Scanning Calorimeter (DSC) Analysis

The thermal properties were analyzed using Perkin Elmer DSC 4000 differential scanning calorimeter (Perkin Elmer, Turkey) operating with Pyris software. Pure nitrogen was used as the purge gas with a flowing rate of 19.8 ml/min. Uncooked samples, prepared as described previously, were weighed approximately 5 mg (dry basis) accurately into 30 µL aluminum DSC pans and then hermetically sealed. Sealed pans were allowed to equilibrate for 4h at room temperature before differential scanning calorimeter (DSC) analysis. An empty aluminum DSC pan was used as a reference. The temperature scan was from 30°C to 110 °C at a rate of 10 °C/min, and the endotherm curve was recorded to calculate the thermal parameters, including gelatinization onset temperature and gelatinization enthalpy. Three replicates of each sample were analyzed.

2.3.2.2 X-Ray Diffraction (XRD) Analysis

The measurements were carried out in the Central Laboratory of the Middle East Technical University with an X-ray diffractometer (Rigaku Ultima-IV X-ray, U.S.). X-ray diffractometer was operated at 40kV, 40 mA Cu-K α radiation at wavelength $\lambda = 0.154$ nm. The scanning region of the diffraction angle (2θ) was from 4° to 40° at 4°/min. Three replicates of each sample were analyzed.

2.3.2.3 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Assessment of the molecules and the bonds that constitute the structure of the samples was achieved using an IR Affinity-1 Spectrometer with Attenuated Total Reflectance (ATR) attachment (Shimadzu Corporation, Kyoto, Japan). Samples were analyzed in the region of 4000-400 cm^{-1} at a resolution of 4 cm^{-1} . Each spectrum was collected at a resolution of 4 cm^{-1} with an accumulation of 32 scans against the air as background. Three replicates of each sample were analyzed.

2.3.2.4 Scanning Electron Microscope (SEM) Analysis

The morphology of starch samples was monitored using a scanning electron microscope (QUANTA 400F, US). Starch samples were coated with 3 nm thickness of gold (Au-Pd) since they have no electrical conductivity. The imaging analyses were carried out in the Central Laboratory of the Middle East Technical University, with magnification between 500 – 4000x.

2.3.3 *In vitro* Digestion Analysis

In vitro digestion simulations were performed according to the method described by (Englyst et al., 2018; 2006) with some modifications. Since model systems without protein content were used in this study, pepsin enzyme (EC 3.4.23.1) was not used for this *in vitro* simulation. Solution I was for creating low pH like the human stomach for the gastric stage without pepsin enzyme and Solution II was to simulate the small intestinal stage with intestinal enzymes (Glucoamylase (EC 3.2.1.3), Pancreatin 8 x USP from porcine pancreas). About 0.5 g and 1 g sample (in dry matter) were weighed into 50 ml screw-cap polypropylene tubes for fiber-free and fiber-containing samples, respectively. Weights of samples were adjusted to provide about 500 mg of available carbohydrate. A tube defined as blank prepared with no sample in it and treated the same as other sample tubes. Flow chart of digestion

simulation steps and free glucose analysis were presented in sections 2.3.3.3 and 2.3.3.3.1, respectively. The amount of released glucose was determined by a D-Glucose Assay Kit (GOPOD Format) (Megazyme, Ireland) for both analyses.

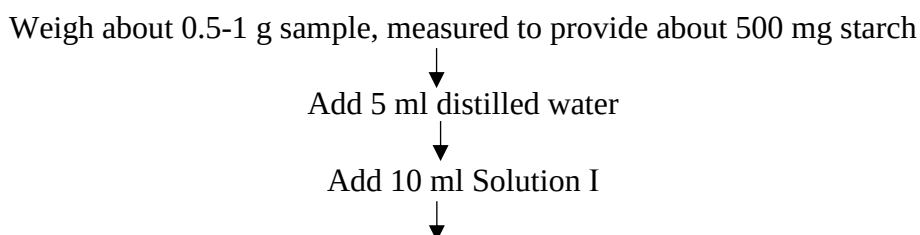
2.3.3.1 Preparation of Solution I

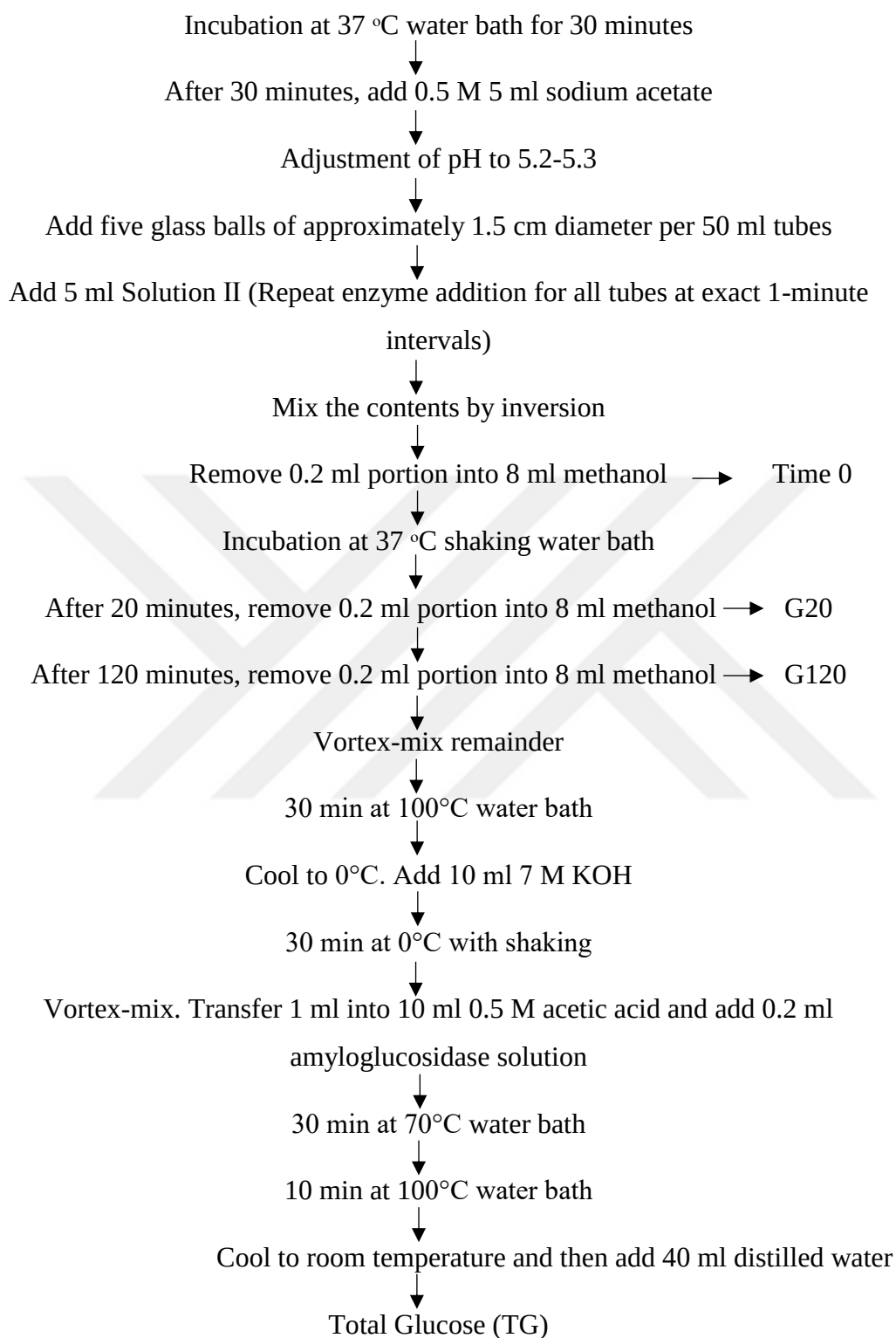
0.6 g guar gum was added into a beaker and wetted with 1 ml ethanol to prevent lumps from forming. After that, 120 ml of 0.05 M hydrochloric acid was added, and the mixture was mixed with a magnetic stirrer (Jeio Tech, MS-52M, Korea). This suspension was kept agitated until usage to keep the guar gum in suspension.

2.3.3.2 Preparation of Solution II

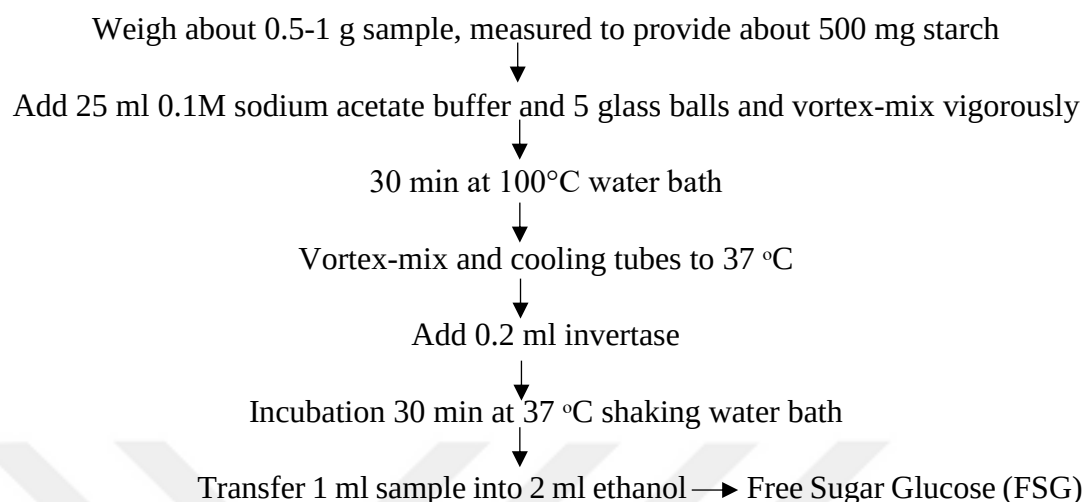
For samples and standards, into each of three 50 ml centrifuge tubes 3.3 g of 8 x USP pancreatin enzyme weighed and suspended in 22 ml 0.1 M calcium chloride solution. The mixtures were mixed using a vortex mixer for 10 min and then centrifuged (Sigma, 2-16KL, Germany) at 1500 g for 10 min. 17 ml of the cloudy supernatant from each of the centrifuge tubes were taken and combined in a beaker. Into the beaker, 4 ml glucoamylase (EC 3.2.1.3, 10,000 U/ml), and 2 ml distilled water were added. The solution was mixed with a magnetic stirrer (Jeio Tech, MS-52M, Korea), and the temperature of the solution was brought to 37 °C in a water bath, a few minutes before use. The Solution II was used within one hour of use due to enzyme activation.

2.3.3.3 *In vitro* Starch Digestion Flow Chart





2.3.3.3.1 Free Glucose Stage Flow Chart



2.3.3.4 Determination of Glucose Amount

The glucose content of the tubes was measured using a Megazyme glucose assay kit (GOPOD (glucose oxidase-peroxidase) Format, Megazyme International Ireland Ltd., Ireland). D-Glucose was measured using the kit based on the glucose oxidase/peroxidase enzymatic procedure. The reactions that form the principle of this enzymic procedure are provided below, and the reagents necessary for the experiment were presented in Table 2.2.

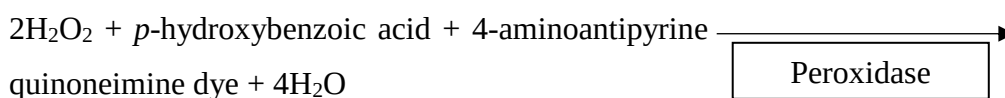
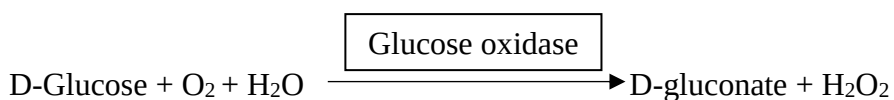


Table 2.2. Reagents of Glucose Assay Kit

GOPOD Reagent Buffer:	Buffer (50 mL, pH 7.4), p-hydroxybenzoic acid and sodium azide (0.095% w/v)
GOPOD Reagent Enzymes:	Glucose oxidase, peroxidase and 4-aminoantipyrine
D-Glucose standard solution:	5 ml, 1.0 mg/ml in 0.2% (w/v) benzoic acid
GOPOD Reagent:	GOPOD Reagent Buffer + 1L distilled water + GOPOD Reagent Enzymes

Before the measurement of released glucose, the solutions of the samples were centrifuged at 500 g for 5 min. After centrifugation, 0.1 ml of the solution was transferred into a tube, and 3.0 ml of GOPOD reagent was added. The tubes were incubated at 47 °C for 20 minutes. Absorbances of the samples and D-glucose standard was determined with a spectrophotometer (The Shimadzu UV-1700, Japan) at 510 nm, against the reagent blank to obtain ΔA_{sample} and $\Delta A_{\text{D-glucose standard}}$. The amount of D-Glucose ($\mu\text{g}/0.1 \text{ mL}$) in the solutions was calculated using the equation below.

$$\text{D-Glucose } (\mu\text{g}/0.1 \text{ mL}) = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{D-Glucose standard (100 } \mu\text{g)}}} \times 100 \quad (\text{Eq.1})$$

2.3.4 Calculations of Starch Fractions

Carbohydrate digestibility parameters, which were rapidly digestible starch (RDS), slowly digestible starch (SDS), and remaining starch (RS) percentages per gram starch in the samples, were calculated according to equations below from the measurement of glucose fractions, where:

FSG: free soluble glucose present in the sample (g)

G20: glucose released from the sample at the first 20 minutes (g)

G120: glucose released from the sample at the 120 minutes (g)

TG: total glucose present in the sample (g)

$$TS = 0.9 \times (TG - FSG) \quad (\text{Eq.2})$$

$$RDS (\%) = (0.9 \times (G20 - FSG)/TS) \times 100 \quad (\text{Eq.3})$$

$$SDS (\%) = (0.9 \times (G120 - G20)/TS) \times 100 \quad (\text{Eq.4})$$

$$RS (\%) = (0.9 \times (TG - G120)/TS) \times 100 \quad (\text{Eq.5})$$

2.3.5 Statistical Analysis

The data were the means of at least three replicate measurements. Analysis of variance (ANOVA) and Tukey's comparison test (95 % confidence interval) was carried out by using the statistical software MINITAB (Version 16.2.0.0, Minitab Inc., Coventry, UK). The statistical calculation tables were presented in Appendix C.



CHAPTER 3

RESULTS AND DISCUSSION

3.1 Physical Properties

3.1.1 Thermal Properties with Differential Scanning Calorimeter (DSC)

Results showed that decreasing the water content to 50 % caused a reduction in the gelatinization enthalpy (J/ g starch) of native wheat starch (Table 3.1). There was no statistically significant ($p>0.05$) difference in the gelatinization enthalpies (J/ g starch) of the samples at high water concentrations (65 and 80%). These results indicated that low water concentration limited the starch granules' swelling and caused some remaining double-helical structures in the granules, usually disrupted at higher water concentrations during heating processes.

The starch-water interactions start mainly in amorphous regions of the granule. Starch granule swells substantially with water absorption and gelatinizes until available water runs out in the medium. Olkku & Rha (1978) stated that when the temperature of the starch-water mixture increases but remains below the gelatinization temperature range, the water mobility is reversibly reduced. This is attributed to the adsorption of water molecules to starch granules. During the gelatinization phase, the mobilization of water increases, which indicates the rapid transfer of soluble starch into solution. After that, a slight increase in the mobilization of water and starch is observed. When the water concentration increases up to 65 and 80%, extensive hydration and swelling of the starch granules facilitate the melting of the starch crystallites during heating. If there is limited water in the medium, only partial melting of crystallites occurs rather than complete gelatinization. The slight

increase in the transition temperatures with the increasing concentration of water from low (50%) to high water concentrations (65 and 80%) (Table 3.1) was parallel with the continued swelling of granules. This slow process observed at high water concentrations is related to the softening, the ability of granules to swell, and sucking water in from the surface to the amorphous growth rings of the starch granules. The range of gelatinization becomes broader depending on the starch/water ratio. Wang & Copeland (2013) stated that broadening reflects the changes from limited swelling to maximum swelling of starch granules and partial dissolution of starch polymers with the increasing water concentration. The absence of available water limits the swelling of the starch granules and restricts access to their internal structures for solubilization. Bertrand et al. (2019) observed low and relatively constant enthalpies at low water concentrations ($\leq 50\%$ weight) and a similar increase in enthalpies as the water content was increased. Increasing transition temperatures with increasing water: starch has also been reported in the literature (Biliaderis, Maurice, & Vose, 1980; Gunasekaran & Altay, 2006; Wang & Copeland, 2012).

The addition of psyllium fiber (1:1) to wheat starch decreased the measured gelatinization enthalpies (J/ g starch) at high water concentrations (80 % and 65 %), and no gelatinization peak was seen at low (50%) water concentration (Table 3.1). There was no statistically significant difference in the measured gelatinization enthalpies with the cellulose fiber addition (Table 3.1). Comparing the gelatinization enthalpy values per gram of “sample” with the values per gram of “starch” in the sample for all water concentrations showed the effect of dilution due to fiber addition (Table 3.1).

The reduction in the measured gelatinization enthalpies (J/g starch) with psyllium fiber addition could be due to the restriction of available water for starch to use during gelatinization, caused by the high water holding capacity of psyllium. Cellulose fiber did not affect the gelatinization enthalpy like psyllium. At low water

concentration, psyllium fiber inhibited the gelatinization entirely, indicating that the water in the psyllium starch mixture could be all bound. However, there was free water available for starch to cause limited gelatinization on the granule surface for the cellulose starch mixture.

Since the enthalpy of transition refers to the disruption of crystal order, reduced gelatinization enthalpies (J/g starch) indicated that psyllium fiber addition restricted the disruption of the crystalline structures by limiting the swelling of the starch granules. The decreases in ΔH values of wheat starch containing psyllium agreed with previous studies that used hydrocolloids such as inulin, xanthan gum, and beta-cyclodextrin (Luo et al., 2017; Tang, Hong, Gu, Zhang, & Cai, 2013; Tian et al., 2009).

The observed onset, peak, and conclusion temperatures during gelatinization of psyllium and cellulose fiber added samples were higher ($p < 0.05$) than the temperature values of starch alone samples at all water concentrations (Table 3.1). The differences were higher for the psyllium fiber added samples. These elevated gelatinization temperatures were possibly due to the restriction of available water for starch gelatinization with the fibers' high hydration ability, causing a competition of free available water between the fibers and starch. Limiting the water concentration by adding non-starch polysaccharides into the matrix increases the onset temperature of the gelatinization process until reaching the temperature that facilitates hydration of the amorphous regions of the starch granule and triggers the start of the gelatinization process. Several authors also stated that the addition of soluble fibers might increase the starting point temperature of gelatinization (Tang et al., 2013; Tester & Sommerville, 2003; Varela, Navarro, & Yamul, 2016).

Polysaccharides have a strong affinity for water molecules due to the presence of multiple hydroxyl (OH) groups. The solubility of a polysaccharide can be understood with the molecule-molecule interaction and molecule-water interaction. For soluble polysaccharides, water-molecule interactions prevail; water molecules form an envelope around the polymer chain, keeping the polysaccharide molecules apart (Guo et al., 2017). However, for insoluble polysaccharides, the intramolecular interactions between polymer segments dominate and eventually result in aggregation and precipitation (Guo et al., 2017). Cellulose chains have a hydrophilic character, tend to interact with themselves and materials containing hydroxyl groups (Khazraji & Robert, 2013). In the water medium, hydrogen bonding dominates the interchain energy, and the van der Waals dispersion forces are responsible for holding the chains together. Water molecules cannot get in between the cellulose chains due to the strong aggregations of chains. Water molecules can surround the chains and leak between the fibers through capillary forces. The increase in the measured gelatinization temperatures of wheat starch with the addition of cellulose could be explained by the fiber's tendency to interact with available water in the medium and limiting water for starch gelatinization. On the other hand, since psyllium is a viscous soluble fiber that has strong water-molecule interactions and the ability to form a gel in starch-water media, it could effectively delay the melting of starch crystallites more than cellulose fiber, which has low water-molecule interactions in aqueous starch suspension (Figueroa et al., 2018; Guo et al., 2017).

Table 3.1. Thermal properties of wheat starch with and without added psyllium and cellulose fibers

Samples	Water Concentration	T _o (°C)	T _p (°C)	T _d (°C)	ΔH (J/g sample)	ΔH (J/g starch)
S		56.97±0.32 ^{cA}	62.64±0.47 ^{cA}	69.75±0.58 ^{cA}	17.34±1.48	17.34±1.48 ^{aA}
S + C	80 %	63.58±0.03 ^{bA}	68.89±0.13 ^{bA}	74.30±0.30 ^{bA}	6.50±1.40	13.00±2.80 ^{abA}
S + P		64.69±0.13 ^{aB}	70.42±0.08 ^{aB}	76.37±0.10 ^{aB}	6.36±1.12	12.72±0.13 ^{bA}
S		57.79±0.12 ^{cA}	62.07±0.10 ^{cA}	70.11±0.43 ^{cA}	15.84±0.38	15.84±0.38 ^{aA}
S + C	65 %	63.16±0.12 ^{bB}	68.49±0.23 ^{bB}	74.11±0.20 ^{bA}	6.57±0.88	13.14±1.76 ^{abA}
S + P		65.75±0.55 ^{aA}	73.14±0.49 ^{aA}	81.67±1.16 ^{aA}	5.99±0.41	11.98±0.82 ^{bA}
S		55.28±0.64 ^{bB}	60.32±0.25 ^{bB}	66.15±0.23 ^{bB}	5.17±0.64	5.17±0.64 ^{aB}
S + C	50 %	62.69±0.04 ^{aC}	67.87±0.01 ^{aC}	74.27±0.28 ^{aA}	2.86±0.19	5.72±0.38 ^{aB}
S + P		n.d.	n.d.	n.d.	n.d.	n.d.
S		n.d.	n.d.	n.d.	n.d.	n.d.

*The data are averages of three measurements with the standard deviation. Different small letters (a,b,c) indicate significant differences in each box in each column (p<0.05). Different capital letters (A, B, C) indicate significant differences in each group (S, S+C, or S+P) in each column (p<0.05). S: wheat starch, S+C: wheat starch and cellulose, S+P: wheat starch and psyllium at 1:1 dry weight base.
n.d.: not detected*

3.1.2 Crystalline Properties with X-Ray Diffraction (XRD) Analysis

XRD analyses were performed to analyze the long-range disruption of starch granules with or without fiber addition (Figure 3.1 - 3.4). Raw wheat starch exhibited a semi-crystalline structure with peaks at 2θ of 15.0° , 17.1° , 18.1° , 19.9° , and 23.0° (Figure 3.1). The strong reflections around 15° , 23° , and the doublet at 17° - 18° are defined as A-type pattern. The weak peak at 2θ of 20° is attributed to the amylose-lipid complex in starch structure. X-ray diffraction patterns of raw psyllium and cellulose fibers also showed their typical profiles (Figure 3.1). Psyllium fiber showed a broad peak at 2θ of 19.7° of less intensity. Similar observations had been obtained by other researchers (Bajpai, Chand, & Agrawal, 2015; Kumar, Pandey, Khan, Kumar, & Kundu, 2019; Rao, Gaikwad, & Shevate, 2017). The evident halo pattern in the diffractograms was indicative of the amorphous nature of psyllium. Cellulose fiber has a partially crystalline structure and displayed typical I-type crystallization with rising diffraction peaks at 2θ angles around 16° , 22° , and 35° .

The X-ray diffraction profile of the starch samples cooked at high water concentrations, 65%, and 80%, contains a broad peak, which means that the starch matrix was amorphous or weakly crystalline. In contrast, the diffractogram of the sample cooked at low (50 %) water concentration depicts some residual peaks, indicating some remaining crystallinity in the samples (Figure 3.2). Even though the heating profiles were different for the DSC analysis and the cooking process, the results were parallel with the obtained DSC data, where similar gelatinization enthalpy values were obtained at higher water concentrations (65% and 80%) and lower gelatinization enthalpy value at the low water (50%) concentration (Table 3.1). Results indicated that, at high water concentrations, crystallinity was lost due to gelatinization. The residual A-type peaks seen for the wheat starch cooked at lower water concentration (50%) indicated that limiting the water content can lead to incomplete starch gelatinization due to limited swelling of granules and leaving residual crystallinity. The ordered three-dimensional structure of amylopectin,

packing of double helices, is related to the crystallinity of starch granules. Amylose structure also has an effect on the crystalline structure. Yu, Wang, Xu, Guo, & Du, (2016) explained starch gelatinization as the transition of starch from a stable equilibrium state to a disordered non-equilibrium state, and as a consequence, the amylose gel network occurs.

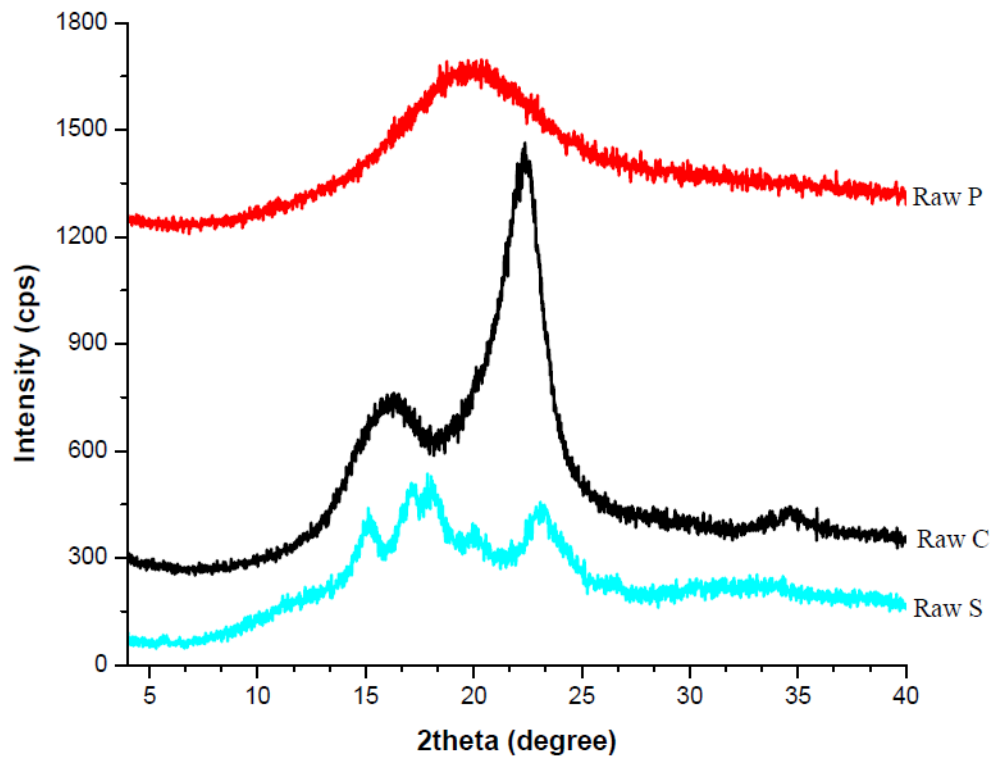


Figure 3.1. X-ray diffractograms of raw starch, cellulose, and psyllium. S: Starch, C: Cellulose, P: Psyllium.

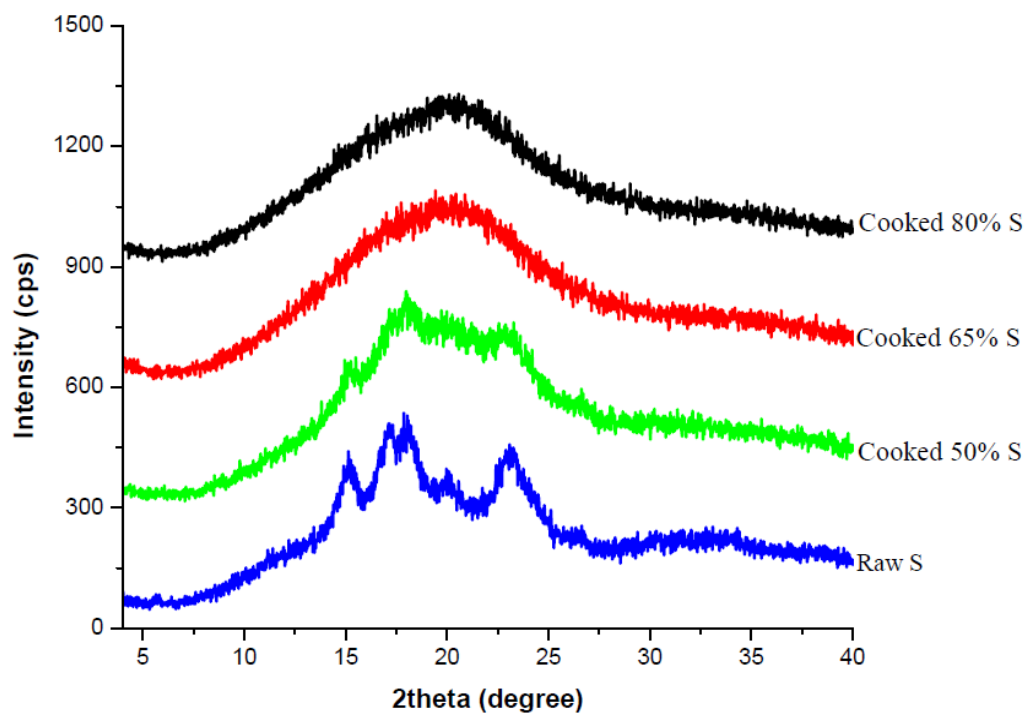


Figure 3.2. X-ray diffractograms of raw and cooked wheat starch (80%, 65% and 50% water concentration). S: Starch.

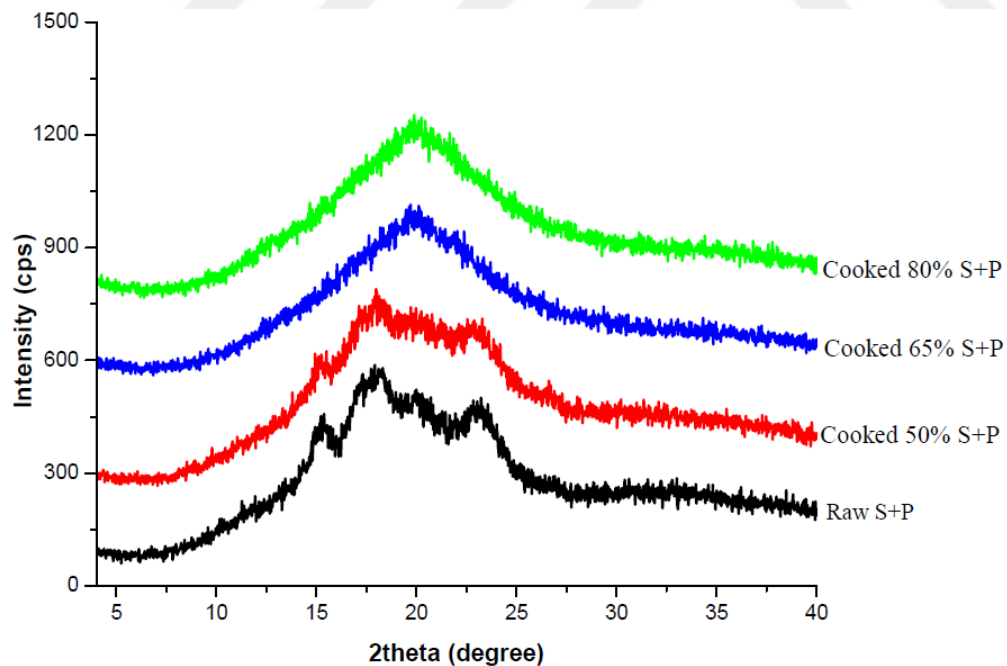


Figure 3.3. X-ray diffractograms of raw and cooked wheat starch-psyllum mixtures (80%, 65% and 50% water concentration). S+P: Starch and Psyllum at 1:1 dry weight base.

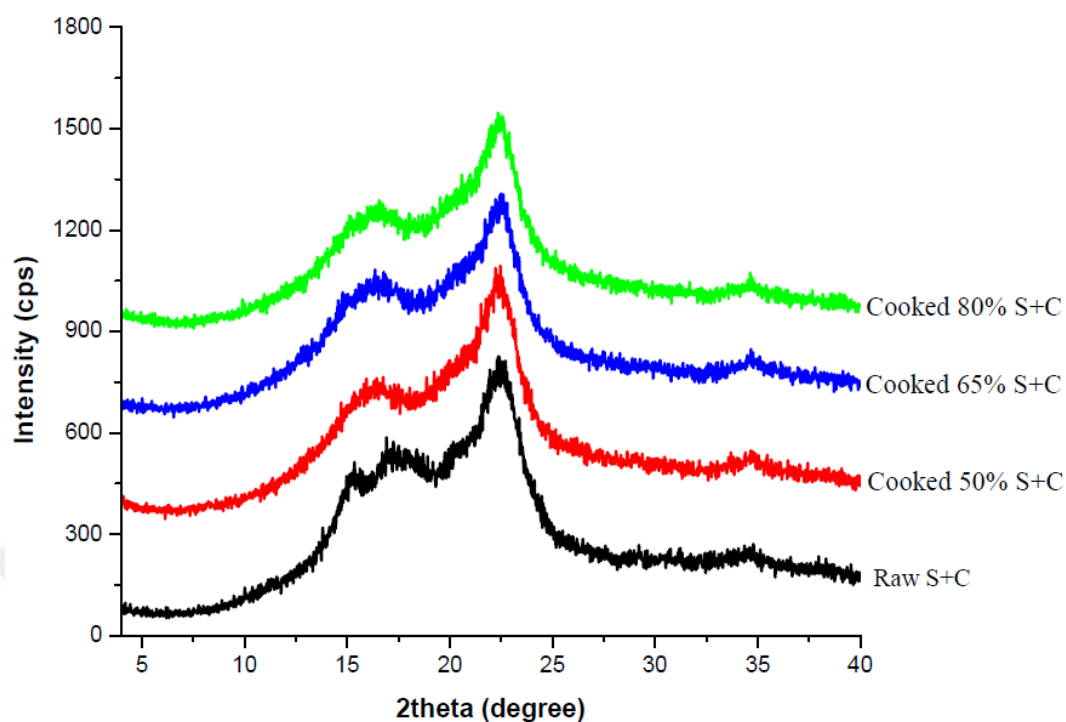


Figure 3.4. X-ray diffractograms of raw and cooked wheat starch-cellulose mixtures (80%, 65%, and 50% water concentration). S+C: Starch and Cellulose at 1:1 dry weight base.

As seen in Figure 3.1, the peak intensities of the psyllium and cellulose fibers were higher compared to wheat starch. Therefore, when the fibers were mixed with the starch, their high-intensity peaks covered some of the starch peaks during the measurements, causing the loss of sharpness of the typical A-type pattern. Only some of the typical A-type peaks could be seen in the diffractograms of the raw mixtures (Figure 3.3 and 3.4). For example, in the raw psyllium fiber starch mixture, the broad, amorphous peak of psyllium concealed the sharpness of the peaks, only the tip of the peaks at 15° , 17° - 18° , 20° , and 23° of the wheat starch could be visible. Similarly, in the cellulose fiber starch mixture, cellulose peaks obscured almost all of the peaks, especially at 17° - 18° and 23° , and only the presence of the peaks at 15° and 23° could be observed (Figures 3.3 and 3.4).

Cooked psyllium-starch mixtures at 65% and 80% water concentrations have wide diffractograms with a sharper peak compared to round diffractograms of the no fiber added cooked starch samples at the same water concentrations (Figure 3.2 and 3.3). However, at 50% water concentration, the XRD diffractograms showed slight differences between the cooked and the raw psyllium-starch mixture (Figure 3.3). The supporting DSC data (Table 3.1) indicated that when compared to wheat starch, psyllium fiber added starch samples showed lower gelatinization enthalpies at higher water concentrations (65 and 80%), whereas no gelatinization peaks were seen at 50% water concentration (Table 3.1). Psyllium fiber has a strong gelling and water absorption capacity. In water media, psyllium fiber swells as a result of developing new inter-and intramolecular hydrogen bonds with water molecules and entanglement of psyllium chains. Psyllium fiber can trap much more water within its chains due to these interactions (Nie, Cui, & Xie, 2018). Data showed that psyllium fiber addition limited the gelatinization and loss of the crystallinity of wheat starch at higher water concentrations (65% and 80%) and inhibited the gelatinization and loss of crystallinity at low water concentrations (50%) (Figure 3.3). Heat and moisture are both essential factors in the gelatinization of starch. Water promotes the effects of heat treatment on starch crystallite disruptions. The long-range crystalline structure is governed by amylopectin molecules. Their side chains form a crystalline structure by hydrogen bonding, and branch points form the amorphous region. When water can penetrate and hydrates the starch chains, hydrogen bonds are broken, and these long-range structures are disrupted. Therefore, wheat starch was expected to degrade as the water content increased. Starch gelatinization is an endothermic process that occurs when starch granule swells substantially with the absorption of water. As seen in Figure 3.2, when there was excess water in media (80% and 65%), extensive hydration and swelling of starch granules occurred, which caused the melting of the starch crystallites and turning into the amorphous matrix. In addition, higher DSC transition temperatures obtained by the psyllium added samples (Table 3.1) compared to starch only samples indicated higher crystallinity and resistance to gelatinization, supporting the XRD findings in this study. The XRD pattern is

affected by many properties such as the length of the amylopectin chain and the formation of new bonds such as H-bonding between –OH groups. The formation of a sharper peak in the psyllium added samples after the gelatinization process could indicate that new chain alignments related to psyllium might have occurred.

The raw and cooked samples with added cellulose fiber showed a diffraction peak around 2θ of 22° , which comes from the partially crystalline region of the cellulose fiber. This diffraction peak had a wide shoulder from 16° to 22° values, and these values incidentally were in the same region of the wheat starch diffraction rising peaks. The gelatinized wheat starch can be seen through the amorphous range in the 2θ region of 10° to 30° . The broad and high-intensity peaks of cellulose fiber (Figure 3.1) covered the diffractograms of the gelatinized starch and limited the quantification of the change in the crystallinity (Figure 3.4). A similar phenomenon was reported by other researchers. Teixeira et al. (2014) observed a characteristic cellulose I-type profile for processed starch-fiber mixture and remarked that the crystallinity of cellulose fiber overlaps with the gelatinized starch.

The difference that was seen in the measured gelatinization enthalpies at 50% water concentration for psyllium and cellulose added wheat starch could still be observed in the XRD diffractograms. The XRD diffractograms of cellulose starch mixtures demonstrated that the slightly visible peaks in the raw mix at 15° and 23° disappeared after cooking, at all water concentrations, indicating loss of crystallinity (Figure 3.4). However, no noticeable reduction was observed in the visible characteristic peaks of wheat starch (Figure 3.3) at 50 % water concentration for psyllium added mix.

3.1.3 Short-range order with Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FTIR spectra of polysaccharides originate from their monomer glucose units' vibrational state in the region between 800 and 1500 cm^{-1} . Absorption peaks in the range of 800–1200 cm^{-1} reflect the short-range order of starch (Liu et al., 2017). The peak in the wavelength around 1047 cm^{-1} represents the ordered crystalline structure, and the peak in the wavelength around 1022 cm^{-1} represents the amorphous structure of the starch in the FTIR spectra. In this study, the wavelength around 1047 and 1022 cm^{-1} observed at 1043 and 1016 cm^{-1} , respectively. Since the band around 1022 cm^{-1} reflects the amorphous regions of the structure, it is sensitive to changes in the degree of order within the granule; it can be emphasized by decreasing the crystallinity (Liu et al., 2017). Hereby, the ratio of 1043 and 1016 cm^{-1} can be used to interpret the crystallinity of the short-range ordered molecules (Flores-Morales, Jiménez-Estrada, & Mora-Escobedo, 2012; Van Soest, de Wit, Tournois, & Vliegenthart, 1994).

Cooking the wheat starch at a high water concentration (80%) caused a reduction in 1043/1016 cm^{-1} ratios, whereas, at other water concentrations (50% and 65%), there was no significant difference from the raw starch (Table 3.2). Natural psyllium and cellulose fiber also showed absorbances at 1043 and 1016 cm^{-1} wavenumbers, and their IR ratios (1043/1016) were higher than native wheat starch (Table 3.2). The cooked fiber starch mixtures had higher IR ratios than starch alone samples because of the fibers' higher IR ratios. When starch-fiber mixtures cooked at different water concentrations compared separately for psyllium and cellulose, it was seen that the IR ratios decreased as the water concentrations increased for both fibers. At higher water concentration, extensive hydration and swelling of the starch granules facilitate the melting of the starch crystallites during heating, leading to the destruction of many of the short-range double helices in the starch granules. On the other hand, at lower water concentrations, not all double helices were disrupted. While XRD detects the double helices' long-range order, FTIR is mainly related to

the level of double helices present in both the crystalline and amorphous regions of the starch granules. The loss of long-range crystallinity may occur before the disruption of short-range molecular order of starch, as stated in other studies (Van Soest, Tournois, de Wit, & Vliegenthart, 1995; Wang, Zhang, Wang, & Copeland, 2016).

Table 3.2 IR ratios of 1043/1016 cm^{-1} of raw materials (wheat starch, psyllium and cellulose fibers) and starch with and without psyllium and cellulose fibers cooked at different water concentrations.

Samples	Water Concentration	1043/1016 cm^{-1} Ratio
S	80 %	0.633±0.017 ^b
S + C		0.728±0.006 ^b
S + P		0.822±0.007 ^b
S	65 %	0.671±0.018 ^{ab}
S + C		0.732±0.002 ^b
S + P		0.829±0.003 ^{ab}
S	50 %	0.694±0.013 ^a
S + C		0.749±0.005 ^a
S + P		0.835±0.004 ^a
Raw S		0.707±0.015 ^a
Raw C		1.005±0.009
Raw P		0.968±0.007

The data are averages of three measurements with the standard deviation. Different small letters (a, b, c) indicate significant differences in each group (S, S+C, or S+P) ($p < 0.05$). S: wheat starch, S+C: wheat starch and cellulose, S+P: wheat starch and psyllium at 1:1 dry weight base.

3.1.4 Granular Morphology with Scanning Electron Microscope (SEM) Analysis

The scanning electron micrographs revealed that raw wheat starch granules have bimodal size distribution with large-disc like or lenticular and small-spherical shaped

granules (Figure 3.5.j). Large disc-like or lenticular granules indicate A-type, and small spherical-shaped granules represent B-type granules. After the heating process, starch granules swelled and merged (Figure 3.5.a,d,g). As the water concentration increases, starch granules become more susceptible to gelatinization and have more cracks on the surface (Chen, He, Fu, & Huang, 2015; Wang et al., 2018). As the water and heat in the cooking process cause gelatinization, they also cause inconsistent swelling and concavities on starch granules' surfaces. Sun, Wu, Bu, & Xiong (2015) described the gelatinized wheat starch structure's appearance as a honeycomb. As the water concentration increased, the gelatinized wheat starch structure was observed with a greater degree of granule swelling and coalescence accompanying by the progressive loss of granular morphology (Figure 3.5.a,d). However, at the limited water concentration (50 %), limited swelling can be observed after cooking (Figure 3.5.g).

In the raw mixture of starch-psyllium and starch-cellulose, the fibrous and rough surface of the fibers and smooth surface of large-small granules of starch observed clearly (Figure 3.5.k,l). The raw starch-psyllium mixture's SEM image showed that the psyllium has a larger surface area (Figure 3.5.l). The starch-cellulose mixture showed that the fibers were poorly inserted into the matrix due to the poor interaction (Figure 3.5.k).

When wheat starch was heated with psyllium fiber, fiber dispersion was observed within the starch matrix (Figure 3.5). During the cooking process, the fibers plucked rather than pulled out. This observation was an indication of the fibers adhering well to the starch matrix (Gironès et al., 2012). The rough, porous fracture surface with many cracks was observed at higher water concentrations (65, 80%). At lower water concentrations, it was possible to see the starch granules that were not disrupted (Figure 3.5.i). It can be attributed to limited available water for the gelatinization process and the high hygroscopic property of psyllium. The observations, revealed

by DSC and XRD data, indicating that starch cooked with psyllium had higher crystallinity and resistance to gelatinization at low water concentration (50%), were also observed with the SEM micrograph (Figure 3.5.i). In water media, psyllium fiber swells as a result of developing new inter-and intramolecular hydrogen bonds with water molecules with its strong gelling and water absorption capacity. As a result, psyllium cause restriction in the hydration of starch by controlling the granule swelling and delaying the gelatinization.

When wheat starch was cooked with cellulose fiber, the smooth surface structure turned into a porous structure (Figure 3.5). Even at a lower concentration (50%) a honeycomb structure could be seen for cellulose added samples (Figure 3.5.h). The weaker binding was seen with longer pulled-out cellulose fibers in the starch matrix.

Both cooked samples with added fibers showed voids and fissures but with a larger number of pulled-out fibers in the case of cellulose added samples compared to the cooked starch-psyllium mixtures indicating a better adhesion between psyllium fiber and the matrix. That might be due to the covalent or non-covalent bonds within the matrix, which results in better interactions.

The behavior of psyllium fiber was different from cellulose fiber when they were in aqueous solutions of starch. This behavior might depend on their solubility and interactions with starch granules. While psyllium is a viscous fiber that can absorb large amounts of water and develops inter-and intramolecular hydrogen bonds, cellulose has low water-molecule interactions between polymer segments. As a result, water molecules cannot enter between cellulose chains effectively. Therefore, cellulose could not delay the complete disruption of the granular structure; hence, the gelatinization process.

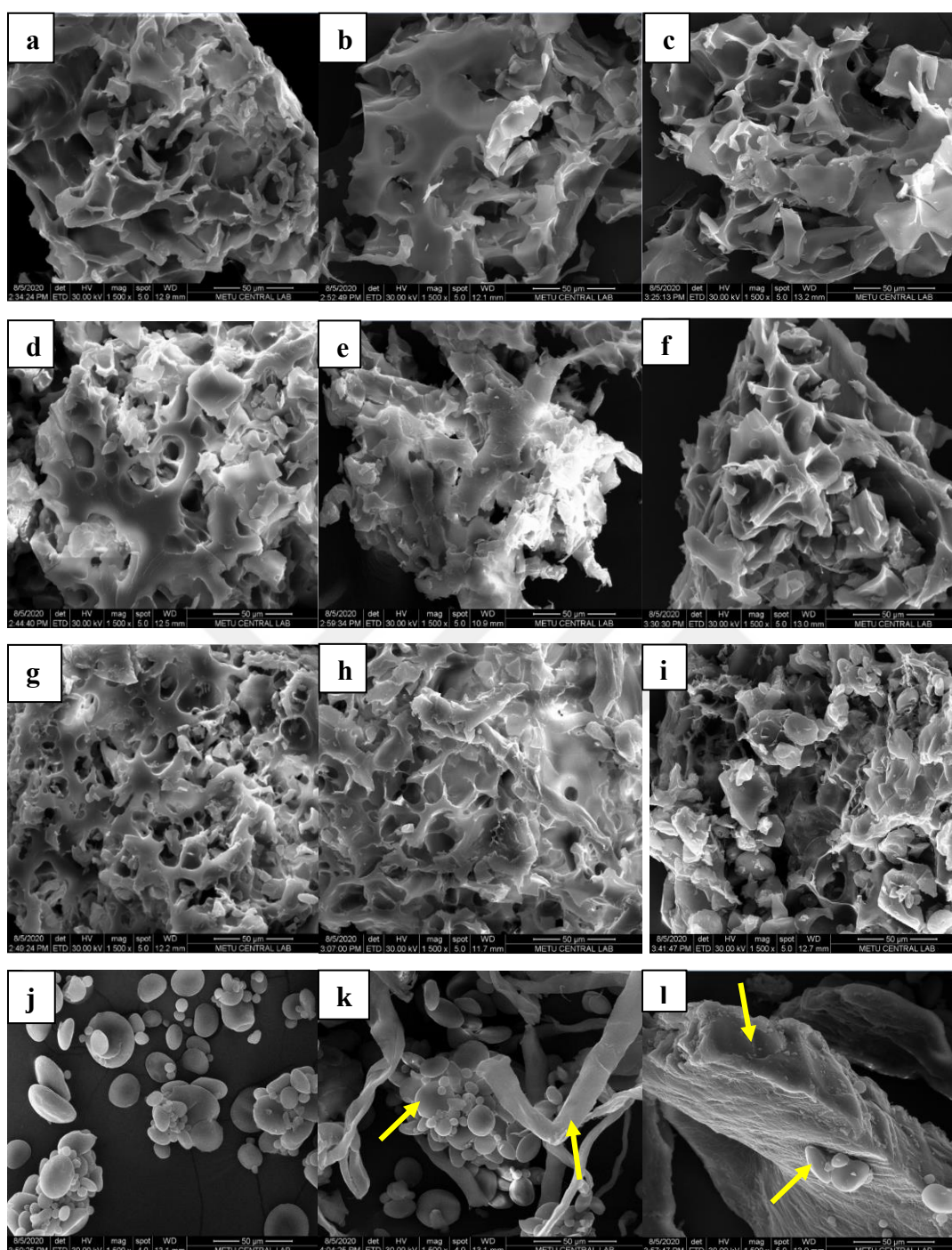


Figure 3.5. Scanning electron micrographs of raw and cooked wheat starch with and without fibers at 1500x magnification. a. Cooked 80%S, b. Cooked 80%S+C, c. Cooked 80%S+P, d. Cooked 65%S, e. Cooked 65%S+C, f. Cooked 65%S+P g. Cooked 50%S, h. Cooked 50%S+C, i. Cooked 50%S+P, j. Raw S, k. Raw S+C, l. Raw S+P

3.1.5 *In vitro* digestibility of wheat starch

Glucose release rates from the wheat starch cooked at higher water concentrations (65 and 80%) were higher than starch cooked at 50% water concentration. The rapidly digestible starch (RDS) ratio which was measured as the digested starch within 20 minutes, increased with the increasing water concentration used during cooking (Table 3.3). The slowly digestible starch (SDS) ratio decreased with increasing the water concentration. Remaining starch ratio (RS) that escapes from digestion decreased as the energy used for gelatinization increased (Table 3.3). This behavior indicated that the starch cooked at a lower water concentration (50%) had more resistance to enzymatic digestion. Increased water concentration during cooking altered the progression of the gelatinization process. Similar results had been reported in several studies conducted so far (Chung, Lim, & Lim, 2006; Dona et al., 2010). The structural changes to an unordered state by gelatinization increased the susceptibility of starch granules to enzymatic degradation (Chen et al., 2019; Holm et al., 1988). Heat and water led to the melting of the long-range crystalline structure (Figure 3.2) and disruption of short-range molecular order (Figure B., Table 3.2).

Fiber addition to starch suspensions could change the measured starch hydrolysis data due to the fibers' dilution effect or other effects like changing the sample matrix. In the study, starch hydrolysis data were presented in two forms to emphasize the dilution effect of fibers on the calculated fractions (Table 3.3). In one form, digestion fractions (RDS, SDS, and RS) were given as percent (%) values in the whole "sample" (dry basis), and in the second form, those values were given as percent (%) of "starch" present in the sample (dry basis). When the RDS, SDS, and RS fractions in the whole "samples" were compared with the data normalized for the "starch" amount present in the samples, it was possible to see that the effect of dilution was influential, especially at these high fiber concentrations (Table 3.3). Discussions for fiber added samples were based on the fractions given relative to starch present in the samples, representing a fair comparison. As seen in Table 3.3, the addition of

both fibers into the starch matrix during cooking decreased the RDS fractions of starch present in the sample at all water concentrations, where the reductions were more pronounced for the psyllium fiber added samples (Table 3.3). Similarly, the SDS fractions of starch present in the samples were higher for almost all fiber added samples than starch samples without the fibers (Table 3.3). The increases in SDS fractions were higher for psyllium fiber added samples cooked at 50 and 65% water concentrations than cellulose fiber added samples. The addition of cellulose increased the RS fractions of starch in the cooked samples only at 50 and 65% water concentrations. RS fractions were higher for psyllium added samples at all water concentrations, and the differences were more pronounced compared to cellulose fiber addition (Table 3.3). The results showed that psyllium and cellulose could reduce RDS and increase SDS and RS fractions for wheat starch when added during cooking. Comparison of the raw samples with and without fibers showed that RDS and SDS fractions were lower and RS fractions were higher for the fiber added starch samples, where the differences were more pronounced for psyllium added samples (Table 3.3).

These results indicated that the matrix formed by psyllium or cellulose fibers affected both the gelatinization and digestion of wheat starch. Besides, type of fiber, cellulose, or psyllium, affected the digestion fractions differently, where psyllium fiber reduced starch hydrolysis more than cellulose fiber. The DSC data (Table 3.1) and the starch hydrolysis data (Table 3.3) together showed that there were many factors affecting the digestion fractions. One of the factors affecting the fractions was the degree of gelatinization. The second one was the enzyme-substrate interaction. Another factor was the dilution effect of the non-digestible compound added.

Limited gelatinization could be due to the swelling of psyllium fiber as a result of developed new inter-and intramolecular hydrogen bonds with water molecules with its strong gelling and water absorption capacity. Psyllium might have restricted water

absorption and swelling of the starch granule. It might have competed for available water with starch granules that could decrease the water mobility of the system and caused a limited internal rearrangement of starch polymers in the granule. Therefore, the resistance of starch granules to amylolysis increased due to limited gelatinization.

When the starch granules gelatinized with the action of heat and water, some of the amylose structure leach out and be deposited on the surface. In the meantime, fiber molecules also go towards the starch granule surfaces, depending on their hydrophilic nature. As a result of this tendency, fiber molecules and leached amylose might have formed a coating around the starch granules that can limit the access of digestive enzymes to starch granules. Also, it has been reported that viscous soluble fibers can increase the viscosity and hence decrease the mobility in the digesta due to their entangling and overlapping properties with starch granules (Zhang, Li, Tian, Song, & Ai, 2019).

The presence of fibers in the medium reduced the digestive enzyme-substrate reactions observed for the raw samples (Table 3.3). Psyllium is a viscous-soluble fiber. Therefore, psyllium chains might have formed aggregates and created high viscous digesta with the help of hydrogen bondings. This enhanced viscosity might have reduced the diffusion rate of digestive enzymes toward the starch granules, which ultimately slowed down the digestion rate. As a result, this viscous matrix created by the psyllium fiber provided a protective barrier for the starch and diminished the susceptibility of the starch to amylolytic attack.

Since cellulose cannot form a gel and has low water-molecule interactions due to the domination of intramolecular interactions between polymer segments, water molecules cannot be adsorbed on cellulose chains effectively. Therefore, it could not restrict the free water in the environment sufficiently and could not establish an

effective barrier around the starch granules as much as psyllium. Different matrix formations around the starch could explain the differences in starch digestibility and gelatinization behavior with psyllium and cellulose fibers. Although psyllium had better water molecule interactions, cellulose fiber also had some inhibition in starch granule swelling and gelatinization and limited the digestive enzymes' mobility.



Table 3.3. Digestion fractions of wheat starch with or without added psyllium and cellulose fibers cooked at different water concentrations. RDS (%): rapidly digestible starch SDS (%): slowly digestible starch and RS (%): remaining starch percentage per gram of starch in the samples.

Samples	Water Concentration	RDS (%) in sample	RDS (%) in starch	SDS (%) in sample	SDS (%) in starch	RS (%) in sample	RS (%) in starch
Cooked S		72.44±0.48	69.72±0.46 ^{aA}	11.06±0.67	10.65±0.65 ^{bD}	20.40±0.14	19.63±0.13 ^{bC}
Cooked S+C	80%	33.93±0.12	66.71±0.24 ^{bA}	6.61±0.30	13.22±0.59 ^{aC}	10.21±0.39	20.07±0.78 ^{bC}
Cooked S+P		23.51±0.55	46.34±1.10 ^{cA}	6.70±0.28	13.36±0.55 ^{aD}	20.45±0.09	40.31±0.17 ^{aD}
Cooked S		67.62±0.14	63.00±0.13 ^{aB}	14.72±0.48	13.72±0.45 ^{bC}	25.00±0.15	23.29±0.14 ^{bB}
Cooked S+C	65%	32.17±0.25	59.75±0.49 ^{bB}	8.07±0.06	14.99±0.11 ^{bB}	13.60±0.50	25.26±0.99 ^{bB}
Cooked S+P		19.45±0.41	37.43±0.81 ^{cB}	9.41±0.44	18.11±0.87 ^{aC}	23.10±0.06	44.46±0.11 ^{aC}
Cooked S		61.36±0.63	57.90±0.60 ^{aC}	16.78±0.13	15.84±0.12 ^{bB}	27.84±0.11	26.26±0.10 ^{cA}
Cooked S+C	50%	28.55±0.18	53.23±0.36 ^{bC}	8.78±0.11	16.37±0.22 ^{bB}	15.20±0.18	30.39±0.35 ^{bA}
Cooked S+P		14.42±0.18	28.06±0.36 ^{cC}	11.75±0.07	22.87±0.14 ^{aA}	25.22±0.23	49.07±0.46 ^{aB}
Raw S		25.10±0.25	24.90±0.25 ^{aD}	57.34±0.48	56.88±0.48 ^{aA}	18.36±0.54	18.22±0.54 ^{cD}
Raw S+C		10.49±0.50	20.98±0.99 ^{bD}	26.73±0.44	53.45±0.88 ^{bA}	12.79±0.35	25.57±0.68 ^{bB}
Raw S+P		2.86±0.04	5.51±0.07 ^{cD}	10.69±0.11	20.59±0.22 ^{bB}	38.37±0.09	73.90±0.18 ^{aA}

The data are averages of three measurements with the standard deviation. Different small letters (a,b,c) indicate significant differences in each box in each column ($p<0.05$). Different capital letters (A, B, C) indicate significant differences in each group (S, S+C, or S+P) in each column ($p<0.05$). S: wheat starch, S+C: wheat starch and cellulose, S+P: wheat starch and psyllium at 1:1 dry weight base.



CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

Information from DSC, XRD, FTIR, and SEM analysis showed that psyllium and cellulose fiber addition and reducing water concentration during cooking reduced the digestibility of wheat starch and limited the gelatinization. The study showed that digested starch fractions were affected by gelatinization, fiber type, and dilution due to the added fiber. The type of fiber or the formed matrix affected both the gelatinization and the accessibility to the digestive enzymes differently. The effect of psyllium fiber on limiting the gelatinization and decreasing starch digestibility was more pronounced than cellulose fiber.

The choice of fibers to use in food products should be based on their functional and physical properties. Therefore, psyllium fiber is an excellent ingredient to use in functional starchy foods with reduced digestibility for a healthy diet. The differences observed *in vitro* studies should be confirmed by *in vivo* data.



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APPENDICES

A. DIFFERENTIAL SCANNING CALORIMETER (DSC) THERMOGRAMS

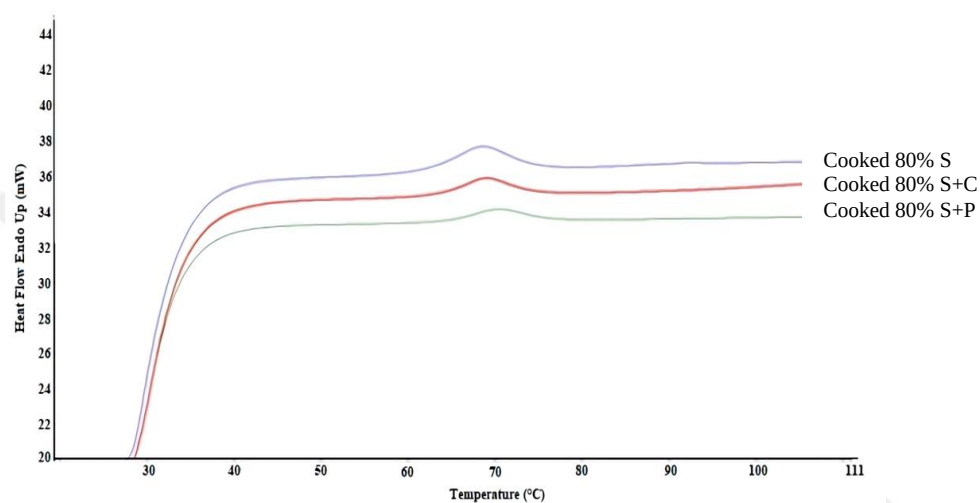


Figure A.1. DSC thermogram of cooked samples at 80% water concentration

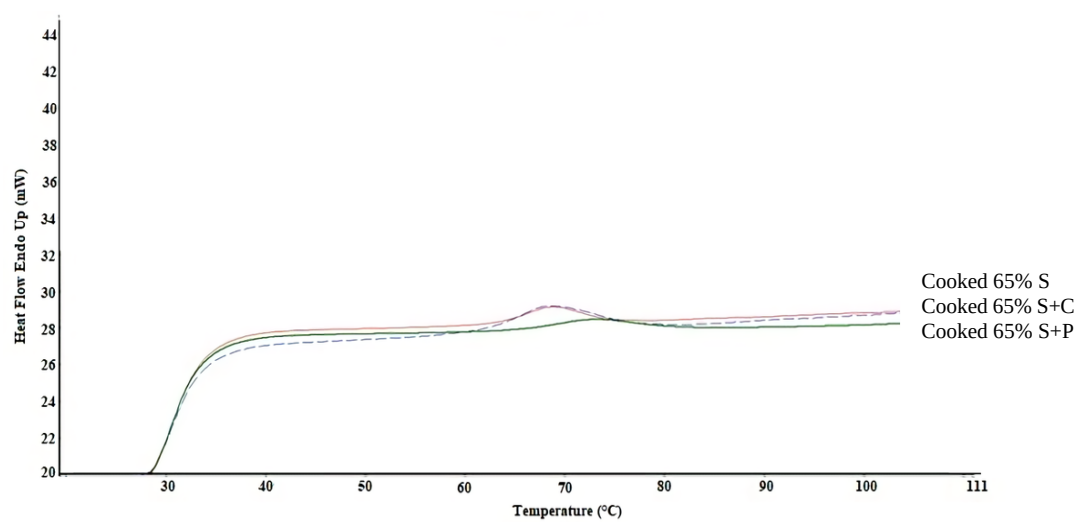


Figure A.2. DSC thermogram of cooked samples at 65% water concentration

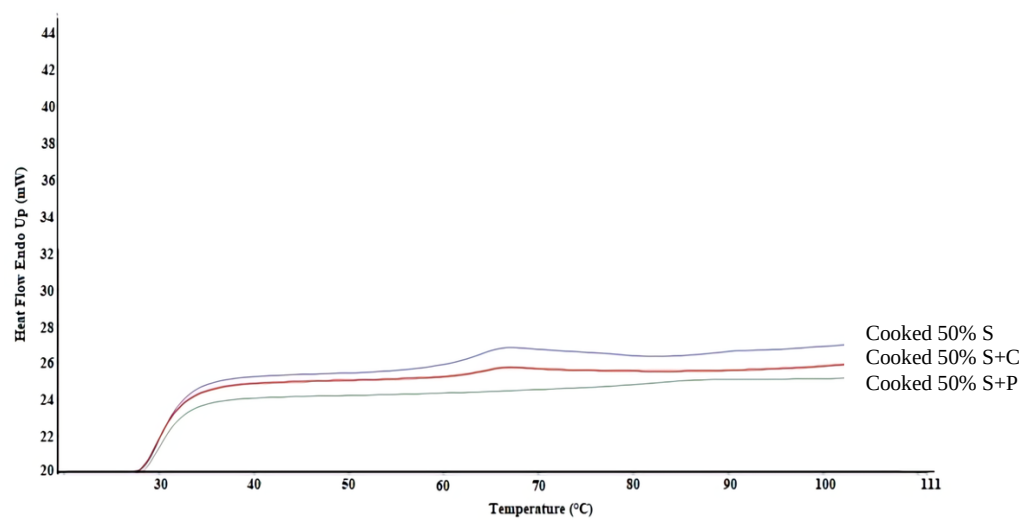


Figure A.3. DSC thermogram of cooked samples at 50% water concentration

B. FOURIER TRANSFORM INFRARED (FTIR) SPECTRA

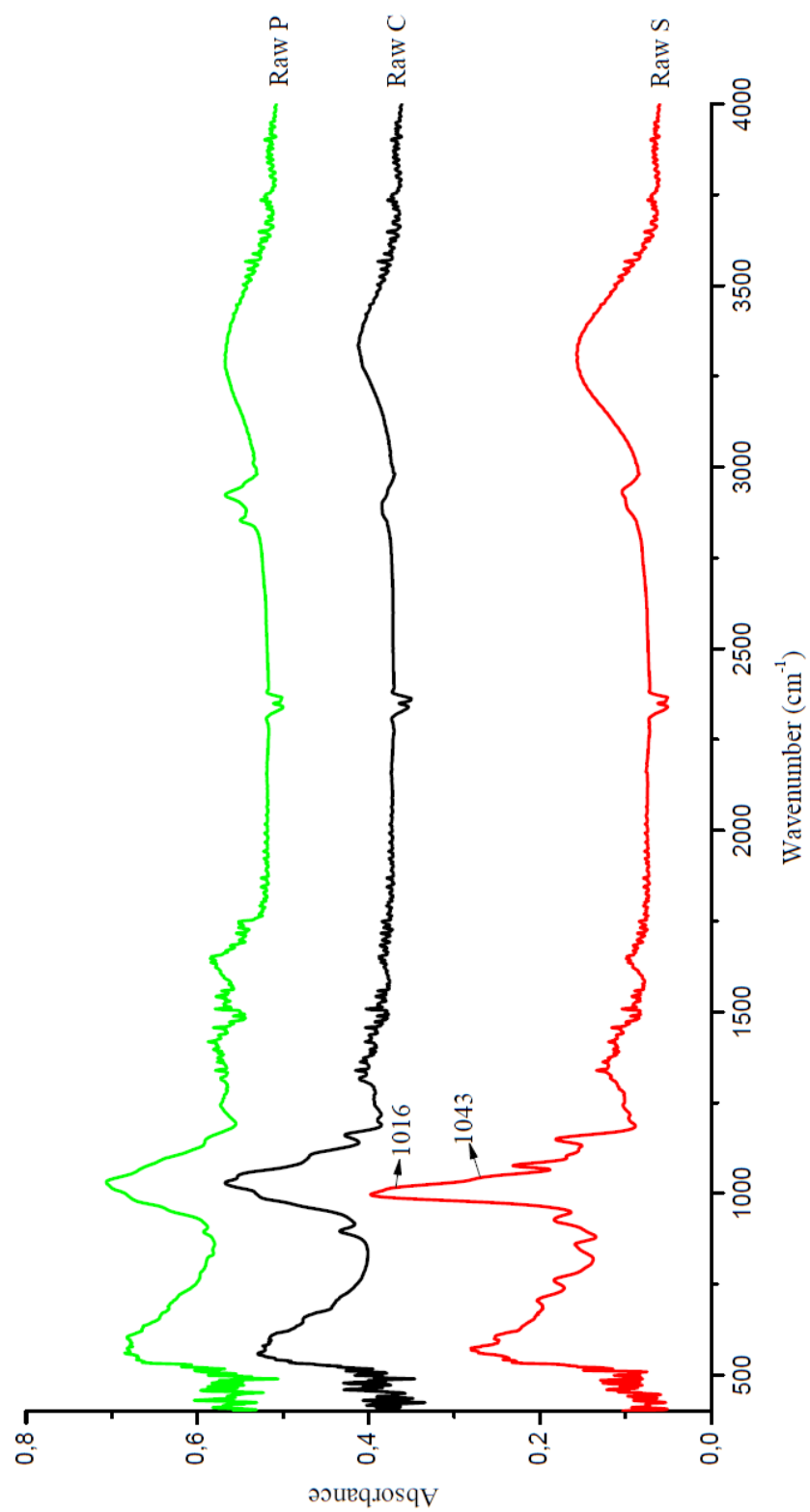


Figure B.1. FTIR spectra of raw starch, cellulose and psyllium

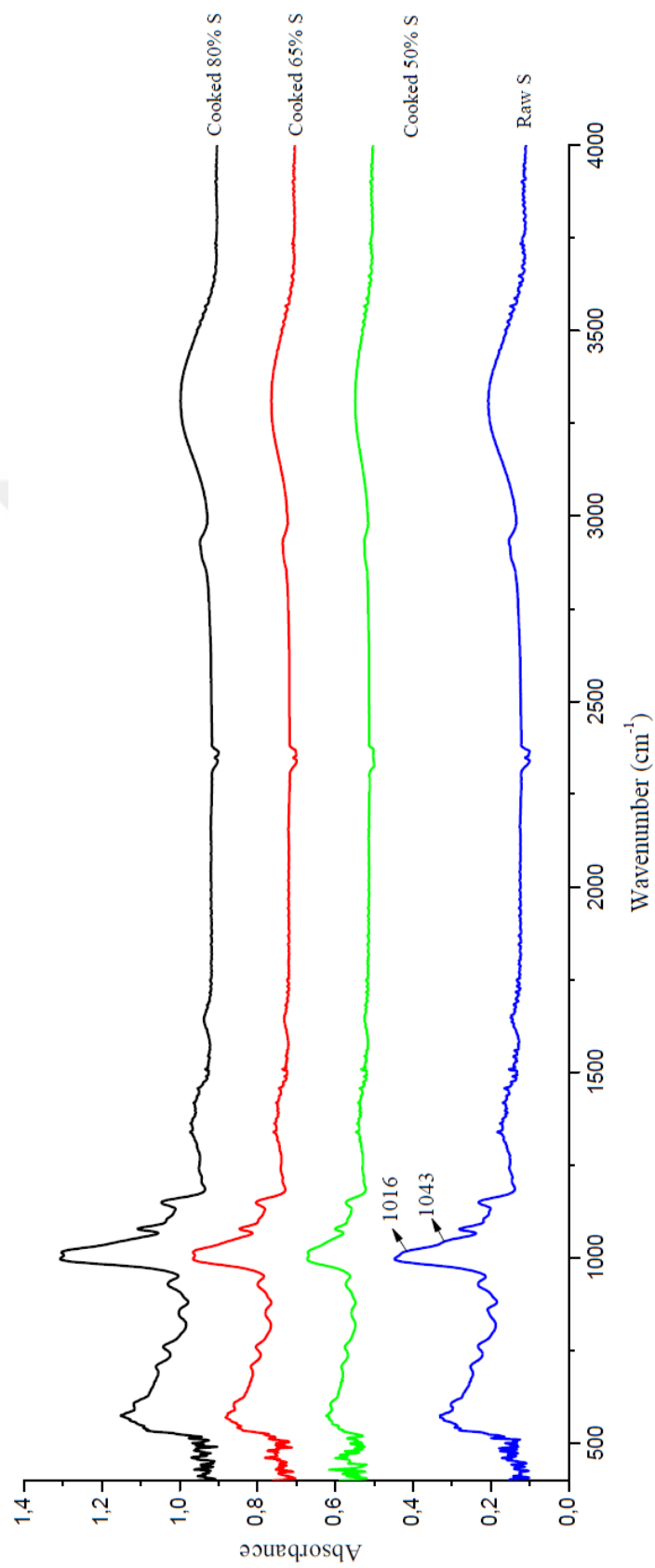


Figure B.2. FTIR spectra of raw and cooked starch

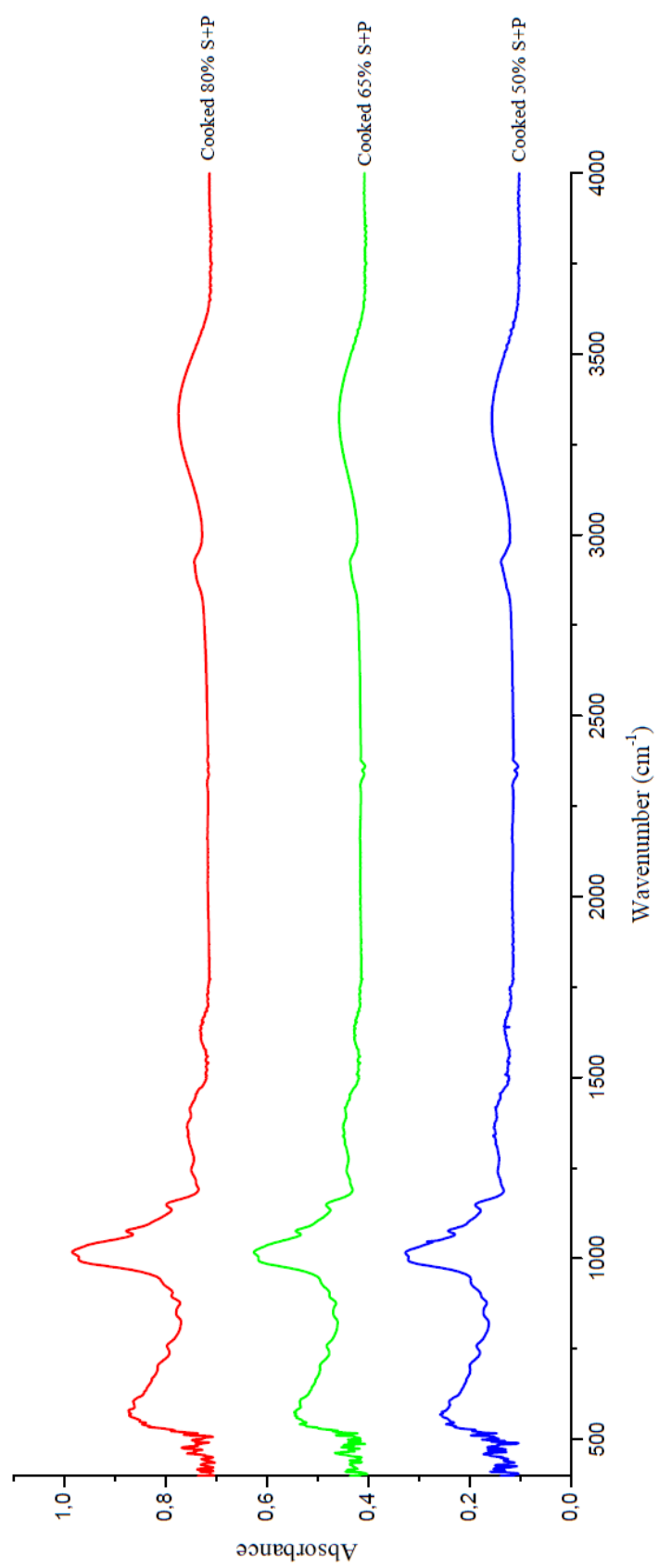


Figure B.3. FTIR spectra of cooked wheat starch-psyllum mixtures at different water concentrations.

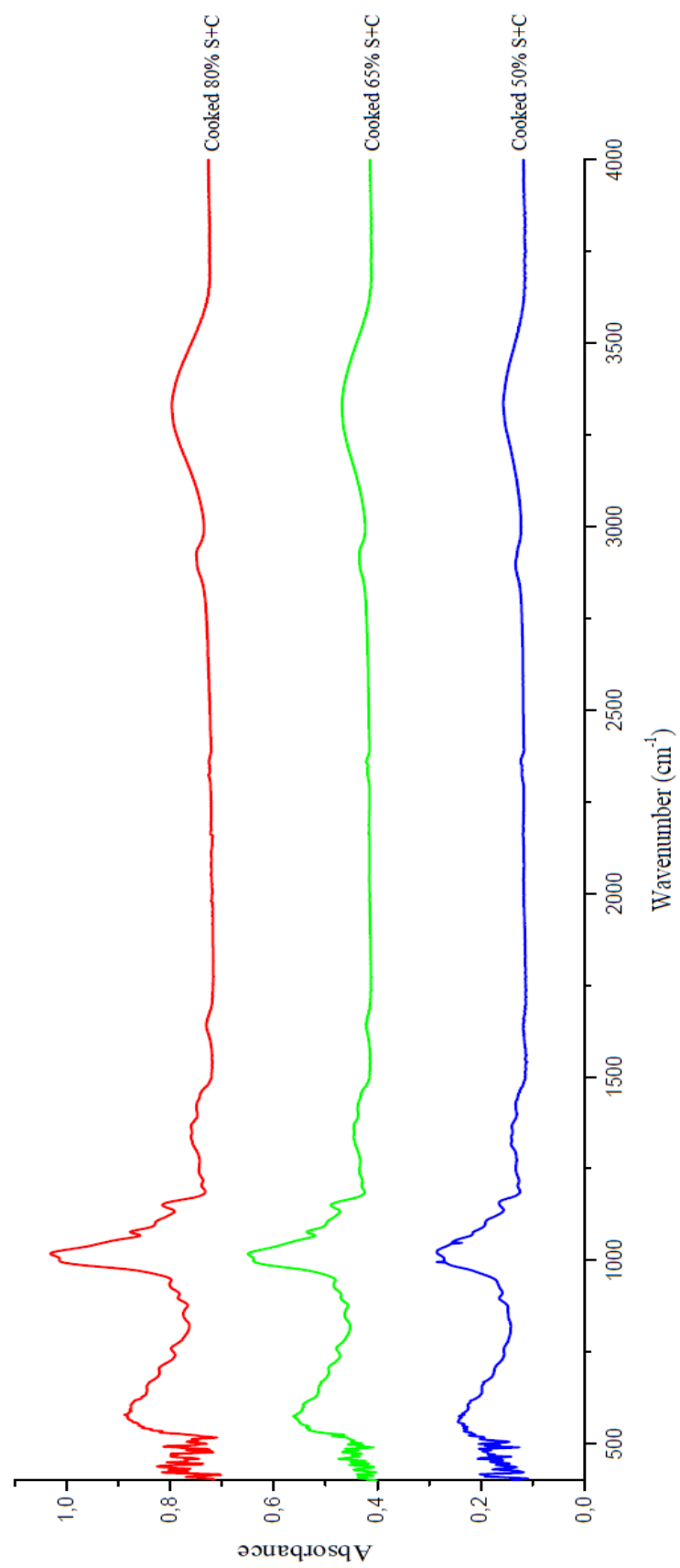


Figure B.4. FTIR spectra of cooked wheat starch-cellulose mixtures at different water concentrations

C. STATISTICAL ANALYSES

Table C.1. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on fiber addition at 80% water concentration uncooked wheat starch

General Linear Model: To versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	uncooked 80%s; uncooked 80%s+c; uncooked 80%s+p

Analysis of Variance for To, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	104,523	104,523	52,261	1304,36	0,000
Error	6	0,240	0,240	0,040		
Total	8	104,763				

S = 0,200167 R-Sq = 99,77% R-Sq(adj) = 99,69%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 80%s+p	3	64,7	A
uncooked 80%s+c	3	63,6	B
uncooked 80%s	3	57,0	C

Means that do not share a letter are significantly different.

Table C.2. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on fiber addition at 80% water concentration uncooked wheat starch

General Linear Model: Tp versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	uncooked 80% <i>s</i> ; uncooked 80% <i>s</i> + <i>c</i> ; uncooked 80% <i>s</i> + <i>p</i>

Analysis of Variance for Tp, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	101,932	101,932	50,966	626,12	0,000
Error	6	0,488	0,488	0,081		
Total	8	102,420				

S = 0,285307 R-Sq = 99,52% R-Sq(adj) = 99,36%

Unusual Observations for Tp

Obs	Tp	Fit	SE Fit	Residual	St Resid
2	63,1100	62,6400	0,1647	0,4700	2,02 R
3	62,1700	62,6400	0,1647	-0,4700	-2,02 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 80% <i>s</i> + <i>p</i>	3	70,4	A
uncooked 80% <i>s</i> + <i>c</i>	3	68,9	B
uncooked 80% <i>s</i>	3	62,6	C

Means that do not share a letter are significantly different.

Table C.3. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on fiber addition at 80% water concentration uncooked wheat starch

General Linear Model: Tc versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	uncooked 80% _s ; uncooked 80% _s +c; uncooked 80% _s +p

Analysis of Variance for Tc, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	68,812	68,812	34,406	236,52	0,000
Error	6	0,873	0,873	0,145		
Total	8	69,685				

S = 0,381401 R-Sq = 98,75% R-Sq(adj) = 98,33%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 80% _s +p	3	76,4	A
uncooked 80% _s +c	3	74,3	B
uncooked 80% _s	3	69,8	C

Means that do not share a letter are significantly different.

Table C.4. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on fiber addition at 80% water concentration uncooked wheat starch

General Linear Model: ΔH versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	uncooked 80% <i>s</i> ; uncooked 80% <i>s</i> + <i>c</i> ; uncooked 80% <i>s</i> + <i>p</i>

Analysis of Variance for ΔH , using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	40,258	40,258	20,129	6,01	0,037
Error	6	20,095	20,095	3,349		
Total	8	60,353				

S = 1,83005 R-Sq = 66,70% R-Sq(adj) = 55,61%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 80% <i>s</i>	3	17,3	A
uncooked 80% <i>s</i> + <i>c</i>	3	13,0	A B
uncooked 80% <i>s</i> + <i>p</i>	3	12,7	B

Means that do not share a letter are significantly different.

Table C.5. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on fiber addition at 65% water concentration uncooked wheat starch

General Linear Model: To versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	uncooked 65%s; uncooked 65%s+c; uncooked 65%s+p

Analysis of Variance for To, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	98,907	98,907	49,453	447,81	0,000
Error	6	0,663	0,663	0,110		
Total	8	99,569				

S = 0,332315 R-Sq = 99,33% R-Sq(adj) = 99,11%

Unusual Observations for To

Obs	To	Fit	SE Fit	Residual	St Resid
5	66,3000	65,7500	0,1919	0,5500	2,03 R
6	65,2000	65,7500	0,1919	-0,5500	-2,03 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 65%s+p	3	65,8	A
uncooked 65%s+c	3	63,2	B
uncooked 65%s	3	57,8	C

Means that do not share a letter are significantly different.

Table C.6. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on fiber addition at 65% water concentration uncooked wheat starch

General Linear Model: Tp versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	uncooked 65% <i>s</i> ; uncooked 65% <i>s</i> + <i>c</i> ; uncooked 65% <i>s</i> + <i>p</i>

Analysis of Variance for Tp, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	185,384	185,384	92,692	917,74	0,000
Error	6	0,606	0,606	0,101		
Total	8	185,990				

S = 0,317805 R-Sq = 99,67% R-Sq(adj) = 99,57%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 65% <i>s</i> + <i>p</i>	3	73,1	A
uncooked 65% <i>s</i> + <i>c</i>	3	68,5	B
uncooked 65% <i>s</i>	3	62,1	C

Means that do not share a letter are significantly different.

Table C.7. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on fiber addition at 65% water concentration uncooked wheat starch

General Linear Model: Tc versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	uncooked 65% <i>s</i> ; uncooked 65% <i>s</i> + <i>c</i> ; uncooked 65% <i>s</i> + <i>p</i>

Analysis of Variance for Tc, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	206,79	206,79	103,39	197,50	0,000
Error	6	3,14	3,14	0,52		
Total	8	209,93				

S = 0,723533 R-Sq = 98,50% R-Sq(adj) = 98,01%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 65% <i>s</i> + <i>p</i>	3	81,7	A
uncooked 65% <i>s</i> + <i>c</i>	3	74,1	B
uncooked 65% <i>s</i>	3	70,1	C

Means that do not share a letter are significantly different.

Table C.8. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on fiber addition at 65% water concentration uncooked wheat starch

General Linear Model: ΔH versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	uncooked 65% <i>s</i> ; uncooked 65% <i>s</i> + <i>c</i> ; uncooked 65% <i>s</i> + <i>p</i>

Analysis of Variance for ΔH , using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	23,535	23,535	11,768	9,02	0,016
Error	6	7,829	7,829	1,305		
Total	8	31,364				

S = 1,14228 R-Sq = 75,04% R-Sq(adj) = 66,72%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 65% <i>s</i>	3	15,8	A
uncooked 65% <i>s</i> + <i>c</i>	3	13,1	A B
uncooked 65% <i>s</i> + <i>p</i>	3	12,0	B

Means that do not share a letter are significantly different.

Table C.9. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on fiber addition at 50% water concentration uncooked wheat starch

General Linear Model: To versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	2	uncooked 50% _s ; uncooked 50% _s +c

Analysis of Variance for To, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	1	82,362	82,362	82,362	400,59	0,000
Error	4	0,822	0,822	0,206		
Total	5	83,185				

S = 0,453431 R-Sq = 99,01% R-Sq(adj) = 98,76%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 50% _s +c	3	62,7	A
uncooked 50% _s	3	55,3	B

Means that do not share a letter are significantly different.

Table C.10. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on fiber addition at 50% water concentration uncooked wheat starch

General Linear Model: Tp versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	2	uncooked 50% _s ; uncooked 50% _s +c

Analysis of Variance for Tp, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	1	85,504	85,504	85,504	2731,75	0,000
Error	4	0,125	0,125	0,031		
Total	5	85,629				

S = 0,176918 R-Sq = 99,85% R-Sq(adj) = 99,82%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 50% _s +c	3	67,9	A
uncooked 50% _s	3	60,3	B

Means that do not share a letter are significantly different.

Table C.11. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on fiber addition at 50% water concentration uncooked wheat starch

General Linear Model: Tc versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	2	uncooked 50% _s ; uncooked 50% _s +c

Analysis of Variance for Tc, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	1	98,902	98,902	98,902	1506,50	0,000
Error	4	0,263	0,263	0,066		
Total	5	99,164				

S = 0,256223 R-Sq = 99,74% R-Sq(adj) = 99,67%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 50% _s +c	3	74,3	A
uncooked 50% _s	3	66,1	B

Means that do not share a letter are significantly different.

Table C.12. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on fiber addition at 50% water concentration uncooked wheat starch

General Linear Model: ΔH versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	2	uncooked 50% _s ; uncooked 50% _{s+c}

Analysis of Variance for ΔH , using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	1	0,4537	0,4537	0,4537	1,64	0,270
Error	4	1,1080	1,1080	0,2770		
Total	5	1,5617				

S = 0,526308 R-Sq = 29,05% R-Sq(adj) = 11,32%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
uncooked 50% _{s+c}	3	5,7	A
uncooked 50% _s	3	5,2	A

Means that do not share a letter are significantly different.

Table C.13. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on different water concentrations for uncooked wheat starch

General Linear Model: To versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	3	uncooked 50% _s ; uncooked 65% _s ; uncooked 80% _s

Analysis of Variance for To, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	2	9,8286	9,8286	4,9143	28,01	0,001
Error	6	1,0528	1,0528	0,1755		
Total	8	10,8814				

S = 0,418887 R-Sq = 90,32% R-Sq(adj) = 87,10%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 65% _s	3	57,8	A
uncooked 80% _s	3	57,0	A
uncooked 50% _s	3	55,3	B

Means that do not share a letter are significantly different.

Table C.14. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on different water concentrations for uncooked wheat starch

General Linear Model: Tp versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	3	uncooked 50% _s ; uncooked 65% _s ; uncooked 80% _s

Analysis of Variance for Tp, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	2	8,7698	8,7698	4,3849	44,84	0,000
Error	6	0,5868	0,5868	0,0978		
Total	8	9,3566				

S = 0,312730 R-Sq = 93,73% R-Sq(adj) = 91,64%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 80% _s	3	62,6	A
uncooked 65% _s	3	62,1	A
uncooked 50% _s	3	60,3	B

Means that do not share a letter are significantly different.

Table C.15. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on different water concentrations for uncooked wheat starch

General Linear Model: Tc versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	3	uncooked 50% _s ; uncooked 65% _s ; uncooked 80% _s

Analysis of Variance for Tc, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	2	28,771	28,771	14,386	75,16	0,000
Error	6	1,148	1,148	0,191		
Total	8	29,920				

S = 0,437493 R-Sq = 96,16% R-Sq(adj) = 94,88%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 65% _s	3	70,1	A
uncooked 80% _s	3	69,8	A
uncooked 50% _s	3	66,2	B

Means that do not share a letter are significantly different.

Table C.16. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on different water concentrations for uncooked wheat starch

General Linear Model: ΔH versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	3	uncooked 50% _s ; uncooked 65% _s ; uncooked 80% _s

Analysis of Variance for ΔH , using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	2	264,21	264,21	132,10	144,41	0,000
Error	6	5,49	5,49	0,91		
Total	8	269,70				

S = 0,956452 R-Sq = 97,96% R-Sq(adj) = 97,29%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 80% _s	3	17,3	A
uncooked 65% _s	3	15,8	A
uncooked 50% _s	3	5,2	B

Means that do not share a letter are significantly different.

Table C.17. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on different water concentrations for uncooked wheat starch-psyllium mixture (1:1)

General Linear Model: To versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	2	uncooked 65% _{s+p} ; uncooked 80% _{s+p}

Analysis of Variance for To, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	1	1,6854	1,6854	1,6854	10,55	0,031
Error	4	0,6388	0,6388	0,1597		
Total	5	2,3242				

S = 0,399625 R-Sq = 72,52% R-Sq(adj) = 65,64%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 65% _{s+p}	3	65,8	A
uncooked 80% _{s+p}	3	64,7	B

Means that do not share a letter are significantly different.

Table C.18. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on different water concentrations for uncooked wheat starch-psyllium mixture (1:1)

General Linear Model: Tp versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	2	uncooked 65% _{s+p} ; uncooked 80% _{s+p}

Analysis of Variance for Tp, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	1	11,098	11,098	11,098	90,04	0,001
Error	4	0,493	0,493	0,123		
Total	5	11,591				

S = 0,351070 R-Sq = 95,75% R-Sq(adj) = 94,68%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 65% _{s+p}	3	73,1	A
uncooked 80% _{s+p}	3	70,4	B

Means that do not share a letter are significantly different.

Table C.19. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on different water concentrations for uncooked wheat starch-psyllium mixture (1:1)

General Linear Model: Tc versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	2	uncooked 65% _{s+p} ; uncooked 80% _{s+p}

Analysis of Variance for Tc, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	1	42,135	42,135	42,135	62,16	0,001
Error	4	2,711	2,711	0,678		
Total	5	44,846				

S = 0,823286 R-Sq = 93,95% R-Sq(adj) = 92,44%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 65% _{s+p}	3	81,7	A
uncooked 80% _{s+p}	3	76,4	B

Means that do not share a letter are significantly different.

Table C.20. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on different water concentrations for uncooked wheat starch-psyllium mixture (1:1)

General Linear Model: ΔH versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	2	uncooked 65% _{s+p} ; uncooked 80% _{s+p}

Analysis of Variance for ΔH , using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	1	0,8214	0,8214	0,8214	2,38	0,198
Error	4	1,3786	1,3786	0,3447		
Total	5	2,2000				

S = 0,587069 R-Sq = 37,34% R-Sq(adj) = 21,67%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 80% _{s+p}	3	12,7	A
uncooked 65% _{s+p}	3	12,0	A

Means that do not share a letter are significantly different.

Table C.21. One way ANOVA and Tukey's Comparison Test with 95% confidence level for To value depending on different water concentrations for uncooked wheat starch-cellulose mixture (1:1)

General Linear Model: To versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	3	uncooked 50% <i>s+c</i> ; uncooked 65% <i>s+c</i> ; uncooked 80% <i>s+c</i>

Analysis of Variance for To, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	2	1,18940	1,18940	0,59470	105,57	0,000
Error	6	0,03380	0,03380	0,00563		
Total	8	1,22320				

S = 0,0750555 R-Sq = 97,24% R-Sq(adj) = 96,32%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 80% <i>s+c</i>	3	63,6	A
uncooked 65% <i>s+c</i>	3	63,2	B
uncooked 50% <i>s+c</i>	3	62,7	C

Means that do not share a letter are significantly different.

Table C.22. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tp value depending on different water concentrations for uncooked wheat starch-cellulose mixture (1:1)

General Linear Model: Tp versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	3	uncooked 50% <i>s+c</i> ; uncooked 65% <i>s+c</i> ; uncooked 80% <i>s+c</i>

Analysis of Variance for Tp, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	2	1,58480	1,58480	0,79240	34,01	0,001
Error	6	0,13980	0,13980	0,02330		
Total	8	1,72460				

S = 0,152643 R-Sq = 91,89% R-Sq(adj) = 89,19%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 80% <i>s+c</i>	3	68,9	A
uncooked 65% <i>s+c</i>	3	68,5	B
uncooked 50% <i>s+c</i>	3	67,9	C

Means that do not share a letter are significantly different.

Table C.23. One way ANOVA and Tukey's Comparison Test with 95% confidence level for Tc value depending on different water concentrations for uncooked wheat starch-cellulose mixture (1:1)

General Linear Model: Tc versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	3	uncooked 50% _{s+c} ; uncooked 65% _{s+c} ; uncooked 80% _{s+c}

Analysis of Variance for Tc, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	2	0,06260	0,06260	0,03130	0,45	0,657
Error	6	0,41680	0,41680	0,06947		
Total	8	0,47940				

S = 0,263565 R-Sq = 13,06% R-Sq(adj) = 0,00%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 80% _{s+c}	3	74,3	A
uncooked 50% _{s+c}	3	74,3	A
uncooked 65% _{s+c}	3	74,1	A

Means that do not share a letter are significantly different.

Table C.24. One way ANOVA and Tukey's Comparison Test with 95% confidence level for ΔH value depending on different water concentrations for uncooked wheat starch-cellulose mixture (1:1)

General Linear Model: ΔH versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	3	uncooked 50% <i>s+c</i> ; uncooked 65% <i>s+c</i> ; uncooked 80% <i>s+c</i>

Analysis of Variance for ΔH , using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	2	108,074	108,074	54,037	14,63	0,005
Error	6	22,164	22,164	3,694		
Total	8	130,238				

S = 1,92198 R-Sq = 82,98% R-Sq(adj) = 77,31%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
uncooked 65% <i>s+c</i>	3	13,1	A
uncooked 80% <i>s+c</i>	3	13,0	A
uncooked 50% <i>s+c</i>	3	5,7	B

Means that do not share a letter are significantly different.

Table C.25. One way ANOVA and Tukey's Comparison Test with 95% confidence level for 1043/1016 cm⁻¹ depending on water concentration for wheat starch without fiber

General Linear Model: 1043/1016 cm⁻¹ versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	4	cooked 50% S; cooked 65% S; cooked 80% S;

Raw S

Analysis of Variance for 1043/1016, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	3	0,0094762	0,0094762	0,0031587	12,55	0,002
Error	8	0,0020140	0,0020140	0,0002517		
Total	11	0,0114902				

S = 0,0158666 R-Sq = 82,47% R-Sq(adj) = 75,90%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
Raw S	3	0,7	A
cooked 50% S	3	0,7	A
cooked 65% S	3	0,7	A B
cooked 80% S	3	0,6	B

Means that do not share a letter are significantly different.

Table C.26. One way ANOVA and Tukey's Comparison Test with 95% confidence level for 1043/1016 cm⁻¹ depending on water concentration for cooked wheat starch-psyllium mixture (1:1)

General Linear Model: 1043/1016 cm⁻¹ versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	3	cooked 50% S+C; cooked 65% S+C;
cooked			80% S+C

Analysis of Variance for 1043/1016, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	2	0,0007460	0,0007460	0,0003730	17,22	0,003
Error	6	0,0001300	0,0001300	0,0000217		
Total	8	0,0008760				

S = 0,00465475 R-Sq = 85,16% R-Sq(adj) = 80,21%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
cooked 50% S+C	3	0,7	A
cooked 80% S+C	3	0,7	B
cooked 65% S+C	3	0,7	B

Means that do not share a letter are significantly different.

Table C.27. One way ANOVA and Tukey's Comparison Test with 95% confidence level for 1043/1016 cm⁻¹ depending on water concentration for cooked wheat starch-cellulose mixture (1:1)

General Linear Model: 1043/1016 cm⁻¹ versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	3	cooked 50% S+P; cooked 65% S+P;
cooked			80% S+P

Analysis of Variance for 1043/1016, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	2	0,0002540	0,0002540	0,0001270	5,15	0,050
Error	6	0,0001480	0,0001480	0,0000247		
Total	8	0,0004020				

S = 0,00496655 R-Sq = 63,18% R-Sq(adj) = 50,91%

Grouping Information Using Tukey Method and 95,0% Confidence

water	N	Mean	Grouping
concentration			
cooked 50% S+P	3	0,8	A
cooked 65% S+P	3	0,8	A B
cooked 80% S+P	3	0,8	B

Means that do not share a letter are significantly different.

Table C.28. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on fiber addition at 80% water concentration

General Linear Model: RDS versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	cooked 80%s; cooked 80%s+c; cooked 80%s+p

Analysis of Variance for RDS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	970,90	970,90	485,45	985,93	0,000
Error	6	2,95	2,95	0,49		
Total	8	973,85				

S = 0,701694 R-Sq = 99,70% R-Sq(adj) = 99,60%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
cooked 80%s	3	69,7	A
cooked 80%s+c	3	66,7	B
cooked 80%s+p	3	46,3	C

Means that do not share a letter are significantly different.

Table C.29. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on fiber addition at 80% water concentration

General Linear Model: SDS versus fiber addition

Factor	Type	Levels	Values
fiber addition	fixed	3	cooked 80%s; cooked 80%s+c; cooked 80%s+p

Analysis of Variance for SDS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
fiber addition	2	13,9876	13,9876	6,9938	19,63	0,002
Error	6	2,1376	2,1376	0,3563		
Total	8	16,1252				

S = 0,596882 R-Sq = 86,74% R-Sq(adj) = 82,32%

Grouping Information Using Tukey Method and 95,0% Confidence

fiber addition	N	Mean	Grouping
cooked 80%s+p	3	13,4	A
cooked 80%s+c	3	13,2	A
cooked 80%s	3	10,6	B

Means that do not share a letter are significantly different.

Table C.30. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on fiber addition at 80% water concentration

General Linear Model: RS versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	cooked 80%s; cooked 80%s+c; cooked 80%s+p

Analysis of Variance for RS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	837,41	837,41	418,71	1899,42	0,000
Error	6	1,32	1,32	0,22		
Total	8	838,73				

S = 0,469509 R-Sq = 99,84% R-Sq(adj) = 99,79%

Unusual Observations for RS

Obs	rs	Fit	SE Fit	Residual	St Resid
8	20,8518	20,0678	0,2711	0,7840	2,05 R
9	19,2838	20,0678	0,2711	-0,7840	-2,05 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
cooked 80%s+p	3	40,3	A
cooked 80%s+c	3	20,1	B
cooked 80%s	3	19,6	B

Means that do not share a letter are significantly different.

Table C.31. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on fiber addition at 65% water concentration

General Linear Model: RDS versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	cooked 65%s; cooked 65%s+c; cooked 65%s+p

Analysis of Variance for RDS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	1162,34	1162,34	581,17	1920,25	0,000
Error	6	1,82	1,82	0,30		
Total	8	1164,15				

S = 0,550138 R-Sq = 99,84% R-Sq(adj) = 99,79%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
cooked 65%s	3	63,0	A
cooked 65%s+c	3	59,8	B
cooked 65%s+p	3	37,4	C

Means that do not share a letter are significantly different.

Table C.32. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on fiber addition at 65% water concentration

General Linear Model: SDS versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	cooked 65%s; cooked 65%s+c; cooked 65%s+p

Analysis of Variance for SDS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	30,686	30,686	15,343	47,01	0,000
Error	6	1,958	1,958	0,326		
Total	8	32,644				

S = 0,571316 R-Sq = 94,00% R-Sq(adj) = 92,00%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
cooked 65%s+p	3	18,1	A
cooked 65%s+c	3	15,0	B
cooked 65%s	3	13,7	B

Means that do not share a letter are significantly different.

Table C.33. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on fiber addition at 65% water concentration

General Linear Model: RS versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	cooked 65%s; cooked 65%s+c; cooked 65%s+p

Analysis of Variance for RS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	820,65	820,65	410,32	1230,57	0,000
Error	6	2,00	2,00	0,33		
Total	8	822,65				

S = 0,577446 R-Sq = 99,76% R-Sq(adj) = 99,68%

Unusual Observations for RS

Obs	RS	Fit	SE Fit	Residual	St Resid
8	26,2435	25,2585	0,3334	0,9850	2,09 R
9	24,2735	25,2585	0,3334	-0,9850	-2,09 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
cooked 65%s+p	3	44,5	A
cooked 65%s+c	3	25,3	B
cooked 65%s	3	23,3	C

Means that do not share a letter are significantly different.

Table C.34. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on fiber addition at 50% water concentration

General Linear Model: RDS versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	cooked 50%s; cooked 50%s+c; cooked 50%s+p

Analysis of Variance for RDS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	1546,32	1546,32	773,16	3731,26	0,000
Error	6	1,24	1,24	0,21		
Total	8	1547,56				

S = 0,455205 R-Sq = 99,92% R-Sq(adj) = 99,89%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
cooked 50%s	3	57,9	A
cooked 50%s+c	3	53,2	B
cooked 50%s+p	3	28,1	C

Means that do not share a letter are significantly different.

Table C.35. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on fiber addition at 50% water concentration

General Linear Model: SDS versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	cooked 50%s; cooked 50%s+c; cooked 50%s+p

Analysis of Variance for SDS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	91,924	91,924	45,962	1656,22	0,000
Error	6	0,167	0,167	0,028		
Total	8	92,091				

S = 0,166587 R-Sq = 99,82% R-Sq(adj) = 99,76%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
cooked 50%s+p	3	22,9	A
cooked 50%s+c	3	16,4	B
cooked 50%s	3	15,8	C

Means that do not share a letter are significantly different.

Table C.36. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on fiber addition at 50% water concentration

General Linear Model: RS versus fiber addition

Factor	Type	Levels	Values
Fiber addition	fixed	3	cooked 50%s; cooked 50%s+c; cooked 50%s+p

Analysis of Variance for RS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Fiber addition	2	886,45	886,45	443,23	3844,15	0,000
Error	6	0,69	0,69	0,12		
Total	8	887,14				

S = 0,339557 R-Sq = 99,92% R-Sq(adj) = 99,90%

Grouping Information Using Tukey Method and 95,0% Confidence

Fiber addition	N	Mean	Grouping
cooked 50%s+p	3	49,1	A
cooked 50%s+c	3	30,4	B
cooked 50%s	3	26,3	C

Means that do not share a letter are significantly different.

Table C.37. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on different water concentrations for cooked wheat starch and raw starch

General Linear Model: RDS versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	4	cooked 50% _s ; cooked 65% _s ; cooked 80% _s ; raw _s

Analysis of Variance for rds, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	3	3570,5	3570,5	1190,2	7351,67	0,000
Error	8	1,3	1,3	0,2		
Total	11	3571,7				

S = 0,402354 R-Sq = 99,96% R-Sq(adj) = 99,95%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
cooked 80% _s	3	69,7	A
cooked 65% _s	3	63,0	B
cooked 50% _s	3	57,9	C
raw _s	3	24,9	D

Means that do not share a letter are significantly different.

Table C.38. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on different water concentrations for cooked wheat starch and raw starch

General Linear Model: SDS versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	4	cooked 50% _s ; cooked 65% _s ; cooked 80% _s ; raw s

Analysis of Variance for sds, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	3	4294,5	4294,5	1431,5	6544,48	0,000
Error	8	1,7	1,7	0,2		
Total	11	4296,3				

S = 0,467690 R-Sq = 99,96% R-Sq(adj) = 99,94%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
raw s	3	56,9	A
cooked 50% _s	3	15,8	B
cooked 65% _s	3	13,7	C
cooked 80% _s	3	10,6	D

Means that do not share a letter are significantly different.

Table C.39. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on different water concentrations for cooked wheat starch and raw starch

General Linear Model: RS versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	4	cooked 50% _s ; cooked 65% _s ; cooked 80% _s ; raw s

Analysis of Variance for rs, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	3	118,896	118,896	39,632	470,37	0,000
Error	8	0,674	0,674	0,084		
Total	11	119,570				

S = 0,290269 R-Sq = 99,44% R-Sq(adj) = 99,22%

Unusual Observations for rs

Obs	rs	Fit	SE Fit	Residual	St Resid
11	18,7600	18,2200	0,1676	0,5400	2,28 R
12	17,6800	18,2200	0,1676	-0,5400	-2,28 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
cooked 50% _s	3	26,3	A
cooked 65% _s	3	23,3	B
cooked 80% _s	3	19,6	C
raw s	3	18,2	D

Means that do not share a letter are significantly different.

Table C.40. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on different water concentrations for cooked wheat starch-psyllium mixture and raw mixture (1:1)

General Linear Model: RDS versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	4	cooked 50% <i>s+p</i> ; cooked 65% <i>s+p</i> ; cooked 80% <i>s+p</i> ; raw <i>s+p</i>

Analysis of Variance for rds, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	3	2771,79	2771,79	923,93	1850,37	0,000
Error	8	3,99	3,99	0,50		
Total	11	2775,78				

S = 0,706627 R-Sq = 99,86% R-Sq(adj) = 99,80%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
cooked 80% <i>s+p</i>	3	46,3	A
cooked 65% <i>s+p</i>	3	37,4	B
cooked 50% <i>s+p</i>	3	28,1	C
raw <i>s+p</i>	3	5,5	D

Means that do not share a letter are significantly different.

Table C.41. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on different water concentrations for cooked wheat starch-psyllium mixture and raw mixture (1:1)

General Linear Model: SDS versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	4	cooked 50%s+p; cooked 65%s+p; cooked 80%s+p; raw s+p

Analysis of Variance for sds, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	3	149,606	149,606	49,869	176,10	0,000
Error	8	2,265	2,265	0,283		
Total	11	151,872				

S = 0,532152 R-Sq = 98,51% R-Sq(adj) = 97,95%

Unusual Observations for sds

Obs	sds	Fit	SE Fit	Residual	St Resid
5	18,9848	18,1108	0,3072	0,8740	2,01 R
6	17,2368	18,1108	0,3072	-0,8740	-2,01 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
cooked 50%s+p	3	22,9	A
raw s+p	3	20,6	B
cooked 65%s+p	3	18,1	C
cooked 80%s+p	3	13,4	D

Means that do not share a letter are significantly different.

Table C.42. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on different water concentrations for cooked wheat starch-psyllium mixture and raw mixture (1:1)

General Linear Model: RS versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	4	cooked 50% <i>s+p</i> ; cooked 65% <i>s+p</i> ; cooked 80% <i>s+p</i> ; raw <i>s+p</i>

Analysis of Variance for rs, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	3	2045,31	2045,31	681,77	9591,55	0,000
Error	8	0,57	0,57	0,07		
Total	11	2045,88				

S = 0,266609 R-Sq = 99,97% R-Sq(adj) = 99,96%

Unusual Observations for rs

Obs	rs	Fit	SE Fit	Residual	St Resid
8	49,5320	49,0740	0,1539	0,4580	2,10 R
9	48,6160	49,0740	0,1539	-0,4580	-2,10 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
raw <i>s+p</i>	3	73,9	A
cooked 50% <i>s+p</i>	3	49,1	B
cooked 65% <i>s+p</i>	3	44,5	C
cooked 80% <i>s+p</i>	3	40,3	D

Means that do not share a letter are significantly different.

Table C.43. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on different water concentrations for cooked wheat starch-cellulose mixture and raw mixture (1:1)

General Linear Model: RDS versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	4	cooked 50% _{s+c} ; cooked 65% _{s+c} ; cooked 80% _{s+c} ; raw _{s+c}

Analysis of Variance for rds, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	3	3680,3	3680,3	1226,8	3481,77	0,000
Error	8	2,8	2,8	0,4		
Total	11	3683,2				

S = 0,593585 R-Sq = 99,92% R-Sq(adj) = 99,89%

Unusual Observations for rds

Obs	rds	Fit	SE Fit	Residual	St Resid
11	21,9700	20,9800	0,3427	0,9900	2,04 R
12	19,9900	20,9800	0,3427	-0,9900	-2,04 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
cooked 80% _{s+c}	3	66,7	A
cooked 65% _{s+c}	3	59,8	B
cooked 50% _{s+c}	3	53,2	C
raw _{s+c}	3	21,0	D

Means that do not share a letter are significantly different.

Table C.44. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on different water concentrations for cooked wheat starch-cellulose mixture and raw mixture (1:1)

General Linear Model: SDS versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	4	cooked 50% _{s+c} ; cooked 65% _{s+c} ; cooked 80% _{s+c} ; raw _{s+c}

Analysis of Variance for sds, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	3	3365,2	3365,2	1121,7	3812,92	0,000
Error	8	2,4	2,4	0,3		
Total	11	3367,6				

S = 0,542398 R-Sq = 99,93% R-Sq(adj) = 99,90%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
raw _{s+c}	3	53,5	A
cooked 50% _{s+c}	3	16,4	B
cooked 65% _{s+c}	3	15,0	B
cooked 80% _{s+c}	3	13,2	C

Means that do not share a letter are significantly different.

Table C.45. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on different water concentrations for cooked wheat starch-cellulose mixture and raw mixture (1:1)

General Linear Model: RS versus water concentration

Factor	Type	Levels	Values
water concentration	fixed	4	cooked 50% <i>s+c</i> ; cooked 65% <i>s+c</i> ; cooked 80% <i>s+c</i> ; raw <i>s+c</i>

Analysis of Variance for rs, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
water concentration	3	160,153	160,153	53,384	98,29	0,000
Error	8	4,345	4,345	0,543		
Total	11	164,499				

S = 0,736987 R-Sq = 97,36% R-Sq(adj) = 96,37%

Grouping Information Using Tukey Method and 95,0% Confidence

water concentration	N	Mean	Grouping
cooked 50% <i>s+c</i>	3	30,4	A
raw <i>s+c</i>	3	25,6	B
cooked 65% <i>s+c</i>	3	25,3	B
cooked 80% <i>s+c</i>	3	20,1	C

Means that do not share a letter are significantly different.

Table C.46. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RDS value (%) depending on fiber addition for raw starch and mixtures (1:1)

General Linear Model: RDS versus fiber addition

Factor	Type	Levels	Values
fiber addition	fixed	3	raw s; raw s+c; raw s+p

Analysis of Variance for rds, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
fiber addition	2	630,66	630,66	315,33	903,09	0,000
Error	6	2,09	2,09	0,35		
Total	8	632,75				

S = 0,590903 R-Sq = 99,67% R-Sq(adj) = 99,56%

Unusual Observations for rds

Obs	rds	Fit	SE Fit	Residual	St Resid
5	21,9700	20,9800	0,3412	0,9900	2,05 R
6	19,9900	20,9800	0,3412	-0,9900	-2,05 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

fiber	addition	N	Mean	Grouping
raw s	3	24,9	A	
raw s+c	3	21,0	B	
raw s+p	3	5,5	C	

Means that do not share a letter are significantly different.

Table C.47. One way ANOVA and Tukey's Comparison Test with 95% confidence level for SDS value (%) depending on fiber addition for raw starch and mixtures (1:1)

General Linear Model: SDS versus fiber addition

Factor	Type	Levels	Values
fiber addition	fixed	3	raw s; raw s+c; raw s+p

Analysis of Variance for sds, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
fiber addition	2	2408,5	2408,5	1204,3	3430,27	0,000
Error	6	2,1	2,1	0,4		
Total	8	2410,6				

S = 0,592509 R-Sq = 99,91% R-Sq(adj) = 99,88%

Grouping Information Using Tukey Method and 95,0% Confidence

fiber addition	N	Mean	Grouping
raw s	3	56,9	A
raw s+c	3	53,5	B
raw s+p	3	20,6	C

Means that do not share a letter are significantly different.

Table C.48. One way ANOVA and Tukey's Comparison Test with 95% confidence level for RS value (%) depending on fiber addition for raw starch and mixtures (1:1)

General Linear Model: RS versus fiber addition

Factor	Type	Levels	Values
fiber addition	fixed	3	raw s; raw s+c; raw s+p

Analysis of Variance for rs, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
fiber addition	2	5490,1	5490,1	2745,0	10471,91	0,000
Error	6	1,6	1,6	0,3		
Total	8	5491,6				

S = 0,511990 R-Sq = 99,97% R-Sq(adj) = 99,96%

Grouping Information Using Tukey Method and 95,0% Confidence

fiber addition	N	Mean	Grouping
raw s+p	3	73,9	A
raw s+c	3	25,6	B
raw s	3	18,2	C

Means that do not share a letter are significantly different.