

EFFECT OF PSYLLIUM AND CELLULOSE FIBER ADDITION ON STARCH
DIGESTIBILITY FOR BREAD AND CRACKER

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STARCH DIGESTIBILITY FOR BREAD AND CRACKER**

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ABSTRACT

EFFECT OF PSYLLIUM AND CELLULOSE FIBER ADDITION ON STARCH DIGESTIBILITY FOR BREAD AND CRACKER

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Understanding the interactions between fibers and other food components gain importance every day due to the increasing number of informed consumers about the health benefits of high fiber-containing foods. The objective of this study was to investigate the effects of fiber addition on the starch digestion behavior of bread and cracker samples. Fiber-added samples (bread or cracker) were prepared by replacing 10 % of the wheat flour with the fibers (psyllium or cellulose). Physical and quality parameters of the samples were measured in addition to *in vitro* digestion simulations. Psyllium fiber was an effective ingredient at the studied concentration to slow down the bread and cracker's digestion rate. However, cellulose fiber was only affected the cracker samples. The high water-holding capacity of the psyllium was the significant factor affecting the starch digestibility. Psyllium fiber reduced starch digestion primarily by hindering the mobility of the enzymes within the digestion medium. Results suggested processing methods, ingredients, and physical properties of the products could affect starch digestion.

Keywords: Bread, Cracker, *In vitro* starch digestion, Dietary fiber, Starch gelatinization

ÖZ

PİSİLYUM VE SELÜLOZ LİFİ İLAVESİNİN EKMEK VE KRAKER ÜRÜNLERİNDE NİŞASTA SİNDİRİLEBİLİRLİĞİ ÜZERİNDEKİ ETKİSİ

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Yüksek lif içeren gıdaların sağlığa faydaları hakkında artan sayıdaki bilinçli tüketici nedeniyle, lifler ve diğer gıda bileşenleri arasındaki etkileşimleri anlamak her geçen gün önem kazanmaktadır. Bu çalışmanın amacı, lif ilavesinin ekmek ve kraker örneklerinin nişasta sindirim davranışı üzerindeki etkilerini araştırmaktır. Lif katkılı numuneler (ekmek veya kraker), buğday ununun %10'u lifler (psilyum veya selüloz) ile değiştirilerek hazırlanmıştır. *In vitro* sindirim simülasyonlarına ek olarak, numunelerin fiziksel ve kalitesel parametreleri de ölçülmüştür. Psilyum lifinin, incelenen konsantrasyonda ekmek ve krakerin sindirim hızını yavaşlatmak için etkili bir bileşen olduğu anlaşılmıştır. Ancak selüloz lifi sadece kraker numunelerini etkilemiştir. Psilyumun yüksek su tutma kapasitesinin nişasta sindirilebilirliğini etkileyen en önemli faktör olduğu gözlemlenmiştir. Psyllium lifi, öncelikle sindirim ortamı içindeki enzimlerin hareketliliğini engelleyerek nişasta sindirimini azaltmıştır. Sonuçlar işleme yöntemlerinin, içerik maddelerinin ve ürünlerin fiziksel özelliklerinin nişasta sindirimini etkileyebileceğini göstermiştir.

Anahtar Kelimeler: Ekmek, Kraker, *In vitro* nişasta sindirimi, Diyet lifi, Nişasta jelatinizasyonu

To my beloved family

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CHAPTER 1

INTRODUCTION

1.1 Functional Foods

As the awareness increased that human health could be supported and even elevated with conscious food consumerism, the significance of the studies on creating functional foods gained importance every day. The functional food concept is defined as any food contributing to human health and physical performance by its nutritious value (Rincon, 2003). A food product can be considered as functional as long as it is derived from natural ingredients, consumable as part of the daily diet, and creates a particular impact on the human body such as prevention, recovery, enhance or control specific body functions (Smith et al., 1996; Rincon, 2003).

A variety of studies done in the field suggested that consuming food with a low glycemic index can help to regulate the blood sugar level, ease weight loss, and lower the risk of coronary diseases (Wolever et al., 1992; Brand-Miller et al., 2003; Sacks et al., 2014; Bustos et al., 2017). However, eating habits, food culture, and the pace of modern life direct people worldwide to consume foods with a high glycemic index more frequently (Rahati et al., 2014). The future of functional foods proceeds to be more specific with the target consumer approach, aiming to meet the needs of the people like have little free time to eat or spend most of the day away from home (Vukasović, 2017). Given the circumstances, studies to decrease the glycemic index of the commonly consumed starchy foods increases their significance for the food industry (Bharath Kumar & Prabhasankar, 2014). Lately, manufacturers have shifted their attention to the food products that would supply satiety for a long time while having high nutrition and low glycemic indexes to meet consumer needs and expectations (Low GI Rice Market Size, Share: Industry Report, 2020-2027, 2020).

To design original food products with all these attributes, understanding how the physical and chemical structures of the foods affect the components released during starch digestion is essential. Also, evaluating the relation between food processes, ingredients, and digestion kinetics greatly influences the desire to produce and optimize innovative food designs.

1.2 Wheat Starch

Carbohydrates include a variety of components, from simple sugars like glucose to complex polysaccharides. Despite the diversity, the human body can absorb only monosaccharides and use them as an energy source. Wheat starch is a polysaccharide composed of glucose molecules and is included in the wheat flour at around 70-80% (Wang et al., 2015, Shevkani et al., 2016). Glucose molecules connect with each other by either α -1, 4 or α -1, 6 glycosidic bonds to form polymer chains. The type of the bond determines the structure of the glucose chains such as α -1, 4 linkage creates linear chains (called amylose) while α -1, 6 creates branched chains (called amylopectin), as shown in Figure 1.1 (Delcour & Hosney, 2010). The distribution and organization of the amylose and amylopectin in starch granules depend on the source plants' type and affect the functionality of the starch (Wang et al., 2013).

Commercial wheat starch includes 18.2-28.8% amylose, a linear polymer with branching of 0.2-0.5% (Singh et al., 2010). As it can be present in double-helical form, amylose also can form an unstable single helical structure in an aqueous solution, which is obtained by the left-handed twisting effect of the α -1, 4 glycosidic bonds between glucose molecules induced by complexing agents (Takeo et al., 1973, Zobel, 1988). Because of this single helix form, amylose is prone to interactions with various chemical compounds like lipids, alcohols, emulsifiers, and flavor compounds present in the environment, making it possible for chemicals to place themselves inside the helical structure (Zobel, 1988, Pérez & Bertoft, 2010). Amylopectin is a

much larger and highly branched macromolecule with various polymer chains than amylose (Shevkani et al., 2016).

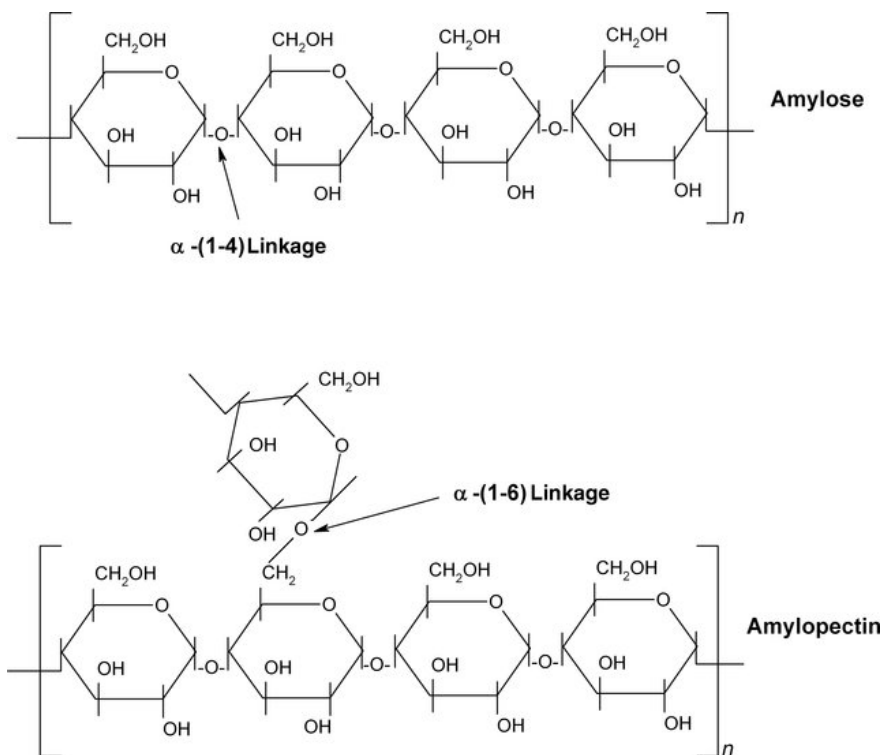


Figure 1.1 Chemical structure of the amylose and amylopectin chains (Habibi et al., 2012).

Blocks of amylose and amylopectin are organized into crystalline and amorphous lamellae with a repeat period of 9–11 nm that make the semi-crystalline growth rings (Jenkins & Donald, 1995, Gallant et al., 1997, Wang et al., 2013). The core of the starch granule, which is amorphous (mostly amylose and disordered reducing ends of amylopectin), is surrounded by alternating amorphous and semi-crystalline growth rings (Vandeputte & Delcour, 2004, Pérez & Bertoft, 2010). Both growth rings include crystalline and amorphous matters (Figure 1.2); however, their proportions make the growth rings diverse (Wang et al., 2013).

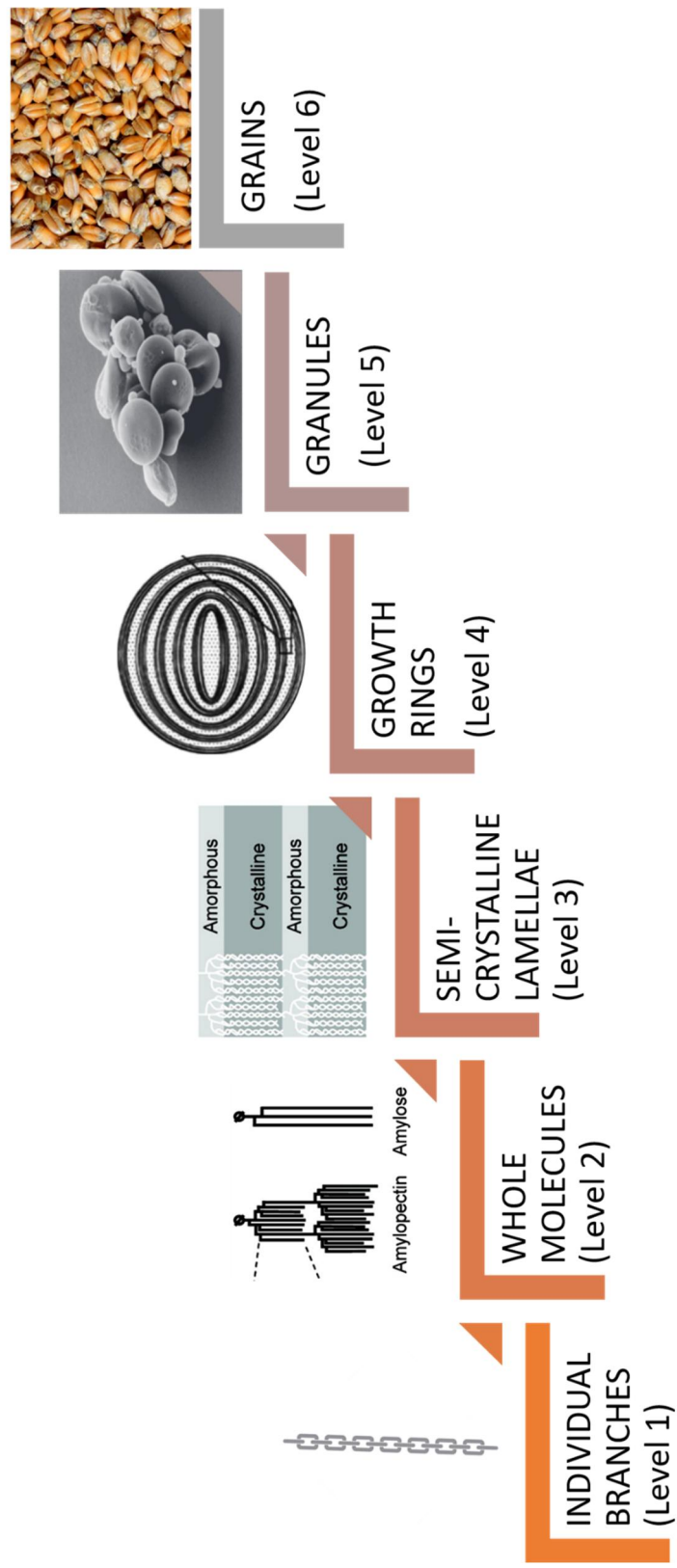


Figure 1.2 Hierarchical structure of wheat starch from individual branches to grains (Tran et al., 2011, Li et al., 2013).

1.3 Starch Gelatinization

The semi-crystalline structure of the starch granules turns into gel form when heat is applied at a specific starch to water ratio. Granules start to absorb the water present in the environment, and as the amorphous shell becomes wet, the hydrogen bonds within the amorphous structure get disrupted. Granules start to swell and undergo glass transition with water absorption by the amorphous region of the starch granules (Biliaderis, 2009). At this point, the change in the structure of the granules is reversible and can be detected by using differential scanning calorimetry (DSC). However, if heating continues, the swelling stress rises to a level that would affect the crystalline region also, causing an irreversible disruption called gelatinization (Atwell et al., 1988, Wang et al., 2013). Because of the glass transition's structural alterations, granules get hydrated, and double helical structures of the crystalline regions dissociate. The energy required for this dissociation is demonstrated as enthalpy change (ΔH) to the DSC thermograms, while the extent of the crystalline perfection reflects as transition temperature (Singh et al., 2010)

The effects of hydrothermal treatments (heating in the presence of water), usually with the application of shear forces, govern the functioning of starch in foods to a great extent (Guo et al., 2018). A variety of the studies in the literature had used DSC to determine the parameters that would alter the gelatinization degree of the food products. Conforti et al. (2012) investigated the effect of the baking conditions on the gelatinization degree and starch digestibility of the biscuits produced with different formulations. The study suggested that the biscuits baked at higher temperatures had a higher degree of gelatinization, correlating with the higher digestibility, due to the possibility of higher water retention within the biscuits.

Additionally, Jia et al. (2020) demonstrated that the addition of a soluble fiber obtained from rice might lower the glycemic index of the biscuits by delaying the starch gelatinization in doughs. Moreover, Fessas and Schiraldi (2000) studied the mechanism of the starch gelatinization in bread dough by simulating the baking of

bread dough in DSC, setting moisture content as a single parameter. Results of these and many other studies found that the progression and extent of gelatinization of a food product rely on parameters related to raw materials such as plant origin, ingredients other than starch (like proteins, lipids, fibers), and production processes like temperature, duration of the process, and shear forces (Cleary & Brennan, 2006, Schirmer et al., 2015). Despite setting parameters of the studies as anything other than moisture content, gelatinization degree can still be rooted in the amount and behavior of free water molecules within the system (Tester & Morrison, 1993).

1.4 Digestibility of Starch

Before being absorbed, polysaccharides and oligosaccharides must be hydrolyzed to monosaccharides. The digestion of the starch starts in the human mouth with salivary amylase and then continues with a much more effective digestive enzyme, pancreatic amylase, in the small intestine (Jones et al., 1983). Maltose, maltotriose, and dextrans, the principal end products of amylase's starch hydrolysis, are hydrolyzed into monosaccharides by a group of digestive enzymes expressed in the small intestine (Gibson & Roberfroid, 1995). The digestion rate of the starch is more rapid than other energy sources, and the monosaccharides' absorption primarily occurs in the upper small intestine. However, depending on the accessible starch amount of the food, absorption of the monosaccharides may last until it reaches the end of the small intestine (Jones et al., 1983, Lee et al., 2012).

The glucose release rate from starchy food has significant importance for human health since it is related to the change in the blood glucose level (Zhou et al., 2014). Based on their enzymatic digestibility, starches are divided into three categories: rapidly digestible starch, RDS; slowly digestible starch, SDS; and resistant starch, RS (Englyst et al., 1992). As the name implies, RDS is the most rapidly hydrolyzed present in foods, elevating the blood glucose level quickly. On the other hand, SDS is slowly hydrolyzed and alters the blood glucose level moderately and stably.

Because of that reason, higher SDS inclusion in starchy foods becomes desirable considering long-term health aspects (Lehmann & Robin, 2007). Unlike these digestible fractions, RS passes through the small intestine remaining undigested since it is resistant to the digestive enzymes. After reaching the large intestine, RS can be fermented by the microflora like dietary fibers, producing short-chain fatty acids and gases such as CO₂ (Stewart & Zimmer, 2018).

A connection between the gelatinization and SDS amount of a starchy product can be logical due to the structural changes in the starch granules caused by gelatinization, as explained in detail in the previous section (Zhang et al., 2006). As starch interacts with the water molecules while heat is applied simultaneously, it forms a gel-like structure and becomes more susceptible to digestive enzymes in the absence of the crystal and granular structure (Wang and Copeland, 2013). This ease of access for the enzymes is reflected as a decrease in the SDS content and an increase in the amount of RDS in the product (Guo et al., 2018). In that respect, the high gelatinization degree of the bread produced by fermentation could imply that the starch included is mostly in RDS form (Bustos et al., 2017). On the other hand, foods that include extrusion, such as pasta, contain low RDS because of their compact structure. Products such as crackers and biscuits, which have additional ingredients such as oil and sugar in their formulations, generally contain moderate RDS (Štěrbová et al., 2016). Therefore, the desirable starch fraction of the food products (SDS) can be preserved or increased by selecting the optimum processing conditions or method of productions (Englyst et al., 2018). Moreover, determining revised formulations of the products with increased dietary fiber amount might also be an effective way to increase the amount of SDS (Englyst et al., 2018).

1.5 Dietary Fibers

It has been shown that reducing glucose release and absorption by adding dietary fiber to foods contributes to health by reducing the risk of metabolic diseases (Wolk

et al., 1999; Ronda et al., 2012; Wyrwisz, 2015). The addition of the dietary fibers to food products by replacing the primary starch source causes a decreasing effect on blood glucose level per portion due to the resistance of fiber to digestion. Moreover, studies show that the dietary fiber present in the product slows down starch digestion due to its behavior during heat treatment or digestion (Aleixandre and Miguel, 2008). Numerous studies suggest that the type of the fiber (water-soluble or insoluble) might also be a parameter on the starch digestion since the interaction between water and fiber could alter the position of the water molecules within the food matrix (Cappa et al., 2013, Dokić et al., 2015, Lauková et al., 2017, Jia et al., 2020).

Psyllium is a member of the plant family called *Plantago ovata*, also known as blackthorn grass (Dhar et al., 2005), and it is included in the water-soluble fibers category. Psyllium cannot be digested in the small intestine, and like other dietary fibers, it has a regulating effect on bowel movements, and therefore it is used to relieve intestinal problems such as constipation (Vries, 2015). It has a high water-holding and gelling capacity, which would cause competition with starch granules to interact with the water molecules (Cappa et al., 2013). Furthermore, the ability of psyllium to increase the viscosity of the solution might hinder the postprandial blood sugar level by affecting the digestion rate with lowering the mobility of the digestive enzymes (Masood and Mirafab, 2010).

Cellulose, on the other hand, belongs to the water-insoluble fiber category. It is also widely used in the production of fabric and paper, as well as in the food industry (Keshk, 2014). As for psyllium, cellulose cannot be digested in the human body; however, its inclusion in the diet on certain levels is often recommended for the digestive system health (Flourie, 1992; Prola et al., 2006). When consumed, cellulose travels through the digestive system without binding to water and proceeds without any structural changes, which allows the shortening of the time waste spent in the digestive tract (Vries, 2015). In other words, although it has a different mechanism

from psyllium, cellulose also has an accelerating effect on bowel movements; it is used for preventive or therapeutic purposes (Eastwood, 1973).

1.6 Glycemic Index

The Glycemic index was first defined in the 1980s to compare the increase in blood sugar levels in two hours after consuming starchy foods (Jenkins et al., 1981). It is widely used to state the blood sugar level, which has a range of 0-100 assigned to the foods individually (Augustin et al., 2015). It is obtained by calculating the area under the glucose response curve of the sample food and proportioning the result with the response of the reference food, which is 50 g of sugar with a glycemic index value of 100 (Fratelli et al., 2018). Foods are divided into three categories depending on their glycemic index as low (1-55), medium (56-69), and high (70 and above) (Atkinson et al., 2008).

For individuals with metabolic problems in the digestion of carbohydrates and fats, low glycemic index diets are essential for increasing their life quality (Campbell et al., 2017). For this reason, daily diets are designed to include fewer carbohydrates that are ready to digest and quickly increase the blood sugar level and replace them with foods with a low glycemic index (Lehmann and Robin, 2007). Therefore, choosing foods such as pasta, whole wheat products, and legumes instead of bread and breakfast cereals positively affects blood sugar balance, as their digestion will take much more time (Englyst et al., 2003).

1.7 The Objective of the Study

The importance of functional food design has been increasing every day. Consumer demand elevates this trend due to increased awareness about the relationship between starchy foods and blood sugar levels, obesity, and heart diseases. In addition, the successful incorporation of dietary fibers into food products has become significant, with medical studies advising to include more fiber in the daily diet to regulate the digestive system. Thereby, the mechanism and extent of starch digestion in model systems and food products had been studied for years by various researchers to determine the effects of different parameters like heat, water content, and ingredients on digestion.

Numerous studies in the literature have proposed a strong relationship between the gelatinization degree of the starch and its digestibility. This connection brings out investigating the gelatinization mechanism of starchy foods such as bread, biscuit, cracker, pasta as a topic of much research either with concerns about the quality or digestibility rates. However, comparing the digestibility of different foods with each other and obtaining a conclusion may not be practical or reliable because of the insufficient number of studies that investigate more than one type of starchy food at the same time. For this reason, bread and cracker products were selected for this study as two different foods with similar formulations yet different processing steps.

Studies have shown that, during heat treatment, dietary fiber and starch may compete for water. Fiber's presence may reduce the degree of gelatinization and consequently affect starch digestion since the amount of water in the medium is limited. Because the free water amount is one of the primary parameters on gelatinization, it was predicted to observe different results depending on the water holding capacity of dietary fibers used, so on whether it is soluble or insoluble in water. Therefore, evaluating the effects of different types of dietary fibers' (psyllium and cellulose) inclusion on the RDS and SDS content of the foods (bread and cracker) produced with different processes was the study's primary objective.

Physical properties of the final products might also be determinant and informative about the starch digestion's mechanism and extent. Therefore, some of the unique quality characteristics of the products, such as specific volume, color, and texture, were also set to be examined for both control (no fiber addition) and fiber added products.

In short, the objectives were to evaluate the effects of fiber (psyllium and cellulose) inclusion on the starch digestibility of bread and cracker. Samples physical and quality parameters were also measured.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

The materials used to prepare bread and crackers were wheat flour, psyllium, powdered cellulose, instant yeast, sodium bicarbonate, salt, sugar, and oil. Powdered cellulose (Jelucel®PF300, JELU-WERK J. Ehrler GmbH & Co. KG, Rosenberg, Germany) with fiber lengths of 32 µm (60%), 100 µm (20%), and 300 µm (2%) and psyllium (Protap Psyllium; minimum clarity; %95) were supplied by Tunckaya Chemicals (Istanbul, Turkey). Wheat flour (Soke Flour, Aydın, Turkey), instant yeast (Dr. Oetker, İzmir, Turkey), sodium bicarbonate, salt, sugar, and oil were bought from local markets.

Pancreatin 8 x USP from porcine pancreas (Sigma-Aldrich Chemical Co., Saint Louis, MO, USA), pepsin from porcine gastric mucosa (Sigma-Aldrich Chemical Co., Saint Louis, MO, USA), invertase from *Saccharomyces cerevisiae* (Fisher Scientific, Hampton, USA), amyloglucosidase from *Aspergillus niger* (Sigma-Aldrich Chemical Co., Saint Louis, MO, USA) and D-Glucose assay (GOPOD) kit (Megazyme, Wicklow, Ireland) were used to perform *in vitro* starch digestion simulation. The other chemicals (guar gum, HCl, CaCl₂, KOH, acetic acid, absolute ethanol, and methanol) used for *in vitro* digestion procedures were analytical grade.

2.2 Methods

2.2.1 Preparation of Bread

The formulation for the control bread (with no additional dietary fiber) was given in Table 2.1. The fiber-enriched bread samples were produced by replacing 10 % of the wheat flour in the standard formulation with either psyllium or cellulose fibers on a dry basis (db).

Bread samples were prepared with a bread maker (K-2710, Arçelik, Turkey) according to the formulation given in Table 2.1. First, the dry mix was prepared by mixing flour, psyllium or cellulose, salt, and sugar in a separate container. Then, instant yeast, the dry mix, and water were transferred into the bread pan with the kneading paddle, respectively. The selected program from the bread maker starts with kneading for 14 minutes. Then, the bread dough was fermented for 30 minutes at 30 °C. Second fermentation was applied for 100 minutes at 30 °C, following a second kneading for 20 minutes. Finally, the bread dough was baked at 200 °C for 50 minutes.

Table 2.1 Standard bread formulation

Ingredient	Amount (g)
Wheat flour	100
Water	70
Instant yeast	3
Salt	1
Sugar	5

2.2.2 Preparation of Cracker

Ingredients and formulation for control crackers (with no additional dietary fiber) were given in Table 2.2. The fiber-enriched cracker samples were produced by replacing 10 % of the wheat flour in the standard formulation with either psyllium or cellulose fibers on a dry basis.

Cracker samples were prepared with a dough mixer (Kitchen Aid 5KPM5, Kitchen Aid Europa Inc., Ohio, USA) according to the formulation given in Table 2.2. First, the dry mix was prepared by mixing flour, psyllium or cellulose, and salt in a separate container. The oil and water were mixed for 30 seconds with the dough mixer. Then, the dry mix was slowly added while mixing for 4 minutes in slow mode. The dough was rested for 10 minutes. Rested dough was sheeted to 2 mm thickness and cut with a 4 cm diameter circular dough cutter. Circular crackers baked in an oven at 175 °C for 30 minutes.

Table 2.2 Standard cracker formulation

Ingredient	Amount (g)
Wheat flour	100
Water	35
Sodium bicarbonate	0.3
Salt	1
Oil	15

2.2.3 Water Holding Capacity (WHC)

Water holding capacities of bread samples, cracker samples, and raw ingredients and raw mixtures were investigated according to Raghavendra et al. (2004) with minor modifications. The WHC was determined in triplicate experiments with two parallel measurements at each time. Samples were weighed as 1.5 g and dissolved in 30 mL

distilled water. Then, they equilibrated at 37 °C for one hour. After equilibration, the tubes were centrifuged at 6,000xg for 10 min, the sediment was weighed (as wet weight) and dried to constant weight (as dry weight) in an oven (50 °C) for 48 hours.

WHC of the samples was calculated as follows:

$$\text{WHC (g/g)} = \frac{(W_W - W_D)}{W_D} \text{ where } W_W \text{ is wet weight, and } W_D \text{ is the dry weight.}$$

2.2.4 Differential Scanning Calorimetry (DSC)

Thermal property measurements of bread dough, cracker dough, baked bread, and baked cracker samples were conducted using a differential scanning calorimeter (Pyris 6 DSC, PerkinElmer, Massachusetts, USA). The moisture content of the dough and baked samples was determined using a moisture analyzer (MS-70, A&D Company, Tokyo, Japan).

Preparation of dough samples for DSC analysis: Bread dough was sampled right after the second fermentation step, and cracker dough was sampled right after the resting step.

Preparation of baked samples for DSC analysis: Baked samples were ground until a fine powder was obtained and tightly packed into plastic bags.

Samples were weighed (5mg) into 30 µl capacity aluminum (Al) DSC pans and moistened with distilled water with a ratio of dry sample to water around 1:3. The DSC pans were hermetically sealed and allowed to reach equilibrium conditions at room temperature for 20 hours.

All samples were heated at a rate of 10°C/min from 10 to 100°C with nitrogen flushing (20 ml/min). An empty pan was used as a reference. Each experiment was carried out in triplicate. For each endotherm, onset (T_o), peak (T_p), and conclusion (T_c) temperatures together with enthalpy change values were determined using the software program (Pyris Manager, PerkinElmer, Massachusetts, USA). Transition

peaks of the samples were taken as the required energy to reach complete gelatinization.

2.2.5 Specific Volume

The baked bread samples were rested for 4 hours at room temperature before being weighed for specific volume analysis. Bread loaves were cut into 25 mm slices after removing the crusts, and then they were cut by using a round stainless steel cutter with 40 mm diameter. After weighing these defined volumes of bread crumbs, the specific volume (cm^3/g) was calculated as bread volume/bread weight.

2.2.6 Porosity

The bread crumb pictures taken by a laser camera with 28MP (Asus Zenfone 3 Laser, Asus, Taipei, Taiwan) were saved as bitmap files, with a 300 DPI resolution in real-color format (RGB, 256 million colors) as described in Cappa et al., 2013. The images were then cropped to the resolution of 310x310 pixels. The cropped images were converted into an 8-bit grayscale image and then thresholded using the software ImageJ (US National Institutes of Health, USA), which allowed converting the images into black and white colors. Pore number and sizes per image were calculated using the histogram tool in ImageJ software.

2.2.7 Color

The values of surface color of bread crumbs and crackers were measured using the colorimeter (Minolta CR- 400, Konica Minolta Sensing, Inc., Osaka, Japan), and expressed as L^* ($L^* = 0$ [black] and $L^* = 100$ [white]), a^* ($-a^*$ = greenness and $+a^*$ = redness), and b^* ($-b^*$ = blueness and $+b^*$ = yellowness). Color measurements were obtained from triplicate experiments with six parallel sample measurements for

bread crumb samples and nine parallel sample measurements for the cracker samples each time.

2.2.8 Texture Profile Analysis

Texture profile analysis (TPA) of bread crumbs was conducted at the fourth hour after baking by the Texture Analyzer (CT3 Texture Analyzer, AMATEK Brookfield Inc., Massachusetts, USA). Bread loaves were cut by hand as 25mm thick slices, and then a 40 mm round stainless steel cutter was used to take crumb samples from the middle. Thus, no crust was included in the measurements. The slices were compressed twice with a 25.4 mm cylindrical probe (TA11/1000) by 40% with a speed of 1.7 mm/s with 5 g trigger load (Flander, Salmenkallio-Marttila, Suortti, & Autio, 2007). TPA analysis for bread crumbs was conducted on triplicate experiments with six parallel sample measurements each time. Hardness, cohesiveness, springiness, and chewiness were determined. Peak force during the first compression cycle was used to determine hardness. The ratio of the area under the second curve to the area under the first curve was used to calculate cohesiveness. The time recorded between the start of the second region and the second probe reversal was divided by the time recorded between the start of the first area and the first probe reversal to determine springiness. Hardness, cohesiveness, and springiness were multiplied to get chewiness.

The hardnesses of crackers were determined by the three-point bending test using a Texture Analyzer (CT3 Texture Analyzer, AMATEK Brookfield Inc., Massachusetts, USA). A single cracker was placed surface up across two supports. Loading force was applied to the center of each biscuit by a wedge. The trigger load was 5 kg, and the pre-test, test, and post-test speeds of the wedge were 1, 1, and 10 mm/s, respectively. Measurements were performed on triplicate experiments with six parallel sample measurements each time. The crackers' hardness was determined as the force needed to be applied to break the cracker.

2.2.9 Scanning Electron Microscopy (SEM)

SEM images were obtained for raw materials (wheat flour, psyllium, and cellulose), bread and cracker dough samples, baked bread and cracker samples, and digested bread and cracker samples.

Preparation of dough samples for SEM imaging: Bread dough was sampled right after the second fermentation step, and cracker dough was sampled right after the resting step. Dough samples were kept at -20 °C for 24 hours and freeze-dried under vacuum (0.3 mBar) for 48 hours (CHRIST Alpha 1-2 LD Plus, Martin Christ, Osterode am Harz, Germany). The freeze-dried pieces of the samples were fractured into sizes of about 1 cm x 1 cm x 0.5 cm and tightly packed into plastic bags.

Preparation of baked samples for SEM imaging: Baked samples were cut into smaller sizes about 1 cm x 1 cm x 0.5 cm and freeze-dried under vacuum (0.3 mBar) for 48 hours (CHRIST Alpha 1-2 LD Plus, Martin Christ, Osterode am Harz, Germany) after keeping at -20 °C for 24 hours. The freeze-dried samples were tightly packed into plastic bags.

Preparation of digested samples for SEM imaging: Bread, cracker, and wheat flour samples were subjected to *in vitro* digestion procedure as explained in Section 2.2.10, and G20 fractions were used for imaging. The tubes (G20) were removed from the water bath 20 min after the digestion started and immediately centrifuged at 5000x g for 5 min at 1 °C (Sigma 2-16KL; Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). After discarding the supernatants, 20 ml methanol was added to each tube and vortex mixed. Then, the tubes were centrifuged again at 5000 g for 5 min at 1°C, and supernatants were discarded (Berg et al., 2012). The pellets, remaining digested samples, were kept at -20 °C for 24 hours and then freeze-dried (CHRIST Alpha 1-2 LD Plus, Martin Christ, Osterode am Harz, Germany). The freeze-dried pieces of the samples were fractured into sizes of about 0.5 cm x 0.5 cm x 0.5 cm and tightly packed into plastic bags.

SEM analysis of the samples was conducted at the Middle East Technical University Central Laboratory. All the samples were mounted on individual stubs and coated with Au-Pd (3 nm), and images of the samples were obtained with a scanning electron microscope (400F Field Emission, QUANTA, Waltham, MA, US) at an accelerating voltage of 30 kV.

2.2.10 *In Vitro* Digestion Analysis

The simulations of the digestion in the stomach and the small intestine were performed according to Englyst et al. (2018). Then, the experiment continued with the total glucose (TG) procedure according to Englyst et al. (2000) to determine any remaining starch in the solution. Any free sugar present in the samples was determined according to the free sugar glucose (FSG) procedure as Englyst et al. (2018). The glucose content of the samples were determined by using a glucose oxidase/oxidase (GOPOD) assay kit (D-Glucose Assay Kit, K-GLUC 08/18 Megazyme, Wicklow, Ireland) and measured with a UV–visible spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan).

Digestion simulation: Samples were minced with a manual mincer (Kitchen Basics, US) with a plaque having 0.9 cm diameter holes to analyze samples as they are “eaten.” For the FSG procedure only, to standardize the results, samples were milled with a grinder (Sinbo SCM-2934, Istanbul, Turkey) before the analysis. The samples’ moisture contents were determined with a moisture analyzer (MS-70, A&D Company, Tokyo, Japan).

Sample weights were determined to contain at least 500 mg starch in each tube and they were weighed into 50 ml falcon tubes to the nearest 0.1 mg. Wheat flour was also weighed in a falcon tube as a reference sample in every batch. Samples were wetted with 5 ml distilled water, and 10 ml of Enzyme Solution I (Table 2.3) was added to each tube. After shaking gently, the tubes were placed in the water bath (JSR, JSSB-30T, Gongju-City, Korea) at 37 °C and incubated for 30 min. After 30 min of incubation, tubes were removed from the water bath, and 5 ml of 0.5 M

sodium acetate buffer (equilibrated at 37 °C) was added. After the addition of the buffer solution, the pH of the blank tube was checked whether it was between 5.2-5.3 or not while keeping the samples tube equilibrated at 37 °C. Then, the first tube was removed from the water bath, five glass balls with 1.5 cm diameter and 5 ml of Enzyme Solution II (Table 2.3) were added to the tube. The tube was secured horizontally in a shaking (80 double strokes/min) water-bath (JSR, JSSB-30T, Gongju-City Korea) at 37 °C, and the timer was started. This step was completed in 1 minute and repeat for all the other tubes within exact 1 min intervals. This was time zero for the procedure. After the timer started, the same sampling procedure was applied for 20th min (G20 data) and 120th min (G120 data).

Table 2.3 Composition of the working solutions.

Enzyme Solution I		Enzyme Solution II	
Compound	Amount	Compound	Amount
Guar gum	0.6 g	Pancreatin	3 g per tube
Ethanol	1 ml	CaCl ₂ (1 M)	20 ml per tube
Pepsin	0.6 g	Amyloglucosidase	4 ml
HCl (0.05 M)	120 ml	Invertase	2 ml

Preparation of Enzyme Solution I: 6 g guar gum was weighed and wetted with 1 ml ethanol. Then 120 ml 0.05 M HCl was added and mixed with a magnetic stirrer (MS-M-S10, Limited Company, Taipei, Taiwan). While mixing, 0.6 g pepsin was added very slowly. This solution was used within 20 min.

Preparation of Enzyme Solution II: 3 g pancreatin was weighed into three 50 ml falcons, and 20 ml 0.1 M CaCl₂ was added to each tube. The tubes were mixed with a magnetic stirrer for 10 min and centrifuged at 1500 g for 10 min (Sigma 2-16KL; Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). 17 ml supernatant was taken from each tube and combined in a beaker. Finally, 4 ml amyloglucosidase and 2 ml invertase were added and mixed with a magnetic stirrer. This solution was used within 1 hour.

Total Glucose (TG) procedure: When the digestion simulation was completed at the end of the 120 min (G120 fraction), all tubes were removed from the water bath (37 °C), and vortex mixed vigorously in case of any large particles remains in the digestion medium. The tubes were placed in a boiling water bath and kept for 30 min to reduce any granular starch by gelatinization. Then, the tubes were removed, vortex mixed, and placed in ice water for 30 min until they were thoroughly chilled. The tubes were mixed by inversion after adding 10 ml KOH solution (7 M) into each tube. They were placed in the shaking water bath containing ice water and kept for 30 min. The procedure was continued by immediately transferring 1 ml of the contents of each tube into the corresponding 50 ml tube filled with 10 ml acetic acid solution (0.5 M). After adding 0.2 ml of amyloglucosidase solution to each tube, they were placed in a water bath at 70 °C and kept for 30 min. Afterward, the tubes were transferred to the boiling water bath and kept for 10 min. Lastly, tubes were cooled down to room temperature, and 40 ml of distilled water was added to each tube, capped, and mixed to determine glucose content.

Preparation of Amyloglucosidase Solution: 0.5 ml amyloglucosidase was diluted to 1:7 (v/v) with distilled water.

Free Sugar Glucose (FSG) procedure: Samples were weighed into 50 ml falcon tubes to the nearest 0.1 mg. Wheat flour was also weighed in a falcon tube as a reference sample in every batch. Five glass balls and 25 ml of sodium acetate buffer (0.1 M) were added to each tube. After capped and vortex mixed, they were placed in a boiling water bath for 30 min. Then, tubes were removed, vortex mixed, and cooled down to 37 °C. Before placing them in a shaking water bath at 37 °C, 0.2 ml of invertase was added and kept for 30 minutes. Finally, 1 ml of the contents were transferred to 2 ml of absolute ethanol and centrifuged at 500 g for 5 min (Sigma 2-16KL; Sigma Laborzentrifugen GmbH, Osterode am Harz Germany) to transfer 1 ml of the supernatant to 5 ml distilled water for glucose content measurement.

Determination of glucose content:

0.1 mL from the tubes (digested, TG, and FSG determination tubes) were dissolved in 8 ml absolute methanol, then vortex mixed and transferred into 3 ml GOPOD reagent (D-Glucose Assay Kit, K-GLUC 08/18 Megazyme, Wicklow, Ireland). They were incubated at 40-50°C for 20 min. After the incubation, absorbances of the solutions were read using a spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan) at 510 nm against a blank reagent containing distilled water. Obtained absorbance values were converted to glucose concentrations by using the equation below:

$$D - \text{Glucose} \left(\mu \frac{\text{g}}{0.1 \text{ mL}} \right) = \frac{\Delta A_{\text{sample}}}{\Delta A_{D-\text{Glucose standard}(100\mu\text{g})}} \times 100$$

Calculations of Digestion Fractions:

Once the amount of the glucose within the sample tubes at different time frames was obtained as explained above, starch fractions were calculated with the equations given below:

$$\text{RDS} = 0.9 \times (\text{G20} - \text{FSG})$$

$$\text{SDS} = 0.9 \times (\text{G120} - \text{G20})$$

$$\text{TS} = 0.9 \times (\text{TG} - \text{FSG})$$

$$\text{RS} = 0.9 \times (\text{TG} - \text{G120})$$

2.2.11 Statistical Analysis

Statistical analysis was conducted by using Minitab statistical software (Minitab Inc., State College, UK). Analysis of variance (ANOVA) was used to observe if there were any significant differences between treatments. Tukey's Multiple Comparison Test was performed for the data with significant differences ($p \leq 0.0$

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Water Holding Capacity (WHC)

Hydration properties of the dietary fibers could affect the final products' texture while influencing the starch granules' gelatinization (Thebaudin et al., 1997). Determining the water holding capacity (WHC) of the raw materials as well as the final products has crucial importance in understanding the position of the water molecules within the food matrix (Stephen & Cummings, 1979; Robertson & Eastwood, 1981).

Psyllium had a very high water holding capacity (16.88 ± 1.02 g/g) among the raw materials, whereas cellulose and wheat flour had 4.53 ± 1.62 g/g and 0.84 ± 0.30 g/g WHC, respectively (Table 3.1). When added in low amounts, as in the study (10 % db), the results showed that cellulose fiber could not alter wheat flour-fiber mixtures' overall water holding capacity. In contrast, psyllium increased the water holding capacity of the raw mix due to its high WHC (Table 3.1). There was no difference between fiber added crackers and cellulose added bread with their no fiber added controls. However, psyllium fiber added bread showed a lower WHC than control bread. However, the WHC of psyllium and cellulose fiber added bread samples were lower than the control bread samples. This result could be related to differences in the structure of the bread samples. Fibers could interrupt the formation of gluten-starch matrix. They cause a reduction in the extensibility that will allow gas cells to expand during fermentation. A higher reduction in the expansion caused by psyllium fiber could be due to its high water holding capacity that lowers the free water in the bread dough, which would be used to develop a gluten matrix (Park et al., 1997). The results suggest that (10%) both psyllium and

cellulose fibers could interact with water contained in the doughs and alter the water distribution in the samples. The water-binding capabilities of psyllium imply that it is a strong candidate for competing with starch granules and gluten to interact with the water molecules during cooking (Park et al., 1997). This competition reduces the available water in especially in the bread samples for proteins present in the matrix, leading to an inadequate gluten network (Raymundo et al., 2014). Therefore, this slightly lower WHC could be due to the limited expansion of the final product caused by the added psyllium limiting the gluten network formation and expansion.

Table 3.1 Water holding capacities of raw ingredients and cooked samples.

	WHC (g/g)	Moisture Content (%)
Control Bread	3.40±0.26 ^a	39.17±0.88
Psyllium Bread	2.23±0.07 ^b	39.10±0.93
Cellulose Bread	2.86±0.01 ^b	36.46±2.56
Control Cracker	2.54±0.10 ^a	5.27±0.24
Psyllium Cracker	2.62±0.26 ^a	4.43±0.41
Cellulose Cracker	2.67±0.49 ^a	4.07±0.13
Wheat flour	0.84±0.30	10.20±0.45
Psyllium	16.88±1.02	8.80±0.22
Cellulose	4.53±1.62	6.18±0.23
Wheat flour + Psyllium (10% db)	2.45±0.01	-
Wheat flour + Cellulose (10% db)	0.59±0.03	-

For each analysis, Mean ± SD, results are the average of three replicates. Values in each box in the same column with different lower case letters are significantly ($p < 0.05$) different.

3.2 Differential Scanning Chromatography (DSC)

All bread and cracker dough samples showed transition temperatures close to wheat flour, where slight differences were seen due to differences in the ingredients and the preparation steps (Table 3.2). Fiber inclusion at this concentration to the bread and cracker dough did not create significant differences in transition temperatures of the bread and cracker dough samples compared to controls in general (Table 3.2). Only, cellulose added cracker had a lower onset temperature than the control. Due to the complete gelatinization of bread samples, it was not possible to compare the transition temperatures of the dough samples with the baked ones. Transition temperatures of the baked cracker samples were higher than the cracker dough samples (Table 3.2). Higher gelatinization temperatures after baking were also observed in other studies with various sets of food samples (Hoover et al., 1994, Jacobs and Delcour, 1998, Laguna et al., 2011). This could be due to the more ordered crystal structure created by the moisture-heat treatment, making baked crackers more resistant to gelatinization (Sui et al., 2015).

Obtained results from DSC could also make it possible to comment on the gluten formation within the food matrix since gluten is a complex structure resulting from the interaction between water molecules and wheat flour protein. Along with the water content, water mobility also has crucial importance over the gelatinization process (MacRitchie et al., 1987; Fessas and Schiraldi 2000; Lapčiková et al., 2019). In that respect, a competition between flour proteins and starch granules is also possible. It was observed that control bread dough had a higher onset temperature than both control cracker dough and wheat flour. According to Fessas and Schiraldi (2000), the onset temperature of the gelatinization peak depends on the starch granules' water content, not the food's water content. Since the competition between wheat flour proteins and starch granules would be more robust for bread dough than cracker dough, there might be more free water in the cracker dough matrix than bread dough. Higher Due to an underdeveloped gluten network, a higher amount of free water could have provided a lower onset temperature for cracker dough samples.

Table 3.2 Transition temperatures of the samples

Sample Type	T _o (°C)	T _p (°C)	T _c (°C)
Control Bread	ND	ND	ND
Psyllium Bread	ND	ND	ND
Cellulose Bread	ND	ND	ND
Control Cracker	63.40±0.43 ^a	73.13±0.68 ^a	79.48±0.59 ^a
Psyllium Cracker	62.56±0.72 ^{ab}	72.30±0.32 ^a	79.10±0.25 ^a
Cellulose Cracker	62.08±0.18 ^b	72.38±0.53 ^a	79.41±0.18 ^a
Control Bread Dough	59.12±0.47 ^{aA}	64.47±0.05 ^{aA}	70.37±0.21 ^{abAB}
Psyllium Bread Dough	59.54±0.76 ^a	64.54±0.52 ^a	71.12±0.43 ^a
Cellulose Bread Dough	59.06±0.88 ^a	64.15±0.59 ^a	69.98±0.48 ^b
Control Cracker Dough	57.66±0.37 ^{aB}	64.10±0.41 ^{aAB}	71.09±0.45 ^{abA}
Psyllium Cracker Dough	57.03±1.06 ^a	63.93±0.63 ^a	72.86±1.17 ^a
Cellulose Cracker Dough	57.27±0.23 ^a	64.03±0.17 ^a	71.01±0.11 ^b
Wheat Flour	57.64±0.40 ^B	63.80±0.11 ^B	69.73±0.53 ^B

For each analysis, Mean ± SD, results are the average of three replicates. Values in each box in the same column with different lower case letters are significantly ($p < 0.05$) different. Values in the same column with different capital letters are significantly ($p < 0.05$) different. T_o: Onset temperature, T_p: Peak temperature, T_c: Conclusion temperature.

Gelatinization enthalpy values of the samples were presented in two forms, one as J/g dry sample weight and the other as J / dry wheat flour weight in the sample. This representation made it possible to see the differences caused in the data due to the dilution effect of the added fibers (Table 3.3). The discussions were based only on the sample's J/ dry wheat flour weight results.

Although excess water was supplied for all the , . This might be related to the mobility of the water within the system (Donovan, 1979). . . . These results could indicate the effects of preparation steps and the ingredients in altering the energy used for starch gelatinization. Oil content in the cracker and the fermentation steps in the bread were the significant differences between the cracker and bread samples.

Slowing down the heat transfer within the food matrix is a well-known effect of the oil, which could restrain the starch granules from reaching the temperature needed for the gelatinization (Takeo et al., 1973, Salma et al., 2006). In addition, it is possible that having oil in the formulation might inhibit the water molecules from interacting with starch granules, acting as a barrier. Furthermore, it is known that in the presence of heat and water, the formation of lipid - amylose complex is possible, which can also inhibit starch gelatinization (Ghiasi et al., 1982).

It is observed that fiber addition at this ratio did not cause any change for the gelatinization enthalpies compared to the control doughs for the bread and cracker samples. However, cellulose-added bread dough used more transition energy than psyllium-added bread dough (Table 3.4).

Measured gelatinization enthalpy values after baking the cracker samples (with or without fibers) suggests that there were still ungelatinized starch granules present in the cracker samples. On the contrary, bread crumbs did not show any gelatinization peak, indicating complete gelatinization during baking. Incomplete gelatinization of crackers during baking could be rooted in faster water evaporation due to their small thickness and large surface area. Transition enthalpy used for the cooked cellulose fiber added cracker (2.48 ± 0.07 J/g dry wheat flour) was interestingly lower than the cooked control cracker (3.42 ± 0.06 J/g dry wheat flour) and psyllium added cracker (3.68 ± 0.01 J/g dry wheat flour). The results showed that the cellulose added crackers contained the lowest, and psyllium added crackers had the highest number of ungelatinized starch granules after baking. These results could be because cellulose is an insoluble fiber and does not cause competition for water, which will leave more free water in the matrix for starch granules compared to psyllium fiber. In addition,

since the fibers were included by replacing the wheat flour, not as addition, there was a higher amount of free water for the starch granules in cellulose added samples compared to control.

Table 3.3 Transition enthalpies of the bread and cracker samples (dough and baked).

Sample Type	ΔH (J/g dry sample)	ΔH (J/g dry wheat flour)
Control Bread	ND	ND
Psyllium Bread	ND	ND
Cellulose Bread	ND	ND
Control Cracker	2.90±0.05 ^a	3.42±0.06 ^b
Psyllium Cracker	2.79±0.01 ^a	3.68±0.01 ^a
Cellulose Cracker	1.88±0.06 ^b	2.48±0.07 ^c
Control Bread Dough	4.88±0.26 ^{abB}	5.37±0.29 ^{abB}
Psyllium Bread Dough	3.98±0.11 ^b	4.87±0.13 ^b
Cellulose Bread Dough	5.18±0.47 ^a	6.36±0.58 ^a
Control Cracker Dough	3.30±0.20 ^{aC}	3.91±0.23 ^{aC}
Psyllium Cracker Dough	3.34±0.51 ^a	4.40±0.67 ^a
Cellulose Cracker Dough	3.71±0.22 ^a	4.90±0.29 ^a
Wheat Flour	6.02±0.63 ^A	6.02±0.63 ^A

For each analysis, Mean ± SD, results are the average of three replicates. Values in each box in the same column with different lower case letters are significantly ($p < 0.05$) different. Values in the same column with different capital letters are significantly ($p < 0.05$) different.

3.3 Specific Volume

As given in Figure 3.1 and Table 3.4, the addition of fibers decreased the specific volume of the bread samples as expected, where similar results were observed in previous studies (Pomeranz et al., 1977; Gomez et al., 2003). Fibers could hinder gas

retention and dough expansion during baking by interfering in the formation of the gluten-starch matrix and changing the viscoelastic properties of the dough. high bakingsyllium fiber added Thisshowa higher reduction in the specific volume of psyllium fiber added bread samples than cellulose added ones. DSC data also supported this behavior of the bread dough samples, where cellulose added dough used higher gelatinization energy than the psyllium added bread dough (Table 3.4).

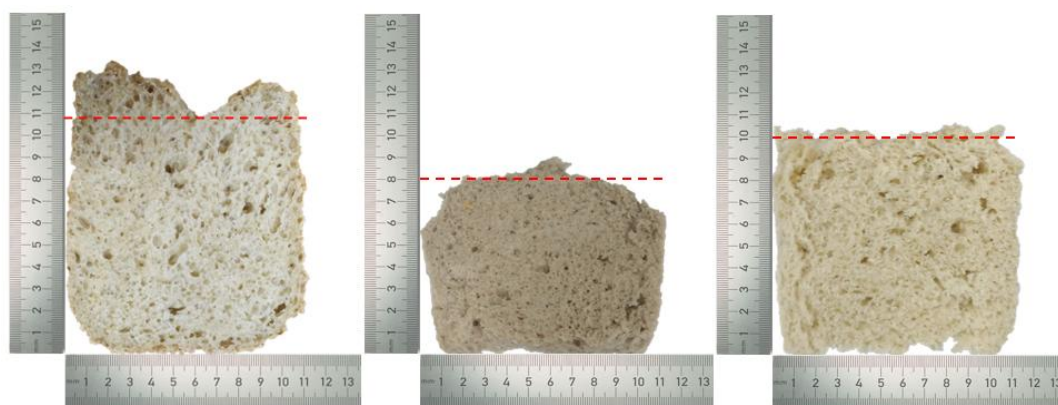


Figure 3.1 Crumb detail: images of finished bread samples. (a) Control bread, (b) psyllium bread, (c) cellulose bread.

Table 3.4 The specific volume of bread samples.

	Specific Volume (cm ³ /100 g)
Control Bread	18.94±0.75 ^a
Psyllium Bread	12.58±1.07 ^c
Cellulose Bread	16.36±1.36 ^b

For each analysis, Mean ± SD, results are the average of six replicates; Values in the same column with different letters are significantly ($p < 0.05$) different.

3.4 Porosity

Control bread had the highest porosity value, while psyllium bread had the lowest porosity determined by the image analysis (Table 3.5, Figure 3.2). Fiber addition adversely affected the bread porosity, whether soluble or insoluble fiber. This result

agrees with specific volumes of the samples (Table 3.4). The gas cells (pores) formed during fermentation by CO₂ and water vapor during baking are responsible for the volume expansion of the bread (Cappa et al., 2013). Psyllium fiber with higher water holding capacity caused lower porosity than cellulose fiber in the bread. By holding the water molecules, psyllium fiber could reduce the amount of available water for the wheat flour proteins (glutenin and gliadin) to interact with. Therefore, proteins could not obtain enough water to form a fully developed gluten network (Brennan & Cleary, 2007). Thus, they cannot supply a strong dough structure, and the dough's gas retention capability weakens and results in a lower crumb porosity.

Similarly, cellulose fiber also created a more minor yet significant decrease in the porosity of the bread crumbs, possibly due to the weakening of the gluten network (Pomeranz et al., 1977). The presence of the cellulose fiber might lead to disruption of the gluten network (Chen et al, 1988). A weakened dough structure might have caused early ruptures of the gas cells (Anil, 2007).

Table 3.5 The porosity of bread samples

	Porosity (%)
Control Bread	47.38±4.56 ^a
Psyllium Bread	18.04±1.91 ^c
Cellulose Bread	34.50±3.32 ^b

For each analysis, Mean ± SD, results are the average of six replicates; Values in the same column with different letters are significantly ($p < 0.05$) different.

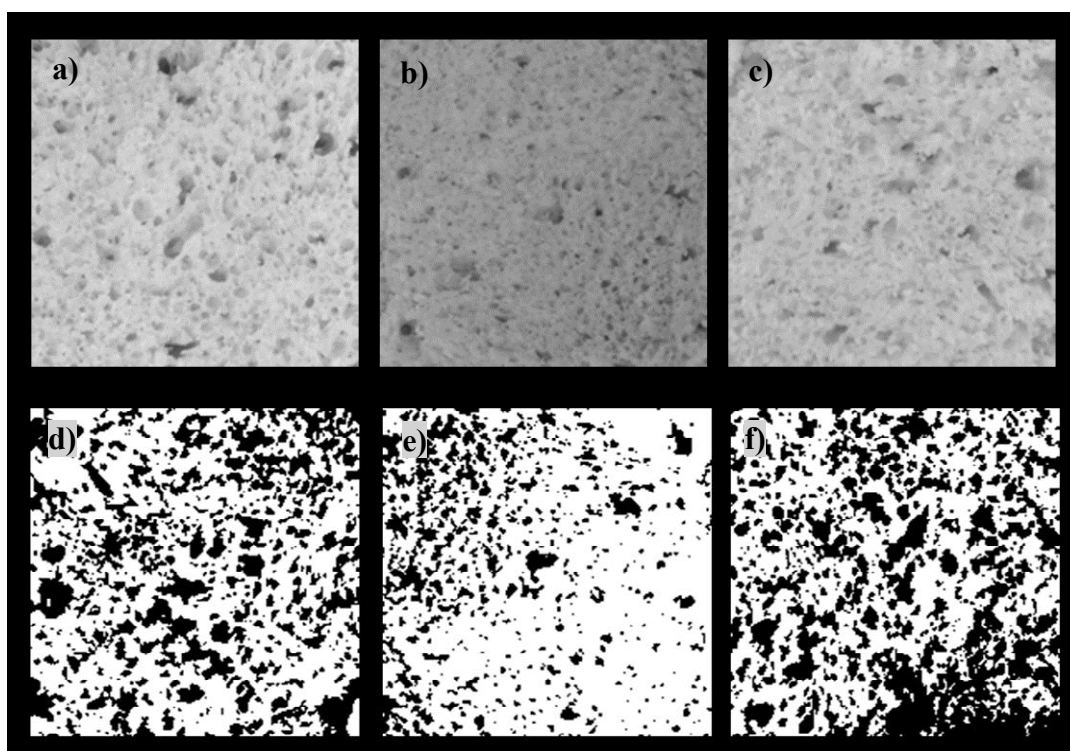


Figure 3.2 Neutralized images of bread crumbs; (a) control bread, (b) psyllium bread, (c) cellulose bread and images adjusted with threshold feature of ImageJ software; (a) control bread, (b) psyllium bread, (c) cellulose bread.

3.5 Color

The inclusion of the psyllium and cellulose fibers reduced the L^* values, where psyllium added bread crumbs had the lowest L^* value (Table 3.6). Psyllium fiber gave a darker color to bread crumbs because of its dark brown color (Park et al., 1997). Similarly, psyllium added bread crumbs had higher a^* and lowered b^* values than control due to the psyllium fiber's color tonality. Fradinho et al., 2019 reported that psyllium had a low L^* value (59.39 ± 0.91) and high a^* , b^* values (6.06 ± 0.18 , 24.22 ± 0.37 respectively). Cellulose bread crumbs did not show a significant difference compared to control bread for both a^* and b^* values.

Table 3.6 Crumb color of the bread samples.

	L*	a*	b*
Control Bread	78.61±1.36 ^a	1.80±0.09 ^b	19.31±0.51 ^a
Psyllium Bread	65.52±0.59 ^c	4.58±0.07 ^a	13.29±0.18 ^b
Cellulose Bread	72.78±1.28 ^b	2.11±0.38 ^b	20.15±1.21 ^a

For each analysis, Mean ± SD, results are the average of three replicates; Values in the same column with different letters are significantly (p < 0.05) different.

Psyllium fiber added crackers had a lower value of L*, meaning they had a darker color than the control cracker (Table 3.7). Lower L* values were also reported by Qaisrani et al. (2012) due to psyllium fiber addition to cookies. Cellulose fiber added crackers were not significantly different from the control cracker samples in terms of lightness (L*). Cellulose added crackers had a lower redness than the control and psyllium added crackers. Control cracker had the highest b* value whereas psyllium fiber added cracker had the lowest b* value.

Table 3.7 The surface color of the crackers.

	L*	a*	b*
Control Cracker	76.40±1.51 ^a	6.02±0.50 ^a	29.09±0.40 ^a
Psyllium Cracker	62.74±0.83 ^b	7.24±0.50 ^a	21.25±1.05 ^c
Cellulose Cracker	78.44±0.53 ^a	4.33±0.06 ^b	26.66±0.39 ^b

For each analysis, Mean ± SD, results are the average of three replicates; Values in the same column with different letters are significantly (p < 0.05) different.



Figure 3.3 The physical appearance of baked cracker samples. (a) Control cracker, (b) Psyllium cracker, (c) Cellulose cracker.

3.6 Texture Profile Analysis

The bread samples' hardness, cohesiveness, and chewiness were significantly affected by the inclusion of psyllium and cellulose fibers, while springiness was only affected by psyllium fiber addition. Hardness is the peak force applied to the sample during the first compression. The hardness of the fiber added samples were significantly higher than the hardness of the control bread. The results correlated well with the specific volume and porosity data. Low porosity and hence low specific volume made the bread crumbs more stiff and hard to compress, resulting in an increased hardness value. Rossell et al. (2001) suggest that the increased crumb firmness could be related to the gas cells' inability to expand due to the thickening of the walls. Lauková et al. (2017) observed a similar effect while investigating the effects of powdered cellulose on bread quality using different fiber lengths, which reports a significant increase in the hardness of the bread substituted with powdered cellulose (5%) regardless of the fiber length. In another study, although the fiber used in bread formulation integrated well within the gluten network, as in the case of powdered cellulose, gluten was still diluted by the fibers, and its gas retention ability was lowered (Morris and Morris, 2012). Furthermore, there are other studies reporting increased crumb hardness that point to a correlation between hardness and other quality parameters of the bread, such as the porosity and loaf volume (Foshcia et al., 2013; Tang et al., 2021). Psyllium having the lowest porosity and loaf volume value supports its highest crumb hardness value.

Psyllium bread had the highest cohesiveness and chewiness values among the crumb samples. The chewiness is a parameter strongly dependent on the hardness of the material. On the other hand, the cohesiveness obtained by the second deformation of the sample represents the extent of deformation that can be made on the materials until it ruptures. As implied by the term, it reflects the strength of the cohesion forces within the material. Psyllium fiber addition had significantly increased the bread's cohesiveness. Thus, bolus formation can be easier than the control and cellulose bread with the forces applied during mastication (Onyango et al., 2010).

Similarly, the addition of cellulose fiber demonstrates a significant increase in cohesiveness which agrees with the findings of Lauková et al. (2017). They investigated the relationship between cellulose fiber lengths and textural properties and reported higher cohesiveness values, especially when cellulose with shorter fiber length was incorporated in bread rolls. Cellulose powder used in this study includes mostly short fiber lengths, 32 μm (60%), 100 μm (20%), indicating cohesiveness.

The springiness of the bread crumb demonstrates the extent of the ability of a material spring back. In other words, it represents the material's elasticity (Onyango et al., 2010). Lower porosity and specific volume values leading to thicker cell walls correlate with the decreased elasticity for the crumbs. Replacement of wheat flour with psyllium fiber significantly decreased the springiness of the bread. Development of the gluten network and the gelatinization of the starch granules, along with the interaction between them, is thought to be the dominant mechanism behind the elastic texture of the bread crumb (Hoseney et al., 1994; Feili et al., 2013). As a soluble dietary fiber, psyllium may interfere with the molecular bonding of the gluten proteins, causing an underdeveloped protein network (Abdullah et al., 2021; Packkia-Doss et al., 2019). The springiness value of the cellulose and control bread was not significantly different. With respect to this, water solubility difference between two fiber could be the dominant mechanism. There may be more free water for the gluten network formation in the cellulose bread since the interaction between water molecules and cellulose fibers is much weaker than the psyllium fiber. Effects of cellulose fiber on the reduction porosity and specific volume were not as much as psyllium fiber. Therefore, the effect of cellulose was lower than psyllium fiber on the textural properties of the bread crumbs.

Table 3.8 Textural characteristics of bread crumbs

Sample Type	Hardness (g)	Cohesiveness	Springiness (mm)	Chewiness (g.cm)
Control Bread	264.33±21.39 ^c	0.77±0.01 ^c	10.37±0.47 ^a	198.33±9.02 ^c
Psyllium Bread	550.80±25.27 ^a	0.84±0.02 ^a	9.31±0.08 ^b	447.86±39.52 ^a
Cellulose Bread	468.33±36.07 ^b	0.81±0.02 ^b	10.17±0.23 ^a	360.50±3.54 ^b

For each analysis, Mean ± SD, results are the average of six replicates; Values in the same column with different letters are significantly ($p < 0.05$) different.

As shown in Table 3.9, the inclusion of the dietary fibers in cracker formulation decreased the hardness of the final products. Similar to the mechanisms explained earlier for fiber added bread samples, the interruption effect of the gluten fibers might be another cause for a more brittle structure. Furthermore, psyllium cracker has the lowest hardness value among all samples. This result correlates with the findings of Ozgoren et al. (2019), who have used Jerusalem artichoke powder (JAP), including inulin which is also a water-soluble fiber source, to increase the fiber content of crackers. Psyllium, as an ingredient, might inhibit the intertwining of the proteins to form a network properly by wrapping the starch granules within the cracker dough (Jia et al., 2020).

Table 3.9 The hardness value of cracker samples

	Hardness (kg)
Control Cracker	3.34±0.26 ^a
Psyllium Cracker	1.25±0.09 ^c
Cellulose Cracker	2.55±0.18 ^b

For each analysis, Mean ± SD, results are the average of six replicates; Values in the same column with different letters are significantly ($p < 0.05$) different.

Degree of the gelatinization is also another factor that is directly proportional with cohesive forces within the dough. According to data (Table 3.3), the crackers cooked with cellulose fiber used a lower energy compared to control and psyllium fiber added crackers for the gelatinization of remaining uncooked starch. However,

psyllium added crackers had the lowest and cellulose added crackers had a lower hardness value compared the control crackers. Thus, it is essential to determine This conflict can be explained by determining which mechanism is the dominant one on the hardness of the crackers. Sozer et al. (2014) demonstrated the effects of wheat bran, which includes insoluble fibers with different particle sizes, on the textural properties of biscuits. The results of that study suggested that the structural factors, like the particle size of the fiber located within the food matrix, are more effective than the status of the starch granules on the hardness of the biscuits based on observing higher hardness values with incorporation with fine particles (68 μm) than the course ones (450 μm) while obtaining a higher degree of gelatinization when finer particles were included. Thus, cellulose having a smaller fiber length, and water insoluble nature provided cracker with cellulose fiber a more stiff structure than psyllium fiber.

3.7 Scanning Electron Microscopy (SEM)

As shown in Figure 3.4, wheat starch (Figure 3.4 a) had a round, elliptical shape with various granule sizes, while cellulose fiber (Figure 3.4 c) had a tubular shape with different fiber lengths. Psyllium fiber (Figure 3.4 b) had an irregular and stacked structure with bigger particle sizes than cellulose fiber. Psyllium's rough surface, as demonstrated in Figure 3.5., could be an improver for the water holding capacity with some capillary effect (Mariotti et al., 2009).

Individual starch granules with all different granule sizes could be observed within the bread dough samples (Figure 3.6). Control bread dough (Figure 3.6 a and b) provided a homogenous structure where only smaller starch granules were visible. Clustered granules on the smooth, gel-like surfaces were observed in different locations of the psyllium fiber bread dough (Figure 3.6 c and d), implying a non-homogenous dough structure. On the contrary, cellulose fiber bread dough (Figure 3.6 e and f) reflects a more homogenous structure, although cellulose fiber disrupted

the continuity of the dough at some places. Additionally, the gluten network was also visible in the SEM images of the bread dough samples.

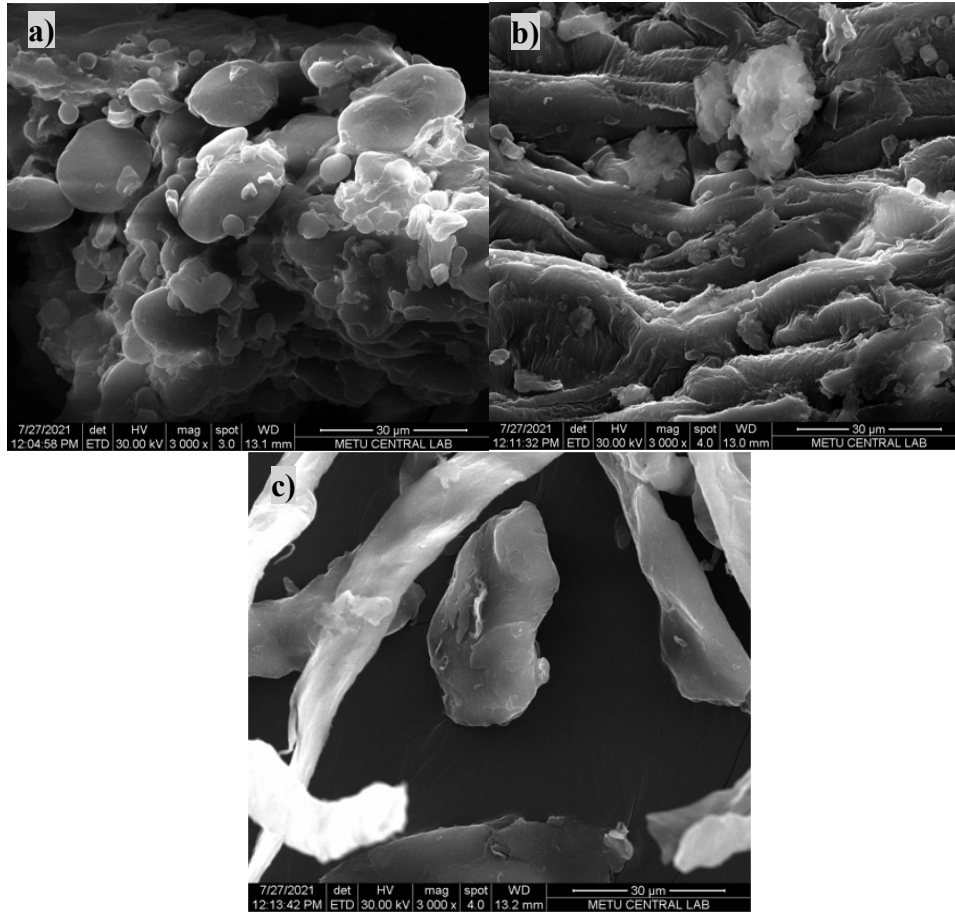


Figure 3.4 SEM images of wheat flour (a), psyllium fiber (b), cellulose fiber (c).

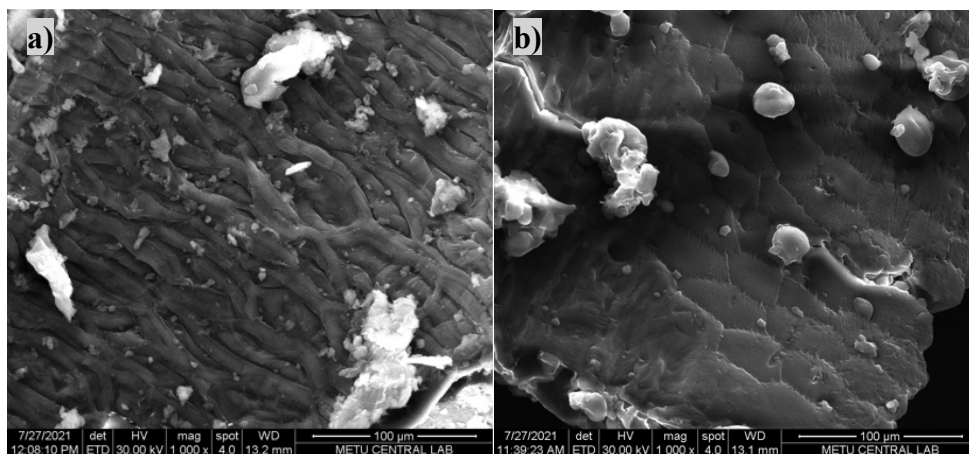


Figure 3.5 SEM images of (a) psyllium fiber (1000x) and (b) psyllium fiber in baked psyllium cracker (1000x).

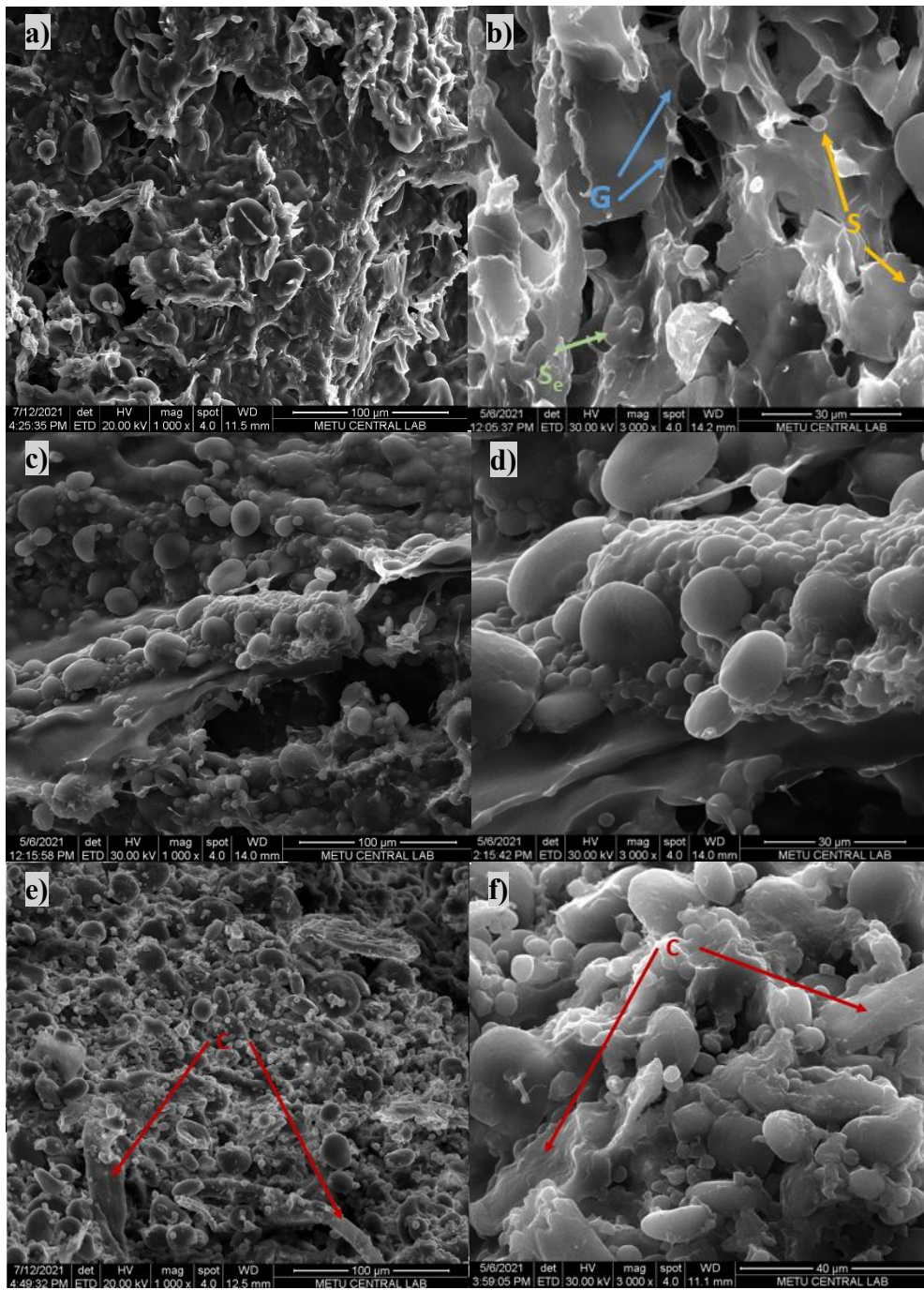


Figure 3.6 SEM images of the control bread dough 1000x and 3000x magnitudes (a and b), psyllium bread dough 1000x and 3000x magnitudes (c and d), cellulose bread dough 1000x and 3000x magnitudes (e and f). S: Starch granule, S_e: Starch granule embedded in the bread, G: Gluten Network, C: Cellulose fiber.

Cracker dough samples represented a more stacked structure (a weak gluten network) rather than a developed one as in the bread dough samples (Figure 3.7). The DSC results (Table 3.2) also supported the underdeveloped gluten network of cracker dough. Cracker dough showed a lower onset temperature than bread dough, hinting at the number of free water molecules for the starch granules. Additionally, individual cellulose fibers were visible in the cracker dough, but it was impossible to differentiate the individual psyllium fibers.

In Figure 3.8., Cross-sections of the bread crumbs' images were given. Even though the DSC analysis of the bread crumbs did not show any endothermic peak, a few ungelatinized starch granules could be observed in all the crumb samples (Figure 3.8). For the control bread crumb, homogenous gas cell distribution can be seen along with only the most minuscule granules of the wheat starch (Figure 3.8 a and b). However, psyllium bread crumbs included larger starch granules that can be observed either as embedded in the complex structure of a gas cell surface or as an individual matter (Figure 3.8 c and d). Along with this observation, the smooth, continuous structure on gas cell surfaces might be related to the gelling capacity of the psyllium.

SEM images of the cracker samples (Figure 3.9) showed a more crumbled and discontinuous structure compared to bread crumbs (Figure 3.8) as expected due to the lack of a genuine gluten network. The overall design of the cracker samples was more stacked than being ordered. On the other hand, unlike the dough state, it was possible to observe visible individual psyllium fiber within the psyllium cracker after baking (Figure 3.9 c). Cellulose fibers were also visibly present in the baked cracker sample (Figure 3.9 e).

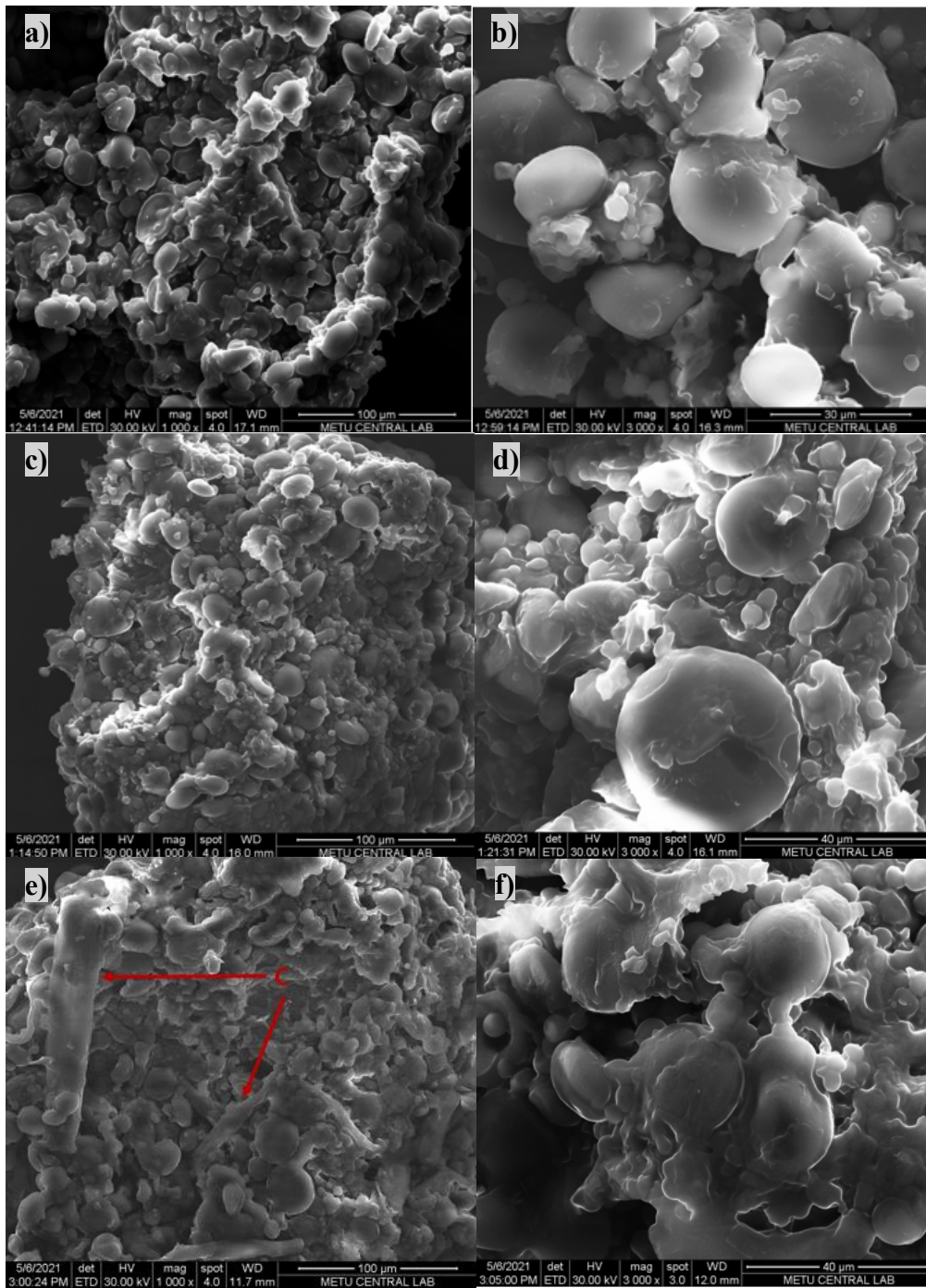


Figure 3.7 SEM images of the control cracker dough 1000x and 3000x magnitudes (a and b), psyllium cracker dough 1000x and 3000x magnitudes (c and d), cellulose cracker dough 1000x and 3000x magnitudes (e and f). C: Cellulose fiber.

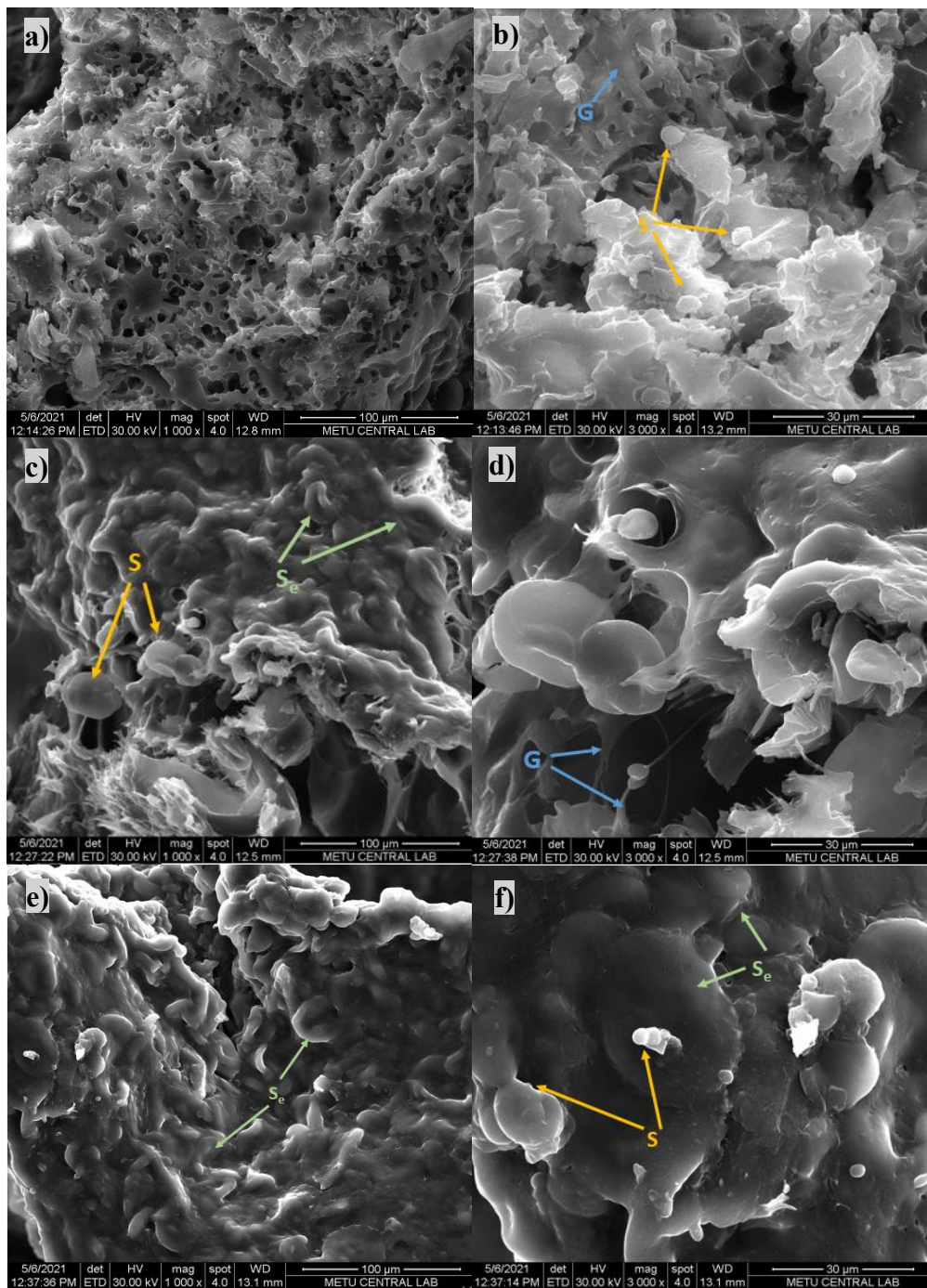


Figure 3.8 SEM images of the control bread crumb 1000x and 3000x magnitudes (a and b), psyllium bread crumb 1000x and 3000x magnitudes (c and d), cellulose bread crumb 1000x and 3000x magnitudes (e and f). S: Starch granule, S_e: Starch granule embedded in the bread, G: Gluten network.

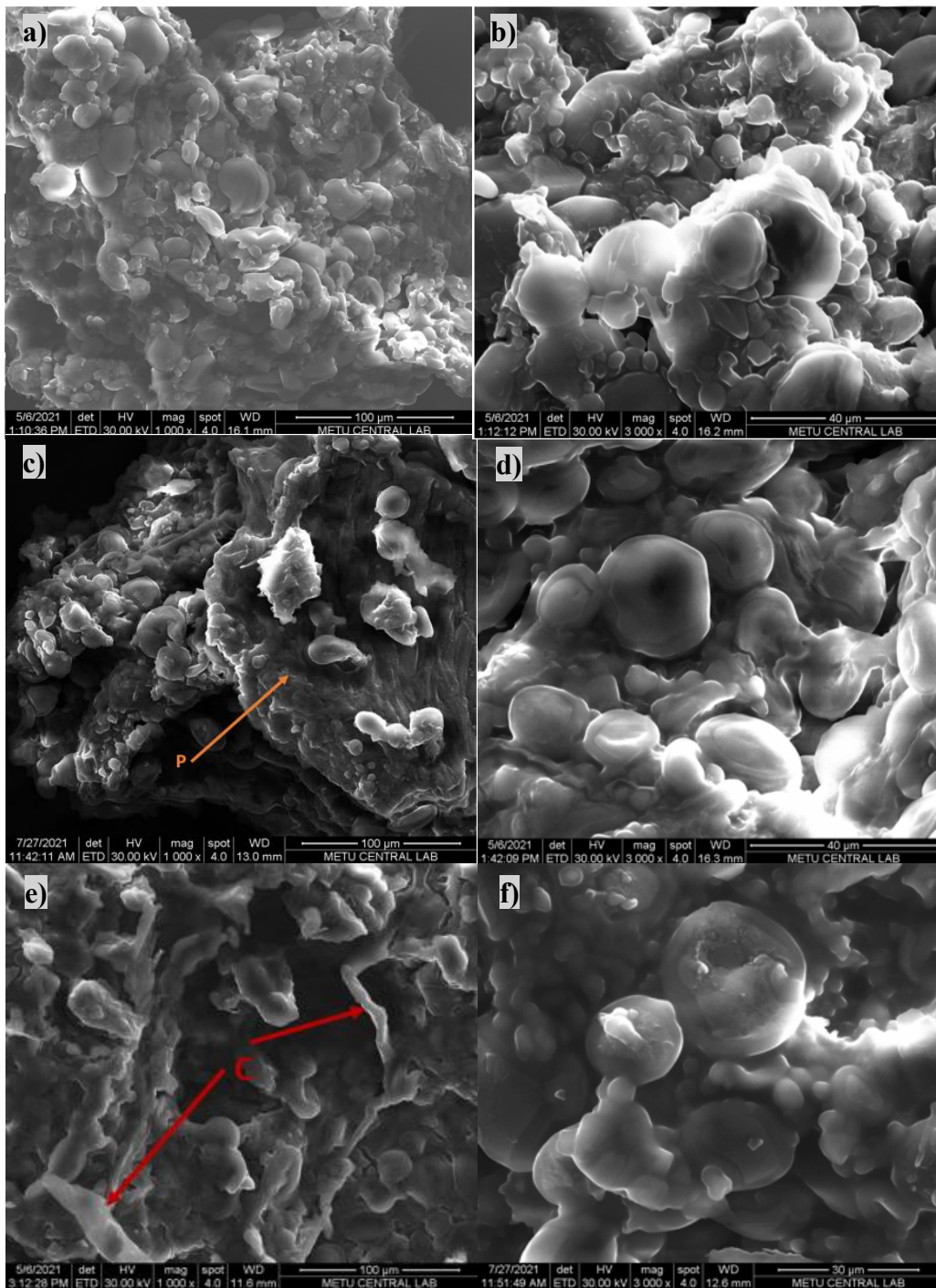


Figure 3.9 SEM images of the control cracker 1000x and 3000x magnitudes (a and b), psyllium cracker 1000x and 3000x magnitudes (c and d), cellulose cracker 1000x and 3000x magnitudes (e and f). C: Cellulose fiber, P: Psyllium.

SEM of digested samples

After 20 min of starch digestion (G20), images of raw wheat flour showed clear enzyme entrances on the starch granule surfaces (Figure 3.10). Similar enzyme entrances on the surface of the granules that did not disperse in the bread crumb and cracker samples were also observed after 20 min of digestion (Figure 3.11 and 3.12).

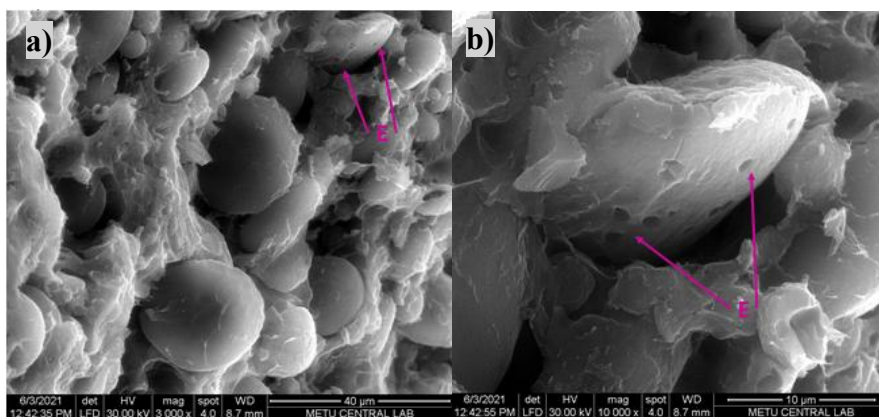


Figure 3.10 SEM images after *in vitro* digestion of the wheat flour 3000x and 10000x magnitudes (a and b). E: Enzyme entrance.

Control and cellulose bread samples after *in vitro* digestion (G20) included only smaller starch granules, while larger starch granules were visible in psyllium bread samples (Figure 3.11). This could indicate that, unlike cellulose, psyllium could be a barrier for the digestion enzyme to break up the large starch granules. Images of the digested cellulose bread showed cellulose fiber was visible, unlike the undigested dough and baked forms. This could represent the success of the protein digestion step applied at the beginning of the *in vitro* digestion. However, a continuous network structure was still visible for the psyllium fiber added crumbs, implying that psyllium fiber could also inhibit protein digestion.

Compared to bread samples, cracker samples included numerous clusters of starch granules even after 20 min of *in vitro* digestion (Figure 3.12). When the images of digested cellulose cracker (Figure 3.12. (f)) and cellulose bread (Figure 3.11 (f)) were investigated together, the cellulose fibers were still embedded within the cracker matrix while becoming loose in the bread matrix after digestion.

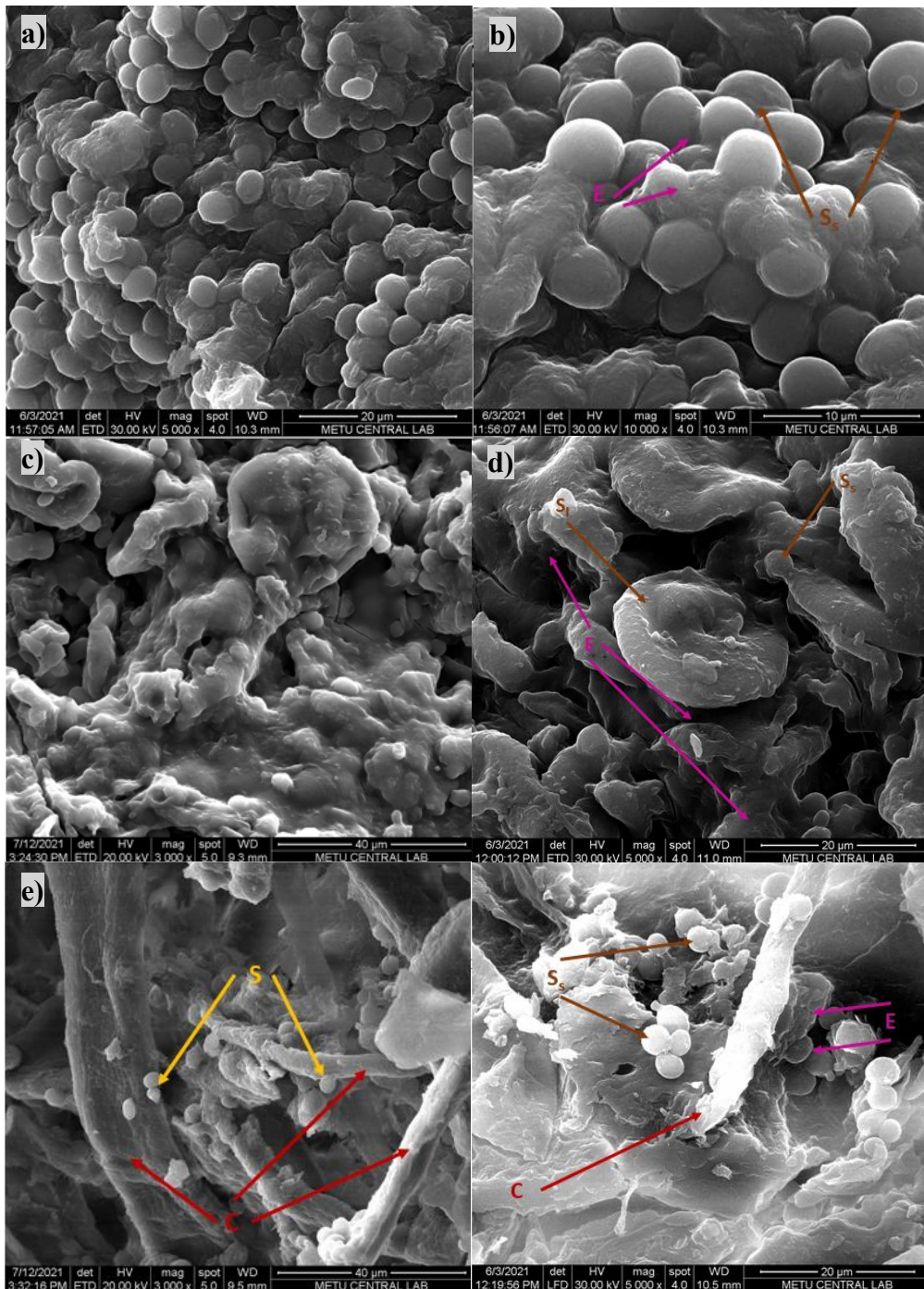


Figure 3.11 SEM images after in vitro digestion of the control bread crumb 5000x and 10000x magnitudes (a and b), psyllium bread crumb 3000x and 5000x magnitudes (c and d), cellulose bread crumb 3000x and 5000x magnitudes (e and f). S: Starch granule, S_s: Small starch granule S_l: Large starch granule, C: Cellulose fiber, E: Enzyme entrance.

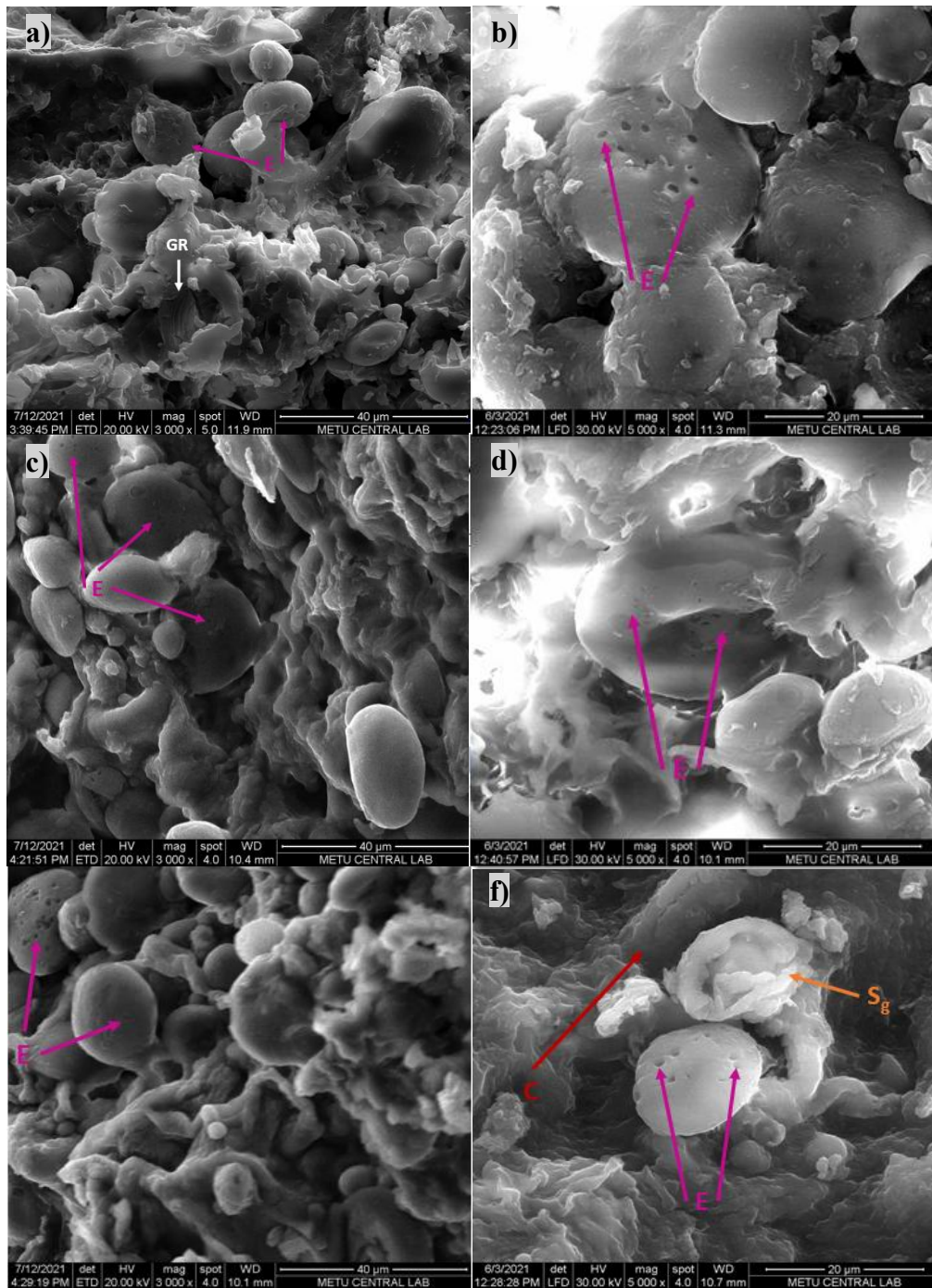


Figure 3.12 SEM images after in vitro digestion of the control cracker 3000x and 5000x magnitudes (a and b), psyllium cracker 3000x and 5000x magnitudes (c and d), cellulose cracker 3000x and 5000x magnitudes (e and f). C: Cellulose fiber, E: Enzyme entrance, S_g: Gelatinized starch, GR: Growth rings.

3.8 *In vitro* Digestion Analysis

Rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) are the starch fractions that can be digested by the human intestine or fermented by the microflora of the large intestine (Englyst et al., 1992). These fractions could be used to demonstrate the extent of starch digestion in different starchy foods in the small intestine (Englyst et al., 2000).

In order to monitor how the dilution of the added fibers on the amount of starch in the product affects the results, Starch digestion fractions were represented in two different forms. In one form, the percentages of the digested starch weight were given as “starch fraction % perdry sample weight (DS)”. In the second form, data was normalized, and they were given as “starch fraction % pertotal starch content of the dry sample weight-measured (TSS)(Table 3.10).

were higher when they were presented as % in TSS, because only 82 % of wheat flour was starch on a dry basis. For clarity, discussions of the results were based on the normalized data (ie., starch fraction % in TSS). RDS fraction (RDS/TSS %) was lower, and SDS fraction (SDS/TSS %) was higher than the control for the psyllium fiber added bread crumbs. Whereas for cellulose fiber added samples, neither fraction was statistically different from the control bread crumbs (Table 3.10). RS fractions (RS/TSS %) were affected by neither psyllium nor cellulose fiber addition. The effect of psyllium fiber on RDS and SDS fractions of bread crumbs could be related to psyllium fiber’s high water holding capacity. The presence of the psyllium would create a competitive environment for the starch granules and could inhibit the swelling of the granules, which would lead to a lower degree of gelatinization (Cappa et al., 2013). However, as seen from the DSC data, all bread crumb samples were completely gelatinized, including the psyllium added samples. Thus, it is possible that the degree of gelatinization was not a significant factor in differing the digestion of crumbs. Psyllium also has a high gelling capacity, resulting in increased viscosity of the solutions. This increased viscosity of the digestion medium could adversely affect the enzymes’ mobility. Thus, the reduced digestion rate could be due to

hindered interaction of the digestion enzyme with the starch in the bread crumb during digestion.

The effect of high viscosity caused by the psyllium fiber could also be seen by TS/TSS data (Table 3.10). Measured total starch (TS) contents were always lower than the calculated starch (TSS) contents in the samples. However, the ratio (TS/TSS) was significantly lower for the psyllium added samples than the control, showing the effect of psyllium on the measured value despite all aggressive steps involved.

Psyllium fiber addition reduced the RDS/TSS % for the cracker samples, but did not cause any significant change for the SDS and RS fractions compared to control. Cellulose fiber addition did not cause any change in the RDS fraction but reduced the SDS fraction, where RS fraction was only higher than the psyllium fiber added crackers (Table 3.10). The effect of psyllium on reducing RDS of the crackers could be due to the high viscosity of the digestion medium caused by psyllium. When the degree of gelatinization values was compared, no statistically significant difference was observed between the control and psyllium fiber added crackers samples (data not shown). On the other hand, cellulose fiber added cracker samples showed a higher degree of gelatinization compared to control and psyllium fiber added samples. The higher degree of gelatinization could explain having higher RDS/TSS %, and lower SDS/TSS % for the cellulose added cracker samples compared to psyllium added crackers.

Textural properties could also create physical barriers and affect the digestibility rate. As mentioned before (Section 3.2.4), psyllium and cellulose fiber added bread crumbs had a higher cohesiveness than the control. Fiber-added samples could form a bolus structure more quickly than the control during mastication due to stronger cohesion forces within the food structure. Since all samples were minced prior to the digestion analysis to simulate the chewing action, digestion started with more stiff samples for the fiber added crumbs, where the psyllium added crumbs had the stiffest samples. As a result, the digestion process could also be hindered to some extent.

When we compared the control samples for bread crumbs and crackers, we saw that bread crumbs had a higher RDS/TSS % and lower SDS/TSS % than cracker samples (Table 3.10). There is a correlation between the degree of gelatinization and the extent of the starch digestion since gelatinized starch becomes more susceptible to the digestion enzymes (Wang & Copeland, 2013). Having a higher digestibility for the bread crumbs showed the effects of processing, where cracker samples had some ungelatinized fractions. SEM images of the digested samples also supported a higher digestion rate for the crumbs. In one example, the cellulose fibers were still embedded within the cracker matrix while they were becoming loose in cellulose fiber (Figure 3.11. f and Figure 3.12. f). Observing individual cellulose fibers was not possible for digested the cracker samples, showing a lower disassociation of the matrix.

Table 3.10 Carbohydrate digestibility parameters

	RDS/DS %	RDS/TSS %	SDS/DS %	SDS/TSS	RS/DS %	RS/TSS	TS/TSS(g)
Control Bread	43.27±1.93 ^{aA}	59.30±2.64 ^{aA}	7.12±1.68 ^{bB}	9.75±2.30 ^{bB}	10.60±2.24 ^{aA}	14.52±3.07 ^{aA}	85.18±0.02 ^a
Psyllium Bread	30.77±2.08 ^b	42.14±2.84 ^b	12.57±1.81 ^a	17.22±2.48 ^a	11.42±1.89 ^a	15.64±2.59 ^a	74.10±0.05 ^b
Cellulose Bread	40.29±2.23 ^a	55.15±3.08 ^a	4.22±1.28 ^b	5.78±1.75 ^b	8.66±0.48 ^a	11.86±0.65 ^a	0.77±0.04 ^{ab}
Control Cracker	39.60±0.96 ^{ab}	52.55±0.53 ^{ab}	13.67±3.02 ^{aA}	18.73±4.14 ^{aA}	10.64±0.36 ^{abA}	14.57±0.49 ^{abA}	0.87±0.03 ^a
Psyllium Cracker	31.15±2.14 ^b	41.96±2.71 ^b	16.18±1.17 ^a	22.17±1.60 ^a	6.90±3.61 ^b	9.45±4.95 ^b	0.75±0.02 ^b
Cellulose Cracker	37.01±1.84 ^a	49.23±2.19 ^a	3.84±1.42 ^b	5.26±1.94 ^b	13.42±2.52 ^a	18.38±3.46 ^a	0.76±0.06 ^{ab}
Wheat Flour	27.25±1.82	30.42±1.90	28.20±2.37	32.68±2.75	17.51±3.24	20.29±3.76	0.86±0.02

For each analysis, Mean ± SD, results are the average of three replicates. Values in each box in the same column with different lower case letters are significantly (p < 0.05) different. Values in the same column with different capital letters are significantly (p < 0.05) different. RDS, rapidly digestible starch; SDS, slowly available starch; RS, resistant starch; TS, total starch.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this study, the advantage of evaluating two different starchy foods with two different types of dietary fibers was used to compare their *in vitro* digestibility values with each other. Psyllium fiber was an effective ingredient at the studied concentration to slow down the bread and cracker's digestion rate. However, cellulose fiber was only affected the cracker samples. The high water-holding capacity of the psyllium was the significant factor affecting the starch digestibility. Psyllium fiber reduced starch digestion primarily by hindering the mobility of the enzymes within the digestion medium. Results suggested processing methods, ingredients, and physical properties of the products could affect starch digestion.

Introducing updated versions of commonly consumed food products with added health aspects holds a high potential for the food industry. Future studies to understand the interaction between fiber, water, and starch should be combined with those that focus on improving the quality and sensory characteristics of the final products to strengthen the outcome.

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APPENDICES

A. DSC Thermograms of the samples

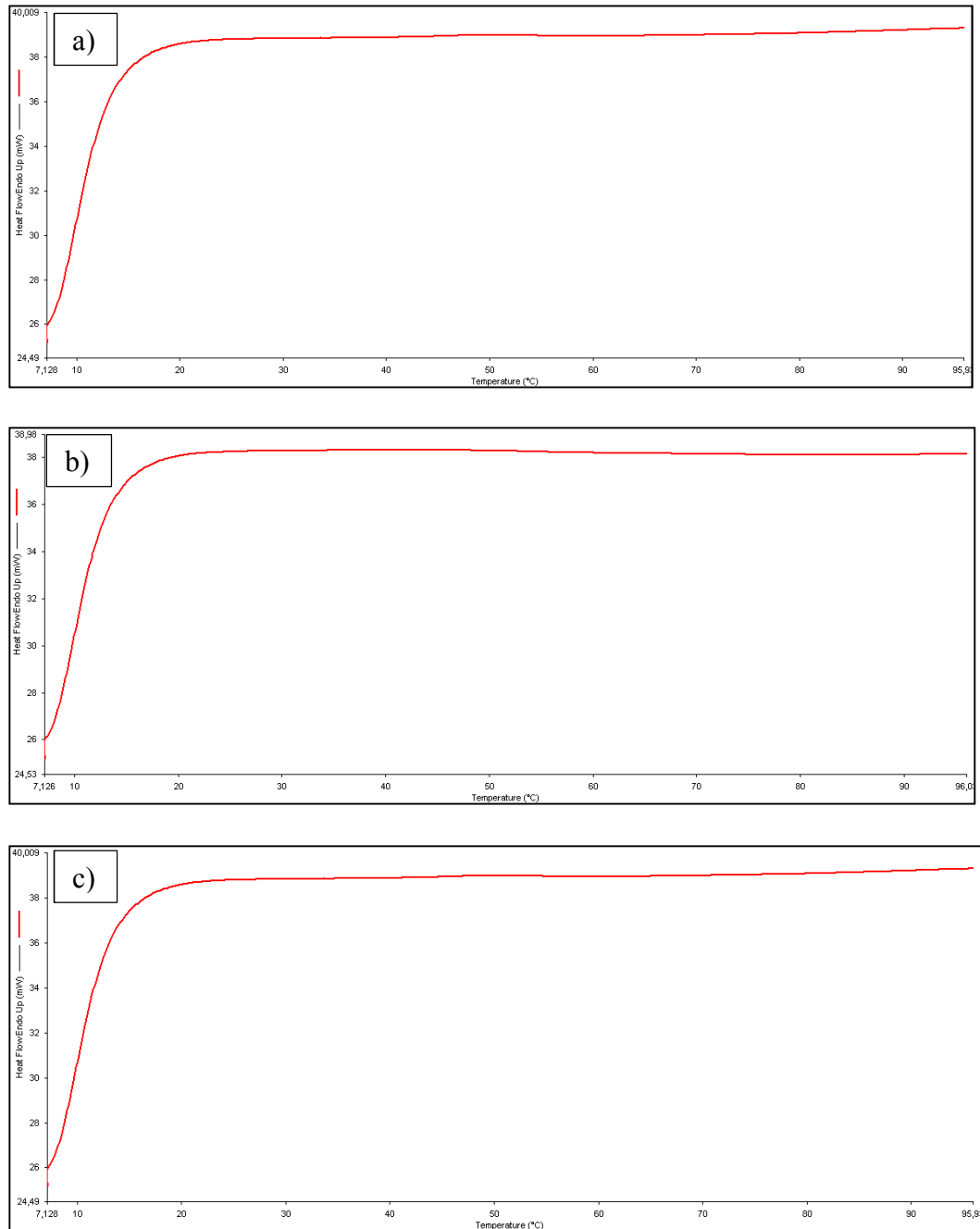


Figure A.1 DSC thermograms of the bread samples; a) control bread, b) psyllium bread, c) cellulose bread.

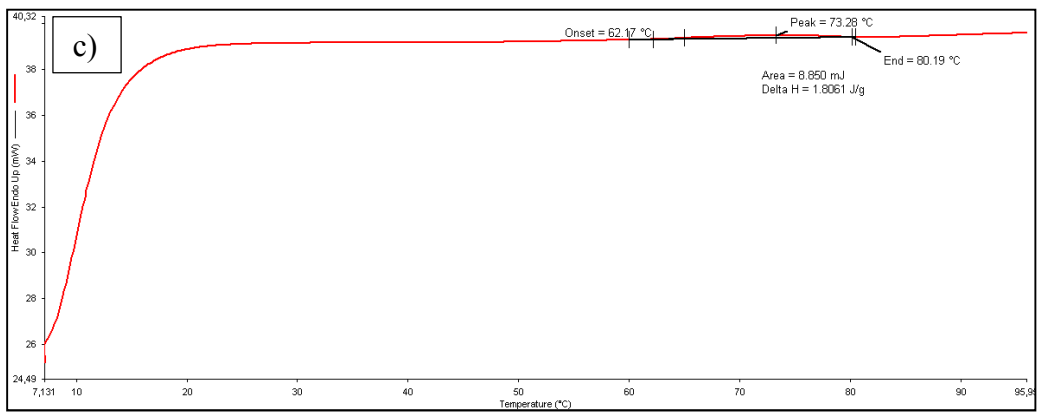
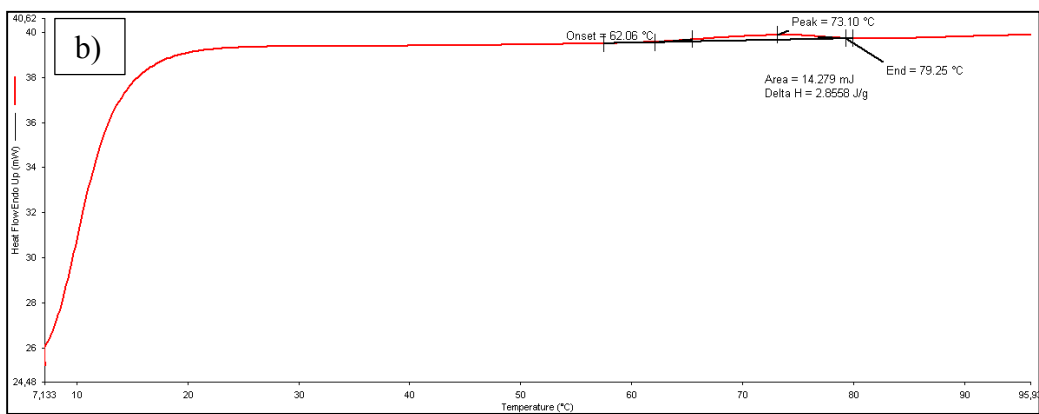
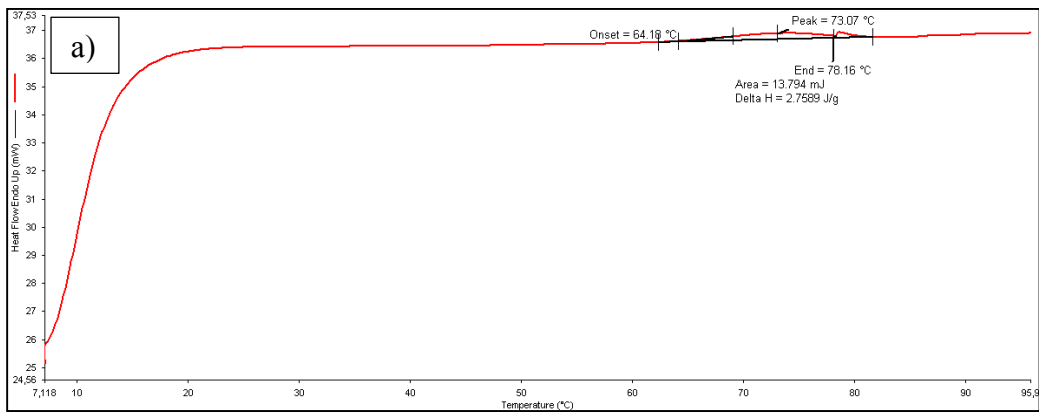


Figure A.2 DSC thermograms of the cracker samples; a) control cracker, b) psyllium cracker, c) cellulose cracker.

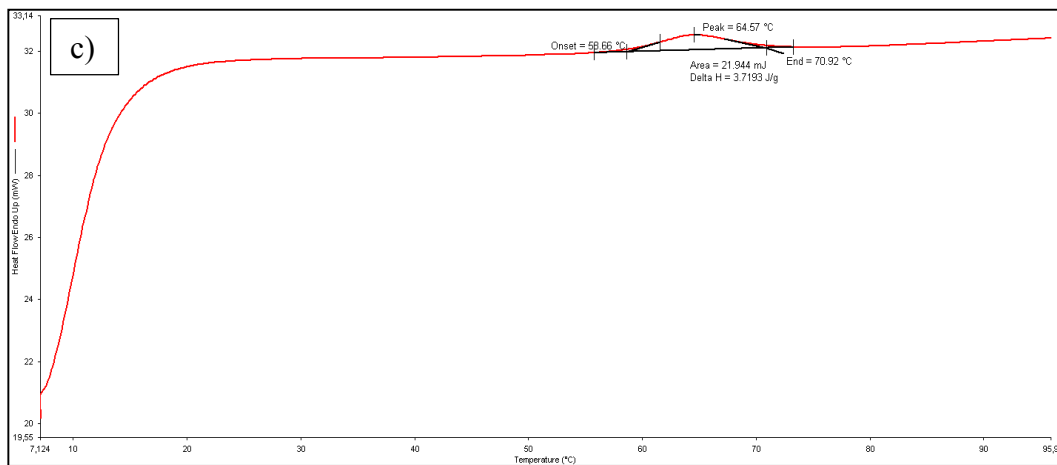
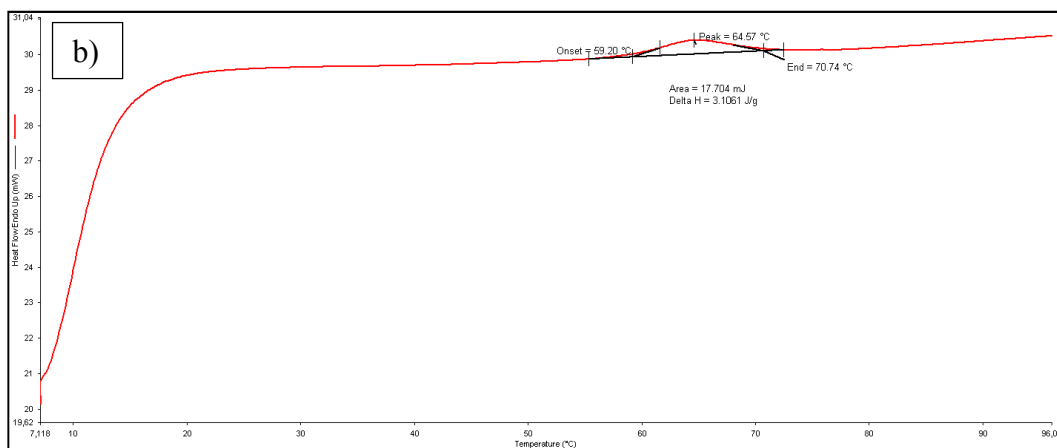
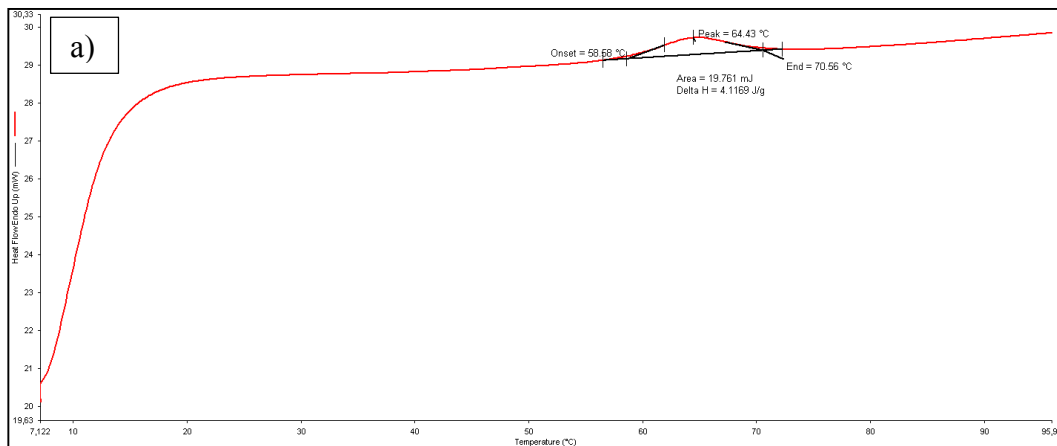


Figure A.3 DSC thermograms of the bread dough samples; a) control bread dough, b) psyllium bread dough, c) cellulose bread dough.

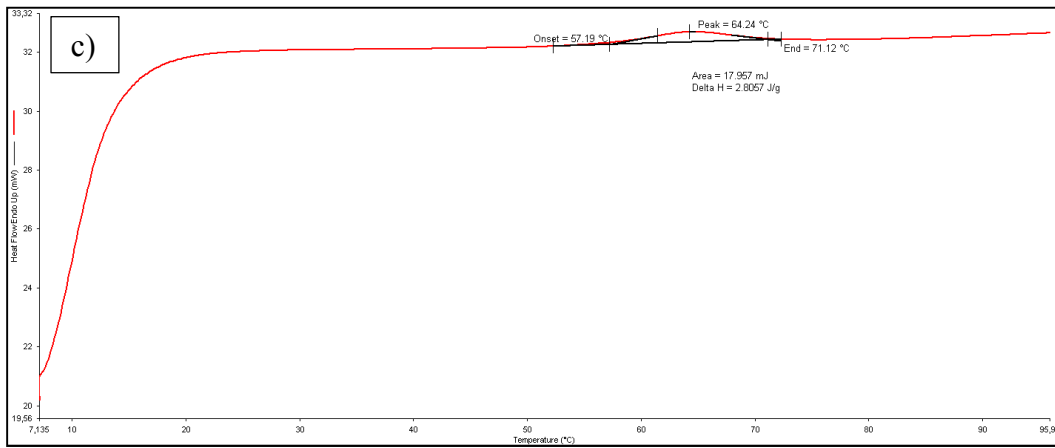
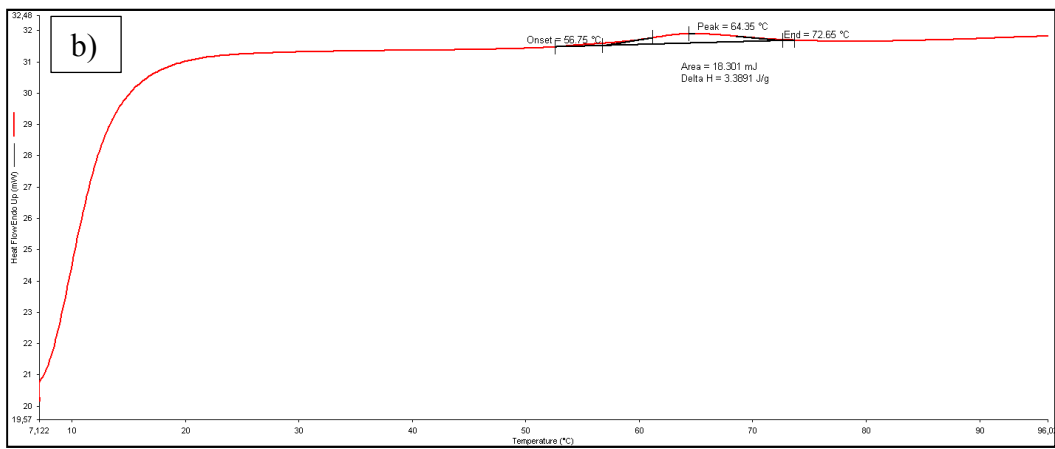
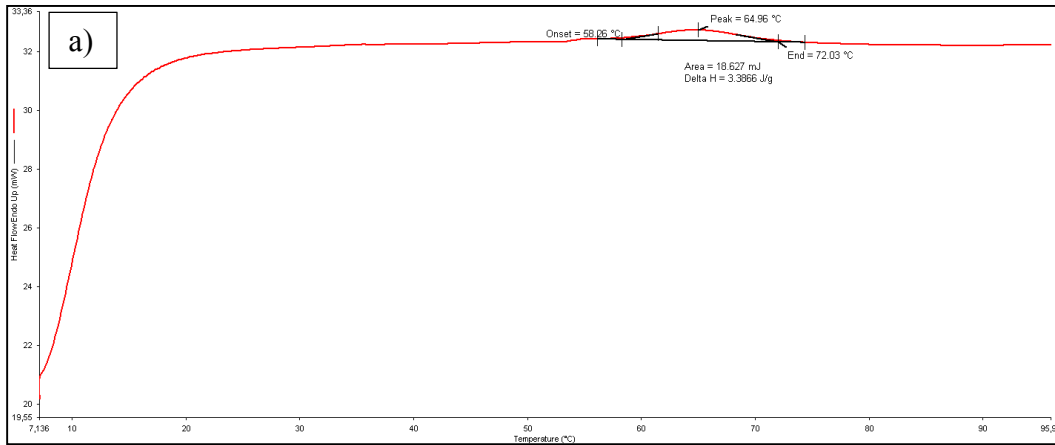


Figure A.4 DSC thermograms of the cracker dough samples; a) control cracker dough, b) psyllium cracker dough, c) cellulose cracker dough.

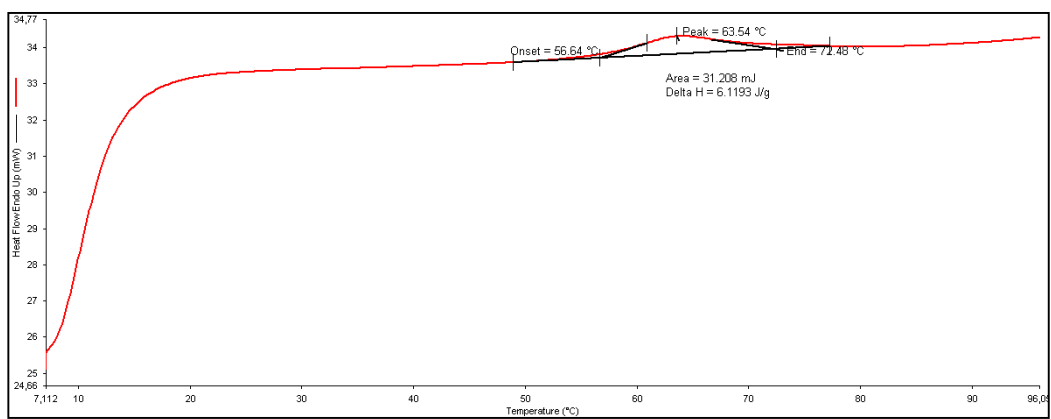


Figure A.5 DSC thermogram of the raw wheat flour