PRODUCTION OF HAZELNUT SKIN MICRO AND NANO FIBERS AND UTILIZATION IN BISCUITS

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

ELİF YILDIZ

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

JANUARY 2014

Approval of the thesis:

PRODUCTION OF MICRO AND NANO FIBERS FROM HAZELNUT SKIN AND UTILIZATION IN BISCUITS

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

PRODUCTION OF MICRO AND NANO FIBERS FROM HAZELNUT SKIN AND UTILIZATION IN BISCUITS

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January 2014, 239 pages

The main objective of this study was to investigate the effect of micro and nano hazelnut skin fibers on biscuit quality, texture, and staling. In the first part of the study, microstructural properties of cacao powder and hazelnut skin samples and rheological, and textural properties of biscuit dough containing different percentages (0%, 10%, 15%, and 20%) of cacao powder or hazelnut skin samples were observed. Flow behaviors of biscuit dough was explained with power law model and shear thinning behavior was observed. Also, elastic and viscous moduli of biscuit dough were evaluated and it was found that the highest elastic modulus and viscous modulus were obtained from dough containing 20% microfluidized hazelnut skin fibers. Moreover, the effects of replacement of flour by cacao powder or hazelnut skin samples on quality of biscuits (moisture content, size, color, and hardness) were determined. The darkest color and the highest moisture content was obtained from biscuits containing 20% microfluidized hazelnut skin fiber. The lowest spreading ratio

was also observed in this sample. Breaking strength increased with increasing percentage of cacao powder or hazelnut skin samples in biscuit samples.

In the second part of this study, staling of biscuit samples was analyzed with X-ray, FT-IR and other quality parameters. Slow starch retrogradation was achieved in biscuits prepared with microfluidized hazelnut skin fiber. It means that staling of biscuits was retarted.

In the last part of this study, phenolic content of cacao powder and hazelnut skin samples were analyzed. The highest value was observed for microfluidized hazelnut skin fiber.

Keywords: Biscuits, Hazelnut skin micro and nano fiber, Microfluidizer, Phenol, Rheology, Staling,

ÖZ

MİKRO VE NANO FINDIK ZARI ÜRETİMİ VE BİSKÜVİLERDE KULLANIMI

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Ocak 2014, 239 sayfa

Bu çalışmanın ana amacı mikro ve nano fındık zarı liflerinin bisküvilerin kalitesi, tekstürel özellikleri ve bayatlaması üzerindeki etkilerini incelemektir. Çalışmanın ilk aşamasında kakao tozu ve fındık zarı çeşitlerinin mikroyapısal görüntüleriyle, farklı oranlarda (%0, 10%, 15%, ve 20%) kullanılmış kakao tozu ve fındık zarı liflerinin kullanımı ile üretilmiş olan bisküvi hamurlarının reolojik özellikleri incelenmiştir. Bisküvi hamurlarının akış yapıları power law modeline göre gerçekleştiği ve kayma ile incelen yapı gösterdiği anlaşılmıştır. En yüksek G' ve G'' değerleri unun %20 oranında mikroakışkanlaştırıcı metoduyla üretilmiş olan mikro ve nano fındık zarı liflerinin eklenmesiyle elde edilmiştir. Bisküvilerin nem, boyut, renk ve kırılma kuvvetleri gibi kalite parametrelerinin bisküvilere kakao tozu ve fındık zarı çeşitlerinin eklenmesiyle nasıl etkilendiği belirlenmiştir. En yüksek nem oranı ve en koyu renk %20 oranında mikroakışkanlaştırıcı tekniğiyle üretilmiş olan mikro ve nano fındık

zarı liflerinin eklenmesiyle elde edilmiştir. Aynı zamanda en düşük bisküvi yayılma oranı aynı bisküvi türünde görülmüştür. Bisküvilerde unun kakao tozu ve mikroakışkanlaştırıcı tekniğiyle üretilmiş olan mikro ve nano fındık zarı liflerinin yerdeğiştirme oranı arttıkça kırılma kuvveti arttığı anlaşılmıştır.

Bu çalışmanın ikinci aşamasında X-ray, FT-IR ve diğer kalite parametreleri kullanılarak bisküvilerin bayatlamaları incelenmiştir. Yavaş nişasta retrogradasyonu mikroakışkanlaştırıcı tekniğiyle üretilmiş olan mikro ve nano fındık zarı liflerinin kullanıldığı bisküvi örneklerinde görülmüştür. Yavaş nişasta retrogradasyonu bayatlamanın gecikmesi anlamına gelmektedir.

Bu çalışmanın son aşamasında ise kakao tozu ve fındık zarı çeşitlerinin fenolik madde içeriği analiz edilmiştir. En yüksek fenolik madde miktarı mikroakışkanlaştırıcı tekniğiyle üretilmiş olan mikro ve nano fındık zarı liflerinde görülmüştür.

Anahtar kelimeler: Bayatlama, Bisküvi, Fındık zarı mikro ve nano lifleri, Fenol, Mikroakışkanlaştırıcı, Reoloji,

To my family Ayla & Nazmi YILDIZ,

Lütfiye Yıldız & Sinan ÖZER,

Fatih BULUT

for their support, encouragement & endless love…

ACKNOWLEDGEMENTS

I am greatly indebted to my supervisor Assoc. Dr. Behiç Mert for his guidance, valuable comments, energy, encouragement and support throughout this study. I would have been lost without him. It would be hard to complete this study without his knowledge, patient and valuable advices.

I would like to extend my deep gratitude to Dr. Ilkem Demirkesen Mert, who worked me as hard as for this study. I have learned a lot from her through this study. During the most difficult times of this study, she gave me moral, support me, gave valuable suggestions, and criticism. Also, she answered my endless questions patiently. I also want to thank Sevil Cikrikci for her cooperative study, endless help, support, encouragement and patience throughout this study.

I also thanks to Assist. Prof. Ilkay Sensoy for her laboratory support. A very special thanks goes to Assist. Prof. Mecit Öztop for encouragement during the most difficult time when writing this thesis.

My sincere appreciation goes to Damla Yaprak Berk, Emin Serhat Yağmur, my best friends, for their endless support and unconditional love. They help me in my stressful days. I have to indicate that without their motivation I cannot complete this study. I would like to express my thanks also to my other best friends Peyman Jafari, Mustafa Can, Mehmet Güzeloğlu, Gökhan Izgin, Serhan Cağan, and Hasan Tokulluoğlu not only for their support and encouragement but also for their help to make me smile during my stressful days and be with me in my every tear. I would like to express my special thanks to Betül Tatar for her help through this study, emotional support and friendship. Also, I would like to express thanks to Sezen Dinçel for her support, helpness and friendship. I would also like to thank our all research assistants at our department, especially Hande Baltacıoğlu, Cem Baltacıoğlu, Betül Tatar, Sezen Dinçel, Sibel Uzuner, Sevil Cikrikci, Bade Tonyalı, Oğuz Kaan Öztürk, Hazal Turasan, Ayça Aydoğdu,

Emrah Kırtıl, Ali Übeyitoğulları, Sinem Yavaş Acar, Önay Burak Doğan, Kübra Ünal, Sertan Cengiz for their friendship and support. Especially, I am greatly indepted to Bade Tonyalı, Ayça Aydoğdu, Çağla Çaltınoğlu Sibel Uzuner, Ali Übeyitogulları, Sezen Dinçel, and Betül Tatar for bearing my endless questions patiently and also I would like to thank Elçin Bilgin, İrem Alaçik, Cağla Çaltınoğlu, Ezgi Ayaz, Basak Övet, and Mutlu Yücekutlu for their friendship during the time I spent at the department. Also, I am greatly indebted to Armagan Cabadag for his help and motivation during this study.

I am thankful and giving all my love and appretiation to my mother Ayla Yıldız, my father Nazmi Yıldız, my sister Lütfiye Yıldız Ozer, and my brother Mustafa Sinan Ozer for their endless support, unconditional love, encouragement, and patience at all stages of my life. I love them so much; I am so lucky through my life because I have this family. They provided me everything in my life and respect my decisions. It my pleasure to express my sincere appreciations to them. It would be hard to imagine to complete this study without their support, love, and patience.

I would like to extend my thanks to my uncle Mehmet Duygun, his wife Berna Duygun, and their son Alptug Duygun and daughter Zeynep Duygun for their support and encouragement through this study and my life. They always help me and near me in my hard and stressful days, so I owe to them. I also would like to thank to Nuran Bulut, Mehmet Bulut, Elif Ozsoy and Murat Bulut for their support and encouragement.

At last but not least, I would like to express my special appreciate to Fatih Bulut for his endless encouragement, his patience and unrequited loved me. He always make me happy, and I am so lucky to meet and hang together with him. I don’t find suitable words to express my feelings.

I dedicate this work to my family member Ayla Yıldız, Nazmi Yıldız, Lütfiye Yıldız Ozer, Mustafa Sinan Ozer, and Fatih Bulut. I love them so much, I would like to extend my greatest appreciations to them again.

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G’	Elastic modulus
G’’	Viscous modulus
CP	Cacao powder
RHS	Raw hazelnut skin
BMHS	Ball milled hazelnut skin
MFHSF	Microfluidized hazelnut skin fiber

CHAPTER 1

INTRODUCTION

1.1. Dietary Fiber

Dietary fiber is defined as the portion of food that is digested by human inadequately and it is derived from plant cellular wall (D.A.T et al., 1990). Dietary fiber consists of carbohydrate components such as polysaccharides, oligosaccharides, and non-carbohydrate components such as lignin, polyphenols, waxes, phytates and associated substances (Kamp et al., 2004). Amount of fiber and type of fiber vary for different types of plant. According to their solubility there are two types of fiber: water soluble and water insoluble fiber (Table 1.1). Pectin and gum which are found inside plant cells are water soluble fibers. In contrast, fibers that are found in cell wall structure such as cellulose and hemicellulose are known as water insoluble fiber (Anderson, Perryman, 2010).

In recent years, The FNB (Food and Nutrition Board of National Academy of the National Academy of Sciences, USA) released following definition to differentiate dietary and functional fiber, and according to FNB, 'Dietary Fiber consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Functional Fiber consists of isolated, non-digestible carbohydrates and lignin that have beneficial physiological effects in human. Total Fiber is the sum of Dietary Fiber and Functional Fiber.'

1.1.1. Sources of Fiber

Cereals, vegetables, fruits and nuts are natural source of dietary fiber and the content of dietary fiber change depending on variety. For example, fiber content of non-starch food is 20-25g/100 g of dry weight (Rodríguez et al., 2006). Fiber content of foods that are contain starch is approximately 10g/100g of dry weight, and vegetable 1.5-2.5 g dietary fiber /100 g dry weight (Rodríguez et al., 2006).

Table 1. 1 Source of dietary fiber (Anderson, Perryman, 2010)

Soluble Fiber	Insoluble Fiber
Beans	Wheat Bran
Out Bran	Whole Grains
Fruits	Vegetables
Vegetables	Beans

Main source of dietary fiber is cereal. Humans being intake 50% of fiber they need from cereal. 30%-40% of fiber need is obtained from vegetables and 16% from fruits. The remaining part of fiber need (%3) is coming from other minor sources (Rodríguez et al., 2006). On the other hand, there are some foods which do not contain dietary fiber such as meat, milk and egg.

Fiber content is affected from form of food (canned, raw, etc…). For example, some process may cause a reduction in fiber content such as, drying and crushing. Also, fiber content of food may be reduced due to the removal of seeds, peels and hulls. Therefore, white bread contains less dietary fiber than whole wheat bread.

Some terms are used to describe fiber content of foods;

- High fiber: 5 g or more per serving
- Good source of fiber: 2.5g to 4.9g per serving
- More or added fiber: at least 2.5g or more per serving than the reference food (Anderson, Perryman, 2010).

1.1.2. Physical Properties of Dietary Fiber

Functional properties of food are affected from dietary fiber ingredients. Therefore, properties of dietary fiber should be examined to develop new products.

1.1.3. Solubility

Solubility of fiber and water soluble fibers are different from each other. Soluble dietary fiber means that it is soluble in humans' digestive system, but solubility means that it is dispersion state of dietary fiber (polymer) in water (Nelson, 2001).

1.1.3.1. Viscosity

Viscosity, resistance to flow of material, is defined as the ratio of shear stress over shear rate. Physical interactions between polysaccharides affect viscosity. Intrinsic characteristics which is explained as the amount of space occupied by the polymer affect viscosity of solution (Guillon & Champ, 2000). Viscosity of high dietary fiber samples are affected from many factors such as molecular weight, fiber length, the concentration of fiber, temperature, shear condition, pH and ionic strength.

Table 1. 2 Factors affect the viscosity of food which contain dietary fiber (Nelson, 2001)

Factor	Impact on Food
Dietary Fiber Concentration	Viscosity increases with increasing dietary fiber concentration
Structure of Dietary Fiber	Viscosity increases with increasing molecular weight and decreasing branching(increasing branching)
Presence of cereal based starch	Viscosity increases with increasing the cereal based starch
pH	Viscosity changes with fiber type
Ionic strength	Viscosity changes with fiber type

Viscosity of foods also depend on the origin of dietary fiber. All of dietary fiber (cereal based, fruit based and plant derived) affect the viscosity, but plant derived dietary fiber, is mostly used in food industry as thickening agent.

1.1.3.2. Water Binding Capacity

The interaction between dietary fiber and water is occurred by polar and hydrophilic interaction, hydrogen bonding and enclosure. The interaction between dietary fiber and water is affected from the flexibility of dietary fiber surface. Water uptake, hydration, adsorption, absorption, binding or holding terms can be used to describe interaction between water and dietary fiber (Chaplin, 2007).

Physiological properties of dietary fibers strongly depend on water holding capacity, and water holding capacity and water binding capacity may vary with dietary fiber types (Chaplin, 2007). Water holding capacity and water binding capacity are different from each other. Water holding capacity indicates the amount of water, dietary fiber holds water within its structure without subjected to any pressure or stress. However, water binding capacity is the amount of water, the system keep after subjecting to stress.

Water binding capacity is measured by two methods. One of them is centrifugation methods which are widely used for foods that contain high dietary fiber. The other one is dialysis method. Not only, microstructure of raw material (particle size, fiber length, porosity etc.) and processing conditions but also, measurement type and environmental conditions such as pH, ionic strength and concentration affects water binding capacity (Nelson, 2001).

1.1.3.3. Oil Binding Capacity

Chemical structure of dietary fiber plays a role on oil binding. Rather than the affinity of fiber molecule to oil, porosity of the dietary fiber is more important for binding process such as frying. If presoaking is done, dietary fiber pores are occupied by water and the oil uptake is reduced (Nelson, 2001).

1.1.3.4. Ion Exchange Capacity

Cations (Calcium, Cadmium, Zinc and Copper) can bind to dietary fiber due to the presence of uranic acid and free carboxylic groups. Cation exchange capacity is known as the ability of binding positively charged materials to dietary fiber. Cation exchange capacity is affected from type of fiber, pH, ionic strength and nature of cation. Also, organic molecules can be absorbed by the dietary fibers and this property can reduce the risk of some type of cancer (Nelson, 2001).

1.1.4. Factors Influencing Physical Properties of Fiber

Physical properties and function of dietary fiber are affected from type of dietary fiber source and type and degree of processing.

1.1.4.1. Type of Dietary Fiber Source

Chemical composition and structure of dietary fiber is important to understand how the polymer (dietary fiber) interacts with itself and other polymers. These

properties of dietary fiber changes with the source. For instance, cellulose molecules are linear, due to this property these molecules form crystalline region by making hydrogen bonding with themselves. Dietary fibers are synthesized in nature so the amount of dietary fiber in given source vary. For example, the content of fiber is different among different oat cultivators. So these differences affect the final product's dietary fiber content. The composition differences led to differences in functional properties among the products (Nelson, 2001).

1.1.4.2. Type and Degree of Processing

Final ingredients functionality, which is important for manufacturer to understand how this ingredients are made up and to select process for obtaining most functional ingredient, is influenced mostly type of processing and degree of process. Dietary fiber production processes which are affect the functionality are milling, bleaching, dehydration/drying, and roasting (Nelson, 2001).

1.1.5. Dietary Fiber Effects on Health

High dietary fiber foods have an important place in the diet. If large part of daily diet contains high fiber foods, they provide numerous benefits to the body. The risk of some diseases is reduced by consuming high dietary fiber. Such diseases are obesity, diabetes, metabolic syndrome, cardio vascular diseases, cancer and intestinal disease.

1.1.5.1. Obesity, Diabetes, Metabolic Syndrome,

Epidemiological studies support that obesity is prevented by consuming dietary fiber (Slavin, 2005). Body weight and body fat is inversely proportional to dietary fiber consumption. Also, diabetes and metabolic syndrome inversely proportional to dietary fiber consumption. The total food intake and calorie intake are related with intake of dietary fiber (Slavin, 2005). Obesity and diabetes are linked to unhealthy diets. However, high dietary fiber diets play an important role to regulate, control and even prevent obesity and diabetes (Kamp et al., 2004).

Table 1. 3 Dietary reference intake values for total fiber by life stage (Slavin, 2005)

Life Stage	Adequate Intake (g/d)	
	Male	**Female**
1-3 y	19	19
4-8 y	25	25
9-13 y	31	26
14-18 y	38	26
19-30 y	38	25
31-50 y	38	25
51-70 y	30	21
≥70 y	21	21
Pregnancy		28
Lactation		29

Unhealthy diets which have high protein, carbohydrates, fat, sugar and salt increase blood glucose, cholesterol and lipid levels (Kamp et al., 2004).

1.1.6. Applications of Dietary Fiber

Dietary fibers can be classified as soluble and insoluble. Due to their different chemical structures, the dietary fibers have different properties such as water holding capacity, gel formation ability and microbiological stability (Kamp et al., 2004).

Dietary fibers are mostly used in food industry as an additive. For instance in dairy industry is commonly used as stabilizers and fat replacers. Also, they can be used in ice-cream, yoghurt and cheese production. In these products texture and structure are affected from the addition of dietary fibers. In low fat dairy products dietary fibers are used to reach same textural and sensory characteristics of high fat products. Producing of high quality, low calorie foods and increasing of dietary fiber consumption are the health benefits of dietary fibers in dairy products (Kamp et al., 2004).

In baked goods and extruded foods dietary fibers are widely used to decrease fat and thus calorie content. Dietary fibers affect some properties of baked goods. For instance in bread production extra addition of dietary fibers led to increasing water absorption of dough and mixing time. But, addition of fiber may affect negatively crumb grain because of insufficient formation of gluten network. Dietary fibers can also be used in the production of cakes, muffins, cookies and extruded products (Nelson, 2001). In cookies (biscuits) higher spreading characteristic is needed. However, the use of high- fiber ingredients in cookies production decreases spreading character of cookies. Also, cookie crumb tenderness increase by the addition of high fiber ingredients and top grain appearance affected from high fiber ingredient addition. Since shallow,

narrow crevices are desirable in cookies but fibers absorb water and cracks not evident of cookie top grain (Nelson, 2001).

In beverage industry also dietary fiber can be used. Total dietary fiber content of beverages can be increase using both soluble and insoluble fiber. Mouthfeel of beverages and flavor are effected from addition of dietary fiber, also, dietary fiber stabilize the system/emulsion/foam (Nelson, 2001).

Dietary fibers can be used in jams, jellies and preserves. Moreover, they used in meat, poultry, seafood and analog products because of water binding capacity of dietary fibers. Also, textural properties of food are affected (Nelson, 2001).

1.2. Phenolic Compounds

The main classes of secondary metabolites in plants is known as phenolic compounds in food. Also, consequences of ingestion of plant food is the production of phenolic compound in animal tissue and non-plant material (Shahidi & Naczk, 1995). In different plant species, various phenolic compounds are investigated (Karakaya, 2004). Phenolic compounds play critical roles in human health (Asami et al., 2003). Moreover, sensory and nutritional quality of foods are effected from the origin and amount of phenolic compounds (Karakaya, 2004). Harmful effects of free radicals and the risks of certain types of cancer, coronary heart disease, diabetes type 2 and inflammation can be reduced by consuming foods which contain phenolic compounds (Alasalvar et al., 2009a). The concentration of phenolic compounds in food is very important. At low concentration oxidative deterioration may be prevented because phenolic compounds may act as antioxidants. However, at high concentration phenolic compounds and their oxidation products may give reaction with food ingredients such as protein, carbohydrates, and minerals

(Karakaya, 2004). Concentration of phenolic compounds also affect the astringency and bitterness of food. Moreover, meat and some cheese products' smoke flavor is important and this flavor is obtained by the help of smoke phenolic (Shahidi & Naczk, 1995). Fruits and vegetables are considered to be protective against certain diseases because of antioxidant content. Specifically antioxidant vitamins are ascorbic acid, alpha-tocoperol and beta-carotene. Moreover, according to some researchers' fruits and vegetables protective actions are due to antioxidant activity of phenolic compounds such as flavonoids, isoflavone, flavones, anthocyanin, catechin and isocatechin, not due to vitamin C, E and beta-carotene. Therefore the antioxidant activity of phenolic compounds are important in foods. Phenolic compound carry out antioxidant activity by the help of their redox properties, which allow them to act as reducing agent, hydrogen donor, singlet oxygen quenchers and metal chelators (Kaur & Kapoor, 2002).

Growth and reproduction of plants are closely related with phenolic compounds. Phenolic compounds act as an anti-feedant and anti-pathogens in plants and also phenolic compounds give color to plant. In injured plants, phenolic compounds may be secreted from plants to protect themselves against pathogens (Shahidi & Naczk, 1995).

Studies show that phenols have different biological activities. Phenolic compounds may be used to prevent cardiovascular disease and cancer, also, they can be useful for the treatment of neurodegenerative and infectious disease. Different biological activities of phenols arise from phenols chemical properties such as; antioxidant, anti-inflammatory, hormonal activity and also phenolic compounds' capacity of affecting enzymatic processes, cell growth and gene expression (Koeffer, 2008).

1.2.1. Phenolic Compounds Contribution to Sensory Characteristics of Foods

Sensory properties of food such as; color, taste (astringency and bitterness) and smell are affected from polyphenols (Koeffer, 2008).

1.2.1.1. Flavor Contribution

Aroma and taste of numerous food products of animal and plant origin may be contributed by phenolic compounds. Some oilseeds possess sour, bitter and astringent flavor characteristics, these are due to presence of phenolic compounds (Shahidi & Naczk, 1995).

1.2.1.2. Taste

Taste is one of the complicated sensory characteristics of food because it depends on the initial sensation of human when food is taken in mouth or sensation of after consumption. Texture, dryness and surface roughness can be sensed in the mouth (Koeffer, 2008).

Mouth is become wrinkled caused by phenolic substances which is present in foods is known as astringency. Acceptance of food is closely related to astringent phenols, this sense depends on the ability of a substance to precipitate salivary proteins (Shahidi & Naczk, 1995).

The concentration of phenolic compounds is important for taste and odor of food product (Shahidi & Naczk, 1995). The bitter taste of food depends on the phenolic compounds which have small molecular weight. Some food cause loss

of mouth lubrication, this is also related with food phenols which results from the reaction of tannins and salivary proteins (Koeffer, 2008).

1.2.1.3. Color

Color of vegetable and vegetable derived product are closely related the phenolic content. Phenolic compounds provide large scale of nuance from yellow to orange, red, purple and even blue (Koeffer, 2008). Different flavonoids give different colors to food. For instance, anthocyanin's can be used as a natural colorant of food and beverages. They can give pink, scarlet, red, mauve, blue and violet color to fruit, vegetable, juices and wines (Shahidi & Naczk, 1995).

1.2.1.4. Smell

Phenolic substances are low volatile substances. Therefore, smell of foods which contain phenols is less important than the other sensory characteristics of food. Spices and herbs smell is due to volatile phenols which are vanillin, eugenol, thymol and carvacrol, etc… In vanilla beans guaiacol, 4-methylguaiacol, acetovanillone and vanilly alcohol are found in low concentration, but they give vanillin smell to food. Also, some volatile phenols such as ethyl and vinyl phenol and guaiacol phenol give desired smell to wine (Koeffer, 2008).

1.2.2. Effects on Enzymatic Activity

Enzymatic activities which includes different metabolic pathways promoting to phenols' biological activities for instance antythrombotic, vessel protective, anti-inflammatory and anticarcinogenic properties can be modulated by the ability of flavonoids (Koeffer, 2008).

Hydrolases, oxidoreductases, DNA synthetases, RNA polymerases, phosohatases, protein phosphokinases, oxygenase, aminoacid oxidases are flavonoids which are used for enzyme inhibition (Koeffer, 2008). These enzymes play crucial role in plants due to the ability to resistance to infection by viruses, fungi, bacteria or mechanical damage (Shahidi & Naczk, 1995).

1.2.3. Antioxidant Effects of Food Phenolic

The definition of antioxidant in food is any substances that have ability of delaying, retarding or preventing the occurrence of rancidity or off-flavor in foods due to oxidation. Off- flavor occurrence can be prevented by extending the induction period. After this period, antioxidant addition tends to be ineffective in retarding rancidity development. Inhibition or retardation of oxidation can be done by antioxidants in two different ways. One of them is scavenging free radicals, the other one is binding to metal ions (Pojorny et al., 2001). Main antioxidant activity of phenolic compounds is scavenging free radicals. Existence of sugar moiety in molecules reduce antioxidant activity of phenolic compounds. Antioxidant activity of food phenols are closely related to their ability to induce phase II enzymes which are glutathione transferase, metallothionein. Researches show that phenolic compounds concentration and environmental conditions effects the antioxidant activity of food phenolics. During radical scavenging, phenolic compounds produce phenoxyl radicals which have ability to oxidize both proteins and lipids. Cell survival, which

means protection of DNA, proteins and lipids from oxidative damage, can be improved by antioxidant activity of polyphenols (Koeffer, 2008).

1.2.4. Antimicrobial effect of Food Phenolic

Phenolic compounds have some antimicrobial effect against *Staphylococcus aureous* and *S. epidermidis* (Koeffer, 2008). The activity of some enzymes such as HIV, influenza virus, and Rhinovirus which are necessary for viral replication with in the cell can be inhibited by flavonoids (Koeffer, 2008).

The immunostimulatory effect of flavonoid treatment exterminate all infectants, include viruses. Antiviral effects are fortification of the cellular membrane and induction of nucleases that attack viral genome which belongs to Interferons. They production is stimulated by flavonoids (Koeffer, 2008).

1.2.5. Health Concern

Cell regulatory pathways such as growth of cell (containing cell division which is important for anticancer activity), apoptosis, DNA damage repair, energy metabolism, hormonal activity, gene expression, inflammation, antioxidant activity, enzyme inhibitor, hormones or anti-hormones, modulator of genes expression are affected from flavonoids (Koeffer, 2008).

Flavonoids are very important antioxidants. They help the reduction of oxidation of low density lipoproteins which protective against cardiovascular disease (Pojorny et al., 2001). Also, some studies show that flavonoids protective against neurodegenerative disorders such as Alzheimers' and Parkinsons' disease (Koeffer, 2008).

1.3. Hazelnut

Turkey is the largest hazelnut producer with it's approximately %70 of the total world's production. Therefore, hazelnut industry is important for Turkey economy. Other major hazelnut producer countries are Italy, U.S. and Spain (Alasalvar et al., 2003). Hazelnut is produced around the world approximately one million tons of per year. Hazelnut belongs to classes of fruits which have hard, smooth shell. The seed of hazelnut is covered by skin or testa which is dark brown pellicular pericarp. Skin or testa can be removed after roasting process (Contini et al., 2008). Hazelnut in the form of natural, blanched or roasted are used to give flavor to products in dairy, bakery, confectionary, candy and chocolate industry (Köksal et al., 2006). In addition they provide desired texture to the bakery, cereal, salad, sauce, dessert and dairy products (Dervisoglu, 2006). The storage stability and quality of hazelnut depends on the phenolic content of hazelnut (Chemistry, 2000).

Although, hazelnut is considered as unhealthy because of its high fat content, it contains unique fatty acid (MUFA), fat soluble bioactives (tocopherols and phytosterols), vitamins (vitamin E), essential minerals (selenium), essential amino acids, antioxidant phenolic, and dietary fibers. Therefore hazelnut is important food for human health and nutrition need (Mercanligil et al., 2007).

The main constitutents of hazelnut is fat which is followed by carbohydrate and protein. The final composition of hazelnut is closely related with harvest time, farming, drying methods, season, geographical origin, environmental factors, and storage and handling condition. Also, variety of hazelnut affects its composition (Alasalvar et al., 2003).

Table 1. 4 Proximate composition of Hazelnut

Composition	(g/100g)
Protein	15.35 ± 0.42
Fat	61.21 ± 0.99
Carbohydrates	17.30 ± 0.48
Moisture	3.90 ± 0.20
Ash	2.24 ± 0.03
Energy	631 kcal in 100g / 2640 kJ in 100g

(Alasalvar et al., 2003)

Hazelnut contains eighteen different minerals with fifteen essential minerals and aluminum, cadmium, and silver. Potassium is the most abundant mineral in hazelnut. It is followed by phosphorus, calcium and magnesium. It is also big source of iron, manganese, copper and selenium. Selenium is a very important antioxidant and hazelnut is good source of it. It prevents free radical generation to protect cell (Alasalvar et al., 2003).

Minerals generally promote human health by making contribution to bones, teeth, soft tissues, hemoglobin, muscle, bond, and nerve cells, Mental and physical well-being closely related with minerals. The risk of cancer which are breast, colon, lung and prostate and heart disease is reduced by hazelnut consumption. In addition, tissue elasticity may be preserved by hazelnut consumption. Mineral composition of hazelnut is affected from variety of hazelnut, geographical origin, harvest year, climate, composition of soil, irrigation, use of fertilizer, and method of cultivation (Alasalvar et al., 2003).

Table 1. 5 Mineral Composition of Hazelnut

Minerals	**(mg/100g)**	**Minerals**	**(mg/100g)**
Aluminum	5.02 ± 0.04	Manganese	3.29 ± 0.06
Cadmium	0.01±0.00	Nickel	1.25 ± 0.03
Calcium	193.4 ± 0.60	Phosphorus	355.7 ± 4.16
Chromium	0.01±0.00	Potassium	761.0 ± 2.65
Cobalt	0.22 ± 0.01	Selenium	0.06 ± 0.00
Copper	1.60 ± 0.02	Silver	0,01 ± 0.00
Iron	4.97 ± 0.07	Sodium	3.13 ± 0.45
Lead	0,03 ± 0,01	Vanadium	0.08 ± 0.00
Magnesium	176.5 ± 0.50	Zinc	1.94 ± 0.10

(Alasalvar et al., 2003)

Hazelnut is also a good source of vitamins such as vitamin B_1, B_6, biotin, folate, B_2, niacin, pantothenic acid, vitamin C and mainly vitamin E [Table1.6] (Alasalvar et al., 2003).

Table 1. 6 Vitamin Composition of Hazelnut

Vitamins	(mg/100g)	% of RDA*
E	24.0 ± 0.54	240.0
Thiamin (B_1)	0.42 ± 0.03	27.9
Riboflavin (B_2)	0.10 ± 0.01	6.1
Pyridoxine (B_6)	0.63 ± 0.04	31.3
Cobalamin (B_{12})	nd	0.0
Niacin	1.94 ± 0.15	10.2
Pantothenic acid	1.12 ± 0.07	11.2
Biotin	0.08 ± 0.01	27.0
Total folate	0.12 ± 0.01	59.0
Ascorbic acid (C)	5.54 ± 0.23	9.2
Carotene	nd	0.0

*Recommended Daily Allowance Percentage (RDA) (Alasalvar et al., 2003)

Hazelnut contains both soluble and insoluble fiber [Table 1.7] (Alasalvar et al., 2003). Soluble fiber such as pectins, gums and hemicellulose can solubilize in water to form viscous solutions and can be degraded by the intestinal microflora. It leads to decrease of total and LDL cholesterol, and the risk of prostate cancer. Fiber which is not solubilized in water is known as insoluble fiber and cannot be degraded in intestines by bacteria. The examples of insoluble fiber are cellulose, some hemicellulose and lignin (Grosvenor & Smolin, 2006). The feeling of fullness is the result of insoluble fiber. Constipation and digestive system cancer risk can be reduced by consuming insoluble fiber (Alasalvar et al., 2003).

Table 1. 7 Dietary Fiber Content of Hazelnut

Dietary Fiber	**(g/100g)**
Insoluble	10.67 ± 0.15
Soluble	2.21 ± 0.10
Total Fiber	12.88 ± 0.24

(Alasalvar et al., 2003)

Table 1. 8 Amino acid Composition of Hazelnut

Composition	***(mg/g protein)***	***(g/100g hazelnut)***
Total essential amino acids	304.9 ± 2.8	4.68 ± 0.13
Total amino acids	987.9 ± 9.3	15.16 ± 0.41

(Alasalvar et al., 2003)

In addition to its amino acid composition (arginine, glutamic acid, aspartic acid, alanine, and asparagine), hazelnut is a good source of organic acids (oxalic, maleic, citric, malic, lactic, and acetic) and sugar (fructose, glucose, sucrose, raffinose, stachyose, myo-inositol) [Table 1.9]. The composition of them may be affected from variety, growing condition, maturity, season, geographic origin, fertilization, soil type, storage condition, amount of sunlight received and harvesting time (Alasalvar et al., 2003).

Table 1. 9 Free Amino Acid, Sugar, and Organic Acid Composition of Hazelnut (Alasalvar et al., 2003)

Composition of Hazelnut	**(g/100g)**
Total Free amino acid	7.152 ± 0.03
Total Sugar	3.58 ± 0.56
Total Organic Acid	1.624 ± 0.032

Hazelnut skin, hazelnut hard shell and hazelnut green leafy cover are the byproducts of hazelnut industry. Their usage in food industry add economic value to waste (Del Rio et al., 2011a). Hazelnut skin as an important source of natural antioxidant can be used in the production of food industry. Phenolic compounds which is exist in hazelnut skin have stronger antioxidant activities. Phenolic compounds can reduce the risk of some diseases such as certain types of cancer, coronary heart disease, diabetes of type-2,and inflammation because they provide protection from free radicals harmful effects (Alasalvar et al., 2009b). Procyanidin B2, (epi)catechin,, gallic acid, quercetin, prtpcatechuic acid are widely found phenolic compounds in hazelnut skin (Del Rio et al., 2011a). They have cardio protective, antimicrobial and neuro protective activities (Monagas et al., 2009).

Hazelnut skin can be used in bakery products to increase the dietary fiber content and they can be named as antioxidant dietary fiber (Anil, 2007).

1.3.1. Micro-fluidization process

Microfluidization is applied to food products to preserve and improve the quality of product. It is the combination of forces which are ultrahigh pressure and has high-velocity impact, high-frequency vibration, instantaneous pressure drop, intense shear and cavitation (Hu et al., 2011). Flow of liquid materials involved in microfluidization process passes through microchannels toward an impingement area at very high temperature. The forces which are acting together reduce mean droplet diameter of the emulsion dispersed phase resulting in more uniform distribution of foods (Pinnamaneni et al., 2003). Therefore, it is different from high hydrostatic pressure (HHP) and conventional valve homogenization.(Hu et al., 2011) While HHP method and conventional process use pressure from 100 to 1500 MPa, microfluidization pressures can be reach up to 200 MPa (Liu et al., 2009). This process can be used for preparation of nanoemulsions and improvement of polyphenol oxidase activity. Microfluidization possess lots of advantages such as having no exogenous chemicals, low treatment temperature, low nutritional composition loss, and short processing time (Hu et al., 2011).

Microfluidization is applied for microbial reduction, mozzarella cheese preparation, enhancement of whey protein, and liposomes nano-emulsion preparation, and dietary fiber improvement (Liu et al., 2009).

Microfluidization process gaining popularity in food industry and also in other areas. This new techniques improve texture, color and stability of products. Also, it gives opportunities to produce more uniform samples (Cikrikci, 2013). This novel technique is used in milk industry for the purpose of homogenization (Mert, 2011). Microfluidization, also, used in the production of cheese (Tunick et al., 2000), yoghurt (Ciron et al., 2011),cream liquors (Lagoueyte & Paquin, 1998), ice cream (Olson et al., 2003), cacao fiber production (Duman, 2013), orange juice (Yüce, 2011), peanut (Hu et al., 2011) and ketchup (Mert, 2011).

Increasing pressure of microfluidization lead to reduction thickening and stabilizing properties of Xanthan gum(Lagoueyte & Paquin, 1998). By using microfluidization process particle size of samples are reduced and they affects the rheological and sensorial properties of food product (Ciron et al., 2011).

Application areas of microfluidization are pharmaceutical, biotechnology, chemical, energy, cosmetic, and food industries.

1.4. Biscuit

Biscuits are the most popular consumed bakery items nearly by all levels of society. Some of the reasons for such wide popularity of it are their ready to eat nature, affordable cost, good nutritional quality, availability in different tastes and having longer shelf life (Sudha et al., 2007). Biscuits are thought as staple foods, snacks, luxury gifts, dietary products, infant foods, dog and cat foods. Also, with the addition of some additives such as chocolate, cream, and marshmallow they can be considered as confectionary product (Manley, 2011). Crackers which are crispy and salty products and cookies which is known as small cake can be considered as biscuits but wafers cannot be considered as biscuits (Edwards, 2007).

Biscuits can be stored for very long times because they have low moisture content, and they are generally made from wheat flour (Manley, 2011). As a result of long storage time and low moisture content fat rancidity can occur in biscuits (Cauvin & Young, 2006). Water content affect biscuits quality. In order to handle crispy structure of biscuits and longer shelf life of biscuits water addition to dough should be limited and most of it must be baked out in the oven during baking. Generally, the moisture content of biscuits should be lower than %10. In the production of cookie the gluten network should be limited, this is provided by the lower amount of water addition. The limited gluten

development minimize changes in biscuits shape after forming and during baking (Cauvain & Young, 2000).

1.4.1. Biscuits Ingredients

Quality of biscuit depends on the ingredients, because they effect both physical and chemical structure of product.

1.4.1.1. Flour

Flour type is important to obtain good quality from baked products. For instance, bread flour is made from hard wheat with high protein content and high starch damage [Table 1.10]. However, in the production of biscuits, flour with low protein content and low starch damage are used. Also, flour treatments are different for biscuit and bread flour. Biscuit flour acts as a reducing agent but bread flour behaves like oxidizing agent. The most important property of biscuit dough is extensibility and dough resistance is not desirable in biscuit production (Edwards, 2007).

Wheat flour is used in the production of biscuits. There are some advantages of using wheat flour in biscuit production. Wheat flour is inexpensive, nutritious and it provides good machining properties for automatic processing (Samuel A. Martz, 1968). There are two types of wheat flour; wholemeal and white. However, generally white wheat flour is preferred in the production of biscuits.

The effects of flour in food products can be explained based upon its composition, protein, starch, fiber content and other physicochemical properties such as size of particle, and quality of protein. The properties of flour

changes with the wheat types and also changes with season for the same type of wheat. Moreover, the wheat milling step affects the properties of flour (Manley, 2011).

There are four protein types in wheat flour; prolamins (gliadins), glutelins (glutenins), albumins, and globulins. However, the major proteins of wheat flour are gliadins and glutenins which are responsible for gluten network formation. Flour quality and dough rheological properties depend on these major proteins. The variations of level of glutenins and gliadins closely related with wheat genetics (Cauvin & Young, 2006).

Flour is composed, from moisture content, starch and other carbohydrates, protein, lipid and crude fiber. The dominated substance is starch in flour and starch water absorption is very important for baked products. Gelatinization is explained by the water absorption of starch. Gelatinization doesn't occur in all baked products. Such as, if the water level is too low like in biscuits, water competition is so high for occurrence of gelatinization.

In biscuit production the main ingredient is flour. Flour does not contribute flavor so much to product but is strongly effects the baked texture, hardness and shape of biscuits. These effects are different for various type of biscuits based upon fat and sugar content of product and also mixing of ingredient while dough preparation (Manley, 2011).The other function of flour in biscuit production is water binding, so low protein and low starch damage flour should be used in the production. The aim of the biscuit production is to produce low moist food. If the water binding capacity is high, the aim cannot be achieved (Edwards, 2007).

Table 1. 10 Comparison of Flours used Bread and Biscuits

Property	Bread Flour	Biscuit Flour
Protein	High	Low
Starch damage	High	Low
Dough extensibility	Low	High
Dough resistance	High	Low
Wheat	Hard	Soft
Flour treatment	Oxidizing agent	Reducing agent

(Edwards, 2007)

The ash content of flour affects color of biscuits and biscuits can be look grey at final stage. If the ash content of flour is high, the function of gluten is weakened during baking process. Final product quality of biscuit is low moisture content and high fat and/or sugar levels. Dough forming process can be different for various types of biscuits [Table 1.11].

Table 1. 11 Comparison of Biscuit Types

	Crackers	**Semi-Sweet**	**Short Dough (High fat)**	**Short Dough (High Sugar)**	**Soft**	**Wafer**
Added water in dough	33%	21%	2-3%	2-3%	15%	140%
Moisture in biscuit	3-4%	1-2%	2-3%	2-3%	3+%	1-2%
Critical ingredients	Flour	Flour	Fat	Fat and sugar particle size	Fat and sugar particle size	Flour and batter
Dough piece forming	A	A	B,C,D,A	B,C,D,A	C,B,D,A	E
Baking time (minutes)	3	5-6	15-25	6	12+	1.5-3

Key: (A; sheet, gauge, cut - B; Rotary mould - C; Wire cut - D; Extrude - E; Deposit) (Cauvin & Young, 2006).

1.4.1.2. Fat

The quality of finished products and machining properties of dough depend on fats type. Also, fats and oil provide coating and filling structural part of the

product (Samuel A. Martz, 1968). In biscuit production fats are essential component. The other functions of fats are contribution to biscuit aeration and contribution to sensory properties of product. Size increasing ability of biscuits during baking process lacks due to incorporation of air while preparation of biscuit. Therefore, after baking process the crumby and short eating character of the product is lost (Cauvin & Young, 2006). Biscuits are stable in the storage period by the help of fats content. Fats are added to biscuits to provide desired texture. If the dough of biscuits coated with fats, developing of gluten network can be blocked. Therefore, less hard product can be obtained (Edwards, 2007). Moreover, eating quality and flavor can be obtained by addition of fats (Manley, 2011). However, fats can be deteriorate in two ways. The first way is lipolysis which means fatty acids split from the glycerol. The other way is oxidative lipolysis which is the addition of oxygen free radicals to double bonds. This is known as oxidative rancidity. Oxidative rancidity can be prevented in different ways. One of this to ensure no double bond exist in fatty acids, and antioxidant can be used to block oxidation. Moreover, ın order to prevent rancidity, UV light and oxygen exposure should be blocked (Edwards, 2007).

In most of biscuit products vegetable fats, and hydrogenated fats, aren't used since the biscuits will be too soft physically and not stable to rancidity (Edwards, 2007). Fats which is used in biscuit should be solid at ambient temperature (Manley, 2011).

1.4.1.3. Sugar

Sugar is also an important component of biscuits because it has s great impact on texture and appearance of the product. It also affects the flavor of biscuits. The level of sugar affects the spreading character of product (Samuel A. Martz,

1968). The level of sugar is high in the production of biscuits which is made from short dough, while semi-sweet biscuit has less sugar content. On the other hand, cracker has least sugar content (Edwards, 2007). Since biscuit has low water content, sugar is not dissolve. Therefore the size of sugar crystal is important. This means that, if the sugar content is high in short dough biscuits, a hard glassy biscuit is handled (Manley, 2011). The usage of glucose syrup may soften the biscuits (Edwards, 2007).

In baked product, the level of sucrose affects the water activity. If the level of sucrose is high, water activity of product will be low. Meaning spoilage free shelf life to product (Cauvin & Young, 2006).

1.4.1.4. Milk Powder

In baked products milk powder is used to improve color, absorb water and control spread property and contributing to flavor. The major protein of milk powder is casein, and this protein leads to formation of porous structure in cookie and crackers. Also they react with reducing sugar ,lactose, providing browning reactions (Samuel A. Martz, 1968).

Dried form of milk is preferred because minimum storage space is occupied and their shelf life is longer than the other form (Samuel A. Martz, 1968). In order to prevent problems, which is accompanying with product volume loss, heat treatment should be done to milk powder (Cauvin & Young, 2006).

1.4.1.5. Salt

The purposes of usage of salt in baked products are contribution of flavor, control of product water activity and providing longer shelf life. The level of salt in dough also affects the gluten network formation (Cauvin & Young, 2006). In baked products the concentration of salt should be 1-1.5% based on the flour weight. If the level of salt more than 2%, the taste of product will be unpleasant. By the addition of salt, less sticky dough is handled since salt toughens the gluten. Yeast fermentation rate is slow down by addition of salt. Furthermore, the action of proteolytic enzymes on gluten is inhibited (Manley, 2011).

1.4.1.6. Leavening Agent

Leavening agents are inorganic compounds that are added to dough to produce carbon dioxide in order to improve texture of biscuits during baking (Manley, 2011). The amount of carbon dioxide is depend on the amount of leavening agent in the mixture of product (Cauvin & Young, 2006).

Sodium bicarbonate is generally used in biscuit production because it is cheap, has food grade purity and also provides different size of particle (Manley, 2011).

In biscuit sodium bicarbonate adjust pH for both dough and resulting product (Manley, 2011).

1.4.1.7. Water

Due to presence of dissolved minerals and organic substances in water, flavor, color and physical attributes of baked products are affected from addition of water (Samuel A. Martz, 1968).

Water which is added to biscuit dough is removed in the oven during baking but the effects of the water quality on biscuits continue (Manley, 2011). The water content in final product is important in terms of eating quality and shelf life. In order to achieve desired handling properties of biscuits the level of water addition should be optimized (Cauvin & Young, 2006).

1.4.1.8. Cacao

Cacao powder is produced from the cake formed of cacao when cacao butter is handled from cacao mass (Manley, 2011). Fat content of cacao powder is vary from 8% to 32%. Cacao powder is generally used in the production of chocolate colored and flavored cake, biscuit and cookie production (Cauvin & Young, 2006).

Good quality of cacao powder properties are fine and has free flowing and 5% of moisture content. The level of water addition to dough should be increase when using cacao powder to regulate rheology of dough or batter. The higher moisture content leads to higher mold activity during storage. In order to store cacao powder for a long time, the storage conditions should be;

- Relative humidity is lower than 50%
- Temperature doesn't exceed 20^0C, the optimum level of temperature is between 15-18^0C

- Food items which has strong odors such as spices, ammonia, cheese should be placed far away from cacao powder (Manley, 2011).

1.5. Objective of the Study

In today's life, demand for health oriented products such as sugar-free, low calorie and high fiber products is increasing. Consumption of food products containing high amount of fiber is increasing to overcome some health problems such as; hypertension, diabetes, colon cancer, cardiovascular diseases, myocardial infarction, atherosclerosis and other chronic ailments (Mercanligil et al., 2007). Hazelnut is a good source of dietary fiber. Hazelnut skin is added to bakery product to increase dietary fiber content of food product (Anil, 2007). Hazelnut skin has some phenolic substances with antioxidant property. The phenolic compounds of these substances reduce the risk of several disease such as cancer, coronary heart disease, diabetes, and inflammation (Alasalvar et al., 2009a).

Turkey is the world's largest producer of hazelnuts, contributing to approximately %70 of the total global production. Therefore, hazelnut has great importance for Turkey's economy. Some works demonstrated that hazelnut skin is a rich and low cost source of natural phenolic antioxidants. The hazelnut skins are by-products of roasting process, respectively, and are now investigated for their consumption in the attempt to add economic value to waste from the hazelnut industry. Hazelnut skin fiber is handled by using microfluidization method, also hazelnut skin size is reduced by using hammer and ball mill process.

In this research, hazelnut skin was used as an additive in biscuit production. Since, biscuits are the most popularly consumed bakery items nearly by all levels of society. Some of the reasons for such wide popularity are their ready

to eat nature, affordable cost, good nutritional quality, availability in different tastes and longer shelf life.

In this research, quality parameters of biscuits were analyzed. In biscuits hazelnut skin which are prepared by using ball mill, hammer mill and microfluidization are used, and also cacao was used to make comparison between biscuits in terms of their quality. Cacao is an accepted bakery good ingredient because of its flavor component, it gives rich brown color to biscuits. Also, textural properties of biscuits are acceptable for consumers. Hazelnut skin fiber can be used in biscuits instead of cacao. It gives color, flavor and phenolic compound to biscuits but there is no research about hazelnut skin fiber addition to biscuits. Rheological and textural properties are the most important quality parameters for food product. These properties were analyzed for all biscuits in this research. Also, size, moisture and color of biscuit were analyzed. Different ingredients and cake samples were analyzed to make qualitative comparison. Microstructural analysis, SEM analysis, were used for this purpose. Shelf life of food products are very crucial quality parameter. Therefore, X-Ray diffraction and FT-IR methods were used to determine staling characteristics of biscuits. Besides of this properties, phenolic content of biscuits were analyzed in this research to decide whether biscuits that is contain hazelnut skin fiber healthier or not.

CHAPTER 2

MATERIAL AND METHODS

2.1. Materials

Biscuit flour, biscuit shortening, not-fat dry milk, sugar, baking powder (sodium bicarbonate), salt and cacao powder were provided from Ulker Biscuit Industry Co. Inc. (Ankara, Turkey). Hazelnut skin was received from Sunset Food Tourism Industry Co. Inc. (Ordu, Turkey) Folin-Ciolcalteu, sodium carbonate, and methanol were taken from Merck Chemical Companies (Deisenhofen, Germany). Sodium carbonate was purchased by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA)

2.2. Methods

2.2.1. Hazelnut Skin Processing

2.2.1.1. Raw Hazelnut Skin Production

Size of hazelnut skin was reduced by using hammer mill. (Thomas- Wiley Laboratory Mill, Model 4, Arthur H. Thomas Company, Philadelphia, PA, USA).

2.2.1.2. Ball Milled Hazelnut Skin Production

Size of hazelnut skin was reduced by using ball mill (Retsch PM 100, Retsch GmbH, Germany).

2.2.1.3. Micro and Nano Hazelnut Skin Fiber Production

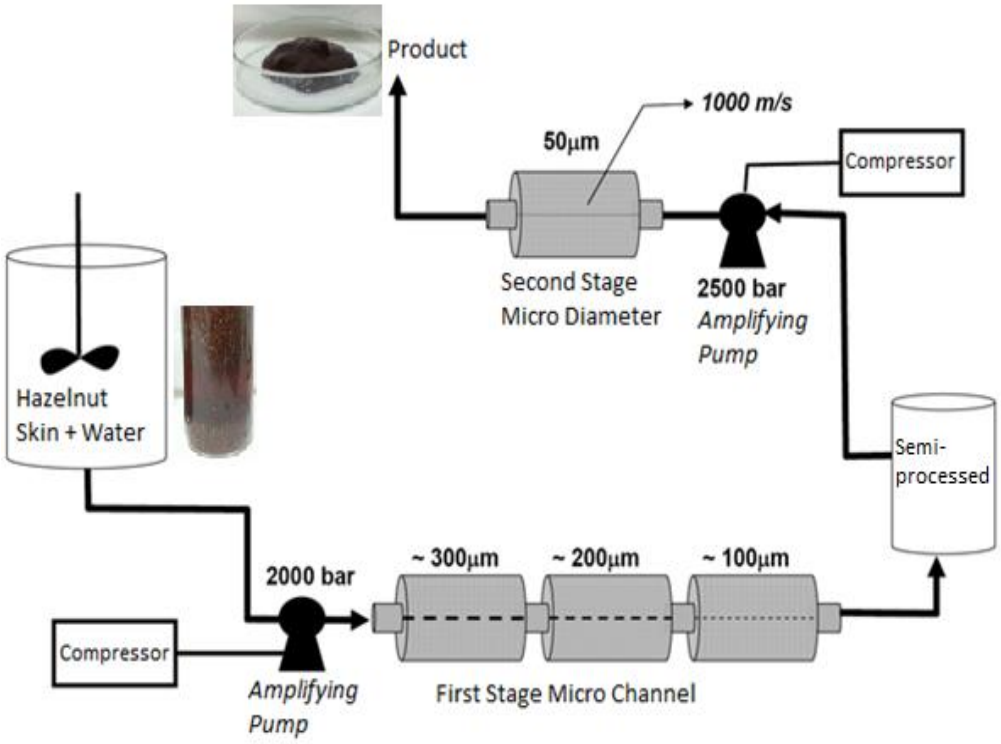

Figure 2. 1 Microfluidization method

Microfluidizer equipment (M-110Y, Microfluidics, USA) was used to produce micro and nano fiber from hazelnut skin. Two main stages exists in microfluidization process. In first stage, water was added to raw material for the purpose of softening. After this stage, slurry like product was handled and it was pumped with 2000 bar pressure through the chamber in order of 300 µm, 200 µm and 100 µm size and collecting them in the output reservoir. Then, slurry like product undergo to second size reduction step of which chamber is 50 µm. At this stage, the slurry velocity is 1000 m/s and shear rate was obtained as higher than 10^7 1/s. After this stage, hazelnut skin was broken down into fibers during passage though channels. Final product water content approximately 12-13%. Part of microdluidized sample put into jars and store at refrigerator, and the other part put into vacuum bags and stored into freezer. After one night these freezed samples were freeze dried to use experiments.

2.2.2. Biscuit Making Procedure

Basic dough recipe on 100 g wheat flour basis consists of 60% sugar, 3% non-fat dry milk, 2.5% sodium bicarbonate, 30% shortening, 0.47% salt, and 21.75% water were used in the experiments.

Control biscuits were prepared according to revised AACC Method 10-52 – Micro Sugar- Snap Cookies method. First of all, dry ingredients which are sucrose, nonfat dry milk, and sodium bicarbonate were mixed by using Kitchen – Aide mixer. After mixing process, shortening was added. Mixing was done for 1min at low speed, medium speed for 1min, high speed for 30 sec, then scrape bowl and paddle, and finalize the mixing process at high speed for 30 sec to get creamy structure. After that, water and salt were added to mixture and mixed for 3 min at medium speed. Mixer was stopped if shortening was stuck on side of bowl and scraped with small spatula. Then, wheat flour was

added to mixture and mixed for 25s at medium speed. Dough was scraped from bowl and gently form into a single dough mass and cut with spatula into equal portions. Biscuits' shape was given at this stage by using gauge strips 6mm thick. Dough was rolled to thickness with one forward and one backward stroke of rolling pin. Dough was cut with cookie cutter (60mm inside diameter), excess dough was discarded and removed from cutter.

Biscuits were prepared by replacing the flour with cacao (10%, 15%, and 20%), raw hazelnut skin (10%, 15%, and 20%), ball milled hazelnut skin (10%, 15%, and 20%), and microfluidized skin fiber (10%, 15%, and 20%).

2.2.3. Baking

Control biscuits and biscuits prepared by replacing flour with cacao powder/ hazelnut skin samples were placed into 200 $\pm 2^0$ C oven and baked for 5 min. Baking time used for each biscuits formulation were determined by conducting experiments based on quality tests of biscuits.

2.2.4. Analytical Tests

2.2.4.1. Dough Rheology Analysis

TA rheometer (AR 200ex, Sussex, UK) was used to conduct rheological measurements of biscuit dough. All measurements were done at 25^0C by using parallel plate geometry (20 mm diameter and 2 mm gap). The approximately 1 g of dough samples were placed between the plates. The oscillation experiments

which is frequency sweep test were conducted under constant deformation. During this test the frequency is changed from 0.1 to 20 Hz while constant shear strain rate (0.2%). The dough was rested at room temperature for 15 min before testing. Finally, results were expressed in terms of elastic (G') and loss (G'') values. All the rheological experiments were performed at triplicate and their averages were reported in the study.

2.2.4.2. Dough Texture Analysis

Biscuit dough were analyzed by using a texture analyzer (The TA.XTPlus, England). Dough samples having 3cm diameter and 1cm height, were placed to texture analyzer. For determination of lubricated extensional viscosity of dough, both side of dough were lubricated with oil before putting to instrument and the dough compressed with 1mm/s pre-test speed, 1mm/s, 2mm/s, 4mm/s and 6mm/s test speed, 10 mm/s post-test speed and %25, %50 strain were settled. The measurement were done in triplicate for three different dough samples. Lubricated biaxial extensional viscosity values of biscuit dough samples calculated by using below equations;

$$\acute{\varepsilon}B = (Uz \,/\, 2 * (h0 \, - \, Uzt)) \tag{2.1}$$

$$\acute{\eta}B = (\sigma B / \acute{\varepsilon}B\ ))) \tag{2.2}$$

$$\sigma B \, = \left(\frac{F}{\pi r2}\right))) \tag{2.3}$$

where;

- $\acute{\varepsilon}_B$ is strain rate,
- U_z is plate velocity,
- h_0 is the initial sample thickness
- $\acute{\eta}_B$ is extensional viscosity

- σ_B is net stretching stress
- F is the force which is required pushing down on the upper plate

(Steffe et al., 1996).

2.2.5. Biscuit Analysis

After baking, biscuits were allowed to cool down for 1h then stored in vacuum packages at 25^0C for different storage times (0, 1, 3, and 7 days). The measurement were done in triplicate for three different dough samples.

2.2.5.1. Color Determination

For color measurements of fresh biscuits, scanner (CanoScan Lide 110, Tokyo, Japan) was used. Biscuits were scanned at 300 dpi resolution. CIE L*, a*, and b* color scale of biscuits were determined by using image pro-plus 6.0 program. Total color change (ΔE) was calculated from following equation;

$$\Delta E = [(L^*-L_0)^2 + (a^*-a_0)^2 + (b^*-b_0)^2]^{1/2}$$

L_0, a_0, and b_0 were reference point which were found from control biscuits.

The data were taken from eight different point of each biscuits and for one dough three different biscuits were analyzed.

2.2.6. Phenolic Content Determination

For phenolic content determination extraction was applied to samples which are raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder.

500mg of sample was put to beaker and 5mL methanol/H_2O (75:25, v/v) was added. The mixture was placed to sonic bath (Ultrasonic Cleaner, US-10 (10P), Lab Companion, Shanghai, China) for 15 minutes and stirred by using magnetic stirring (Jeio Tech-Multi Channel Stirrer, MS52- M) was done immediately. Sonic bath extraction and vortex mixing was done in duplicate. Finally, the mixture was centrifuged (Sigma Laborzentrifugen GmbH; Osterode am Harz, Germany) for 10 min 1000 rpm. Supernatant were taken and stored at -80^{0}C (Beko, 7103 DF,Istanbul, Turkey) for analyzes (Del Rio et al., 2011b).

Folin-Ciocalteu method (Aida, 2011) was used to determine total phenolic content of samples. This method depends on the color change of samples and in the presence of the sodium carbonate phenol reduces the Folin-Ciocalteu reagent.

There are two solutions which were used to determine phenolic content of samples. First of all solutions were prepared. One of the solution was mixture of 10% (v/v) of Folin- Ciocalteu's phenol reagent and 90% of distilled water. The other solution was composed of 7.5% (w/v) of sodium carbonate in distilled water. 2.5 mL of 10% Folin-Ciocalteu's phenol reagent was mixed with 0.5 mL diluted sample. Then this mixture was stored at dark place for 5 min. After 5 min, 2mL sodium carbonate solution was assed to mixture and this was stored at dark place for 1h. Finally samples were analyzed with spectrophotometer at 760nm.

To prepare standard curve 0.01 g gallic acid was mixed with 50 mL methanol/water mixture (50:50 v/v). In total phenol determination method

samples were replaced with different concentration of gallic acid solution (20ppm, 40ppm, 60ppm, 80ppm, 100ppm, 120 ppm, 160ppm, and 200ppm) and same procedure was applied to draw curve.

Samples result data which were taken from UV/VIS spectrophotometer T 70, (PG Instruments LTD, UK) were put to standard curve to find total phenol content of samples.

2.2.7. Staling Analysis

Control biscuits and other biscuits prepared with replacement of flour with 10% cacao powder, 10% raw hazelnut skin, 10% ball milled hazelnut skin, and 10% microfluidized hazelnut skin fiber were baked. After these baking process, biscuits were removed from oven and cool them for 1h at room temperature. After 1h biscuits were packed into vacuum pack and stored at 25 ± 2^0C for staling analysis.

For FT-IR and X-RAD experiments, biscuits stored different times (0, 1, 3 and 7 days) were frozen at -80^0C (Beko, 7103 DF, Istanbul, Turkey) and then freeze-dried (Christ, Alpha 1-2 LD plus, Germany) for 48h at a pressure lower than 1mbar to prevent outside of the desired staling. Biscuits' size reduction was done with a coffee grinder (Sinbo, SCM-2909, Istanbul, Turkey)

2.2.7.1. Size Determination

For size determination of biscuits vernier was used. The measurement were done in triplicate for three different dough samples.

2.2.7.2. Moisture Content Determination

Moisture content of biscuits which were stored at different storage times (0, 1, 3, and 7) was determined by using Moisture Analyzer (IR-35, Denver Instrument). Temperature of instrument was settled to 106^0C (approved method 10-42). Results were expressed on a wet basis. The measurement were done in triplicate for three different dough samples.

2.2.7.3. Biscuit Texture Analysis

Stored biscuits were analyzed by using texture analyzer (The TA.XTPlus, England). In texture profile analysis the force required for breaking of biscuits were measured. The biscuits were placed to top of the platform of which gap distance was 5cm and the other probe was used to break biscuits. Pre-test speed was 1mm/s, post-test speed was 1mm/s and test speed was 1mm/s of texture analyzer.

2.2.7.4. FT-IR

IR Affinity-1 Spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used to perform FT-IR experiments. This instrument use Diamond w/KRS-5 lens single reflection ATR plate. (MIRacle ATR, Pike Technologies, Madison, WI, USA) The analysis was performed at the middle region of IR, 600-4000cm^{-1} at a resolution of 4 cm^{-1} with 32 scan. The samples were put into crystal surface which provides sample and ATR crystal contact. This analysis was done in duplicate. PeakFit version 4.12 software was used to analyze data.

2.2.7.5. X-Ray Diffraction Method

CuKa (λ=1.54056) radiation on a Ultima IV X-Ray diffractometer (Rigaku, Japan) at 40kV and 40 mA was used to make X-Ray diffraction analysis. The scanning region of the diffraction angle (2θ) was 5^0-45^0 with scanning speed of 2^0/min. PeakFit version 4.12 software was used to analyze data. XRAD analysis were done at Central Laboratory of METU (Ankara, Turkey).

2.2.8. Microstructure Analysis

Screening electron microscopy (SEM) was used to determine microstructure of biscuits. Biscuits were broken into small pieces (cubes in about 2.5cm dimension). They were immersed into liquid nitrogen and then freeze dried.

Magnification levels of which sample observed were 100X, 250X, 1000X and 4000X. SEM analysis were done at Central Laboratory of METU (Ankara, Turkey). Scanning electron microscope (QUANTA 400F Field Emission SEM, Eindhoven, Holland) at an accelerating voltage of 20kV was used to examination and recording of images of samples. Sputter Coater Device (Polaron Range, East Sussex, England) was used to coat samples with gold-palladium to render them electrically conductive.

2.2.9. Statistical Analysis

In order to examine whether there was a significant difference between biscuits sample types, the replacement ratios (10%, 15%, and 20%) and storage times (0, 1, 3, and 7 days) analysis of variance (ANOVA) was performed ($p \leq 0.05$). MINITAB (Version 16) software was used for this analysis. If there were

significant differences between samples, means were compared by the Tukey Single Range test ($p \leq 0.05$).

CHAPTER 3

RESULTS AND DISCUSSION

In the first part of study, SEM analyses of cacao powder, raw hazelnut skin, ball milled hazelnut skin, and hazelnut skin fiber produced by microfluidization process were examined. After that, rheological and textural properties of biscuit dough prepared by replacing the flour with cacao powder (10%, 15%, and 20%), raw hazelnut skin (10%, 15%, and 20%), ball milled hazelnut skin (10%, 15%, and 20%), and microfluidized hazelnut skin fiber (10%, 15%, and 20%) were investigated and the effects of them on viscoelastic properties, and biaxial extensional viscosity of dough were determined.

In the second part of study, quality parameters which were moisture content, physical properties (size), color, and breaking strength of fresh biscuit samples were evaluated. Also, microscopic structures of fresh biscuit samples were analyzed by SEM images.

In the third part of study, the effect of different formulations (flour replacement percentages, types of raw material) and storage times on staling of biscuits were analyzed. X-Ray diffraction and FT-IR analysis were also performed for staling analyses.

In the last part of study, quantitative information about phenolic compounds in cacao powder, and hazelnut skin samples were analyzed.

3.1. Scanning Electron Microscope (SEM) Images of Hazelnut Skin and Skin Fibers and Cacao Powder

SEM was used to characterize cacao powder, raw hazelnut skin handled by hammer mill, ball milled hazelnut skin, and hazelnut skin fiber produced by microfluidization process. Hazelnut skin samples particle sizes were different from each other and these differences can be observed with SEM analyses.

Figure 3.1 belongs to cacao powder at 1000x magnification. In Figure 3.1 there was lack of lattice network. Cacao powder had small, and scaly particles. Figure 3.2 which is SEM images of raw hazelnut skin. It had larger block structure which was broken into smaller particle after ball milling process. Figure 3.3 which is SEM images of ball milled hazelnut skin was found to be similar to Figure 3.1. The size of hazelnut skin particles were decreased by ball milling process and so, particles distributed more homogenously.

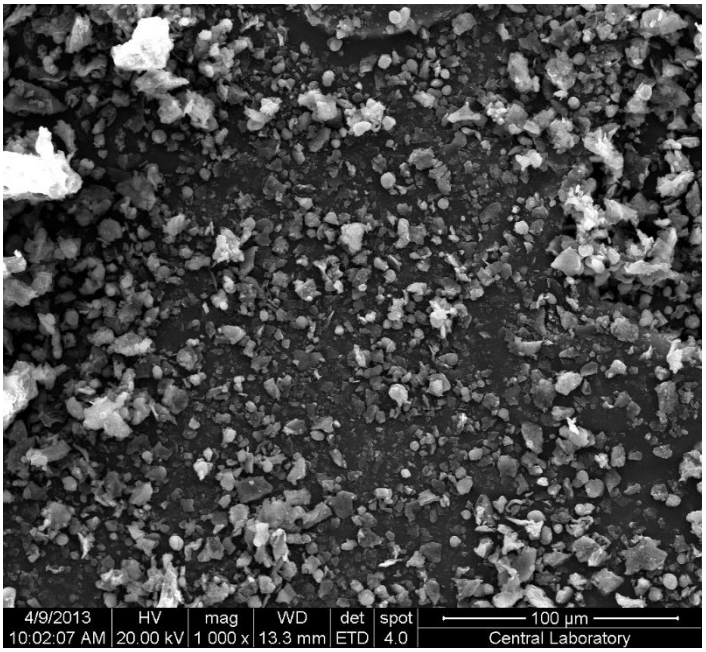

Figure 3. 1 SEM image of cacao powder. Magnification: 1000x.

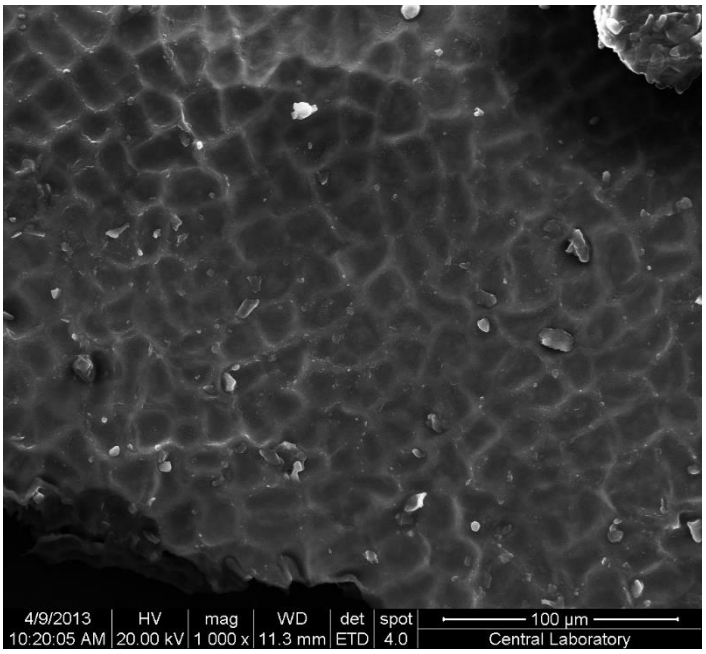

Figure 3. 2 SEM image of raw hazelnut skin. Magnification: 1000x.

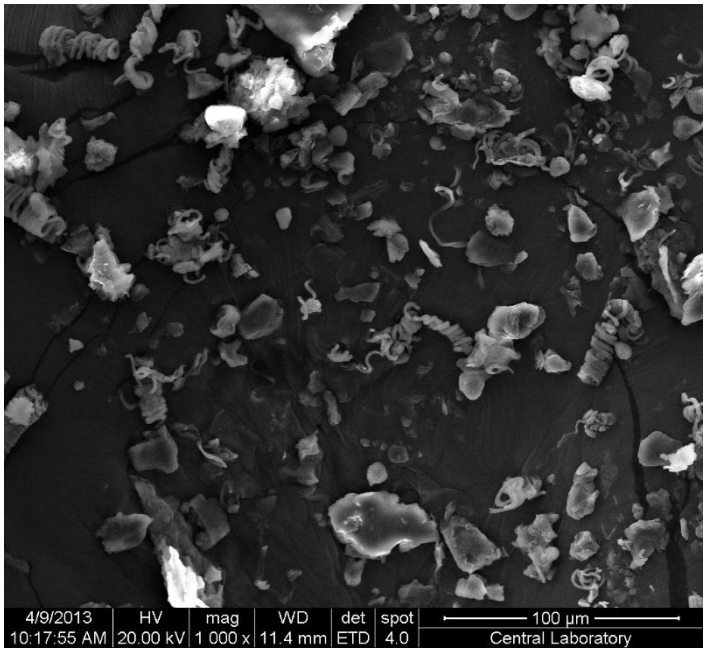

Figure 3. 3 SEM image of ball milled hazelnut skin. Magnification: 1000x.

Figs. 3.4-3.6 show SEM images of microfluidized hazelnut skin fibers with different magnification levels. More homogenous structure was observed in Figs. 3.4-3.6 compared to the other SEM images which belongs to cacao powder, raw hazelnut skin, and ball milled hazelnut skin. By using microfluidization process micro and nano hazelnut skin fibers were obtained. As can be seen in Figure 3.6, the structure of microfluidized hazelnut skin fiber longer, but thinner, and had higher surface area. According to these images microfluidized hazelnut skin fiber samples fragmentation were higher and distributions were more uniform. Hu et al. (2011) observed that high pressure microfluidization treatment led the disintegration of large clumps into a small size. It was also stated that before microfluidization treatment, complex heterogeneous structure with slumps consisting of different size and shapes was seen in SEM images. Also, Tunick et al. (2000) highlighted that high pressure

caused smaller size of fat globules in the cheese making from milk. Also, Mert (2011) observed similar result on ketchup type product and stated that microfluidization process affected the rheological properties of samples. Microfluidization process increases the surface area of fibers leading to increases in water holding capacity of hazelnut skin fiber. SEM images can be used to analyze other results.

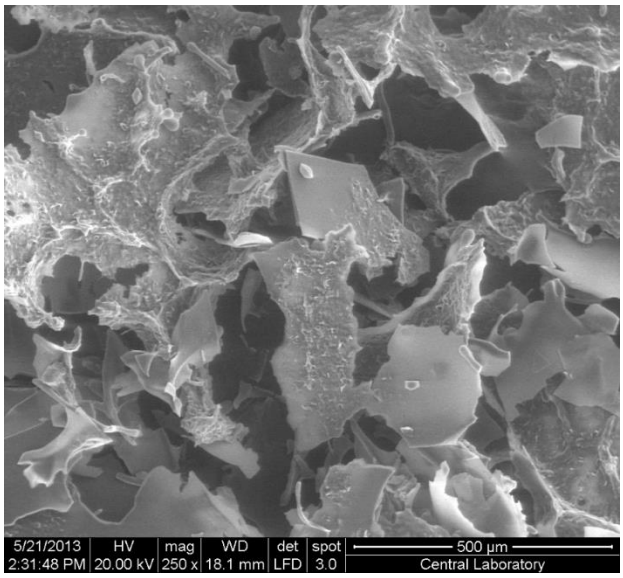

Figure 3. 4 SEM image of hazelnut skin fiber processed by microfluidizer. Magnification: 250x.

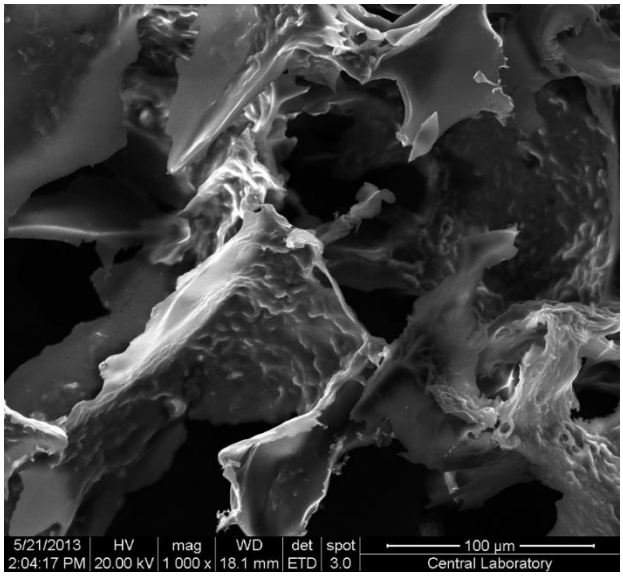

Figure 3. 5 SEM image of hazelnut skin fiber processed by microfluidizer. Magnification: 1000x.

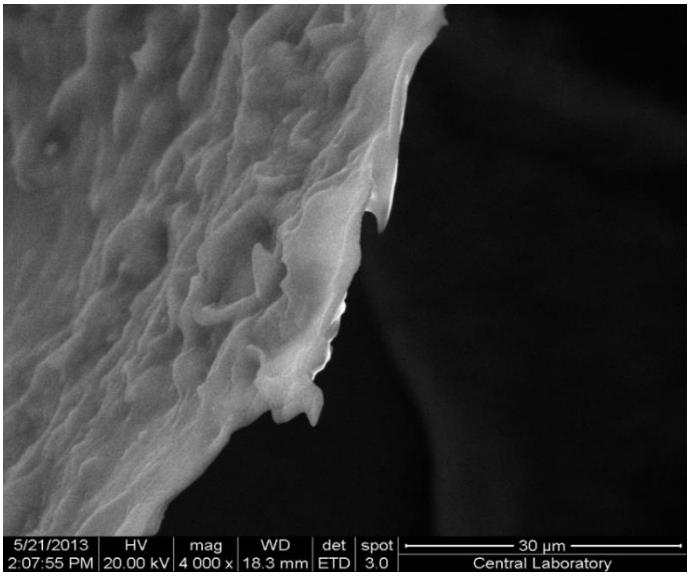

Figure 3. 6 SEM image of hazelnut skin fiber processed by microfluidizer. Magnification: 4000x.

3.2. Biscuit Dough Analysis

3.2.1. Dough Rheology

Quality of final biscuits depends on viscoelastic properties of biscuit dough. Deforming the dough and watching it spring back can be explained by elastic behavior of dough (Edwards, 2007). Elastic (G') and viscous (G") moduli of biscuit dough were represented on Figs. 3.7–3.20.

Elastic modulus (G') is defined as the stress component in phase with the shear strain (Bourne, 1982). Another explanation of elastic modulus which is also known as storage modulus measure of energy stored in the material and recovered from it per cycle (Singh et al., 2003). On the other hand, viscous modulus (G") is defined as the stress component 90^0 out of phase with the shear strain (Bourne, 1982). Measurement of energy dissipate which is also defined as viscous modulus known as loss modulus (Singh et al., 2003).

Rheological and textural properties of biscuit dough are affected by the change of ingredients and rheological properties strongly depend on water content of dough (Seremesic et al., 2012).

Seremesic et al. (2012) clarified that increasing water content of dough decreases elastic and viscous moduli. However, water content of dough don’t lead to change of overall dough and gluten network structure (Pedersen et al., 2004).

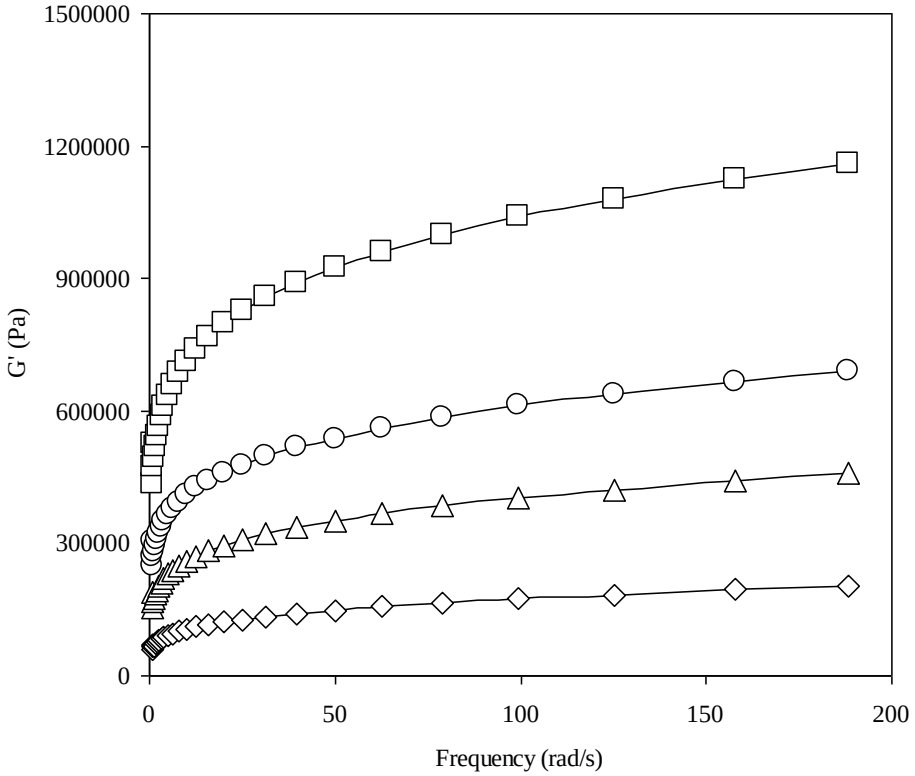

Figure 3. 7 Elastic modulus of dough samples prepared with the replacement of flour by cacao powder at different levels (0%, 10%, 15%, and 20%) where (◊): Control, (Δ): Cacao powder 10%, (○): Cacao powder 15%, (□): Cacao powder 20%.

The graph of elastic modulus of biscuit dough samples prepared by the replacement of flour with cacao powder at 0%, 10%, 15%, and 20% was given in Figure 3.7. According to Figure 3.7, the replacement of flour by cacao powder led to increases in elastic modulus of biscuit dough. Further increases in elastic modulus was observed at higher replacement levels. This might be due to the fact that cacao powder holds more water readily than flours, thus

increasing cacao powder content increases elastic modulus values. Increasing elastic modulus indicates increasing strength of biscuit dough samples.

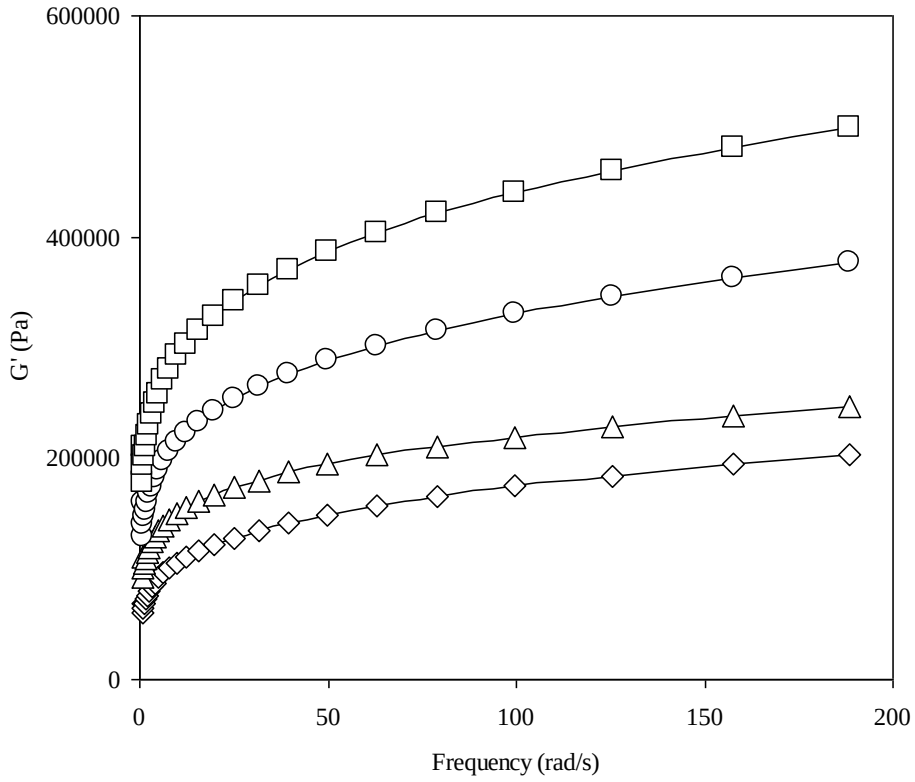

Figure 3. 8 Elastic modulus of dough samples prepared with the replacement of flour by raw hazelnut skin at different levels (0%, 10%, 15%, and 20%) where (◊): Control, (Δ): Raw hazelnut skin 10%, (○): Raw hazelnut skin 15%, (□): Raw hazelnut skin 20%.

Elastic modulus values of biscuit dough samples prepared by the replacement of flour with raw hazelnut skin at 0%, 10%, 15%, and 20% were given in Figure 3.8. Similar to cacao powder, raw hazelnut skin also resulted in harder biscuit dough samples when replaced with flour at 10%, 15%, and 20%. These results are shown in Figure 3.8.

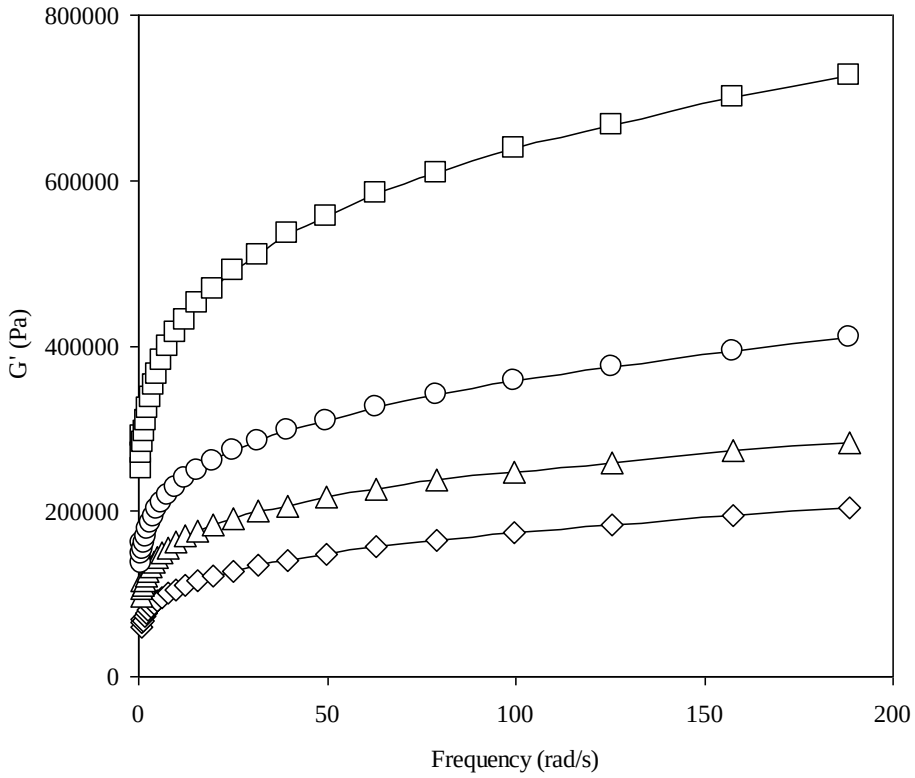

Figure 3. 9 Elastic modulus of dough samples prepared with the replacement of flour by ball milled hazelnut skin at different levels (0%, 10%, 15%, and 20%) where (◊): Control, (Δ): Ball milled hazelnut skin 10%, (○): Ball milled hazelnut skin 15%, (□): Ball milled hazelnut skin 20%.

The effects of replacement percentage of flour with ball milled hazelnut skin on elastic modulus values of biscuit dough were presented in Figure 3.9. Replacement of flour by ball milled hazelnut skin elevated elastic modulus of dough as can be seen in Figure 3.9. The highest elastic modulus values was obtained in the dough samples containing 20% ball milled hazelnut skin (w/w, based on weight flour used), probably because of their high water holding capacity as in the case of cacao powder.

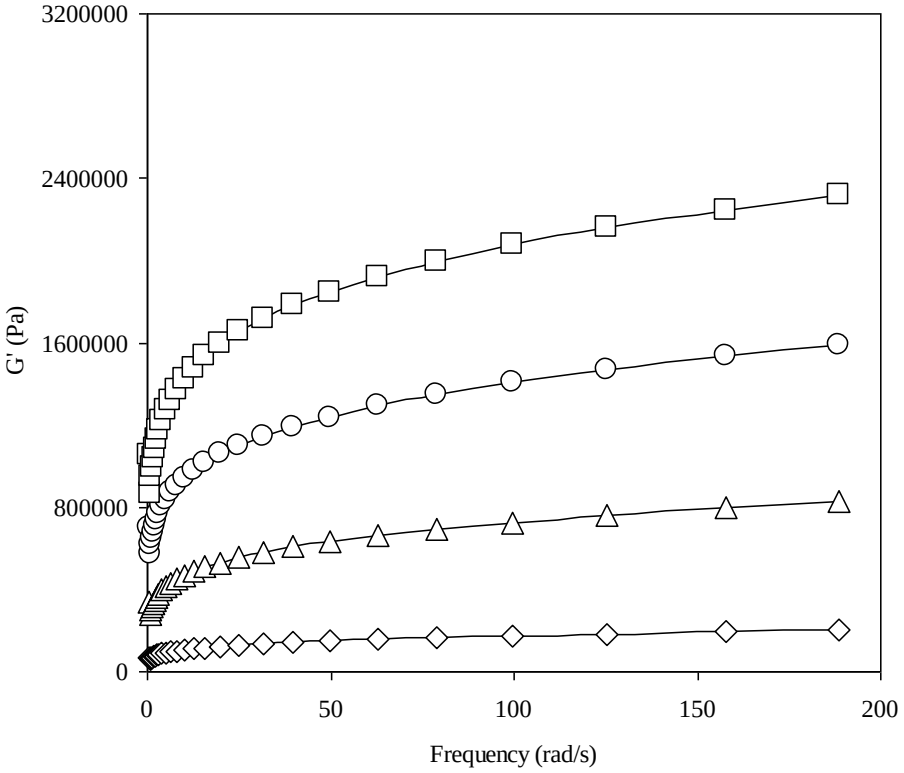

Figure 3. 10 Elastic modulus of dough samples prepared with the replacement of flour by microfluidized hazelnut skin fiber at different levels (0%, 10%, 15%, and 20%) where (◊): Control, (Δ): Microfluidized hazelnut skin fiber 10%, (○): Microfluidized hazelnut skin fiber 15%, (□): Microfluidized hazelnut skin fiber 20%.

The effects of replacement percentage of flour with microfluidized hazelnut skin fiber on elastic modulus values of biscuit dough were presented in Figure 3.10. As indicated in Figure 3.10, replacement of flour by microfluidized hazelnut skin fiber also caused to increases in elastic modulus of biscuit dough.

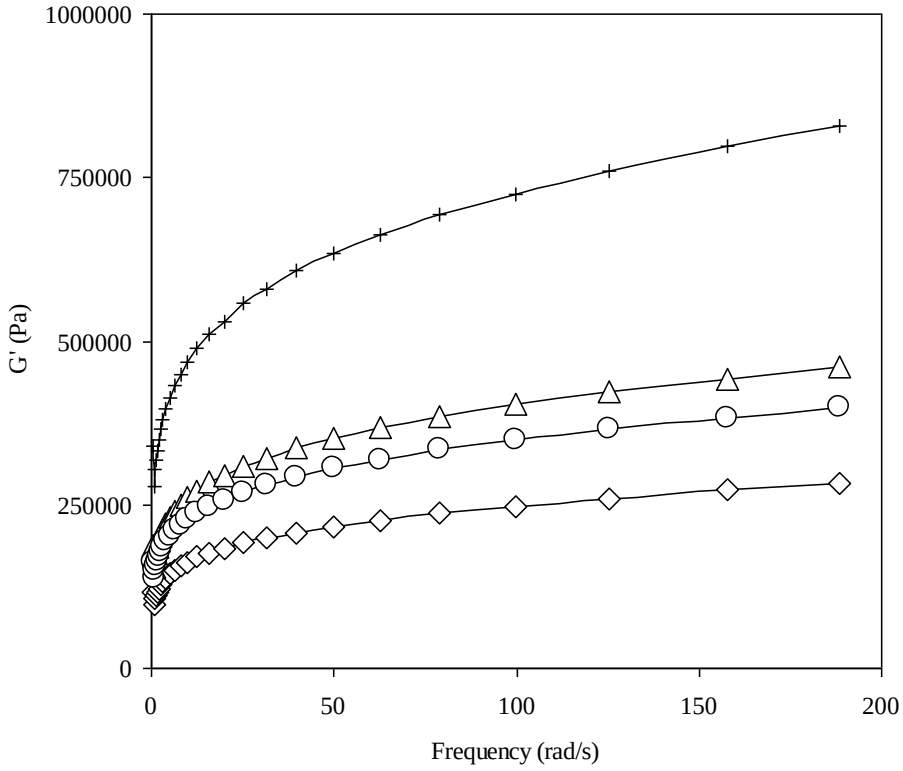

Figure 3. 11 Elastic modulus of dough samples containing different types of hazelnut skin samples, and cacao powder where (◊): Ball milled hazelnut skin 10%, (Δ): Cacao powder 10%, (○): Raw hazelnut skin 10%, (+): Microfluidized hazelnut skin fiber 10%.

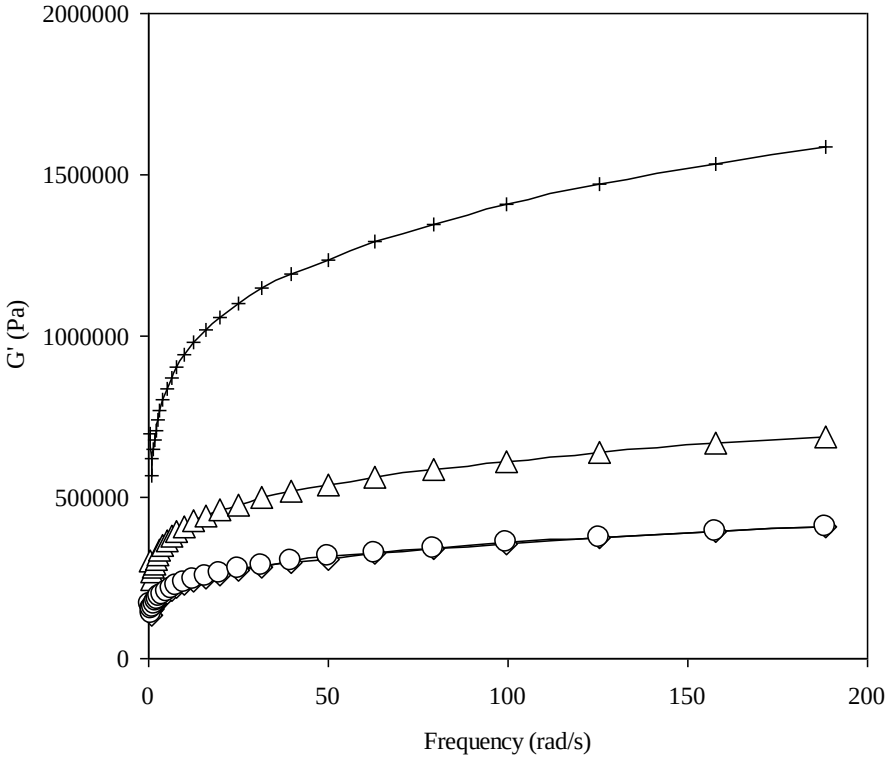

Figure 3. 12 Elastic modulus of dough samples containing different types of hazelnut skin samples, and cacao powder where (◊): Ball milled hazelnut skin 15%, (Δ): Cacao powder 15%, (○): Raw hazelnut skin 15%, (+): Microfluidized hazelnut skin fiber 15%.

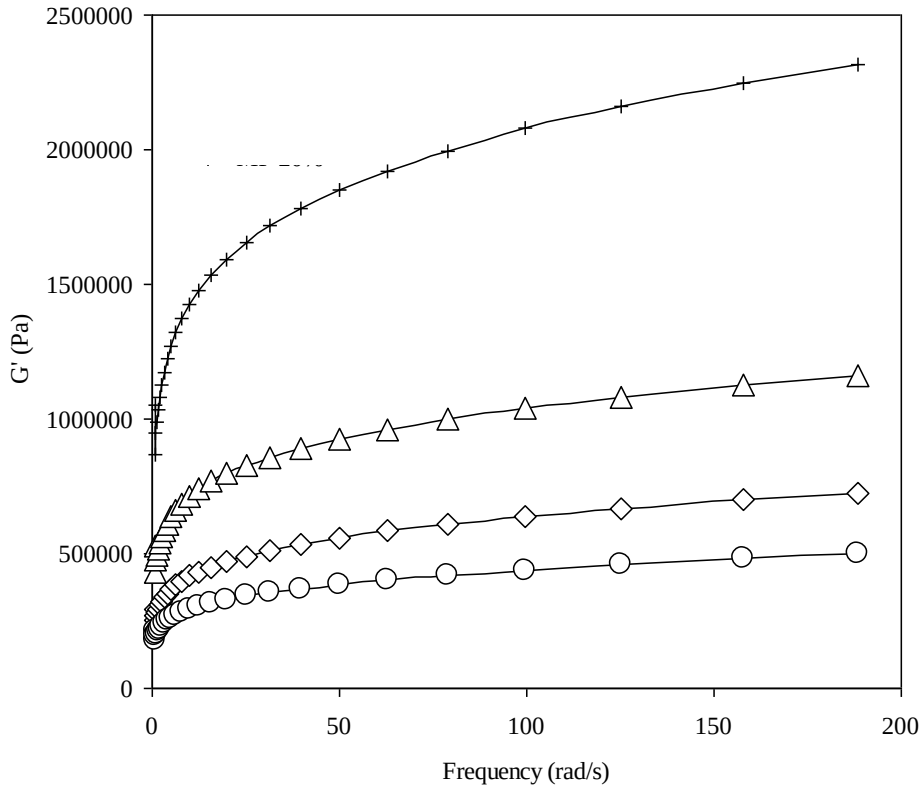

Figure 3. 13 Elastic modulus of dough samples containing different types of hazelnut skin samples, and cacao powder where (◊): Ball milled hazelnut skin 20%, (Δ): Cacao powder 20%, (○): Raw hazelnut skin 20%, (+): Microfluidized hazelnut skin fiber 20%.

The elastic modulus graphs in Figs. 3.11-3.13 show biscuit dough samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20%. According to Figs. 3.11–3.13, biscuit dough containing microfluidized hazelnut skin fiber had the highest elastic modulus. These might be due to high surface area and longer but thinner structures of microfluidized hazelnut skin fiber. Smaller particle formation increased surface area of fibers

which led to increases in water holding capacity of fibers, thus the highest elastic modulus values was obtained from samples which prepared with the replacement of flour by microfluidized hazelnut skin fiber among all biscuit dough samples. It can be concluded that smaller particle size affected the elastic behavior of biscuit dough.

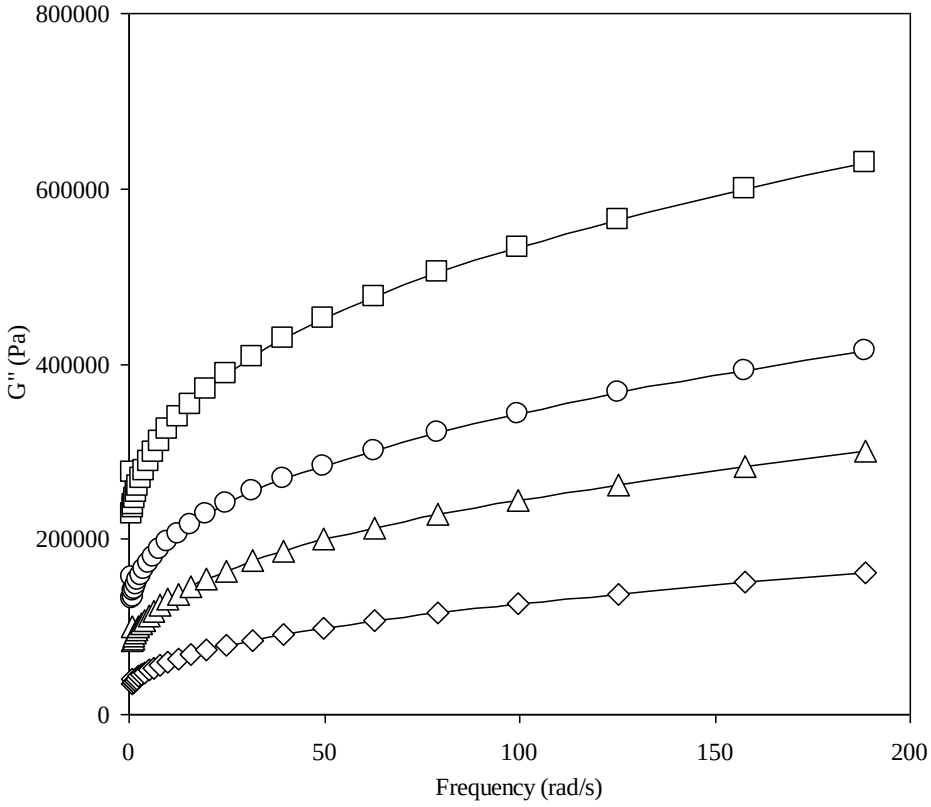

Figure 3. 14 Viscous modulus of dough samples prepared with the replacement of flour by cacao powder at different levels (0%, 10%, 15%, and 20%) where (◊): Control, (Δ): Cacao powder 10%, (○): Cacao powder 15%, (□): Cacao powder 20%.

The effects of replacement percentage of flour with cacao powder on viscous modulus values of biscuit dough were presented in Figure 3.14. According to Figure 3.14, similar to elastic modulus of dough samples the replacement of flour by cacao powder led to increases in viscous modulus of dough samples. Further increases was observed when higher replacement levels were used.

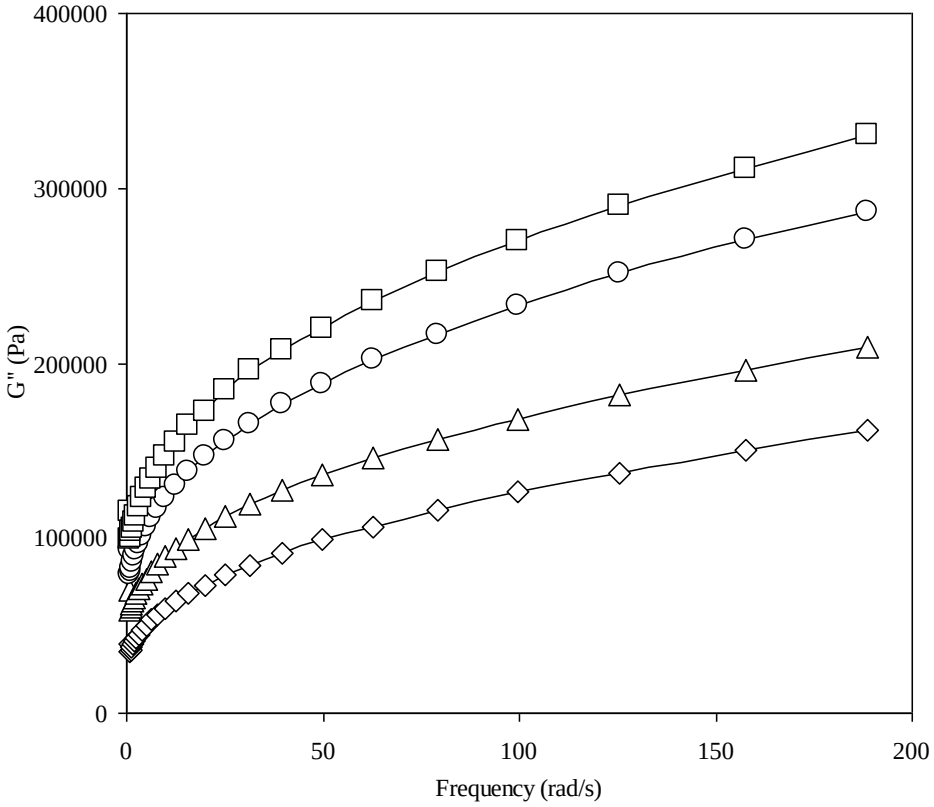

Figure 3. 15 Viscous modulus of dough samples prepared with the replacement of flour by raw hazelnut skin at different levels (0%, 10%, 15%, and 20%) where (◊): Control, (Δ): Raw hazelnut skin 10%, (○): Raw hazelnut skin 15%, (□): Raw hazelnut skin 20%.

The effects of raw hazelnut skin and ball milled hazelnut skin on viscous modulus of biscuit dough samples were presented in Figs. 3.15-3.16. Similarly, the replacement of flour by raw hazelnut skin and ball milled hazelnut skin led to increases in viscous modulus of biscuit dough. Higher replacement levels resulted in higher increases in viscous modulus of biscuit dough samples. Thus, the lowest viscous modulus value was obtained from control samples which prepared without any addition of hazelnut skin samples or cacao powder (Figs. 3.14-3.17). On the other hand, the highest viscous modulus was obtained from dough samples prepared with 20% replacement levels.

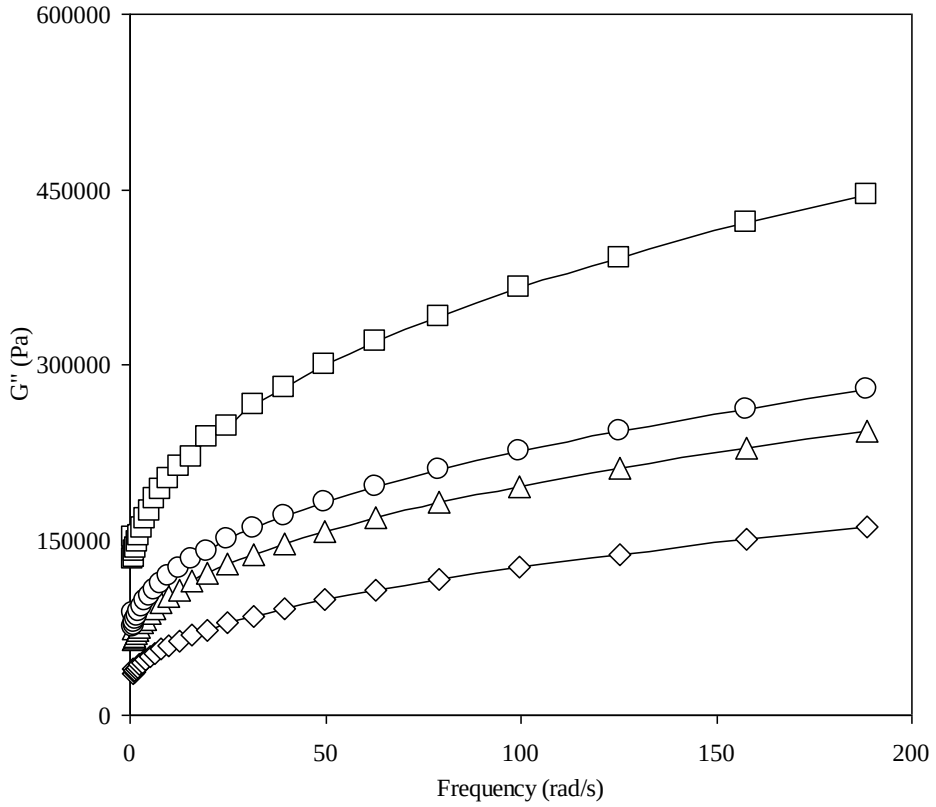

Figure 3. 16 Viscous modulus of dough samples prepared with the replacement of flour by ball milled hazelnut skin at different levels (0%, 10%, 15%, and 20%) where (◊): Control, (Δ): Ball milled hazelnut skin 10%, (○): Ball milled hazelnut skin 15%, (□): Ball milled hazelnut skin 20%.

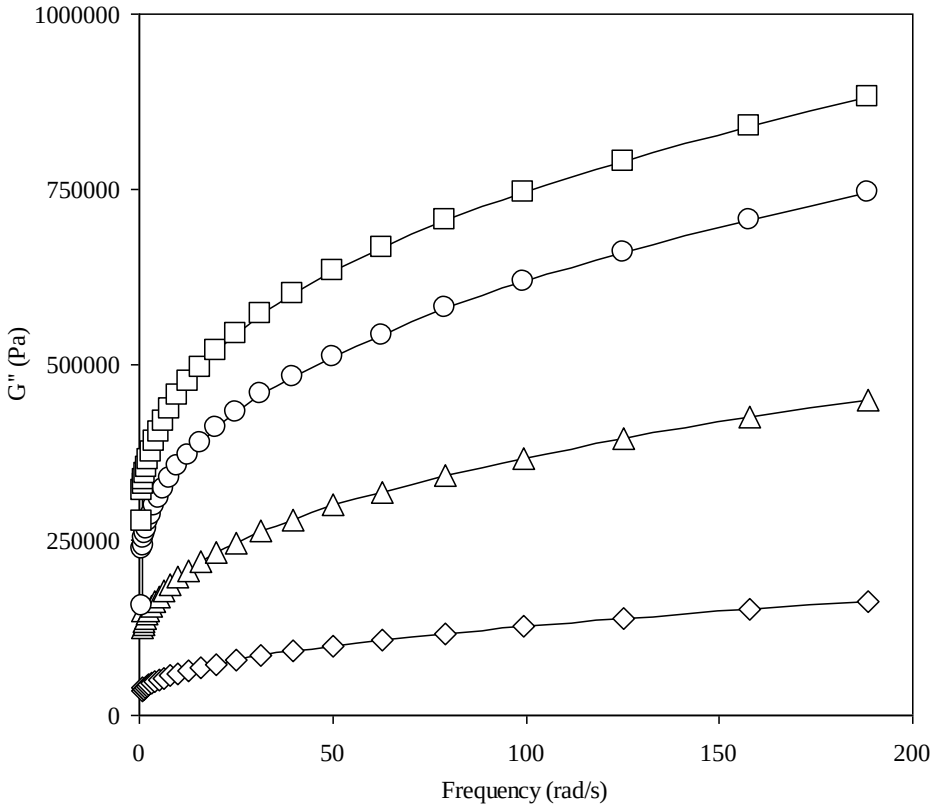

Figure 3. 17 Viscous modulus of dough samples prepared with the replacement of flour by microfluidized hazelnut skin fiber at different levels (0%, 10%, 15%, and 20%) where (◊): Control, (Δ): Microfluidized hazelnut skin fiber 10%, (○): Microfluidized hazelnut skin fiber 15%, (□): Microfluidized hazelnut skin fiber 20%.

The graph of viscous modulus of biscuit dough samples prepared by the replacement of flour with microfluidized hazelnut skin fiber at 0%, 10%, 15%, and 20% was given in Figure 3.17. Similarly, replacement of flour by microfluidized hazelnut skin fiber led to increases in viscous modulus value of biscuit dough samples due to high water holding capacity of microfluidized

hazelnut skin fiber as in the case of elastic modulus of biscuit dough samples containing microfluidized hazelnut skin fiber (Figure 3.9).

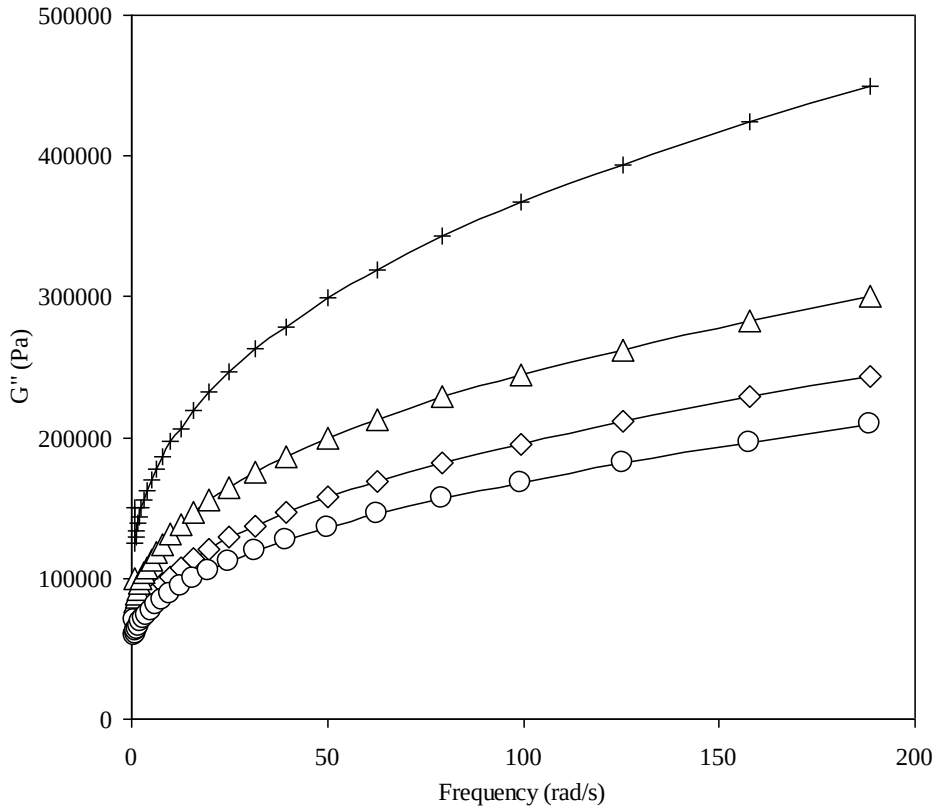

Figure 3. 18 Viscous modulus of dough samples containing different types of hazelnut skin samples, and cacao powder where (◊): Ball milled hazelnut skin 10%, (Δ): Cacao powder 10%, (○): Raw hazelnut skin 10%, (+): Microfluidized hazelnut skin fiber 10%.

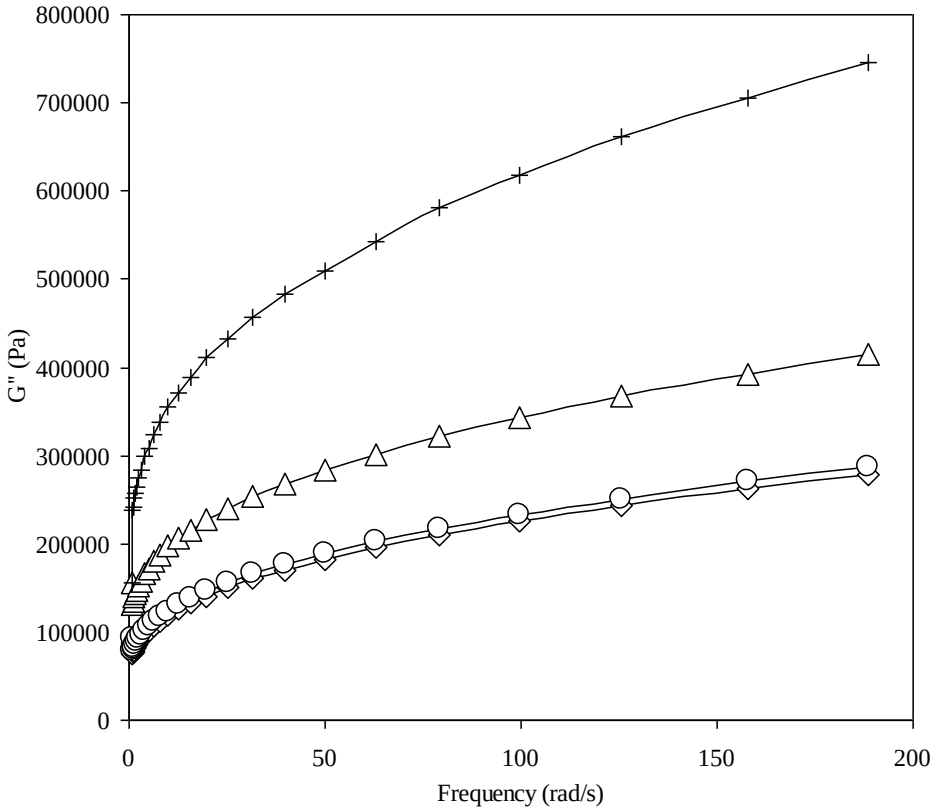

Figure 3. 19 Viscous modulus of dough samples containing different types of hazelnut skin samples, and cacao powder where (◊): Ball milled hazelnut skin 15%, (Δ): Cacao powder 15%, (○): Raw hazelnut skin 15%, (+): Microfluidized hazelnut skin fiber 15%.

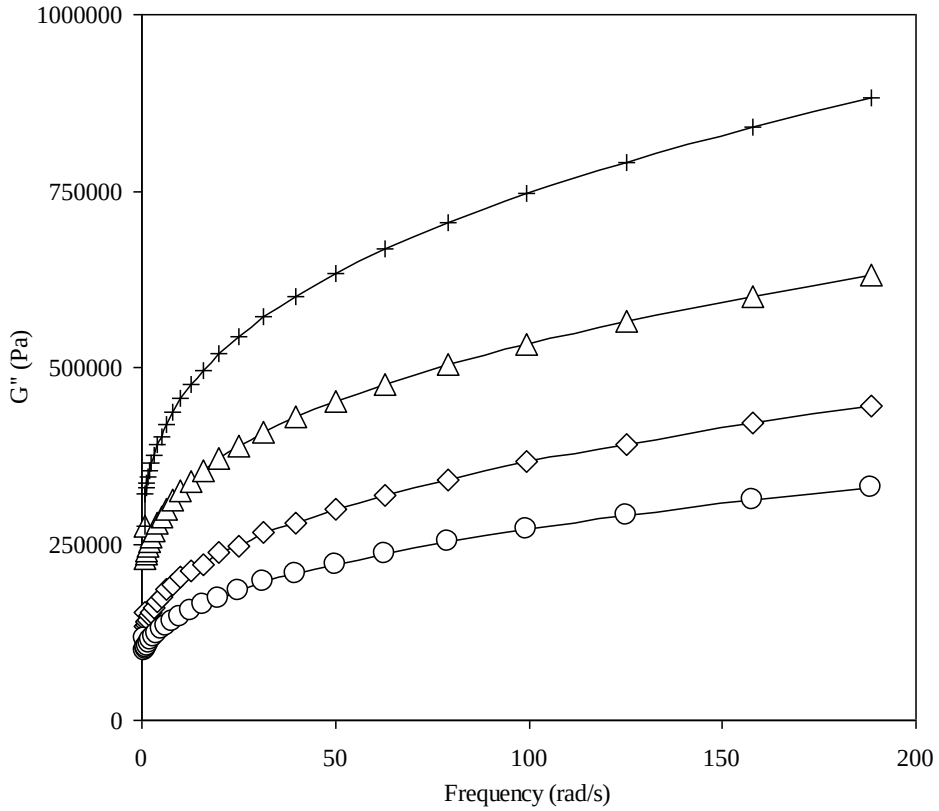

Figure 3. 20 Viscous modulus of dough samples containing different types of hazelnut skin samples, and cacao powder where (◊): Ball milled hazelnut skin 20%, (Δ): Cacao powder 20%, (○): Raw hazelnut skin 20%, (+): Microfluidized hazelnut skin fiber 20%.

The viscous modulus graphs in Figs. 3.18-3.20 show biscuit samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20%. According to Figs. 3.18-3.20 among all biscuit dough samples, the highest viscous modulus values was obtained from microfluidized hazelnut skin fiber containing dough samples.

Elastic and viscous moduli values of biscuit dough samples were shown in Figs. 3.7-3.20. In all samples, both elastic and viscous modulus values increased with increasing replacement percentage of cacao powder, and hazelnut skin samples. Seremesic et al. (2012) shared that the application of resistance starch which is defined as a dietary fiber to biscuit dough led to increases in elastic and viscous moduli of biscuits dough. Also, Sahin (2011) found that increasing wheat bran amount in cracker dough caused increases in elastic behavior of samples. Moreover, the study of Duman (2013) showed that cacao fiber attributed to elevated elastic and viscous behavior of the cake batter. Thus, the highest storage and loss modulus were obtained from cake batter containing cacao fiber.

It was observed that elastic modulus values were higher than viscous modulus values. This means that biscuit dough had a solid, viscoelastic behavior under the testing conditions (Sivaramakrishnan et al., 2004). As the replacement percentage of flour by hazelnut skin samples or cacao powder increases, elastic and viscous modulus were increased. The highest elastic and viscous modulus values were obtained from biscuit dough samples containing 20% microfluidized hazelnut skin fiber. The observed elastic and viscous modulus values were in the following increasing order: control, raw hazelnut skin, ball milled hazelnut skin, cacao powder, and microfluidized hazelnut skin fiber. The lowest elastic and viscous modulus were obtained from control biscuit dough samples among all samples by referring to having the most viscous structure. Duman (2013) found similar results in her study. It was observed that presence of cacao fiber and increasing concentration of cacao fiber led to increases in storage and loss modulus. In the study of Cikrikci (2013), it was found that cacao powder, raw hazelnut skin, ball milled hazelnut skin, and microfluidized hazelnut skin fiber addition to cake batter caused increases in storage and loss modulus. Also, sharp increase in G' and G'' was seen by the application of microfluidized hazelnut skin fiber to cake samples. The results of Cikrikci (2013) were in agreement with the results obtained during this study. It has been

demonstrated, the highest G' and G'' were obtained from cake batter prepared by replacing the flour with 20% microfluidized hazelnut skin fiber (w/w based on wheat flour used). These results were related to hazelnut skin fiber water holding capacity, which is also found to be in agreement with our study. It can be explained by the entanglement of fibers in flour which led to increases elastic and viscous modulus values of dough (Demirkesen et al., 2010). Entanglement can be explained as a network structure of dough and microfluidized hazelnut skin fiber forms a network in biscuit dough samples and this gives the familiar structure of dough. It can be concluded that formation of finely divided fibrous structure was enhanced by microfluidization process which led to long fiber with greater surface area.

3.2.2. Dough Texture

The extensibility of dough was analyzed by using texture analyzer. Both sides of biscuit dough, which were lubricated with oil to minimize the friction effect on stress, were contacted with disk and plunger. Lubrication with oil was done because nonlubricated surface needed higher force than lubricated surface to achieve same degree of compression. Therefore, the surface was required to be lubricated to get more accurate rheological data from compression test (Bourne, 1982). During this study, strain rate increases and changing in viscosity profile was analyzed. Lubricated squeezing flow was used to observe the effect of the replacement of flour by cacao powder or hazelnut skin samples and percentages of replacement levels on biaxial extensional viscosity of biscuits dough. This method was used to several semi-solid food products such as cheeses (Lee & Inglett, 2006), peanut butter (Lee & Inglett, 2006), and wheat flour doughs (Lee & Inglett, 2006).

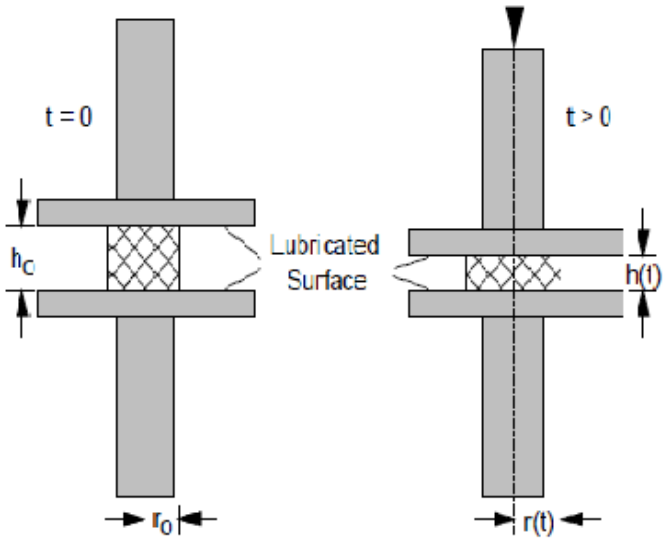

Figure 3. 21 Dough biaxial viscosity measurement method (Steffe, Ph, & Press, 1996)

Lubricated squeezing flow between parallel plates can be used to find biaxial extensional viscosity of biscuit dough samples. Figure 3.21 shows dough biaxial extensional viscosity measurement system where the lower plate is fixed and the upper plate moves vertically downward. During squeezing, the height of the sample decreasing with increasing dough contact area with plate. During squeezing also the cylindrical shape is maintained. Dough samples are lubricated with a lower viscosity liquid (Steffe et al., 1996). In this study, lubrication was done with oil which may appear to be self-lubricating. Proper lubrication is important to get more accurate results from biaxial extensional viscosity experiment. If dough does not lubricated properly, more force would be needed to achieve same degree of deformation.

Biaxial extensional viscosity of biscuit dough samples was calculated from the net stretching stress and the strain rate which depending on the degree of fill between the plates during analysis.

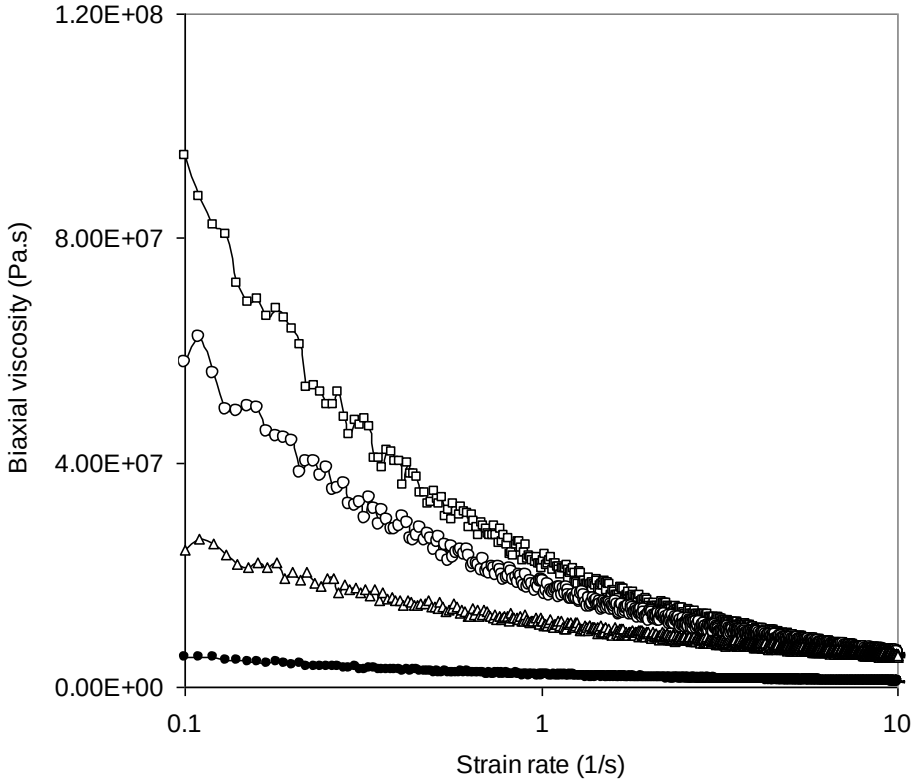

Figure 3. 22 Biaxial extensional viscosity of dough samples prepared with the replacement of flour by cacao powder at different levels (0%, 10%, 15%, and 20%) where (●): Control, (Δ): Cacao powder 10%, (○): Cacao powder 15%, (□): Cacao powder 20%.

The effects of cacao powder addition on biaxial extensional viscosity values of biscuit dough samples are presented in Figure 3.22. According to Figure 3.22, at the lowest strain rate the highest biaxial extensional viscosity value was obtained from biscuit dough containing cacao powder with 20% replacement of flour. Also, increasing strain rate caused decreases in viscosity. Demirkesen, (2013) study showed that viscosity decreases with increasing shear stress due to disruption of interactions between the components for pseudo-plastic materials.

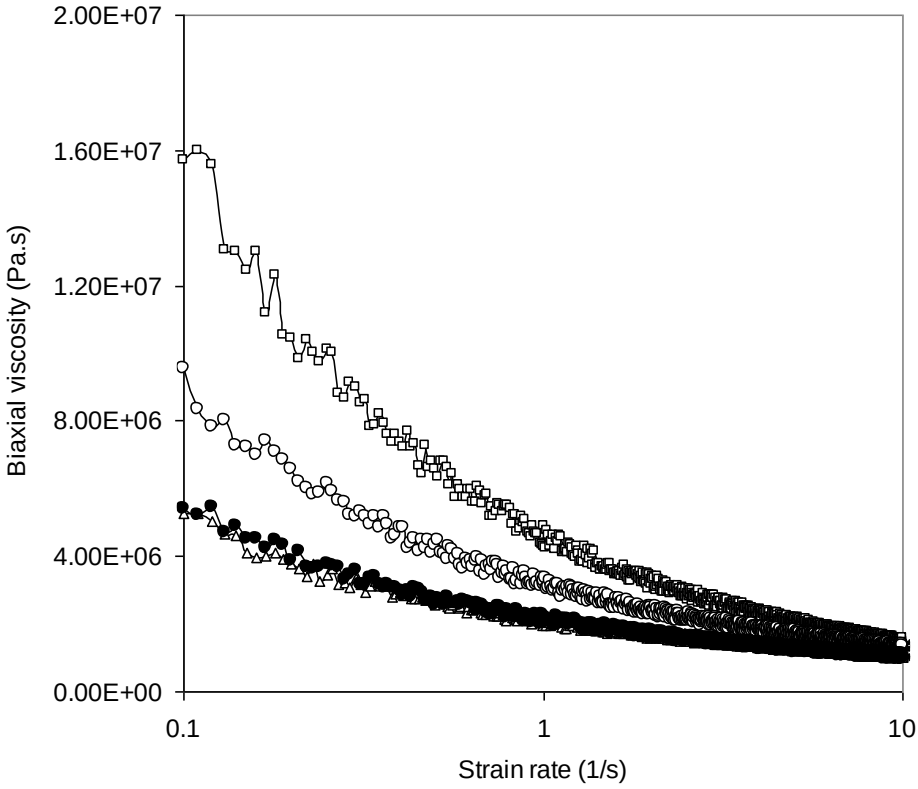

Figure 3. 23 Biaxial extensional viscosity of dough samples prepared with the replacement of flour by raw hazelnut skin at different levels (0%, 10%, 15%, and 20%) where (●): Control, (Δ): Raw hazelnut skin 10%, (○): Raw hazelnut skin 15%, (□): Raw hazelnut skin 20%.

Figure 3.23 compared biaxial extensional viscosity of biscuit dough samples prepared with the replacement of flour by raw hazelnut skin at different levels (0%, 10%, 15%, and 20%). At the lowest strain rate, the biaxial extensional viscosity value of control biscuit samples and biscuit dough prepared with raw hazelnut skin with 10% replacement of flour were relatively same and their decreasing magnitude of biaxial extensional viscosity with increasing strain

rate was approximately equal to each other. At the lowest strain rate, biaxial extensional viscosity value of biscuit dough was closely related with amount of raw hazelnut skin exist within the dough, but increasing strain rate caused to decreases in dependency of biaxial extensional viscosity to ingredients of biscuit dough (Figure 3.23).

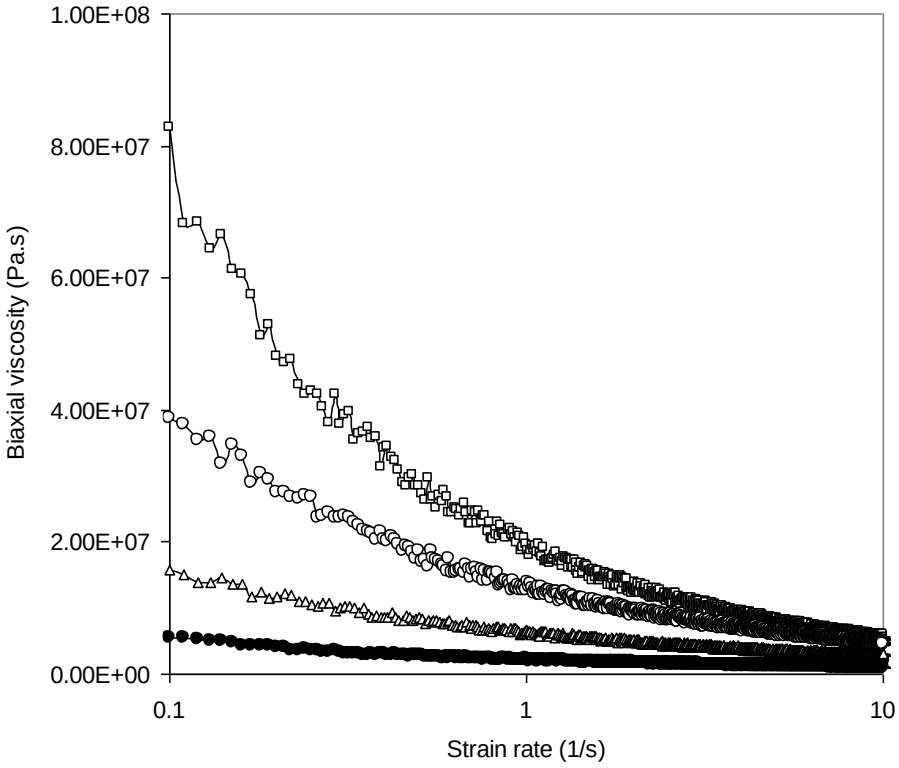

Figure 3. 24 Biaxial extensional viscosity of dough samples prepared with the replacement of flour by ball milled hazelnut skin at different levels (0%, 10%, 15%, and 20%) where (●): Control, (Δ): Ball milled hazelnut skin 10%, (○): Ball milled hazelnut skin 15%, (□): Ball milled hazelnut skin 20%.

The impacts of ball milled hazelnut skin addition on biaxial extensional viscosity values of biscuit dough samples are shown in Figure 3.24. At the lowest strain rate, the highest biaxial extensional viscosity was obtained from biscuit dough containing ball milled hazelnut skin with 20% replacement of flour. As expected, increasing concentration of ball milled hazelnut skin led to increases in biaxial extensional viscosity of biscuit dough samples. Also, increasing strain rate led to decreases in biaxial extensional viscosity (Figure 3.24).

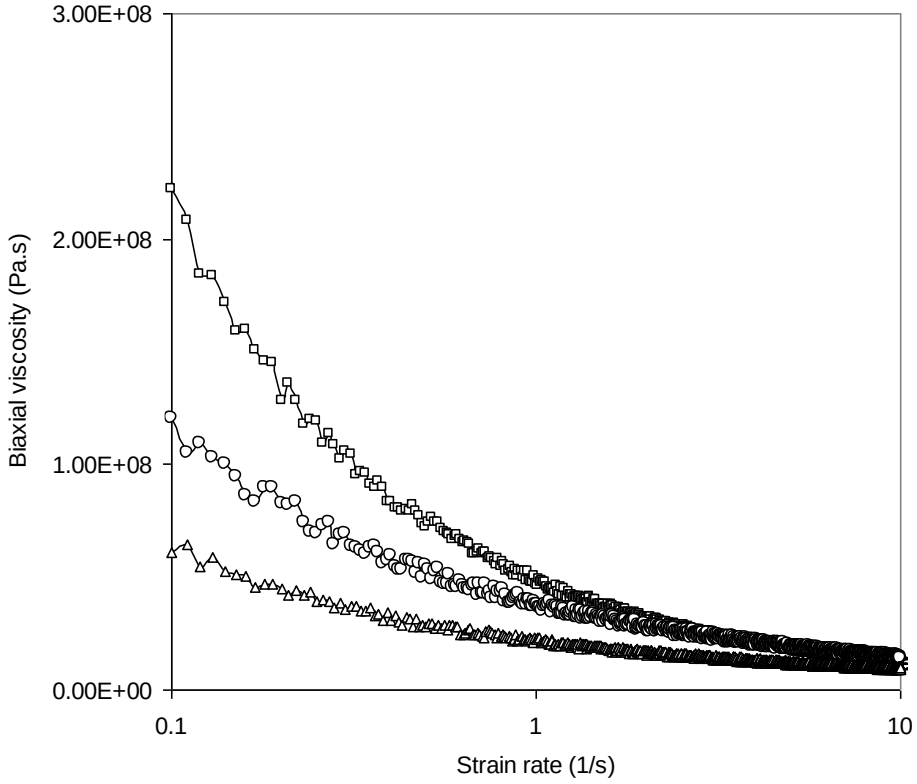

Figure 3. 25 Biaxial extensional viscosity of dough samples prepared with the replacement of flour by microfluidized hazelnut skin fiber at different levels (10%, 15%, and 20%) where (Δ): Microfluidized hazelnut skin fiber 10%, (○): Microfluidized hazelnut skin fiber 15%, (□): Microfluidized hazelnut skin fiber 20%.

The graph of biaxial extensional viscosity of biscuit dough samples prepared by the replacement of flour with microfluidized hazelnut skin fiber at 0%, 10%, 15%, and 20% is given in Figure 3.25. According to Figure 3.25, biscuit dough which was prepared by replacing the flour with microfluidized hazelnut skin fiber (20%) had the highest biaxial extensional viscosity value at the lowest

strain rate and biaxial extensional viscosity was decreased with increasing strain rate.

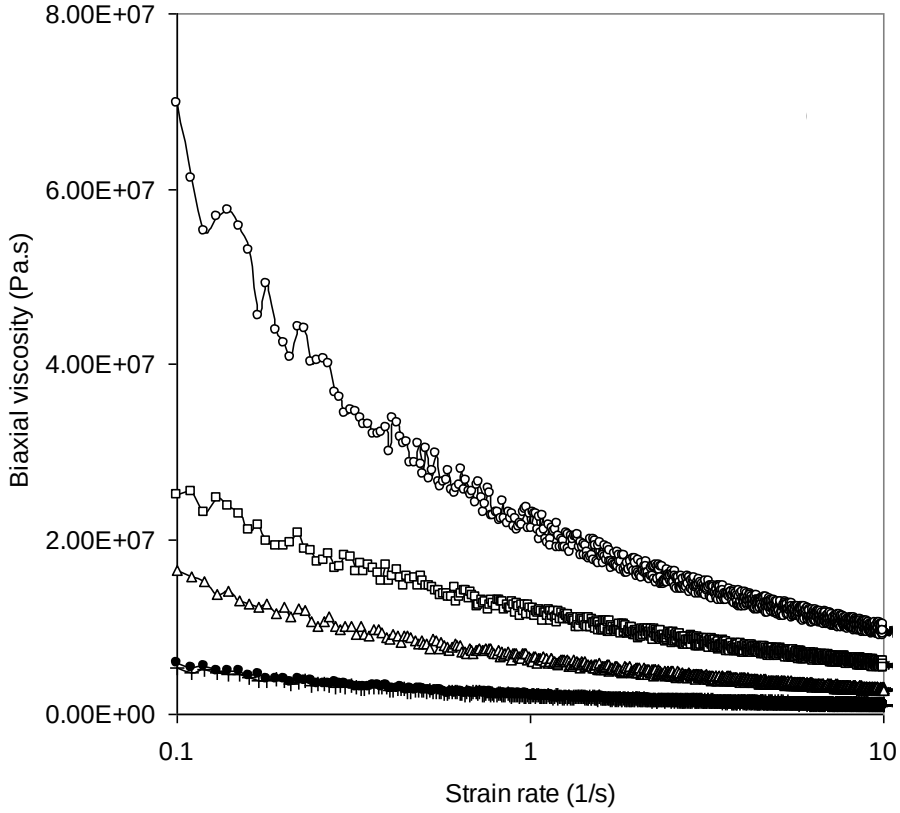

Figure 3. 26 Biaxial extensional viscosity of dough samples containing different types of hazelnut skin samples, and cacao powder where (●): Control, (○): Microfluidized hazelnut skin fiber 10%, (□): Cacao powder 10%, (Δ): Ball milled hazelnut skin 10%, (+): Raw hazelnut skin 10%.

The biaxial extensional viscosity graph in Figure 3.26 shows biscuit samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, and 10%. As shown in Figure 3.26, among all samples prepared with 10% flour replacement levels, the highest biaxial extensional viscosity was obtained from samples containing microfluidized hazelnut skin fiber. On the other hand, the lowest biaxial extensional viscosity values was obtained from control samples followed by raw hazelnut skin containing biscuit dough samples. Processing with microfluidizer increased the viscosity of hazelnut skin fiber noticeably. This can be explained by water holding capacity of fiber. Microfluidized hazelnut skin fiber had smaller particle size and branched structure, so it can be said that higher surface area was obtained after microfluidization process which can be seen in Figs. 3.4-3.6. This was resulted in increases in water holding capacity resulting in high viscosity values. Similar result was observed in the study of Duman (2013).

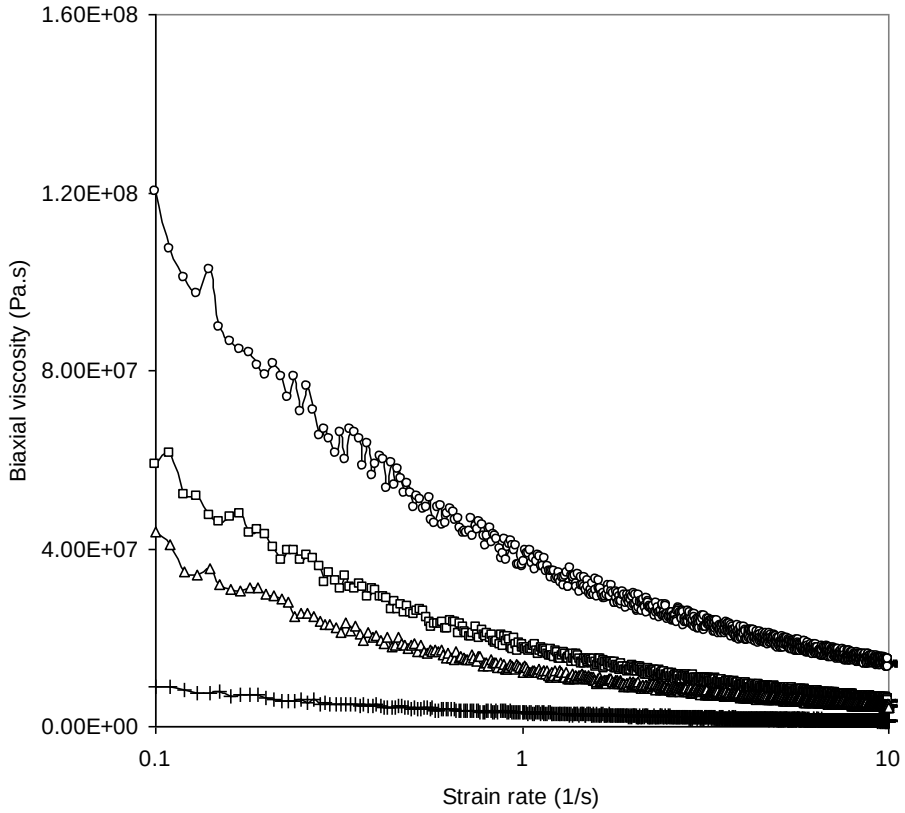

Figure 3. 27 Biaxial extensional viscosity of dough samples containing different types of hazelnut skin samples, and cacao powder where (○): Microfluidized hazelnut skin fiber 15%, (□): Cacao powder 15%, (Δ): Ball milled hazelnut skin 15%, (+): Raw hazelnut skin 15%.

The biaxial extensional viscosity graph in Figure 3.27 shows biscuit samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 15%. As in Figure 3.27 biaxial extensional viscosity value was the highest for biscuit dough containing microfluidized hazelnut skin fiber among all biscuit dough samples prepared with 15% flour replacement.

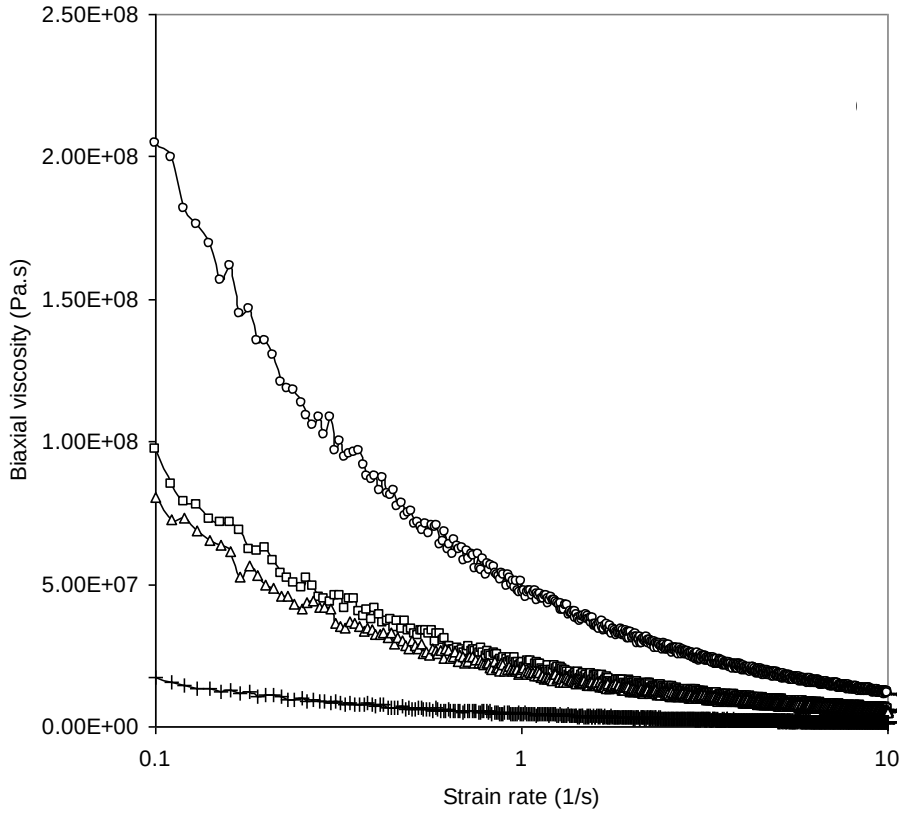

Figure 3. 28 Biaxial extensional viscosity of dough samples containing different types of hazelnut skin samples, and cacao powder where (○): Microfluidized hazelnut skin fiber 20%, (□): Cacao powder 20%, (Δ): Ball milled hazelnut skin 20%, (+): Raw hazelnut skin 20%.

Figure 3.28 shows the biaxial extensional viscosity graph of biscuit samples prepared by 20% replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder. Similarly, the highest biaxial extensional viscosity was obtained from dough samples made of 20% replacement of flour by microfluidized hazelnut skin fiber. On the other hand, the lowest biaxial extensional viscosity was observed in dough

samples prepared with 20% replacement of flour by raw hazelnut skin. According to Figs. 3.22–3.28 the highest value of biaxial extensional viscosity was obtained from biscuit dough prepared by replacing the flour with 20% microfluidized hazelnut skin fiber and the lowest value was obtained from control biscuit dough samples. Biaxial extensional viscosity is also called as extensional viscosity which is important for biscuit quality such as spreading ratio. As the extensional viscosity increased, spreading ratio decreased in this study, which can be seen in part 3.2.2 where the lowest diameter of biscuits was obtained from biscuits made of 20% replacement of flour by microfluidized hazelnut skin fiber. HadiNezhad & Butler (2008) suggested that the correlation between dough extensional viscosity and cookie final diameter is important. The study shows that large differences are observed in extensional viscosity of cookie dough which were done with soft wheat and hard wheat flours. Biscuits prepared by 20% replacement of flour by microfluidized hazelnut skin fiber had the lowest diameter value, this can be explained by its biaxial extensional viscosity. Biscuit dough containing 20% microfluidized hazelnut skin fiber had the highest biaxial extensional viscosity and it was inversely proportional to spreading character of biscuits. Ktenioudaki et al. (2010) depicted that dough rising property was limited by increasing biaxial extensional viscosity. There was a negative correlation between biaxial extensional viscosity and loaf volume.

The shear stress versus shear rate data for all formulations containing hazelnut skin samples or cacao powder at 25^0C were fitted Power Law model. Figs. 3.29-3.30 show the power law model parameters for dough samples. According to Figs. 3.29-3.30 the biscuit dough samples obeys Power Law which is valid for Non-Newtonian fluid. They can be whether shear thinning or shear thickening.

$$\tau = K\,(\dot{\gamma})^{n} \qquad (3.1)$$

Where

K = the consistency coefficient

n = flow behavior index

For shear thinning (pseudo-plastic) fluids flow behavior index (n) smaller than 1, and for shear thickening fluids flow behavior index (n) larger than 1 (Şahin & Şümnü, 2006). Figs. 3.29-3.31 show the extensional consistency coefficient, extensional thinning index, and maximum extensional viscosity of biscuit dough samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20%. According to Figure 3.30, the biscuit dough showed pseudo-plastic behavior because flow behavior index values of biscuit dough ranging from 0.45 to 0.75. The lowest flow behavior index was obtained from biscuit dough samples prepared by replacing the flour with 20% microfluidized hazelnut skin fiber. According to Şahin & Şümnü (2006) viscosity decreases with increasing strain rate for pseudo-plastic fluids. By looking Figs. 3.22-3.28 increasing strain rate led to decreases in biaxial extensional viscosity as expected. Similar result was observed in Lee & Inglett (2006) study, power law relationship between biaxial extentional viscosity and strain rate was found for cookies dough which have different level of fat. Biaxial consistency coefficient was used for observing deformation relationship of biscuit dough. The higher biaxial consistenceny ceoefficient indicates that resistance to dough deformation is higher which also affects the raisen of dough. On the other hand, the lower biaxial consistenceny ceoefficient indicates that dough can be easily deformed under stress (Lee & Inglett, 2006). According to Figure 3.29, the highest biaxial consistency coefficent belonged to biscuit dough prepared by replacing the flour with 20% hazelnut skin fiber.

It can be said that control biscuit samples deformed easily when compared with biscuit dough samples containing other hazelnut skin samples or cacao powder. According to Figure 3.31, the highest extensional viscosity was obtained from biscuit dough containing 20% microfluidized hazelnut skin fiber and the lowest was obtained from control biscuit samples.

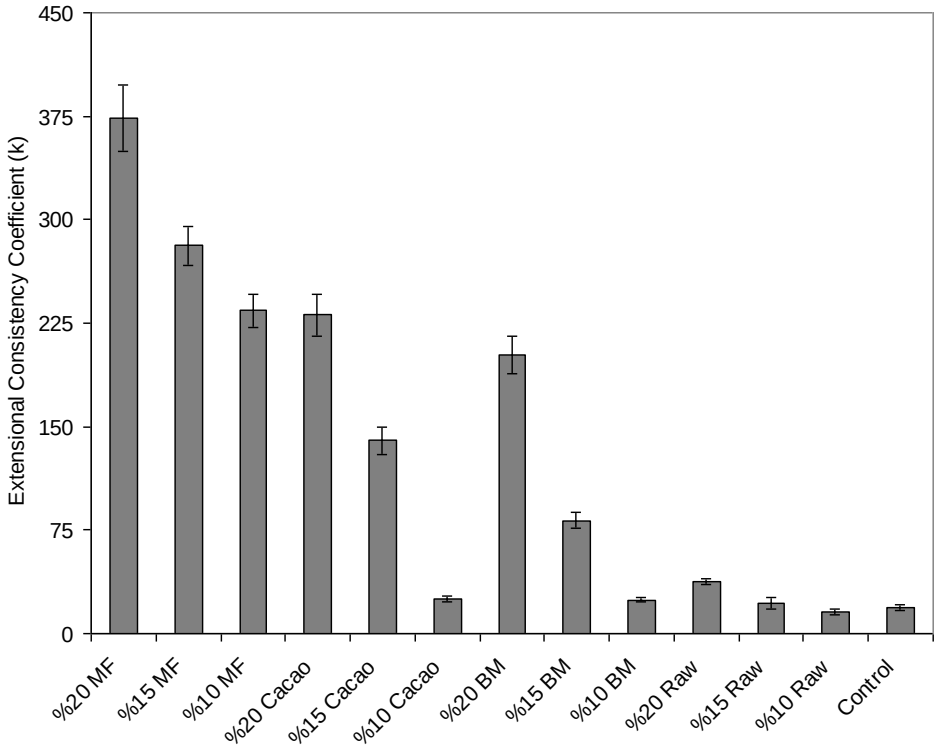

Figure 3. 29 Extensional consistency coefficient of biscuit dough samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20%. Bars indicate standard deviation of the replicates.

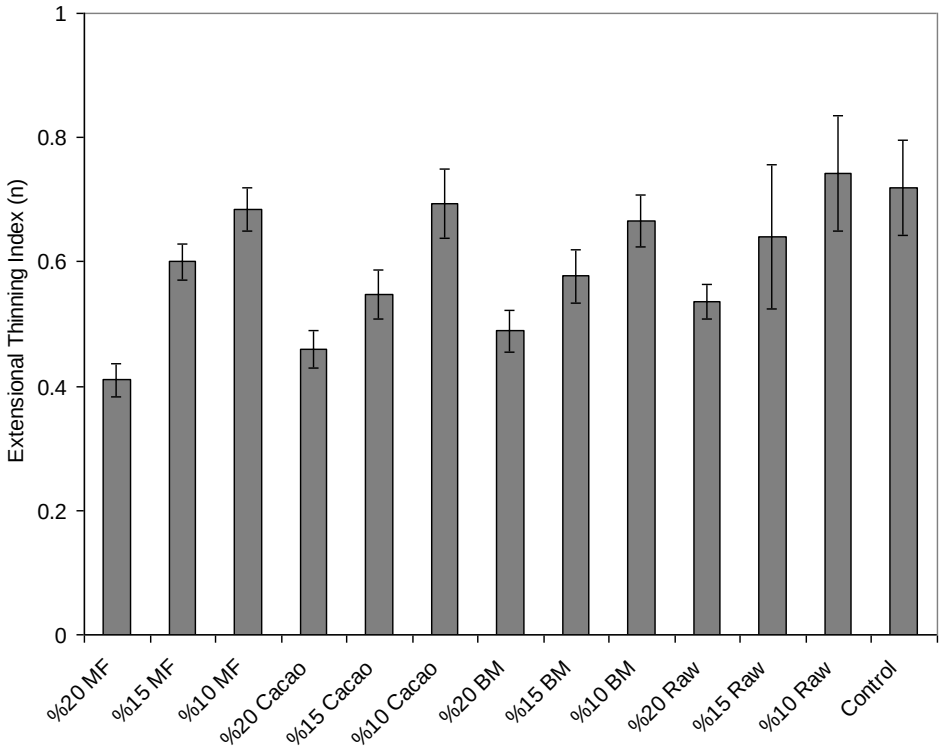

Figure 3. 30 Extensional thinning index of biscuit dough samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20%. Bars indicate standard deviation of the replicates.

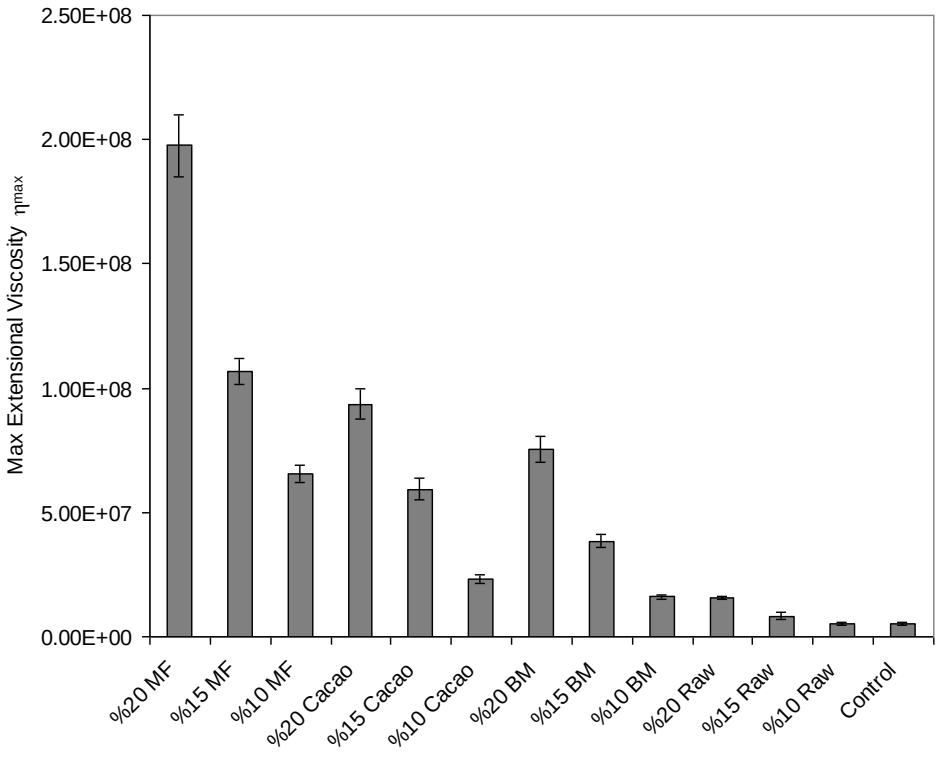

Figure 3. 31 Maximum extensional viscosity of biscuit dough samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20%. Bars indicate standard deviation of the replicates.

3.3. Evaluation of Biscuits

3.3.1. Biscuit Moisture Content

The effect of replacement of flour by hazelnut skin samples or cacao powder on moisture content were presented in Figure 3.32. According to ANOVA

results, there were no significant differences between biscuits prepared by replacing the flour with raw hazelnut skin, ball milled hazelnut skin, and cacao powder, the microfluidized hazelnut skin fiber containing biscuit samples was significantly different from other biscuit samples. Also, there was significant differences between flour replacement percentages ($p \leq 0.05$). Moisture content values of biscuit samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20% were presented in Figure 3.32. According to Figure 3.32, the replacement of flour by microfluidized hazelnut skin fiber led to increases in moisture content of biscuits. This was an expected result, because addition of microfluidized hazelnut skin fiber caused to increases in water holding capacity. The moisture content of biscuit samples were increased with increasing flour replacement percentages. The highest moisture content of biscuit samples were obtained by replacing flour with 20% microfluidized hazelnut skin fiber. The lowest level of moisture content was obtained from 10% replacement of flour with ball milled hazelnut skin. Biscuit samples containing the smallest particle size (cacao powder, raw hazelnut skin, ball milled hazelnut skin, and hazelnut skin fiber produced by microfluidization process) have the highest moisture content. Sowbhagya et al. (2007) stated that the hydration properties increases with decreasing particle size. Since the smaller fiber particles enhances the surface area for better water absorption, they have higher swelling capacity. Also, they have higher packing density.

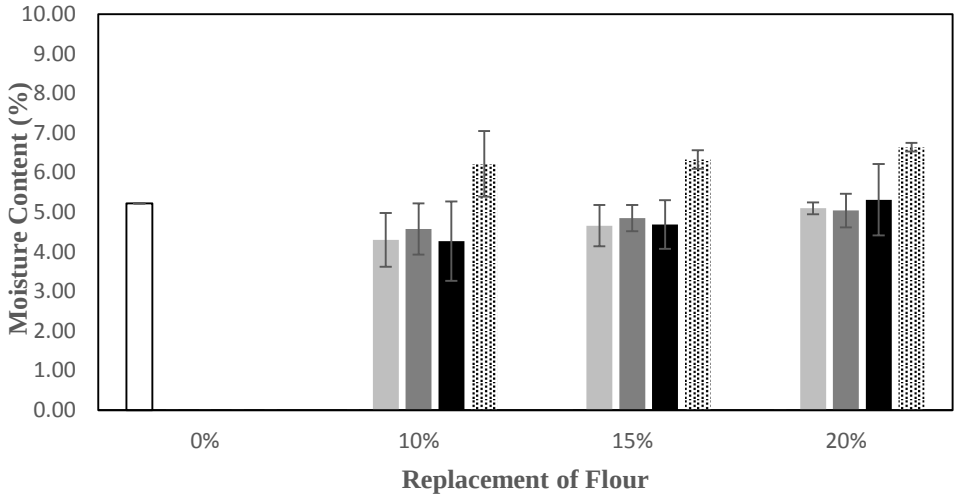

Figure 3. 32 Moisture content values of biscuit samples where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark grey bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates

3.3.2. Physical Characteristics of Biscuits

Biscuits prepared by replacing the flour with hazelnut skin samples and cacao powder (0%, 10%, 15%, and 20%) were evaluated for their various physical characteristics (thickness and diameter values). According to ANOVA results, there were significant differences between biscuit samples containing microfluidized hazelnut skin fiber, ball milled hazelnut skin, and cacao powder in terms of thickness and diameter values of biscuit samples. However, there was no significant difference between biscuit samples containing ball milled hazelnut skin and raw hazelnut skin, and between raw hazelnut skin and cacao

powder in terms of thickness value of biscuit samples. Moreover, there was a significant difference between biscuit samples containing hazelnut skin samples and cacao powder in terms of their diameter value. Also, percentages of flour replacement significantly affected both thickness and diameter values of biscuits ($p \leq 0.05$).

Thickness values of biscuit samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20% were presented in Figure 3.33. According to Figure 3.33, the thickness values of biscuits increased with the increasing amount of cacao powder or hazelnut skin samples in the biscuit samples. As can be seen in this figure, the highest thickness values of biscuits was observed in biscuit samples containing microfluidized hazelnut skin fiber. This was an expected result, since biscuits became harder when thickness was getting larger (Sudha et al., 2007). This results were in agreement with texture analysis results for biscuit samples except biscuit samples containing microfluidized hazelnut skin fiber. Since moisture content also affect the hardness of biscuits, the hardness value of biscuits containing microfluidized hazelnut skin fiber had the lowest value.

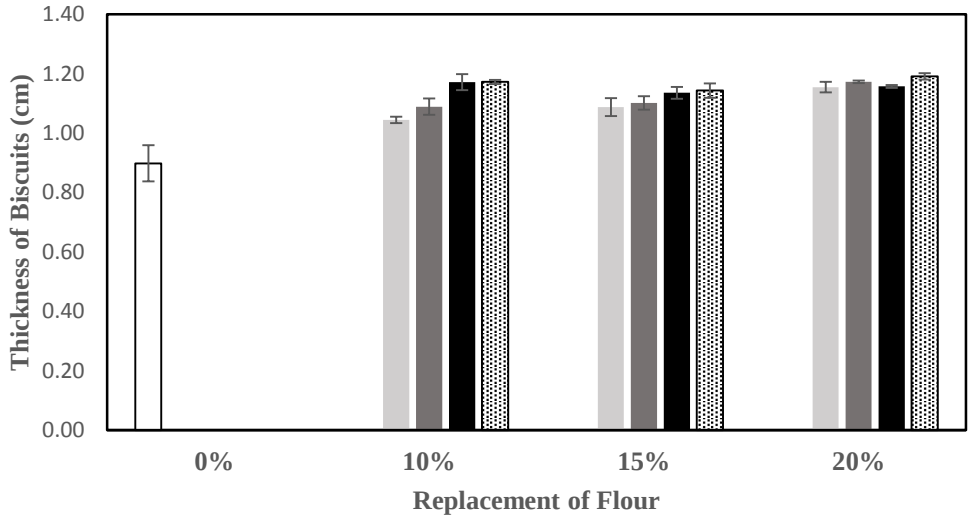

Figure 3. 33 Thickness of biscuit samples where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark grey bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

Diameter values of biscuit samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20% were presented in Figure 3.34. According to Figure 3.34, if incorporation of cacao powder or hazelnut skin samples increases, spreading character of biscuits decreased. Manohar & Rao (1997) study showed that hardness of biscuit increase when spreading character of biscuit samples decreased. This was an expected result, because hardness of biscuit samples increases by increasing thickness and spreading character inversely proportional to thickness. According to Figs. 3.33-3.34, average thickness values of control samples was 0.90 ± 0.06 cm which was the lowest value and the average diameter values of them was 7.21 ± 0.03 cm which was

the highest value. The average thickness value of biscuit samples prepared with by replacing the flour with 20% microfluidized hazelnut skin fiber was 1.19 ± 0.01 cm which was the highest average thickness value and the average diameter values of them was 6.21 ± 0.04 cm which was also the lowest average value. Ajila et al. (2008) observed that the addition of mango peer powder (to enhance fiber content) to biscuits led to decreases in spreading ratio (diameter) of biscuit samples. However, according to Ajila et al. (2008) study no significant difference was observed up to 10% level of fiber addition. Differences are observed after addition of 15% and 20% of fiber, this might be due to dilution of gluten found in wheat flour. Also, the hardness of biscuits increased with increasing percentage of flour replacement. The expected results were obtained in this study which can be seen in 3.3.4. Fresh Biscuit Breaking Strength.

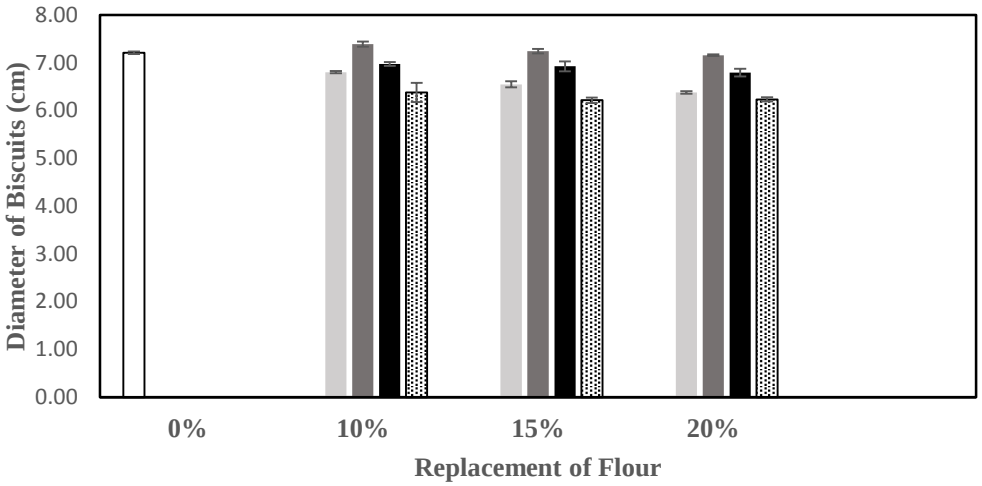

Figure 3. 34 Diameter of biscuit samples where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates

Yadav et al. (2012) reported that the increasing number of hydrophilic sites which is available for competing for the limited free water in biscuits leads to decreases in spreading character of biscuits. According to Figure 3.34, increasing flour replacement percentage led to decreases of diameter values of biscuit samples. Therefore, it can be concluded that number of hydrophilic sites decreases with the increasing flour replacement percentage.

3.3.3. Biscuit Color Determination

The effect of cacao powder or hazelnut skin samples addition on color of biscuits is shown in Figure 3.35. According to ANOVA results, both biscuit types and the flour replacement percentages affected the color results, significantly ($p \leq 0.05$). Addition of cacao powder or hazelnut skin samples to biscuit samples as an ingredient gave darker color to biscuit samples. According to Figure 3.35 the darkest color which means having the highest ΔE value was obtained from biscuits containing microfluidized hazelnut skin fiber at all flour replacement percentages (10%, 15%, and 20%). At 10%, 15%, and 20% flour replacement with cacao powder, ΔE (taking the control biscuit color as reference) values were obtained as 24.90 ± 3.24, 27.96 ± 2.16, and 29.16 ± 2.87, respectively. Whereas, biscuits prepared with raw hazelnut skin gave ΔE values as 14.33 ± 3.93, 20.78 ± 1.63, and 21.06 ± 3.67 at the same replacement levels. On the other hand, biscuits containing ball milled hazelnut skin gave ΔE values as 20.54 ± 1.28, 22.52 ± 0.46, and 22.84 ± 3.33 with the increasing flour replacement percentages, while biscuits prepared with hazelnut skin fiber handled by microfluidization process had ΔE values as 24.23 ± 1.81, 32.2 ± 1.85, and 32.43 ± 2.78 at same flour replacement levels. ΔE values of biscuits prepared with microfluidized hazelnut skin fiber were significantly the highest among the ΔE values of the other biscuit samples ($p \leq 0.05$). Similarly, Cikrikci (2013) observed that hazelnut skin fiber processed by microfluidization gave the darkest color to cake samples. The color changes of biscuits was due to original color of cacao powder and hazelnut skin samples. The use of hammer mill, ball mill and microfluidizer in processing of hazelnut skin samples break the storage cells of polyphenols. For this reason after microfluidization process and ball milling process color of hazelnut skin samples become darker, and so they gave darker color to biscuit samples. Duman (2013) observed similar result that cacao fiber which was obtained from microfluidization process gave darker color to cake than cacao powder. Both cacao powder and hazelnut skin samples are rich in polyphenols. The modification of polyphenols may result in the

formation of brown substances that affect the color of samples (Shahidi & Naczk, 1995). Maillard and caramelization reaction between sugar and proteins of products also contribute color development (Anil, 2007). Moreover, other factors affect the color of products i.e. time & temperature of baking, composition, humidity in oven etc. (Yadav et al., 2012). ΔE values of biscuit samples significantly increased as the flour replacement percentage with cacao powder, raw hazelnut skin, ball milled hazelnut skin, or microfluidized hazelnut skin fiber increased ($p \leq 0.05$). Figure 3.36 shows photographs of biscuit samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20%.

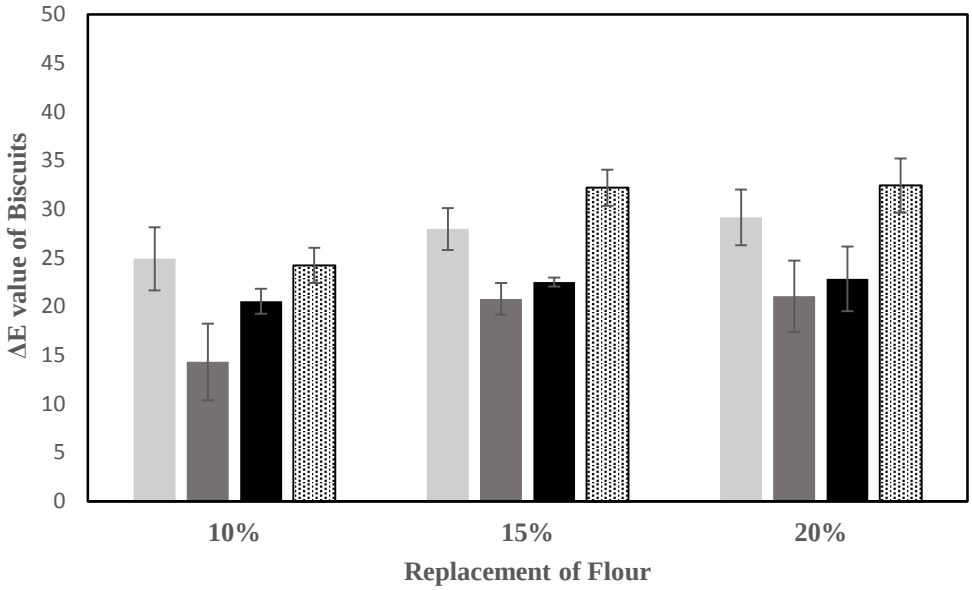

Figure 3. 35 ΔE values of biscuit samples where (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

Figure 3. 36 Photographs of biscuit samples. The photographs from top to bottom represent biscuit samples with cacao powder, raw hazelnut skin, ball milled hazelnut skin, and microfluidized hazelnut skin fiber, respectively and the samples aligned with control, 10% flour replacement, 15% flour replacement, and 20% flour replacement.

3.3.4. Fresh Biscuit Breaking Strength

Breaking strength of biscuits can be described by using hardness values obtained from texture analyzer. Texture is a sensorial properties so, only human being can describe. However, some physical parameters such as; hardness, cohesiveness, viscosity, springiness ,and adhesiveness can be detected and interpreted in terms of sensory perception by using texture analyzer.

The force required to attain a given deformation is defined as hardness. The sensory perception of hardness is defined as the force necessary to compress a substance between molar teeth or between tongue and palate (Szczesniak, 2002). The average peak force is the measure of biscuit hardness and the area from origin to absolute positive is the breaking strength (Yadav et al., 2012).

The effect of flour replacement percentages and biscuit types on hardness values of fresh biscuits was shown in Figure 3.37. ANOVA results showed that hardness values were significantly affected by biscuit types and percentages of flour replacement. Fresh biscuits containing cacao powder, raw hazelnut skin, ball milled hazelnut skin, and microfluidized hazelnut skin fiber were significantly different from each other ($p \leq 0.05$). Control biscuits had the lowest hardness values which was 1466 ± 228.20 g force. The hardness of biscuit samples increased with increasing amount of cacao powder and hazelnut skin samples. Hardness of biscuits containing cacao powder had higher hardness values as compared to hazelnut skin samples containing biscuit samples. Among the biscuits prepared with replacement of flour by hazelnut skin samples and cacao powder the lowest hardness value was obtained from biscuits containing microfluidized hazelnut skin fiber. Thus, the fiber source is important factor affecting the hardness of bakery products. This is consistent result with previous several fiber studies. Wang et al. (2002) also found that the hardness of bread samples containing different fibers was increased by addition of carob fiber and pea fiber. However, Inulin led to decreases in hardness value

of bread samples. Sudha et al. (2007) obtained similar results for biscuits containing different cereals. These results indicated that source of fiber affects the functional properties of fiber. As can be seen in Figure 3.37, as the fiber concentration increased in the biscuit samples, significantly harder structure was obtained ($p \leq 0.05$). Microfluidized hazelnut skin fiber addition into biscuits caused to significant differences between hardness values of biscuit samples. Hardness values of biscuit samples containing microfluidized hazelnut skin fiber as 1969.90 ± 704.15 g force, 2946,19 ± 142.28 g force, and 3511.84 ± 31.90 g force for the flour replacement at 10%, 15%, and 20% levels ($p \leq 0.05$).

Hardness values of biscuits was also affected from the replacement of flour by hazelnut skin prepared by hammer mill or ball mill. According to ANOVA results, there were significant differences between hammer milled and ball milled hazelnut skin containing biscuit samples and flour replacement levels ($p \leq 0.05$). As can be seen in Figure 3.37, as the percentages of hammer milled hazelnut skin and ball milled hazelnut skin amount increased in the biscuit samples, hardness values of biscuit samples increased significantly ($p \leq 0.05$).

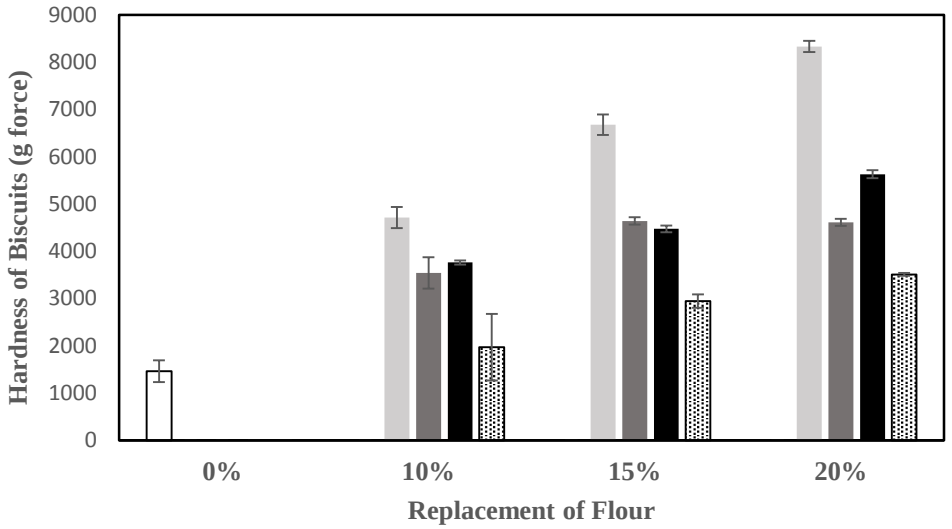

Figure 3. 37 Hardness values of biscuit samples where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

3.3.5. SEM Images of Biscuits

In order to obtain qualitative information about biscuit samples, SEM images of biscuits were used. Figs. 3.38–3.41 depict the SEM images of biscuit types at 250x and 1000x magnification levels. Fresh biscuits were prepared by replacing the flour with 10% cacao powder or hazelnut skin samples. 10% flour replacement percentage was chosen because better quality parameters were obtained when this replacement percentage was used.

Figure 3.38 presents the SEM images of control biscuit samples at 250x and 1000x magnification levels. Starch granules are birefringent and this property can be easily demonstrated by using optical microscope. Origin of the starch affect the shape of granules (Edwards, 2007). Gelatinization of wheat starch granules led to loss of spherical shape of starch granules in biscuit samples. In control biscuit samples spherical shape of most of starch granules were destroyed due to interaction between starch molecules and water. The remaining part of starch was unchanged due to incomplete disintegration of starch granules (Figure 3.38). Demirkesen et al. (2012) observed that swelling and gelatinization were affected from processing time and short processing time may cause disintegration of starch granules.

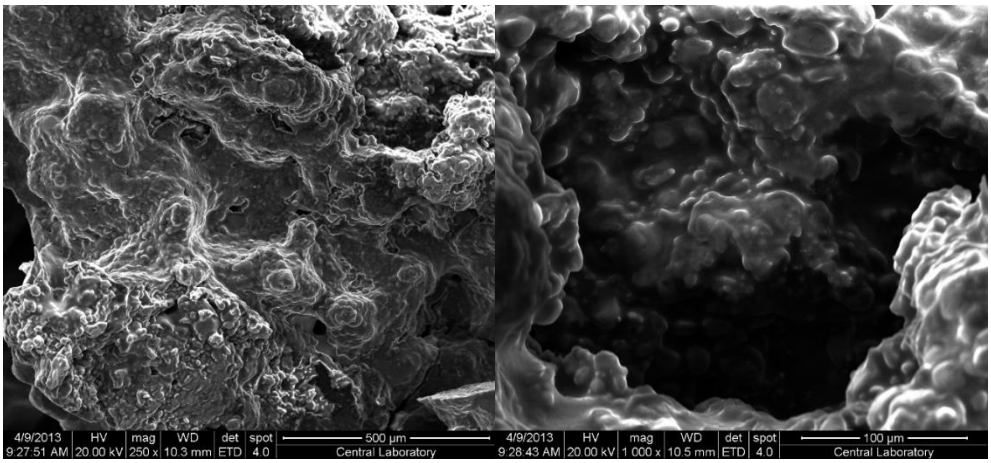

Figure 3. 38 SEM image of control biscuit samples. Magnification: 250x, 1000x.

Figs. 3.39-3.41 depict SEM images of biscuit samples containing cacao powder, raw hazelnut skin, and ball milled hazelnut skin. According to Figs. 3.39-3.41 starch molecules interact with water molecules partially.

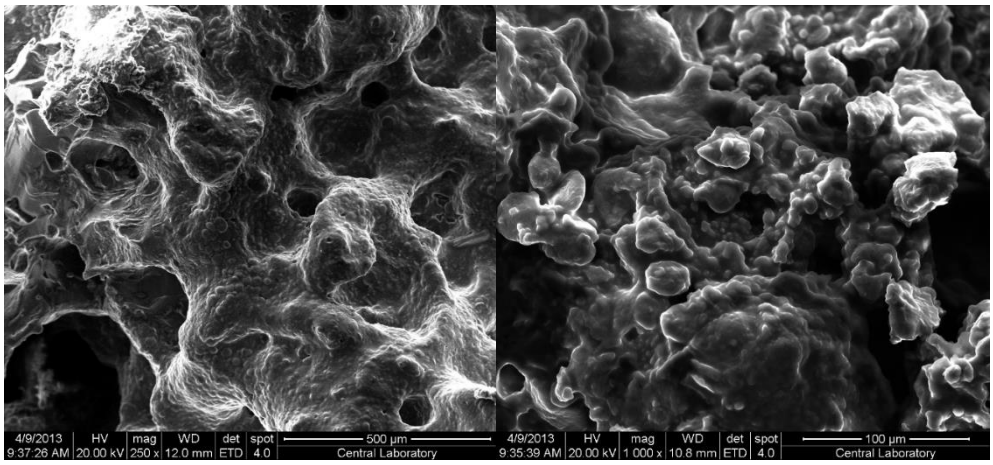

Figure 3. 39 SEM image of biscuit containing cacao powder with 10% replacement of flour. Magnification: 250x, 1000x.

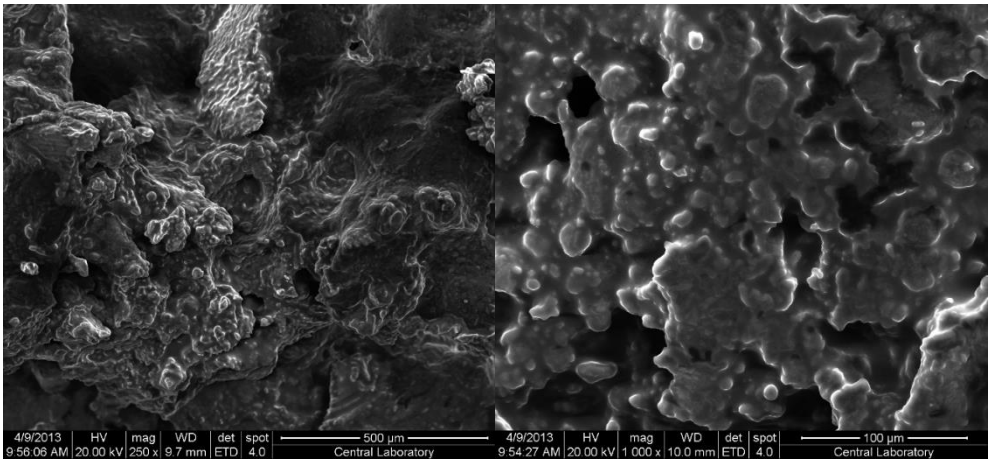

Figure 3. 40 SEM image of biscuit prepared with replacement of 10% of flour by raw hazelnut skin. Magnification: 250x, 1000x.

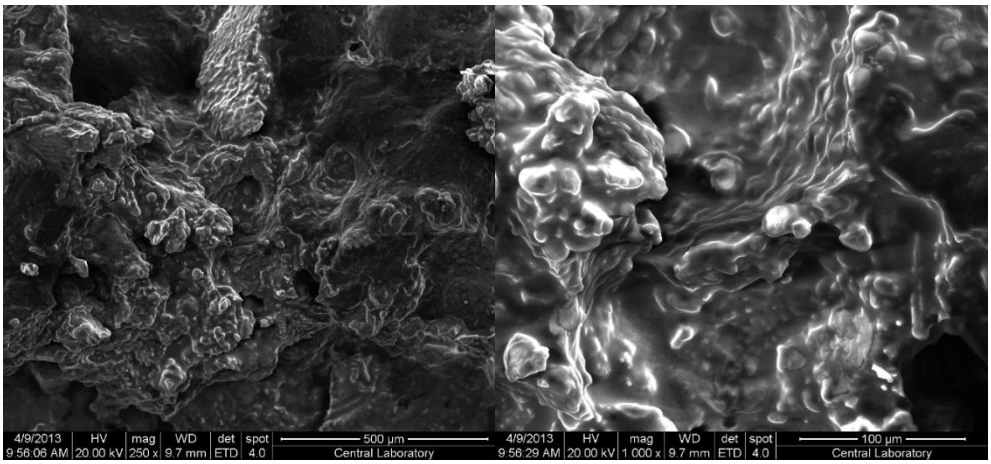

Figure 3. 41 SEM image of biscuit prepared with replacement of 10% of flour by ball milled hazelnut skin. Magnification: 250x, 1000x.

Figure 3.42 shows SEM images of biscuit samples containing microfluidized hazelnut skin fiber. According to Figure 3.42, most of the starch granules were not destroyed due to incomplete disintegration of starch granules. This was an expected result. Demirkesen et al. (2012) stated that increasing fiber content affects the degree of disintegration of starch granules. Water holding capacity of fiber is high and high fiber content eliminates available free water needed for interaction with starch granules. Therefore, starch granules in biscuit samples did not gelatinize. Also, high fiber content might cause increase gelatinization temperatures. This might be another reason of incomplete disintegration of starch granules.

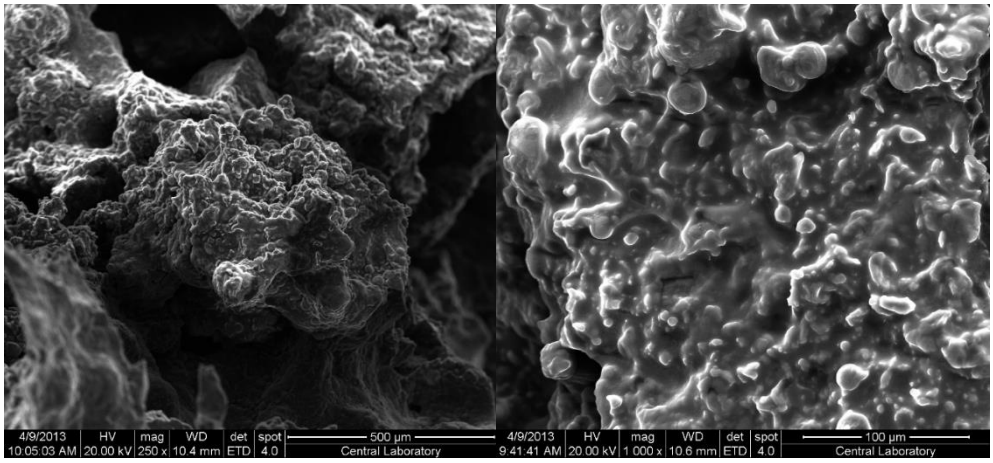

Figure 3. 42 SEM image of biscuit prepared with replacement of 10% of flour by microfluidized hazelnut skin fiber. Magnification: 250x, 1000x.

3.4. Staling Analysis of Biscuit

During storage period changes in crust or product crispness, crumb and crust moisture, crumb firmness, taste, and aroma take place in bakery products. The type of product determine the importance of each these changes in baked products.

Staling is defined as the changes that occur during storage (Cauvain & Young, 2000). Staling of bakery products affected from retrogradation, storage temperature, moisture migration, and moisture redistribution among components. In this study, biscuits containing cacao powder or hazelnut skin samples were analyzed in terms of their staling characteristics.

3.4.1. Physical characteristics of biscuit

According to ANOVA results, there were significant differences between biscuits types and replacement percentages of flour, but there were no significant differences between storage time in terms of thickness and diameter values of biscuit samples ($p \leq 0.05$).

Figs. 3.43-3.44 show the effects of storage times and biscuit types on thickness and diameter values of biscuit samples. According to Figs. 3.43-3.44, at 10% replacement of flour there was no change in thickness and diameter values of biscuits due to staling.

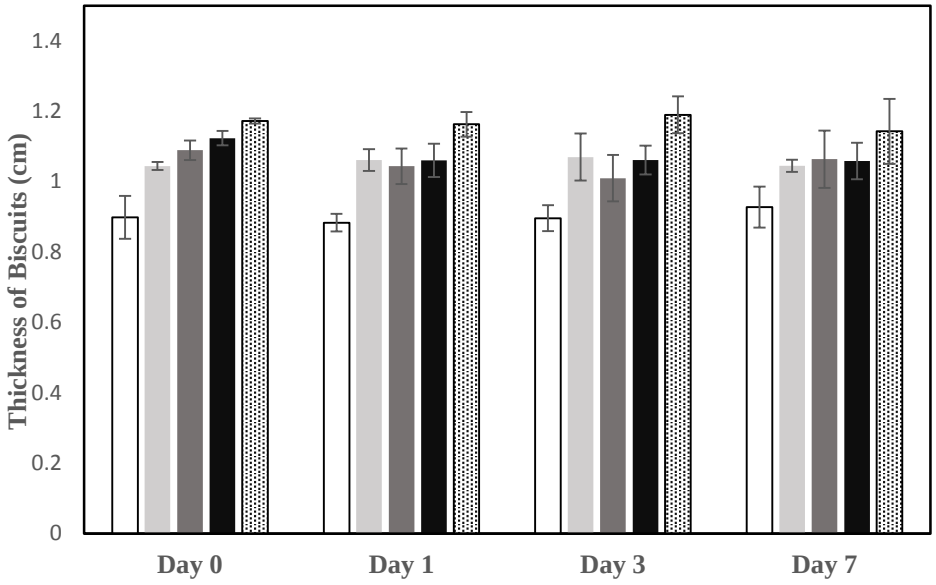

Figure 3. 43 Thickness values of biscuit samples with 10% replacement of flour stored for 0, 1, 3, and 7 days where (white bar): control biscuits (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

ANOVA results showed that there were no significant differences between thickness values of biscuit samples containing raw hazelnut skin, ball milled hazelnut skin, and cacao powder, but biscuits containing microfluidized hazelnut skin fiber was significantly different from other types of biscuit samples. Also, there was no significant difference between 1st day, 3rd day, and 7th day of storage, but they were significantly different from each other at 0th day of storage ($p \leq 0.05$).

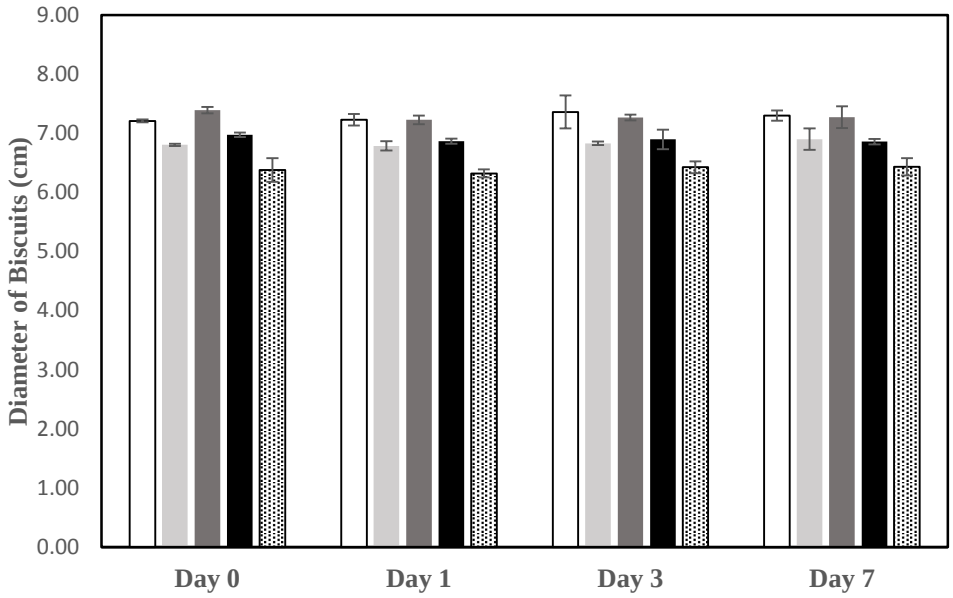

Figure 3. 44 Diameter values of biscuit samples with 10% replacement of flour stored for 0, 1, 3, and 7 days where (white bar):control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

Storage times or flour replacement percentages did not significantly affect the diameter values of biscuit samples ($p \leq 0.05$).

The effects of storage times and biscuit types on thickness and diameter values of biscuits were shown in Figs. 3.45-3.46. According to Figs. 3.45-3.46, during storage period the thickness and diameter values of all biscuit samples were approximately same for 15% flour replacement.

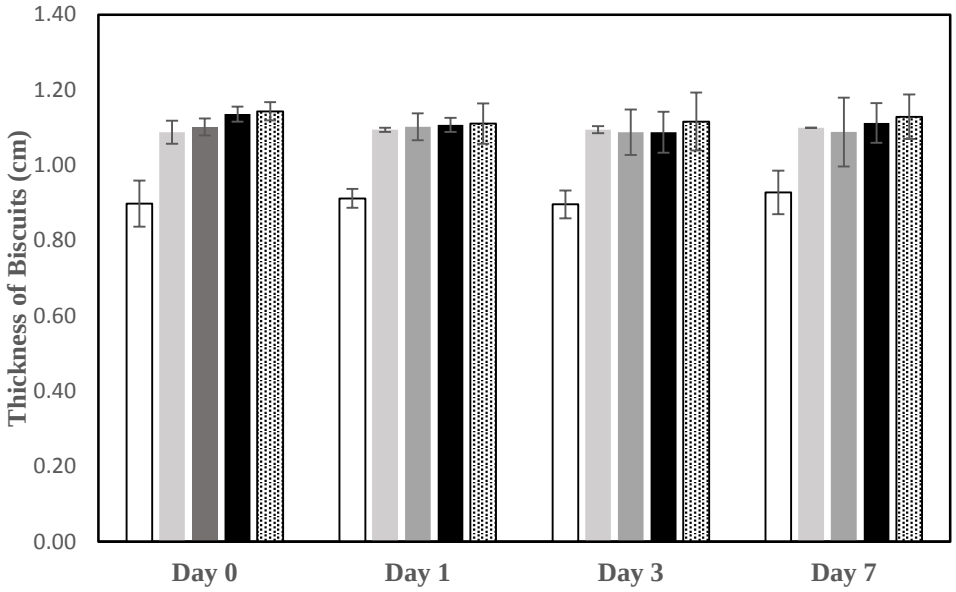

Figure 3. 45 Thickness values of biscuit samples with 15% replacement of flour stored for 0, 1, 3, and 7 days where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

ANOVA results showed that at 15% flour replacement, there were no significant differences between biscuit types and storage times in terms of thickness values of biscuit samples ($p \leq 0.05$).

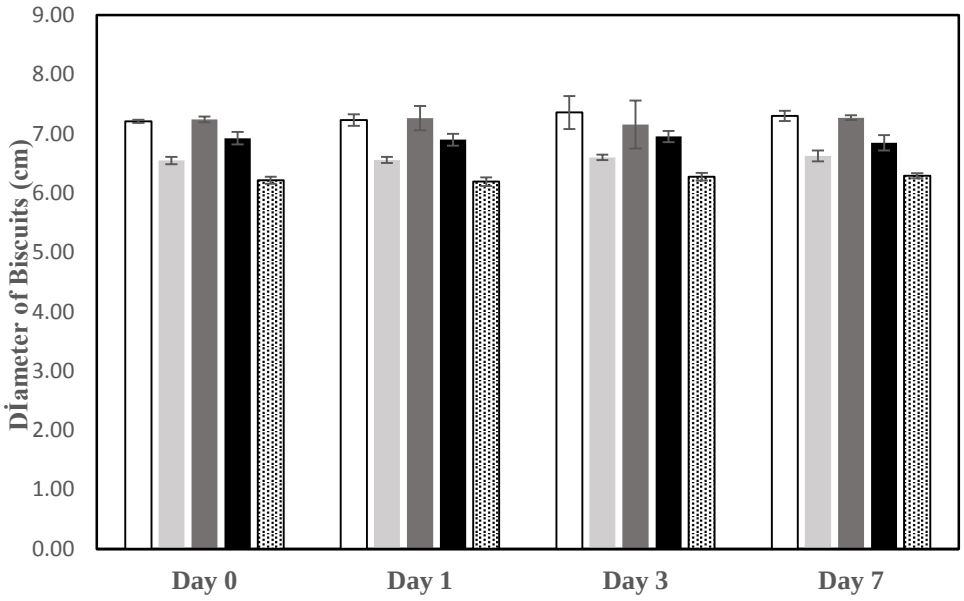

Figure 3. 46 Diameter values of biscuit samples with 15% replacement of flour stored for 0, 1, 3, and 7 days where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

According to ANOVA results, there were significant differences between biscuit types, but there were no significant differences between storage times for 15% replacement of flour in terms of diameter values ($p \leq 0.05$)

The effects storage times and biscuit types on thickness and diameter values of biscuit samples were shown in Figs. 3.47-3.48. According to Figs. 3.47-3.48 thickness values of biscuit samples containing hazelnut skin samples or cacao powder prepared with 20% flour replacement were approximately same during storage period.

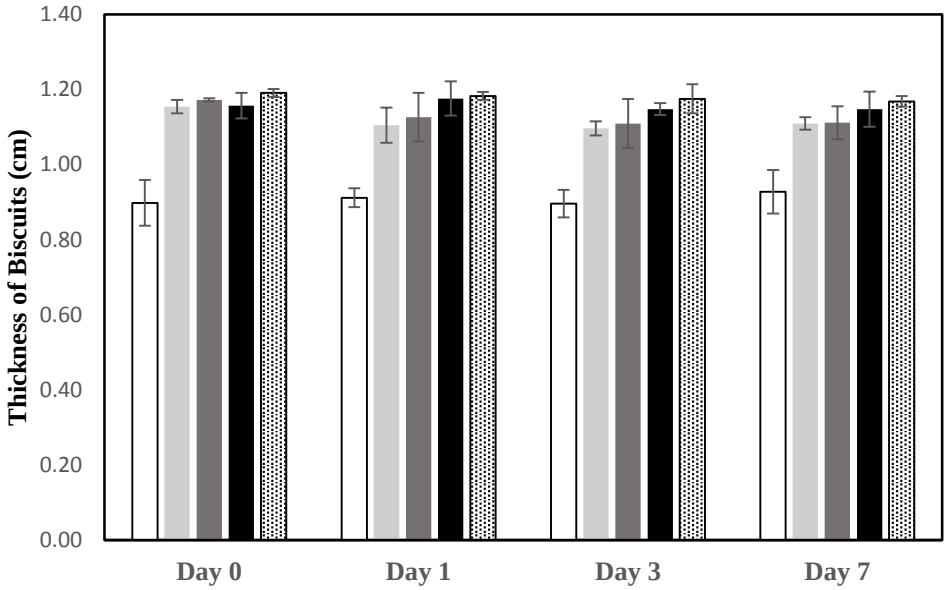

Figure 3. 47 Thickness values of biscuit samples with 20% replacement of flour stored for 0, 1, 3, and 7 days where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

According to ANOVA results, there were no significant differences between biscuits containing cacao powder, raw hazelnut skin, and ball milled hazelnut skin; but there were significant differences between microfluidized hazelnut skin fiber and the other biscuit samples. Also, there were no significant differences between storage times in terms of biscuit samples thickness values at 20% flour replacement ($p \leq 0.05$).

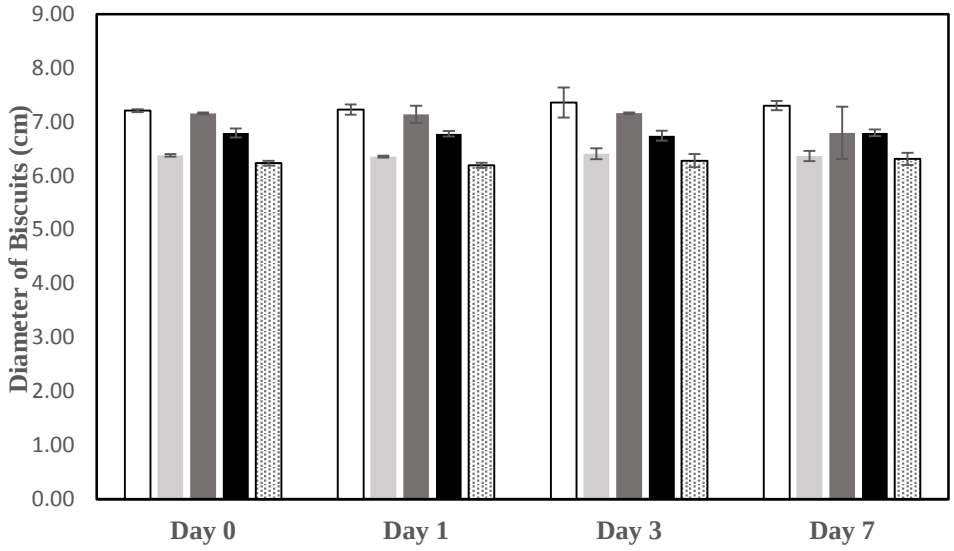

Figure 3. 48 Diameter values of biscuit samples with 20% replacement of flour stored for 0, 1, 3, and 7 days where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

ANOVA results showed that there were significant differences between biscuit types but there were no significant differences between storage times in terms of biscuits diameter at 20% flour replacement ($p \leq 0.05$).

By considering ANOVA results, it can be concluded that staling mechanism did not affect the thickness and diameter values of biscuit samples ($p \leq 0.05$).

3.4.2. Biscuit moisture content

Biscuit moisture content is an important quality parameter. Taste and textural properties of biscuit samples are affected from moisture content of them. For instance, biscuits' burnt taste is due to too low moisture content. Textural properties of biscuits are also affected from moisture content. If the moisture content is too high, then the structure of biscuits will not be crispy and staling can be more rapid than expected. In addition, the flavor changes can be occur (Manley, 2011). The effects of presence of hazelnut skin samples or cacao powder and flour replacement percentages on moisture content of biscuit samples were analyzed at different storage times. Based on ANOVA results, biscuits containing microfluidized hazelnut skin fiber and cacao powder significantly different from biscuits containing raw hazelnut skin and ball milled hazelnut skin. Moreover, moisture contents were dependent on flour replacement percentages ($p \leq 0.05$). Moisture content of biscuit samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20% at different storage time (0 day, 1 days, 3days, and 7days) were presented in Figs. 3.49-3.51. According to Figure 3.49 the addition of microfluidized hazelnut skin fiber to biscuits caused to increases of moisture content compared to other biscuits types at 0^{th} day of storage period. This may be due to the fact that hazelnut skin fiber obtained by microfluidization process increased water holding capacity noticeably. According to ANOVA results, there were no significant differences between storage times (0 day, 1 days, 3 days, and 7 days) of biscuit samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin fiber, and cacao powder at 0%, 10%, 15%, and 20% levels ($p \leq 0.05$). Therefore, it can be concluded that there are other factors affecting hardness of biscuit samples. Staling of biscuit samples may be due to starch retrogradation which was explained in more detailed in part 3.4.4 and 3.4.5.

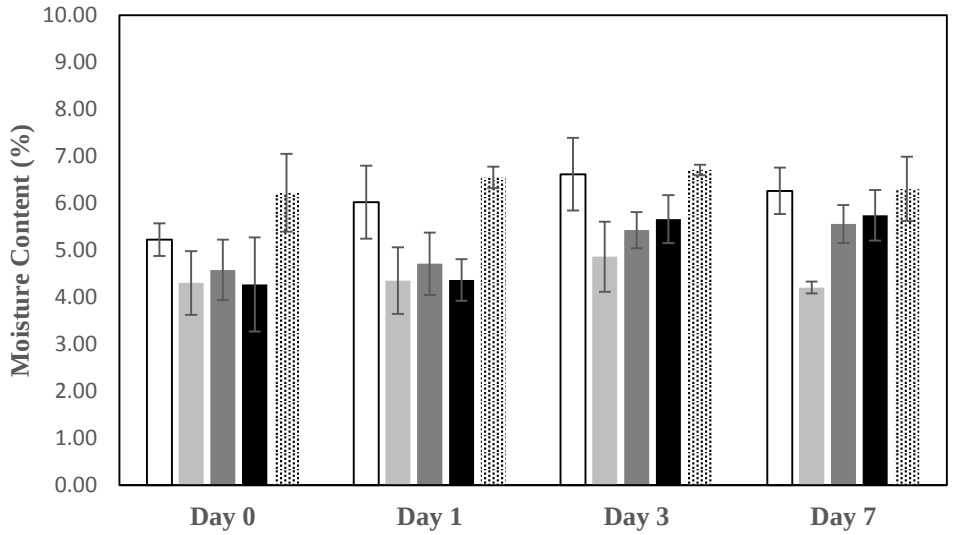

Figure 3. 49 Moisture content of biscuit samples with 10% replacement of flour stored for 0, 1, 3, and 7 days where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

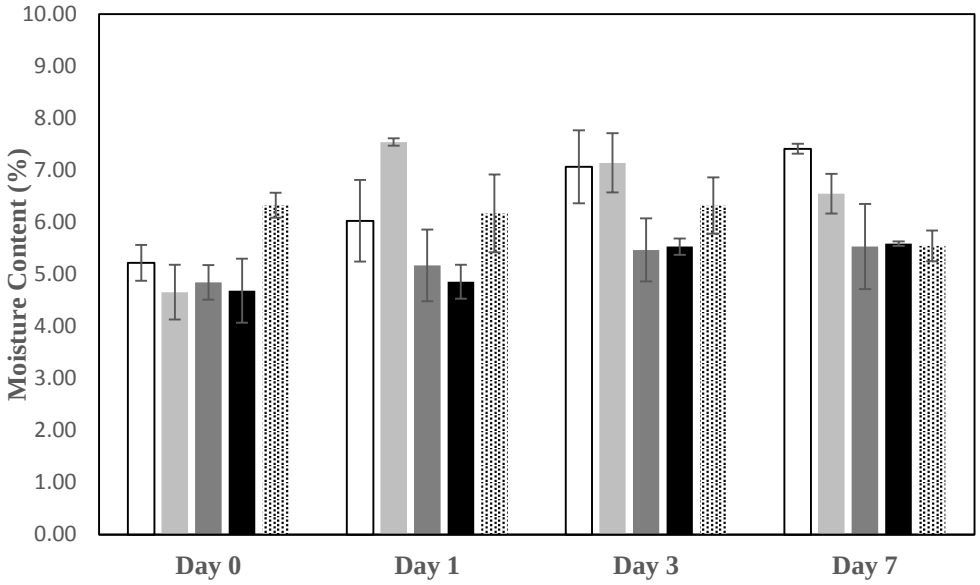

Figure 3. 50 Moisture content of biscuit samples with 15% replacement of flour stored for 0, 1, 3, and 7 days where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

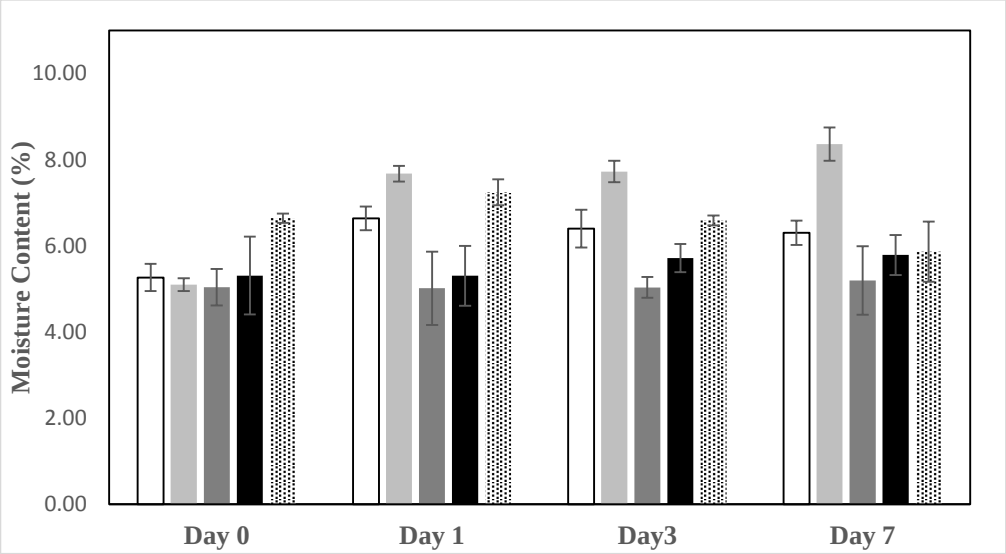

Figure 3. 51 Moisture content of biscuit samples with 20% replacement of flour stored for 0, 1, 3, and 7 days where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white blue): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

3.4.3. Texture Profiles of Stored Biscuits

The results for hardness parameters of the biscuits at 0, 1, 3, and 7 days of storage can be seen in Figs. 3.52-3.54. The hardness values of biscuit samples increased with time through 7 days of storage. According to ANOVA results, hardness values of biscuit samples significantly affected from flour replacement percentages, biscuit types, and storage times ($p \leq 0.05$). The hardness values of biscuit samples increased with increasing storage time for all types of biscuit samples. The hardness results of control biscuits were1466.46 ± 228.20 g force at the 0^{th} day of storage and 5366.02 ± 347.23 g

force at the end of 7^{th} day of storage. Hardness values of biscuit samples containing cacao powder prepared with 10% of flour replacement increased from 4717.35 ± 222.94 g force to 7646.95 ± 475.82 g force at the end of 7^{th} day of storage. At the same percentage of flour replacement by raw hazelnut skin, hardness values was measured as 3543.54 ± 329.15 g force at 0^{th} day of storage and at the end of 7^{th} day of storage it was measured as 6728.61 ± 196.78 g force. The hardness values of ball milled hazelnut skin and microfluidized hazelnut skin fiber were 3764.06 ± 45.89 g force and 1969.90 ± 704.14 g force at the 0^{th} day of storage, but they were measured as 8532.03 ± 224.08 g force and 6632.11 ± 64.58 g force at the end of 7^{th} day of storage. One reason for the increase in hardness during storage may be due to starch retrogradation. During starch retrogradation the structure of amylose and amylopectin change, and starch crystallinity increases with increasing storage time (Ribotta et al., 2004). Sozer et al. (2011) study shows that both hardness and toughness values also increase by the increasing level of starch crystallinity. As a result, the expected result was observed that during storage period.

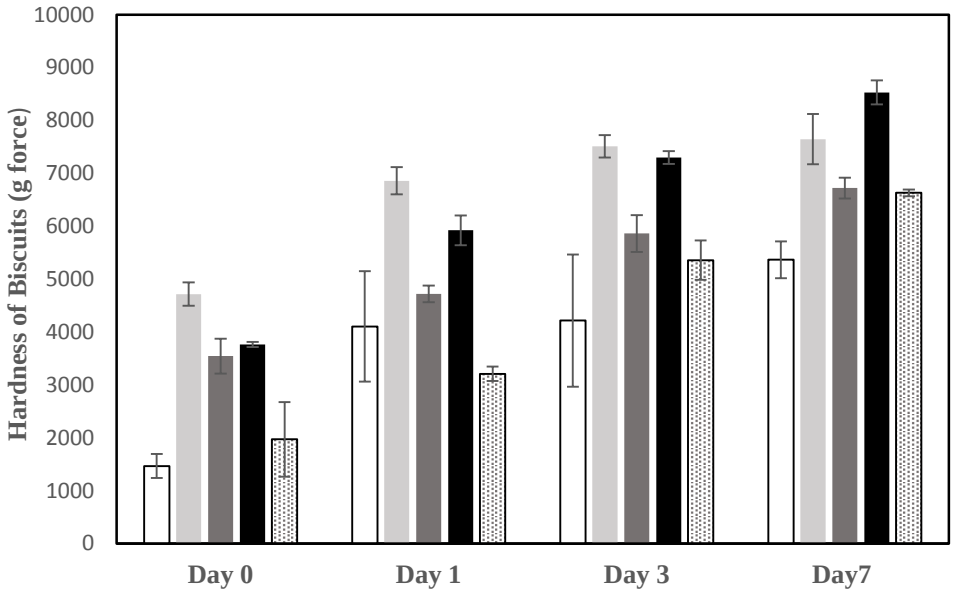

Figure 3. 52 Hardness values of biscuit samples with 10% replacement of flour stored for 0, 1, 3, and 7 days where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

Figure 3.53 depicts the hardness values of biscuit samples during storage period. The hardness results of biscuits containing cacao powder prepared with 15% of flour replacement were increased from 6679.83 ± 215.23 g force at the 0^{th} day of storage to 8266.32 ± 644.89 g force at the 7^{th} storage day. At the same percentage of flour replacement the hardness values of raw hazelnut skin and ball milled hazelnut skin were 4642.35 ± 77.38 g force and 4477.20 ± 71.33g force at the 0^{th} day of storage, but they were measured as 7692.71 ± 238.31 g force and 8710.97 ± 133.33 g force at the end of 7^{th} day of storage. Biscuits prepared with hazelnut skin fiber gave hardness value as 2946.19 ± 142.29 g

force at 0th day of storage and at the end of 7th day of storage it was measured as 7040.74 ± 32.32 g force. According to Figure 3.53, the lowest hardness values was observed from biscuit samples containing microfluidized hazelnut skin fiber. This might be due to water holding capacity of microfluized hazelnut skin fiber. During 7 days of storage the lowest hardness values was obtained from microfluidized hazelnut skin fiber containing biscuit samples because of hydrogen bonds between fiber and starch. As can be seen in Figure 3.53, the hardness values of samples increase with time through 7 days of storage.

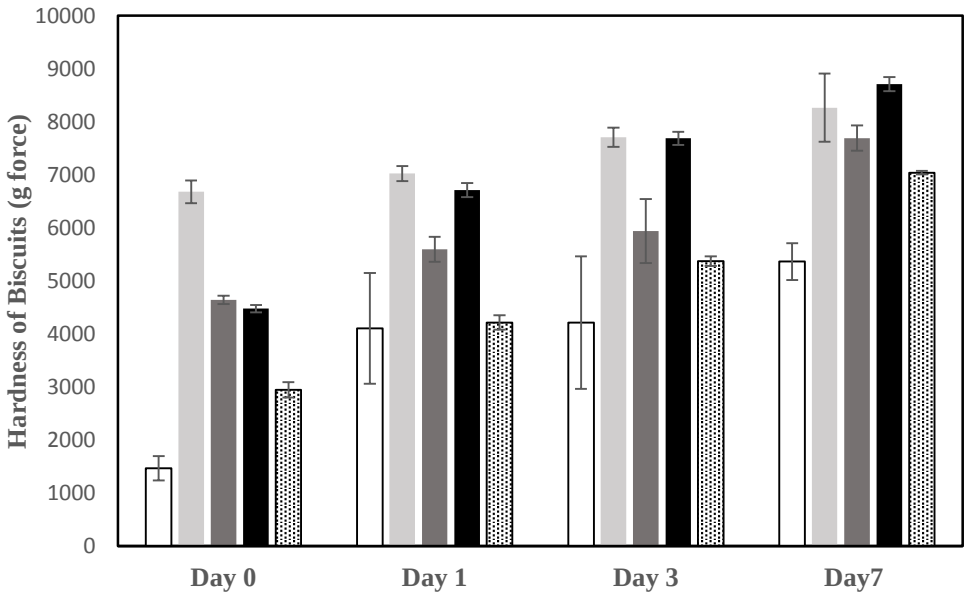

Figure 3. 53 Hardness values of biscuit samples with 15% replacement of flour stored for 0, 1, 3, and 7 days where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

The effects of storage times on hardness values of biscuit samples were presented in Figure 3.54. Hardness values of biscuits prepared with 20% of flour replacement increased from 8333.98 ± 116.66 g force to 9829.91 ± 74.90 g force at the end of 7^{th} day of storage. At the same percentage of flour replacement by raw hazelnut skin hardness value was measured 4614.85 ± 76.61 g force at 0^{th} day of storage and at the end of 7^{th} day of storage it was measured as 8280.40 ± 352.48 g force. The hardness values of ball milled hazelnut skin and microfluidized hazelnut skin fiber were 5631.97 ± 85.51 g force and 3511.84 ± 31.90 g force at the 0^{th} day of storage, but they were measured as 8715.38 ± 238.09 g force and 8016.18 ± 110.72 g force at the end of 7^{th} day of storage.

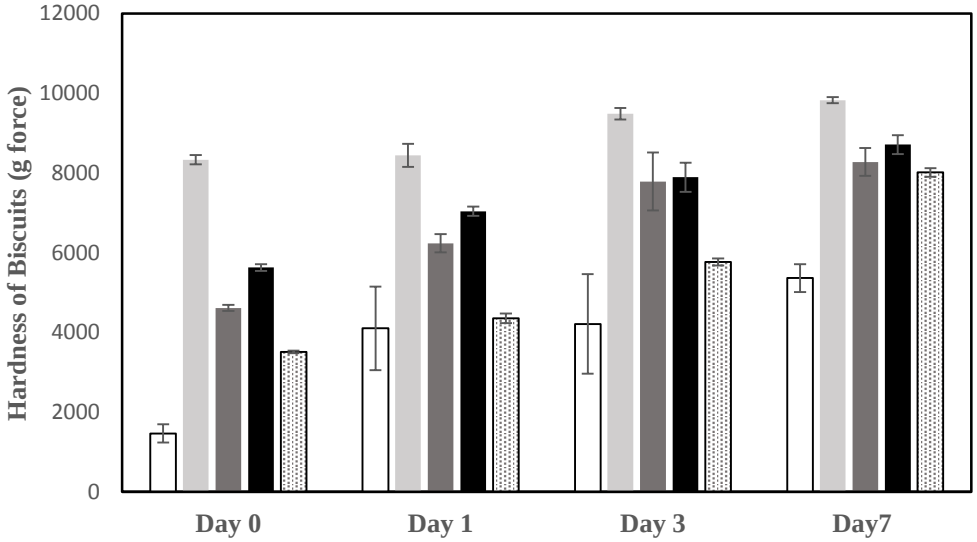

Figure 3. 54 Hardness values of biscuit samples with 20% replacement of flour stored for 0, 1, 3, and 7 days where (white bar): control biscuits, (light gray bar): biscuits prepared with cacao powder, (dark gray bar): biscuits prepared with raw hazelnut skin, (black bar): biscuits prepared with ball milled hazelnut skin, (dotted white bar): biscuits prepared with microfluidized hazelnut skin fiber. Bars indicate standard deviation of the replicates.

Water migration is the important concept when analyzing staling characteristics of biscuits. The changes are due to whether loss of water (i.e. crumb firming), or gain of water (i.e. loss of crispness). In biscuits, water migrates from atmosphere to product because water activities and moisture contents are so low. High water content of biscuits led to fracture of biscuits easily without subject to strong external forces. In cooling time of biscuits moisture content at the edges of biscuits are lower than at the center of biscuits, but as time passes water migrates from center to edge part, thus stresses are developed within the product which causes breaking of biscuits or forming cracks which leads to weakness of product (Cauvain & Young, 2000). Water holding capacity of

biscuit samples containing microfluidized hazelnut skin fiber was higher than other types of biscuit samples. Therefore, the hardness value of biscuits prepared by replacing the flour with microfluidized hazelnut skin fiber was the lowest among other biscuit samples.

Staling, increase in hardness, is the most important quality problems among all baked products. Staling of biscuits containing cacao powder or hazelnut skin samples may be due to retrogradation or recrystallization of starch, starch-protein redistribution, moisture redistribution among component, or gelatinization during baking. Generally, staling mechanism of baked products related with starch-water relation. Staling was related to starch-protein redistribution, retrogradation or recrystallization of starch ,and reorganization of starch polymers within the amorphous region in baked products (Demirkesen et al., 2013). Both retrogradation and recrystallization in the baked products, water movement in micro scale is involve. This situation is valid for starch-protein redistribution. Thus, the degree of water availability is important for starch-protein redistribution, retrogradation/recrystallization of starch. In baked products changes are occur due to crystallinity of the wheat starch during storage. According to Gray & Bemiller (2003), starch-protein interactions affect the staling. During baking process gelatinization occur in the oven. Gelatinization cause expel of starch molecules from the granules and they swell and distort the structures. That changes in starch molecules are the main reason of staling of bakery product (Köksel, 2009). According to Figs. 3.52-3.54 starch retrogradation might be delayed by addition of microfluidized hazelnut skin fiber because of hydrogen bonds between fiber and starch. Hydrogen bonds prevents starch-starch interaction which led to decreases organized starch availability for starch crystallization. It can be concluded that biscuit samples prepared with microfluidized hazelnut skin fiber had lower retrogradation of starch and so lower tendency of staling because of water binding ability of fiber.

3.4.4. X-Ray Diffraction

X-ray diffraction analysis has been used to examine staling mechanism of biscuit samples in particular starch granules crystalline structure. Diffraction diagrams of aged biscuit samples which was stored for 1day, 3 days, and 7 days were given in Figs. 3.55–3.62. Biscuit samples were prepared replacement of flour with 10% of cacao powder or hazelnut skin samples. Since generally better quality was obtained at 10% flour replacement, this ratio was used to determine staling mechanism of biscuit samples.

X-ray diffraction was used to observe crystalline structure change during staling. During staling, starch retrogradation occurs and it involves change in structure amylose and amylopectin. For the first 1 hour of storage amylose recrystallization occurs. Recrystallization of amylopectin is observed more slowly than amylose recrystallization (Demirkesen et al., 2013b). Therefore, X-ray diffraction can be used to determine molecular reorganization of starch granules (Gray & Bemiller, 2003).

Concentric layers that contains crystalline micelles in starch granules are normally arranged perpendicularly to the layer plane. Partially crystalline starch granules give distinct X-ray diffraction patterns. X-ray diffraction detects regularly repeating double helices of molecule structure rather than irregular packed structure. Therefore, role of starch granules in staling can be easily seen by X-ray diffraction. Fresh baked bread starch granules exist is in the form of amorphous but during storage they start to recrystallize. Changing starch granules from amorphous to crystalline state is known as retrogradation. The changes of crystallinity of starch granules which is associated with staling may not develop parallel with rheological properties. The peak of amylopectin retrogradation area is increased by the increasing of storage time. The peak intensities of starch crystallinity is increased with increasing storage time

(Ribotta et al., 2004). Both hardness and toughness values also increase by the increasing level of relative crystallinity (Sozer et al., 2011).

Figs. 3.55-3.59 show the X-ray diffraction profiles of biscuit samples which were stored for 1day, 3 days, and 7days. It is noticeable that as the storage time increased, the peak intensities of X-ray diffraction diagrams also increased. Also, Demirkesen et al. (2013b) proposed that peak intensities increases by increasing starch crystallinity during storage period.

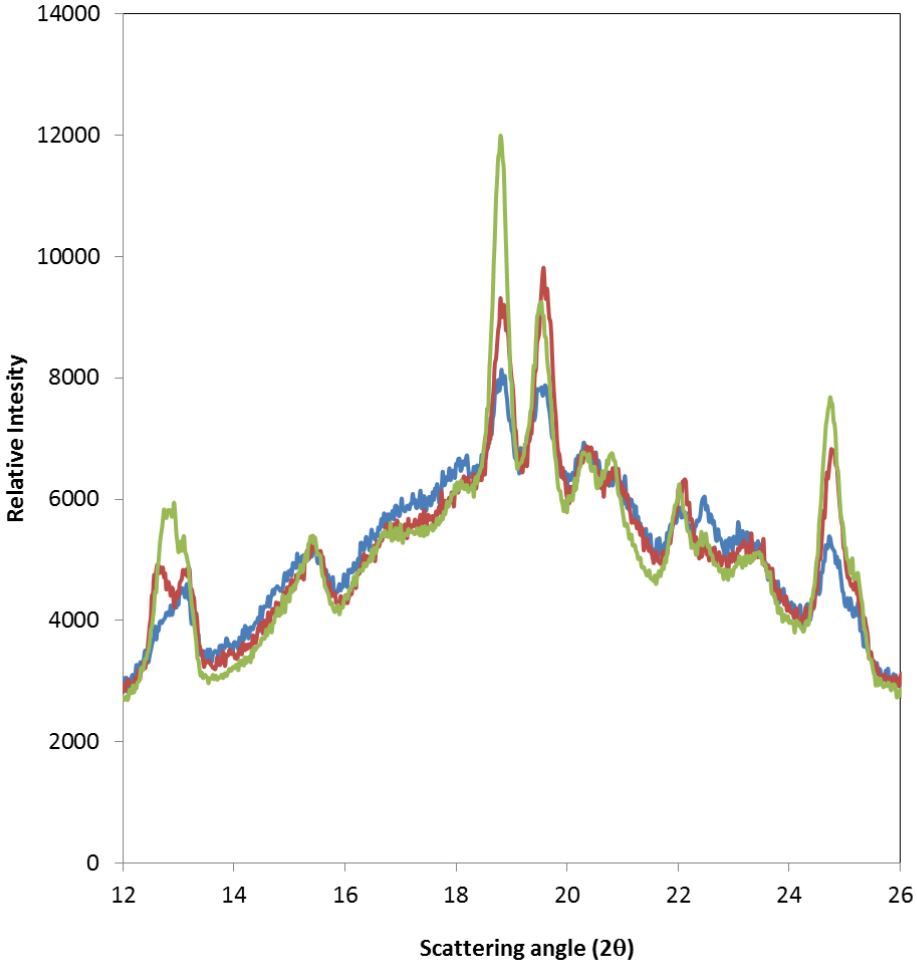

Figure 3. 55 X-ray diffraction diagrams of control biscuit samples stored at different storage times where (blue color): 1 days storage, (red color): 3 days storage, (green color): 7 days storage.

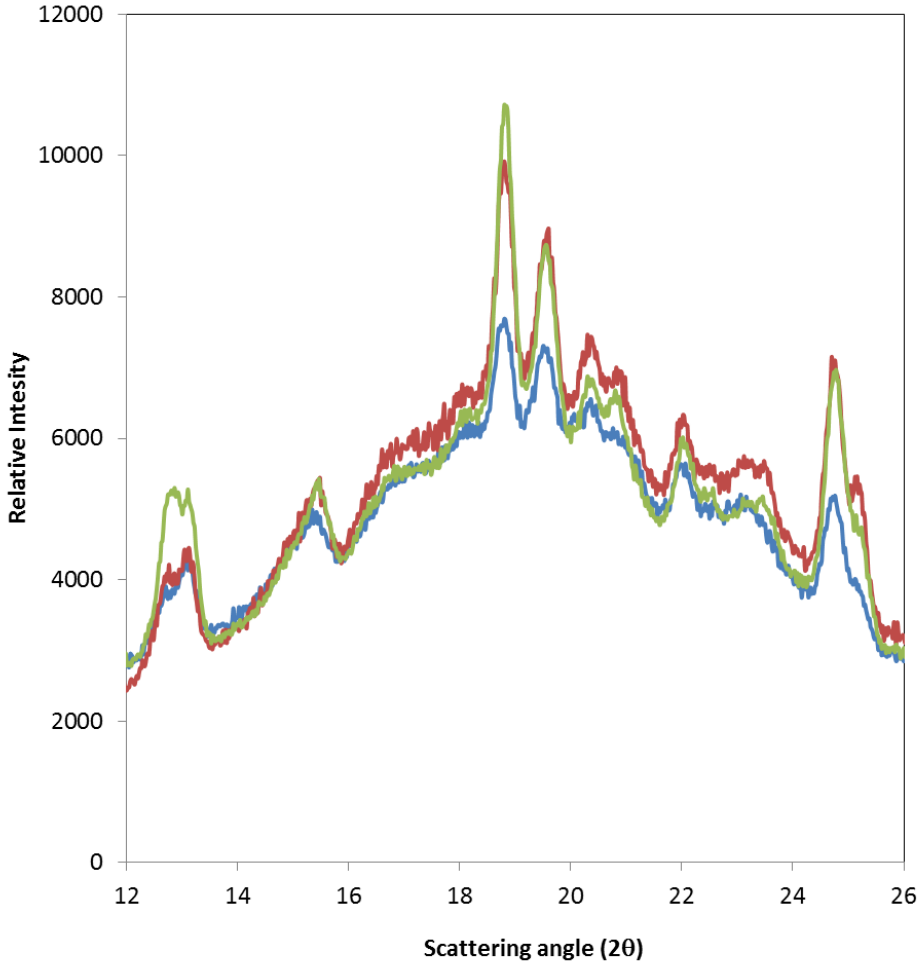

Figure 3. 56 X-ray diffraction diagrams of biscuit samples prepared with 10% replacement of flour with cacao powder and stored at different storage times where (blue color): 1 days storage, (red color): 3 days storage, (green color): 7 days storage.

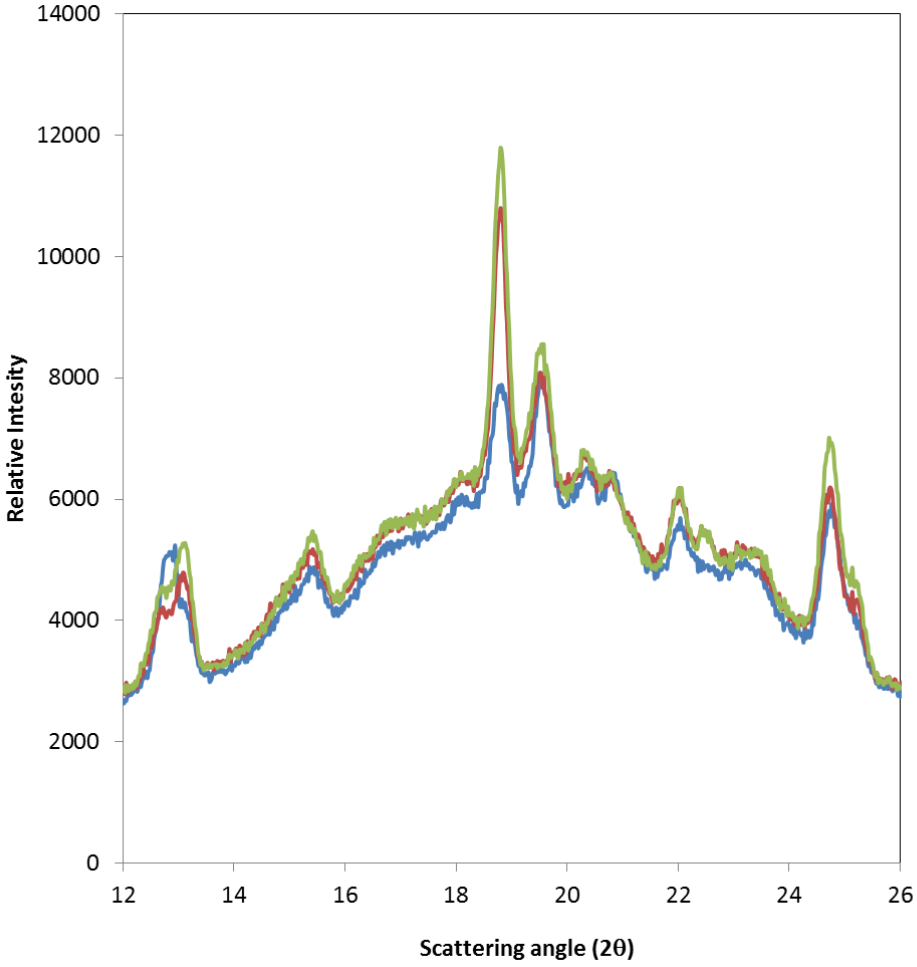

Figure 3. 57 X-ray diffraction diagrams of biscuit samples prepared with 10% replacement of flour with raw hazelnut skin and stored at different storage times where (blue color): 1 days storage, (red color): 3 days storage, (green color): 7 days storage.

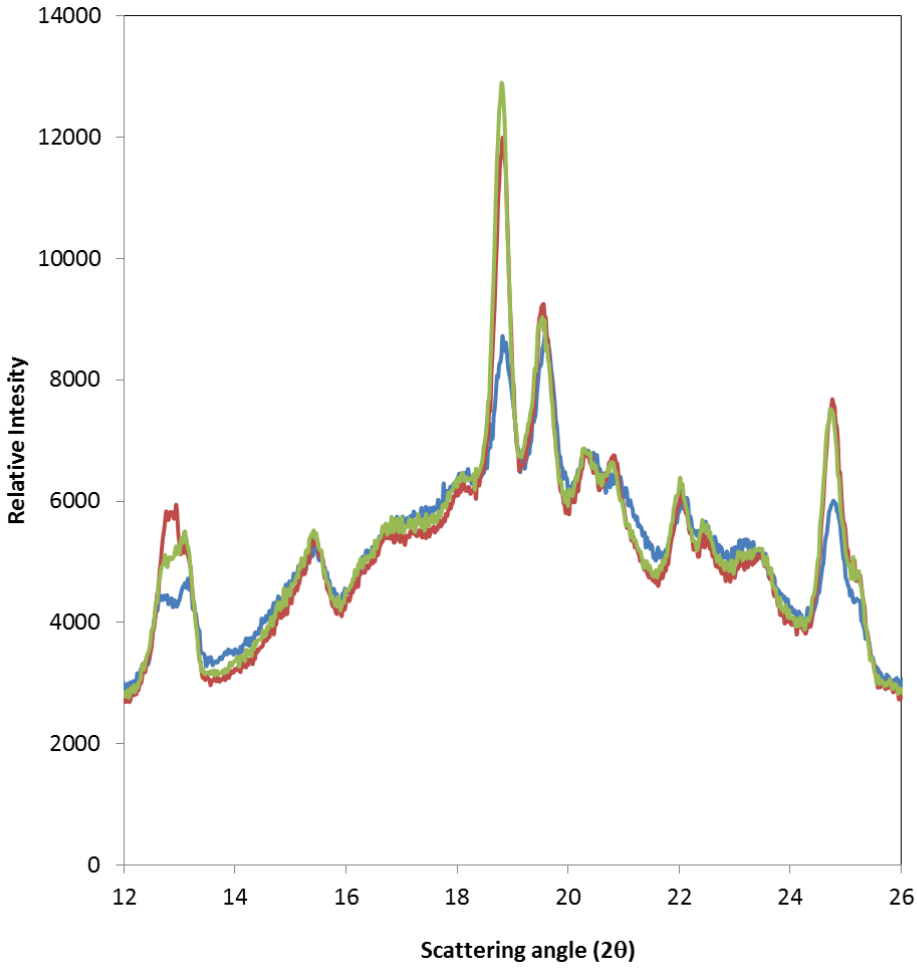

Figure 3. 58 X-ray diffraction diagrams of biscuit samples prepared with 10% replacement of flour with ball milled hazelnut skin and stored at different storage times where (blue color): 1 days storage, (red color): 3 days storage, (green color): 7 days storage.

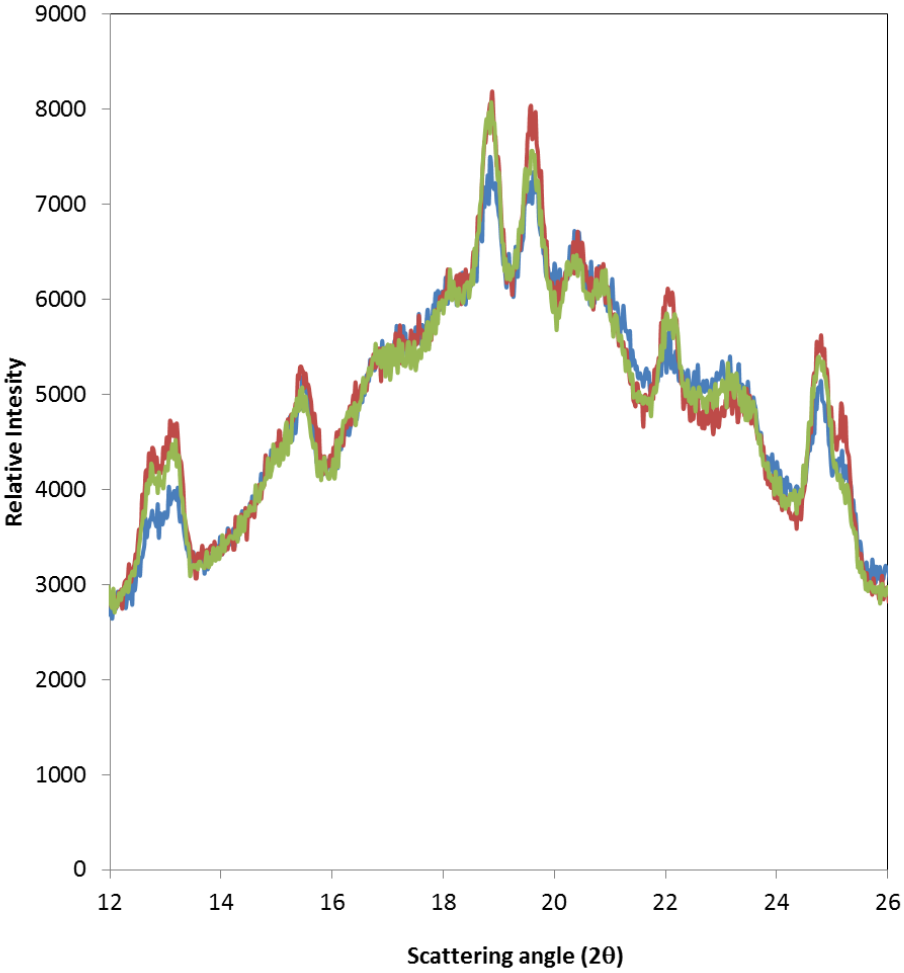

Figure 3. 59 X-ray diffraction diagrams of biscuit samples prepared with 10% replacement of flour with microfluidized hazelnut skin fiber and stored at different storage times where (blue color): 1 days storage, (red color): 3 days storage, (green color): 7 days storage.

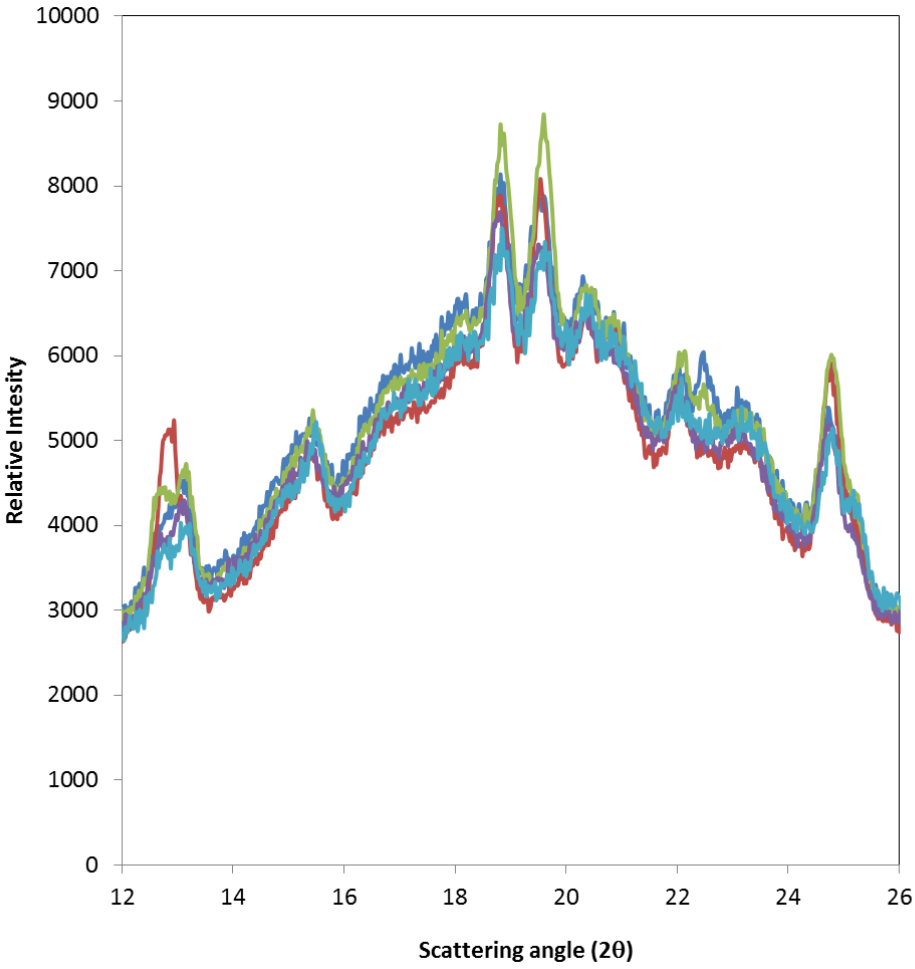

Figure 3. 60 X-ray diffraction diagrams of biscuit samples with 10% replacement of flour stored for 1 days (Dark Blue: control biscuits; Red: biscuits containing raw hazelnut skin; Green: biscuits containing ball milled hazelnut skin; Purple: biscuits containing cacao powder; Light blue: biscuits containing microfluidized hazelnut skin fiber).

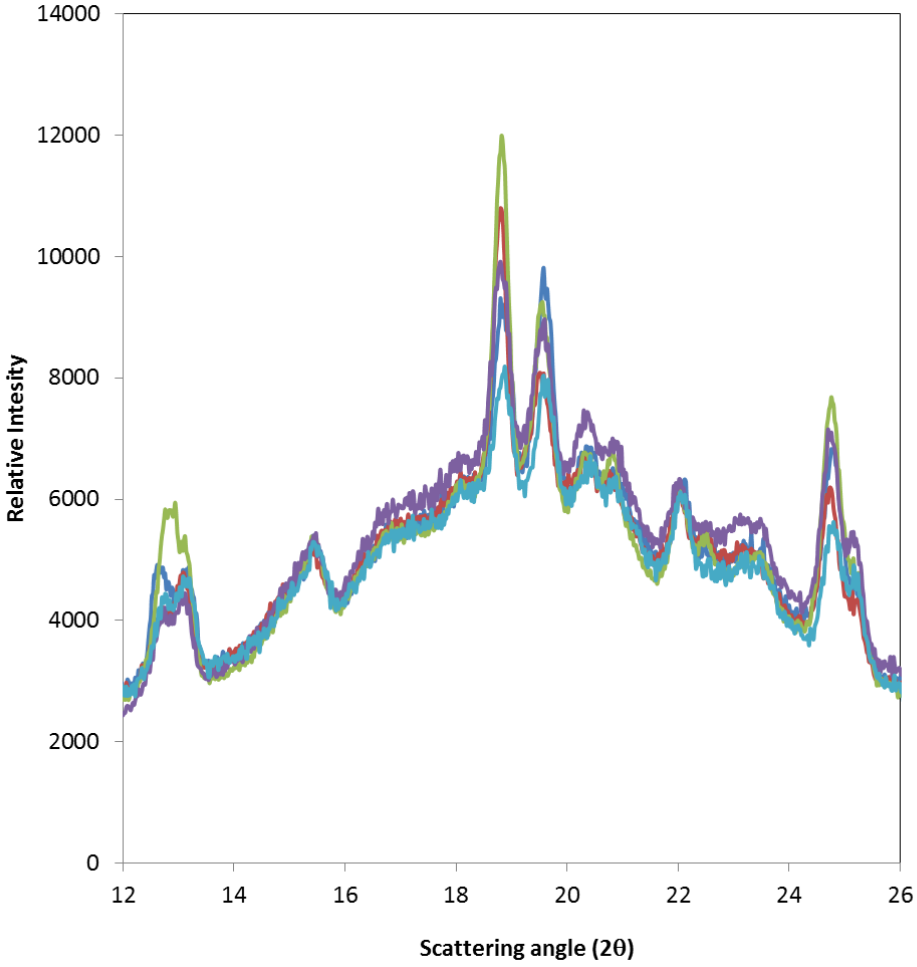

Figure 3. 61 X-ray diffraction diagrams of biscuit samples with 10% replacement of flour stored for 3 days (Dark Blue: control; Red: biscuits containing raw hazelnut skin; Green: biscuits containing ball milled hazelnut skin; Purple: biscuits containing cacao powder; Light blue: biscuits containing microfluidized hazelnut skin fiber).

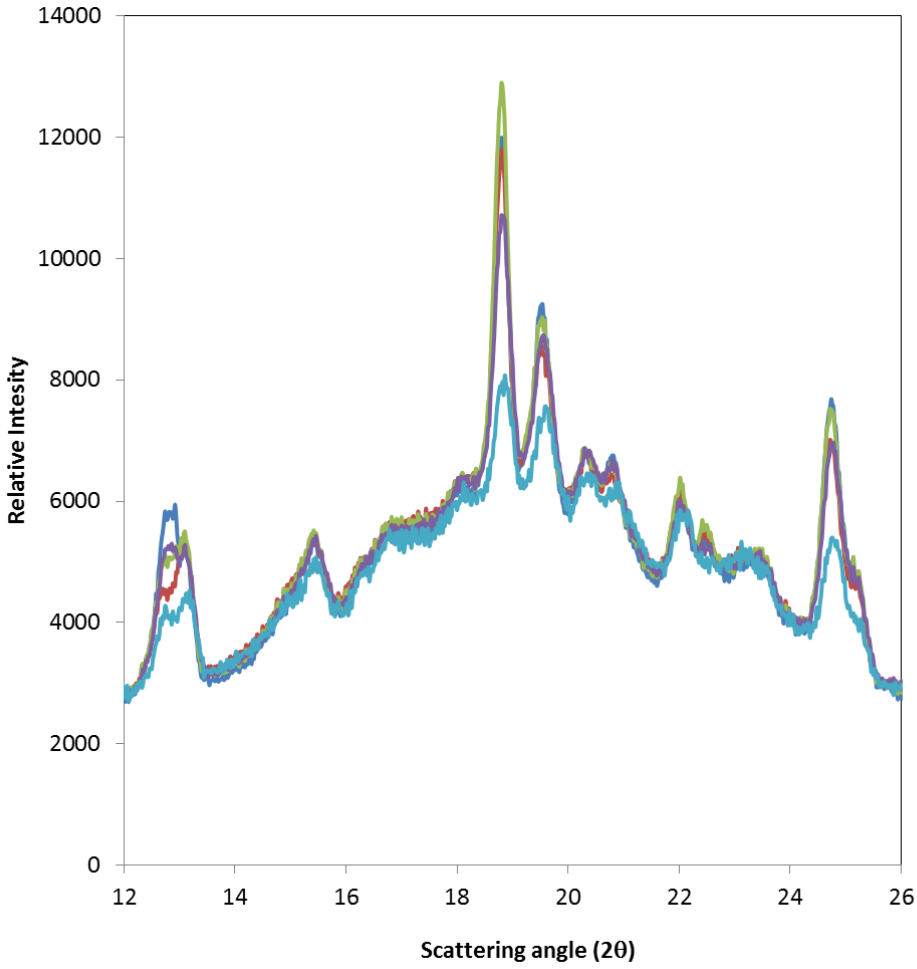

Figure 3. 62 X-ray diffraction diagrams of biscuit samples with 10% replacement of flour stored for 7 days (Dark Blue: control; Red: biscuits containing raw hazelnut skin; Green: biscuits containing ball milled hazelnut skin; Purple: biscuits containing cacao powder; Light blue: biscuits containing microfluidized hazelnut skin fiber).

There are different type of patterns; A type pattern, B type pattern, C type pattern, and V type pattern. Cereal starches (rice, wheat, and corn) exhibit A type pattern, B type pattern is observed by tubes, fruit, and high amylose corn.

Legume seed starches show C-type pattern which is intermediate between A and B types. Moisture is important, samples containing more than 43% moisture show B-type pattern. However, A-type pattern is observed for samples contain less than 29% moisture. Intermediate value between 29% and 43% moisture content exhibit C-type pattern. V-type pattern is related to amylose-lipid complexes. V- type pattern which is seen starch-lipid complexes is meta stable. (Karim et al., 2000).

Helical inclusion cause V-type structure interaction of amylose and fatty acids and it is observed for fresh breads which is stored only one hour. The peak at 20^0 indicates V-type structure (Demirkesen et al., 2013b). This peak does not change during storage (Demirkol, 2007). In this study, it was seen that during storage the peaks observed at 20^0 remained unchanged. Moreover, peaks at around 18^0 and 24^0 were seen in X-ray diffraction diagrams and the peak intensities increased during storage. They probably indicated B-type structure. Demirkol (2007) stated that during storage peak at 15.8^0, 17.7^0-18^0 and 22.8^0 signify that B-type structure. B type pattern shows the differences of the branched amylopectin molecules of starch within the branched amylopectin molecules of starch physical orientation. Also, Demirkesen et al. (2013b) proposed that B-type structure peaks are due to the crystallization of the amorphous starch melt, mostly of the amylopectin fraction, and increased during storage. As a result, the expected result were seen which was that during storage period V- type structure did not change however, the peak intensities of B- type structure increased.

A- , B-, and C-type of structure depend on the origin of starch granules. B-type crystalline regions are formed during storage period due to high water content rather than A- and C- type structure. In B-type structure recrystallization of amylopectin cause more water migration into crystalline region (Demirkesen et al., 2013b). A-type crystal contains eight water molecule whereas 36 water molecules is contained in B- type crystal. During storage starch retrogradation

cause gel structure formation which is linked with crystal development due to amylose and amylopectin interchain association. (Demirkol, 2007). The crystalline portion of A-type pattern starch structure is described with the X-ray diffraction peaks at 14.2^0, 17^0, 18^0, and 23.1^0, These peak intensities which reflects the A-type structure decreased or disappeared during 7 days storage (Osella etl al., 2005). In this study, during 7 days of storage the peaks at around 23^0 getting smaller, which was also the expected result. B-type crystallinity degree depend on water content and storage time. As increases in the water content and storage time, the peak intensity of A- type pattern also decreased. The ratio of degree of crystallization of B-type structure to V-type structure increased by the increasing storage period (Osella et al., 2005).

According to Figs. 3.63-3.65, when different biscuit samples were examined, the similar peak intensities were observed. However, the lowest peak intensities was observed from biscuit samples containing microfluidized hazelnut skin fiber. This means that addition of microfluidized hazelnut skin fiber to biscuit samples retard staling. Demirkol (2007) also stated that retardation of staling is resulted of decreased in crystallinity values of all samples.

Total mass crystallinity grades of different biscuit samples at different storage times can be seen in Figure 3.63. Storage time affect the total mass crystallinity values of biscuit samples because increasing level of crystallinity was observed by increasing storage time. The highest total mass crystallinity value was observed at 7 days storage of biscuit samples. The lowest crystallinity values was observed from biscuit samples containing microfluidized hazelnut skin fiber because addition of hazelnut skin fiber caused decreases in concentration of starch of biscuit samples. Also, water holding capacity of biscuit samples containing microfluidized hazelnut skin fiber was higher than other types of biscuit samples. In the presence of microfluidized hazelnut skin fiber, starch retrogradation might be delayed because of hydrogen bonds between fiber and starch. Starch-starch interaction is prevented by hydrogen bond, so organized

starch availability decrease for crystallization. Higher fiber content and lower starch content help to decrease of staling tendency (Demirkesen et al., 2013b). Therefore, according to results it can be concluded that biscuit samples containing hazelnut skin fiber produced by microfluidization process had lower retrogradation of starch and so lower tendency of staling because of water binding ability of fiber.

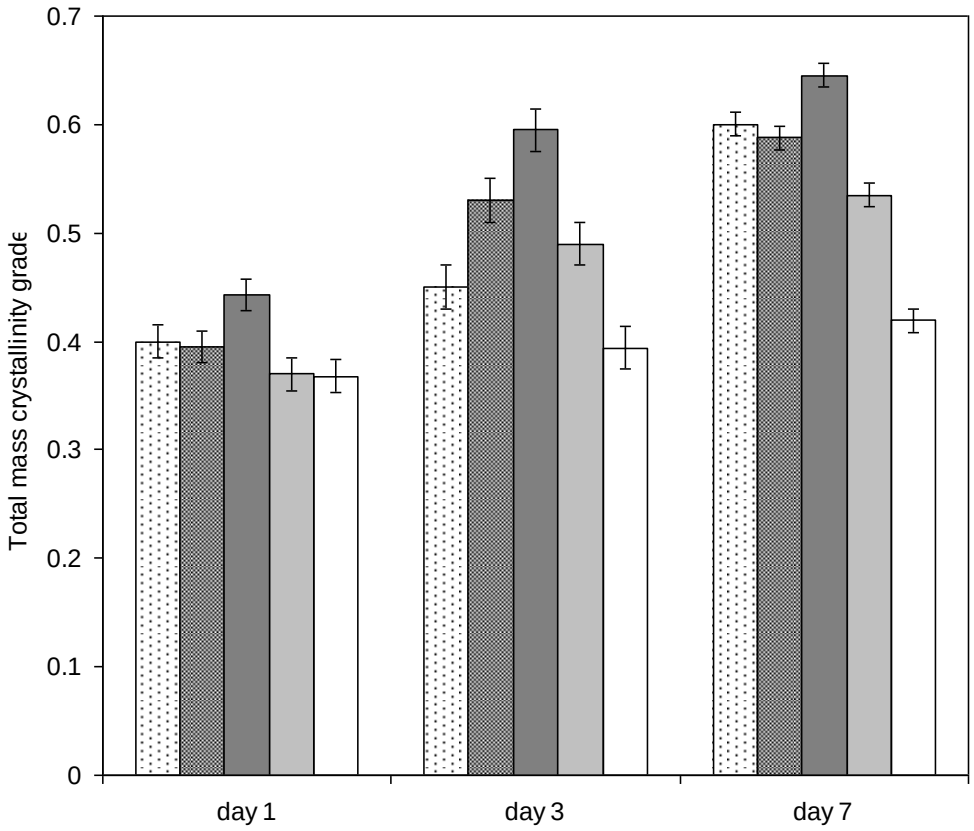

Figure 3. 63 Total mass crystallinity grades of different biscuit samples at different storage times. (dotted bar): control biscuit samples, (dashed bar): biscuit samples prepared with 10% replacement of flour with raw hazelnut skin, (dark gray bar): biscuit samples prepared with 10% replacement of flour with ball milled hazelnut skin, (gray bar): biscuit samples prepared with 10% replacement of flour with cacao powder, (white bar): cake sample prepared with 10% replacement of flour with hazelnut skin fiber produced by microfluidization process.

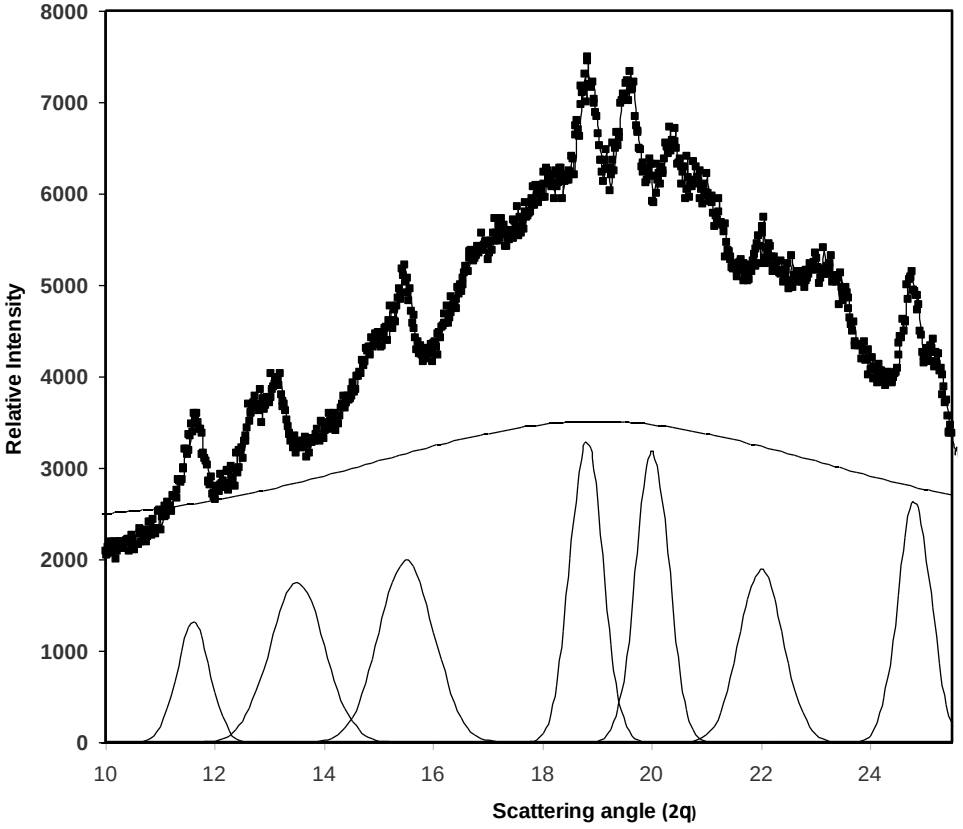

Figure 3. 64 Example of typical X-ray diffraction diagrams of biscuit samples

3.4.5. FT-IR

FT-IR spectroscopy is also used for determination of starch retrogradation. Starch granules consist of amylose and amylopectin as mentioned before. Gelation occurs due to heating of starch granules in water above their critical temperature value and inverse process which is returning of system to an ordered state is known as retrogradation. Gelation and retrogradation are important factors that affects processing conditions and staling of food products (Wilson et al., 1990). Retrogradation of starch strongly depends on amylose/ amylopectin content, lipid content, and amylopectin fine structure. Therefore,

origin of starch affects the retrogradation kinetic (Ottenhof et al., 2005). FT-IR spectra is used to monitor the starch retrogradation by analyzing band narrowing and band intensities changes. Conformation range reduction and smaller distribution of bond energies results from band narrowing, and variations in specific starch conformations cause band intensities change (Demirkesen et al., 2013a).

Fourier transform infrared spectroscopy gives an information about the disorder to order transition in the starch reaction, since carbohydrate polymers (i.e. starch) are bonded with hydrogen bond. Hydrogen bonding network changes of system may be observed in the FT-IR spectra. The spectra of different biscuit samples are depicted in Figs. 3.65–3.75. Ordered or crystalline structure and amorphous structure of starch were observed at 1047 cm^{-1} and 1022 cm^{-1} IR absorbance band. Also, the band at 995 cm^{-1} is sensitive to water. The IR absorbance bands at 1082 and 1153 cm^{-1} are associated with C-O bond stretching of C-O-H group, the peaks at 1022 and 1047 cm^{-1} are ascribed to C-O stretching of C-O-C group in the anhydroglucose ring, and the absorbance at 930 cm-^{1} is associated with C-H bending. Reduced amount of amorphous material gives more organized starch due to starting of retrogradation (Ji et al., 2010).

During storage, short range molecular order was developed in starch samples. The degree of short range molecular order was determined by FT-IR measurements. Absorbance value of ordered phase starch assigned ~1045cm^{-1} which is the major peak of FT-IR spectra of biscuit samples. Absorbance at ~1151 cm^{-1} was assigned for internal standard. During gelatinization and retrogradation, 1045:1151 cm^{-1} ratio can be used to probe the degree of short range molecular order in starch (Ottenhof et al., 2005). C-O and C-C stretching vibrations is result of 800- 1200cm^{-1} region band series. The absorbance at 1635 cm^{-1} band is related with O-H and so it gives an idea about moisture content of food samples. The band ratio (1635:1151 cm^{-1}) gives an information

about moisture content of retrograded starch samples because of linear correlation of them. Loss of moisture content cause decreases in starch retrogradation kinetics (Bello-Pérez et al., 2005).

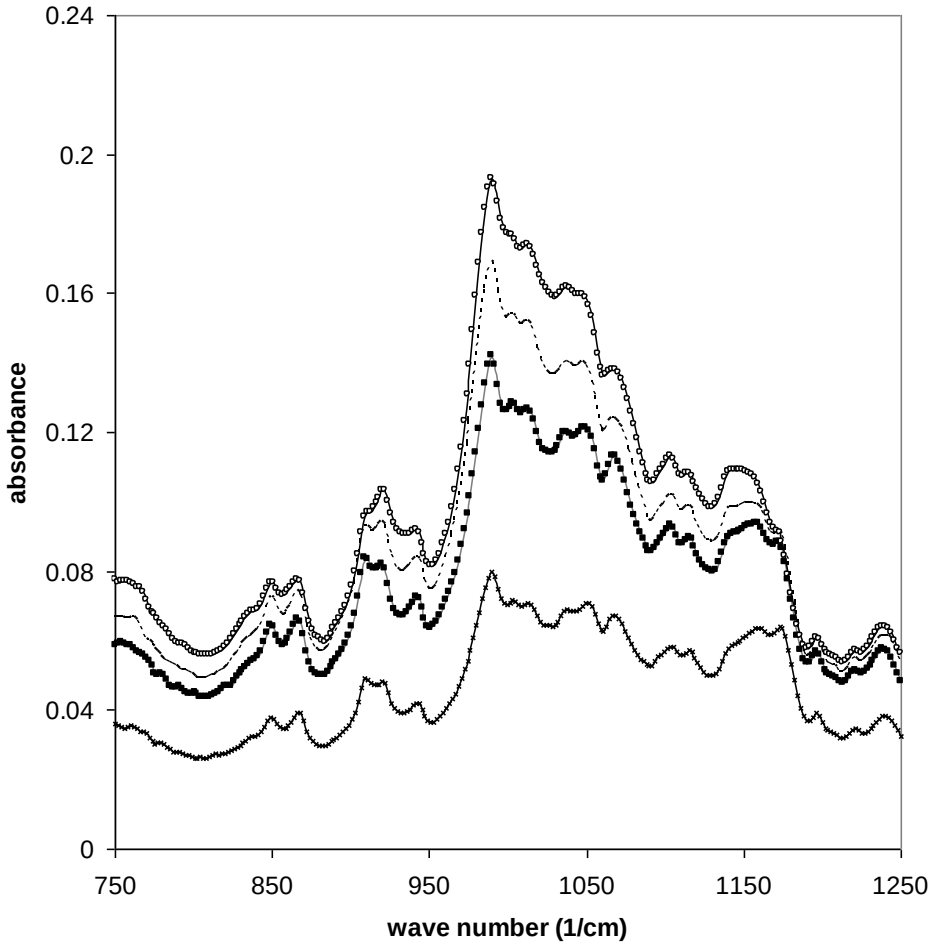

Figure 3. 65 FTIR spectrum of control biscuit samples stored at different storage times (black with dash line): 0 day, (black line with square shape): 1 days, (dashed line): 3 days**,** (black line with round shape): 7 days.

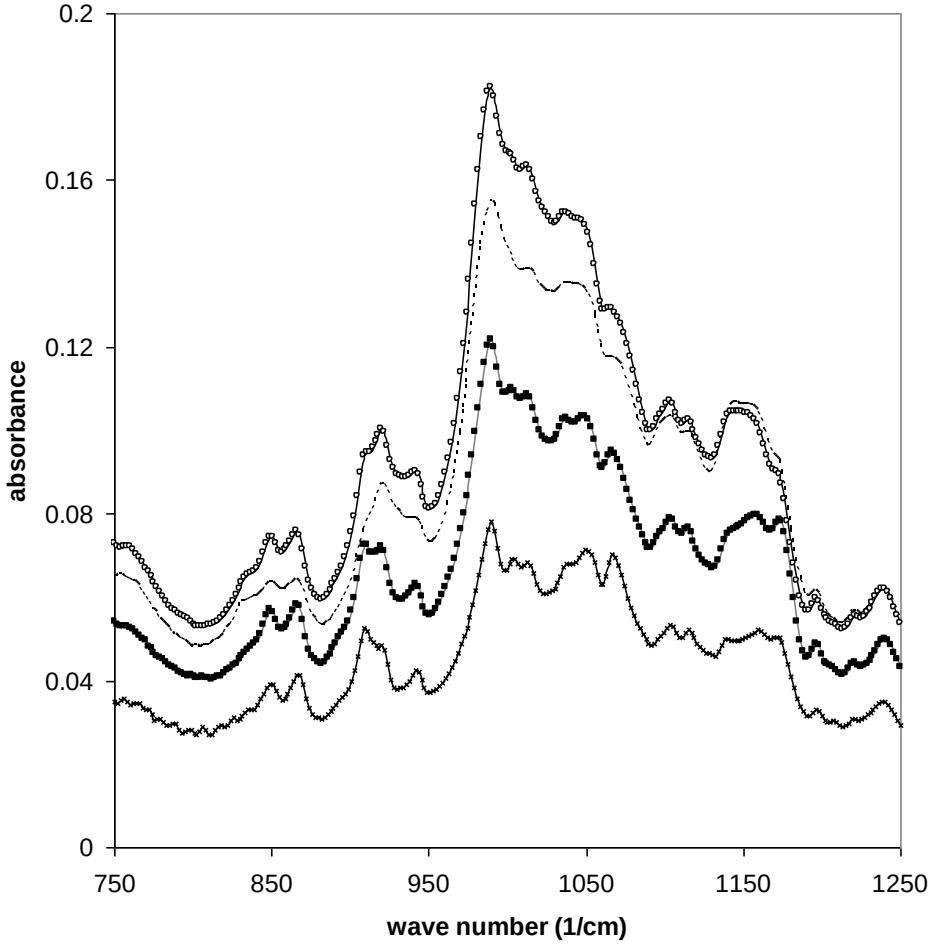

Figure 3. 66 FTIR spectrum of biscuit samples prepared with 10% flour replacement with cacao powder and stored at different storage times (black with dash line): 0 day, (black line with square shape): 1 days, (dashed line): 3 days, (black line with round shape): 7 days.

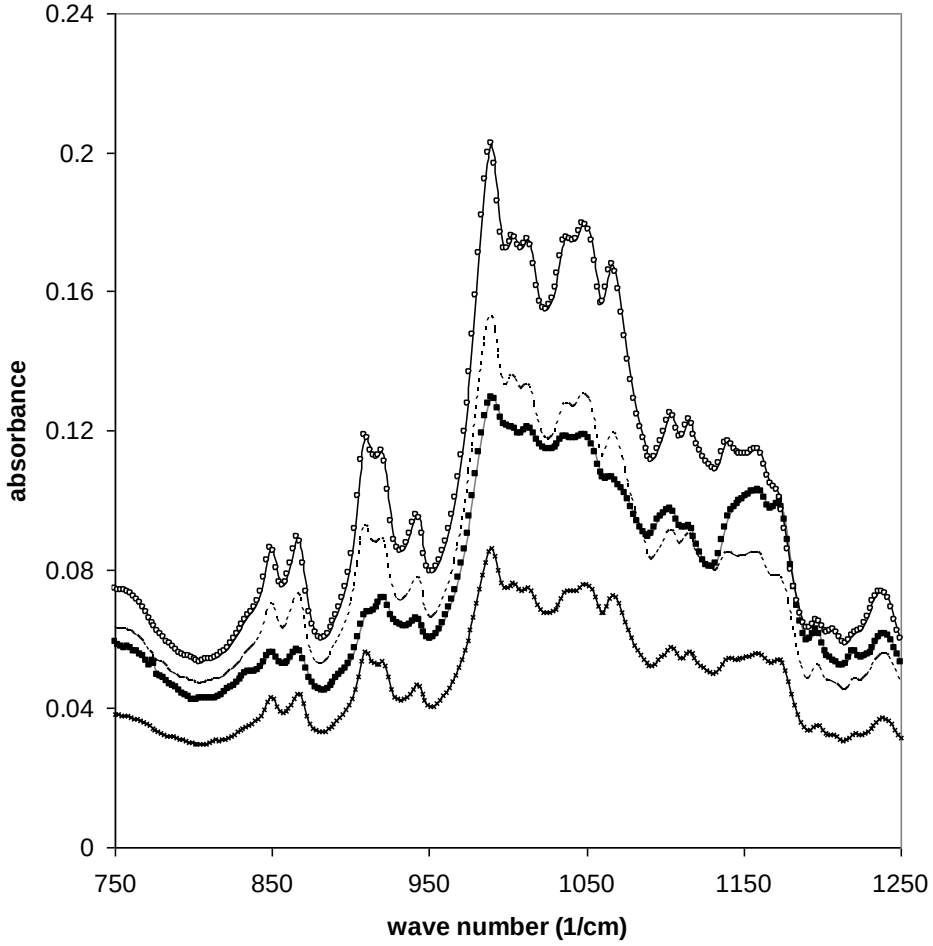

Figure 3. 67 FTIR spectrum of biscuit samples prepared with 10% flour replacement with raw hazelnut skin and stored at different storage times (black with dash line): 0 day, (black line with square shape): 1 days, (dashed line): 3 days, (black line with round shape): 7 days.

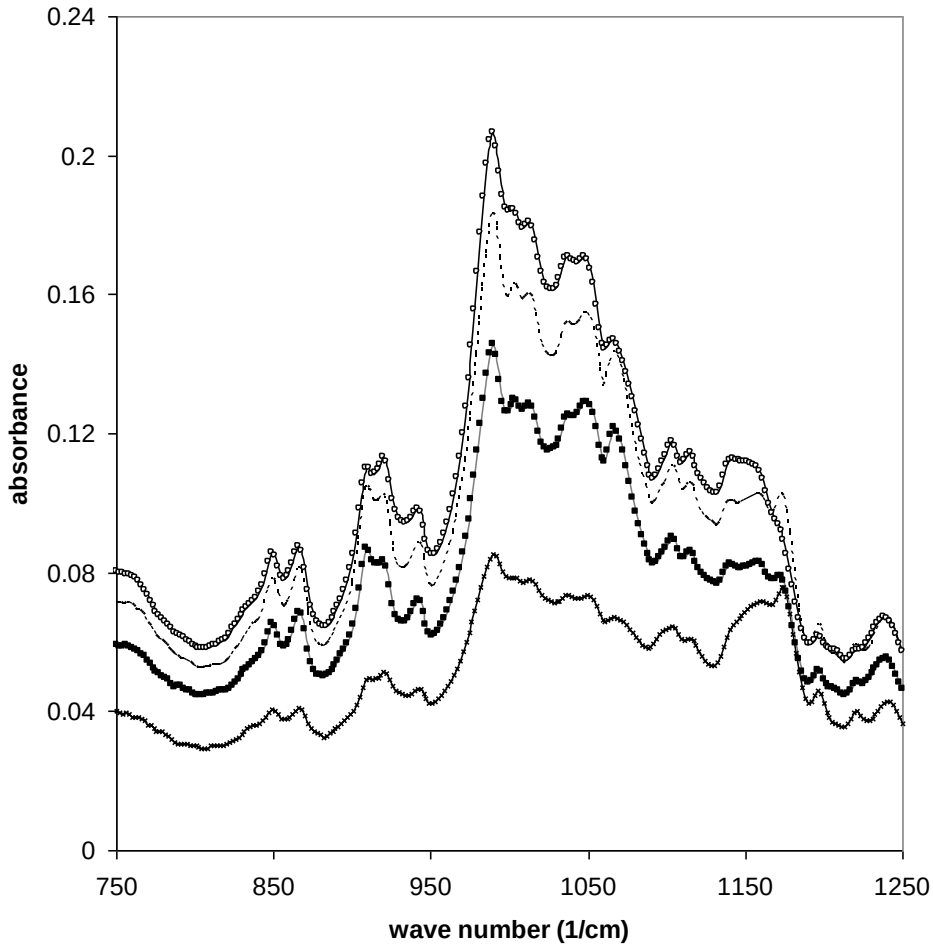

Figure 3. 68 FTIR spectrum of biscuit samples prepared with 10% flour replacement with ball milled hazelnut skin and stored at different storage times (black with dash line): 0 day, (black line with square shape): 1 days, (dashed line): 3 days, (black line with round shape): 7 days.

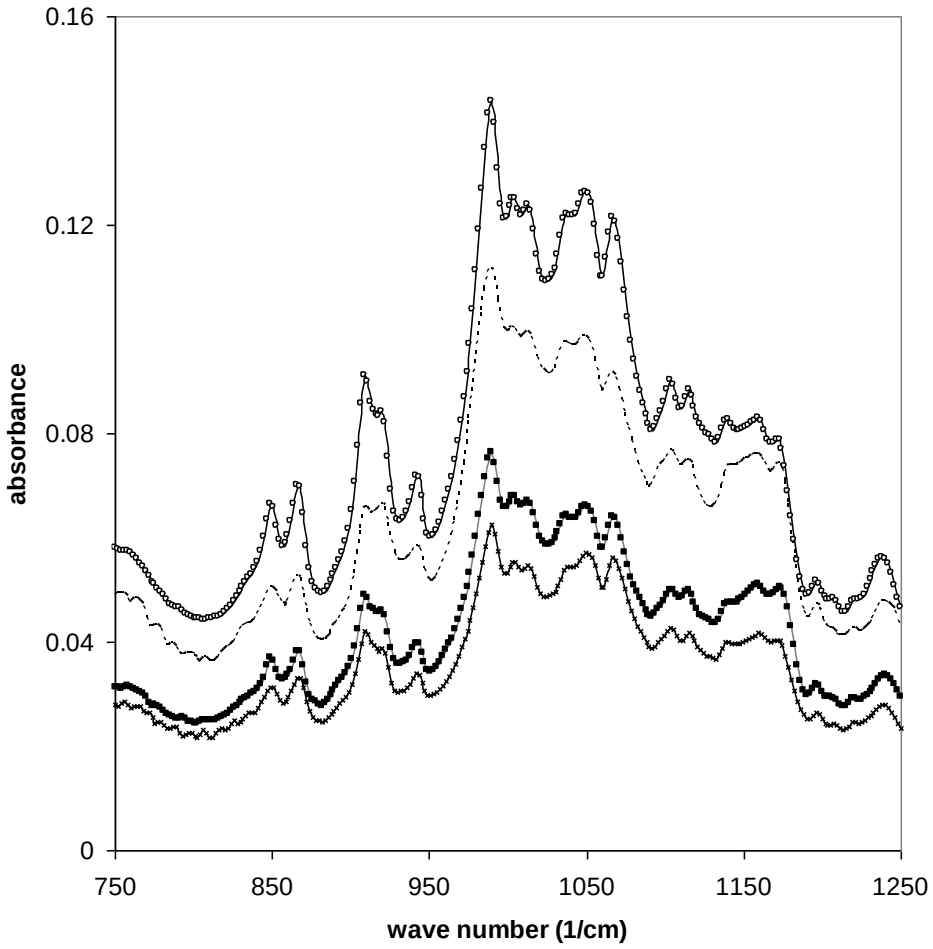

Figure 3. 69 FTIR spectrum of biscuit samples prepared with 10% flour replacement with microfluidized hazelnut skin fiber and stored at different storage times (black with dash line): 0 day, (black line with square shape): 1 days, (dashed line): 3 days, (black line with round shape): 7 days.

According to Figs. 3.62–3.66, FT-IR peak intensities at 1045 cm^{-1} increased with increasing storage time for all biscuit samples.

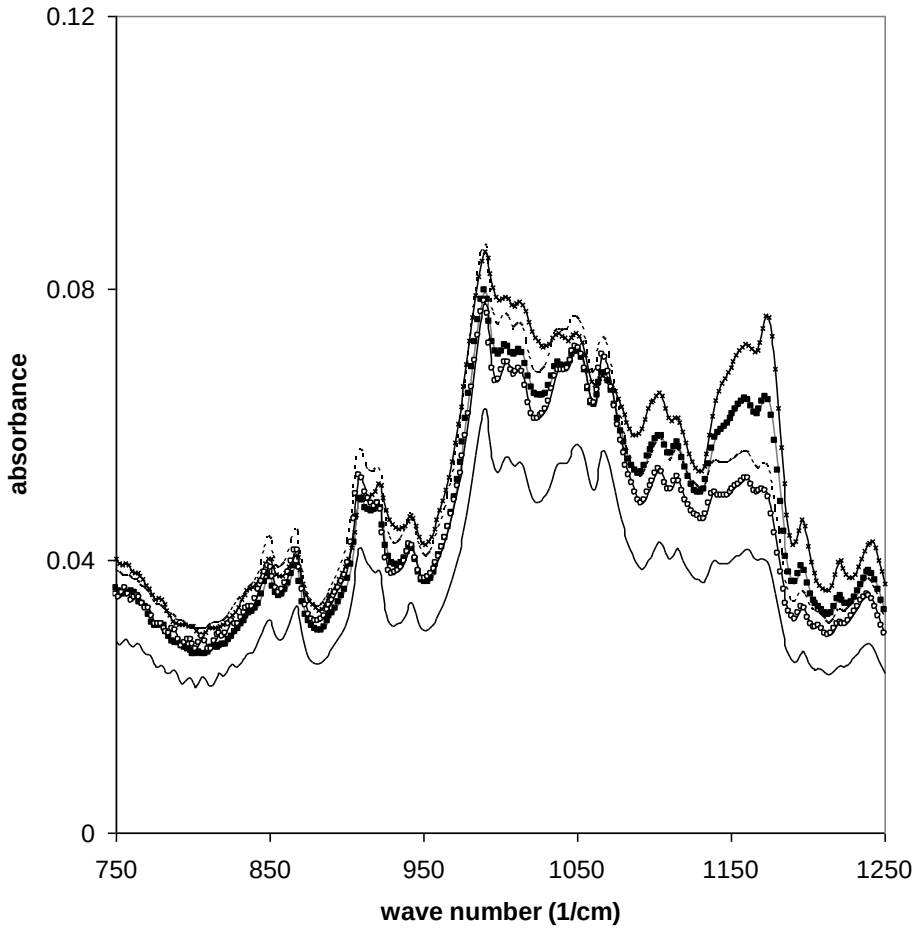

Figure 3. 70 FTIR spectrum of biscuit samples prepared with 10% flour replacement stored for 0 day (black line with black round shape): control, (Black line with white round shape): cacao powder, (dashed line): raw hazelnut skin, (black line with dashed line): ball milled hazelnut skin, (black line): microfluidized hazelnut skin fiber.

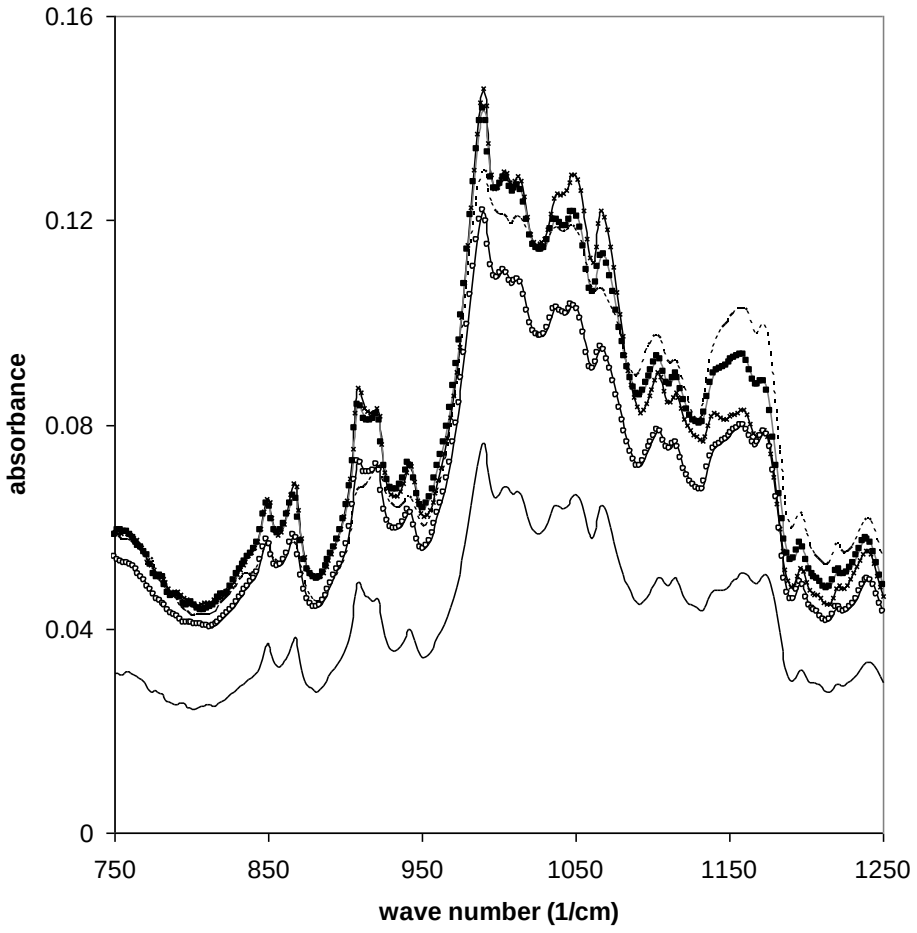

Figure 3. 71 FTIR spectrum of biscuit samples prepared with 10% flour replacement stored for 1 days (black line with black round shape): control, (Black line with white round shape): cacao powder, (dashed line): raw hazelnut skin, (black line with dashed line): ball milled hazelnut skin, (black line): microfluidized hazelnut skin fiber.

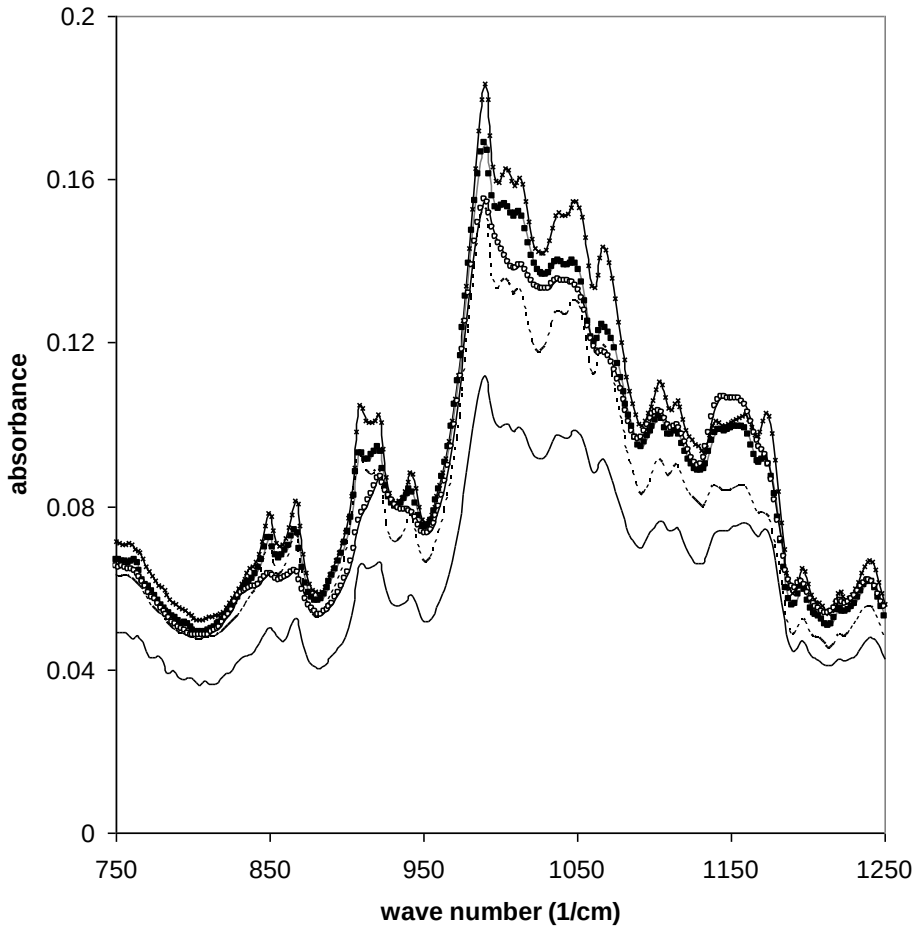

Figure 3. 72 FTIR spectrum of biscuit samples prepared with 10% flour replacement stored for 3 days (black line with black round shape): control, (Black line with white round shape): cacao powder, (dashed line): raw hazelnut skin, (black line with dashed line): ball milled hazelnut skin, (black line): microfluidized hazelnut skin fiber.

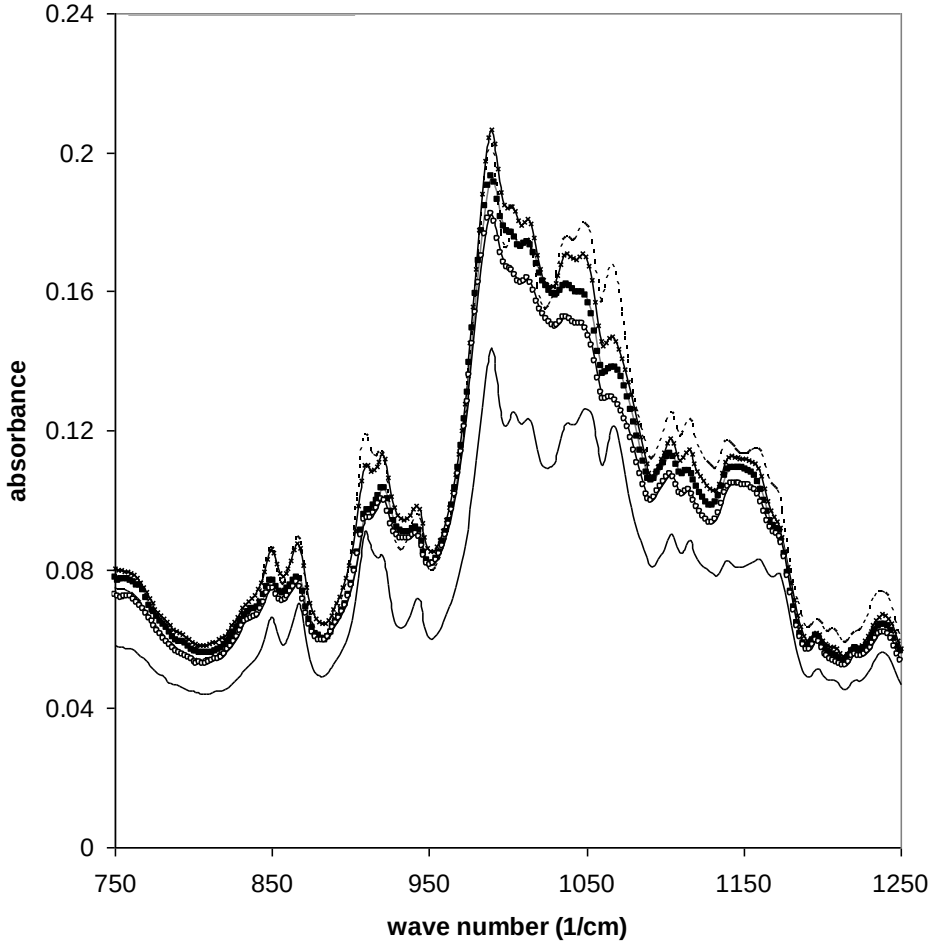

Figure 3. 73 FTIR spectrum of biscuit samples prepared with 10% flour replacement stored for 7 days (black line with black round shape): control, (Black line with white round shape): cacao powder, (dashed line): raw hazelnut skin, (black line with dashed line): ball milled hazelnut skin, (black line): microfluidized hazelnut skin fiber.

According to Figs. 3.70–3.73, the lowest FT-IR spectra intensity was obtained from biscuit samples containing microfluidized hazelnut skin fiber. The peak

intensities at around 1,045 cm-1 was used to analyze starch retrogradation, in other words staling of biscuit samples.

According to Figs. 3.65-3.73 storage times affected the FT-IR spectra intensity of biscuit samples. Increasing signal intensity was observed by increasing storage times. The highest signal intensity was observed at 7 days storage of biscuit samples. The lowest signal intensity was observed from biscuit samples containing microfluidized hazelnut skin fibers. Biscuit samples prepared with microfluidized hazelnut skin fiber water holding capacity was higher than other types of biscuit samples. Therefore, according to results it can be concluded that biscuit samples containing microfluidized hazelnut skin fiber had lower starch retrogradation and so lower tendency of staling.

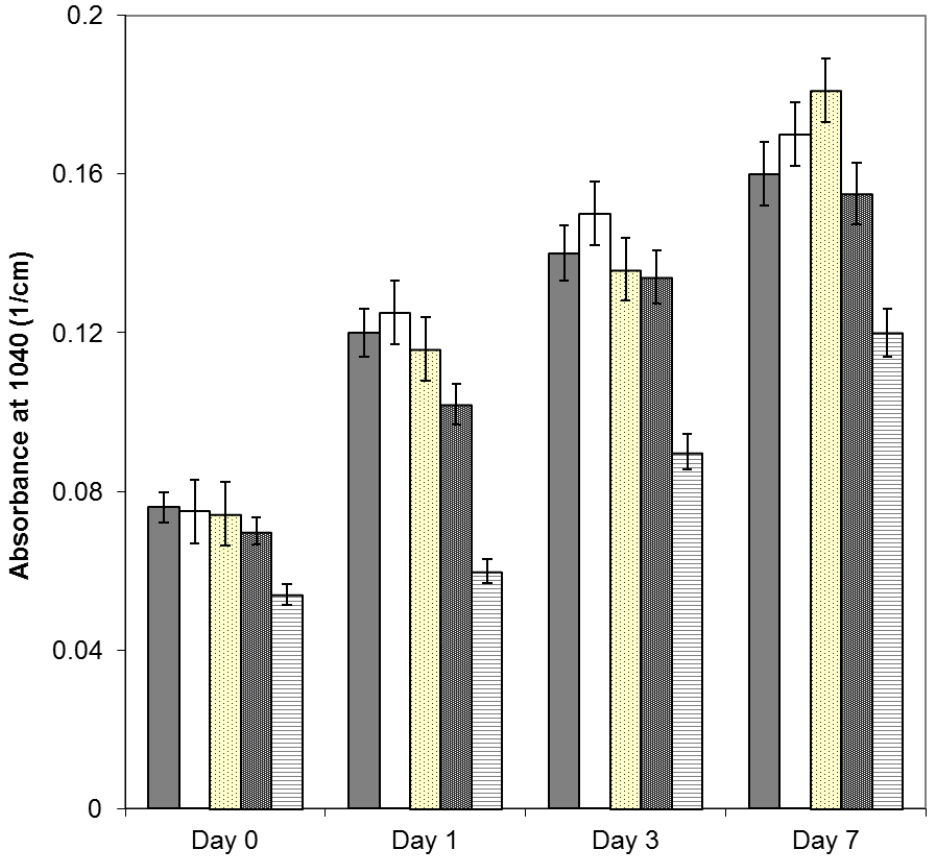

Figure 3. 74 The integral area value of FTIR spectrum peaks absorbance appearing around 1041 cm^{-1} of biscuit samples containing cacao powder/hazelnut skin samples at different storage times (0 day, 1 days, 3 days, and 7 days). (light gray bar): control, (dark gray bar): cacao powder, (light brown bar): raw hazelnut skin, (white bar): ball milled hazelnut skin, (white lined bar): microfluidized hazelnut skin fiber.

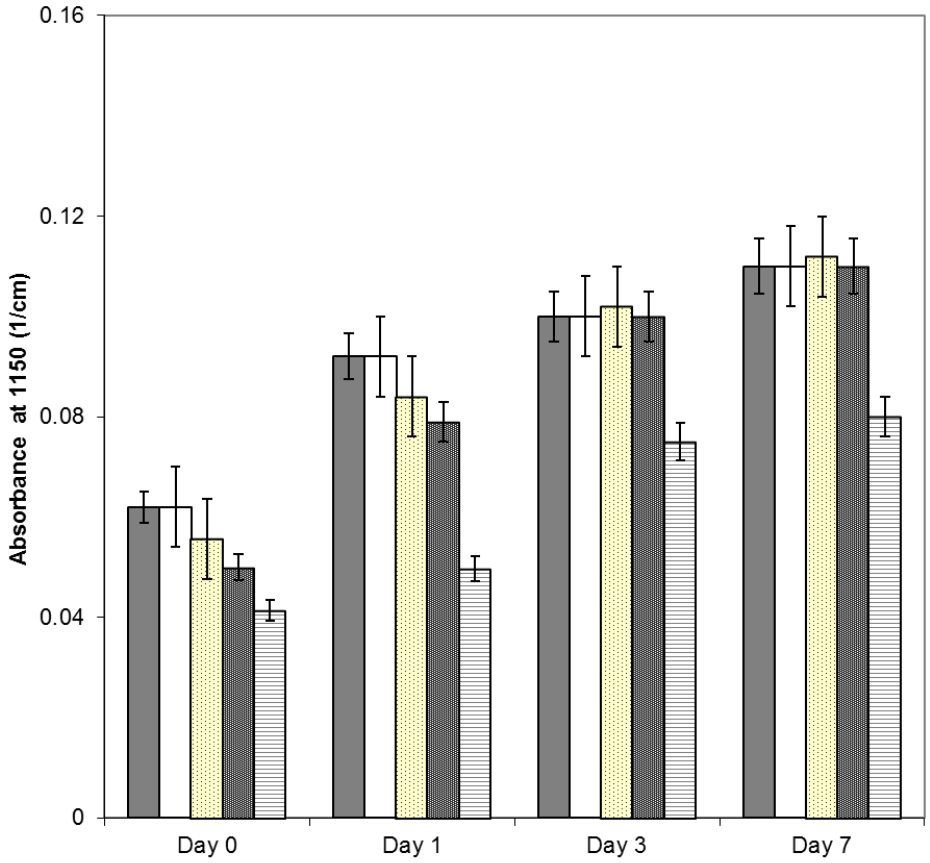

Figure 3. 75 The integral area value of FTIR spectrum peaks absorbance appearing around 1150 cm^{-1} of biscuit samples containing cacao powder/hazelnut skin samples at different storage times (0 day, 1 days, 3 days, and 7 days). (light gray bar): control, (dark gray bar): cacao powder, (light brown bar): raw hazelnut skin, (white bar): ball milled hazelnut skin, (white lined bar): microfluidized hazelnut skin fiber.

The integral area values of biscuit samples FT-IR spectra are shown Figs. 3.74–3.75 at 1040 cm^{-1} and 1150 cm^{-1}. The ratio of peaks appearing increased as the storage time increased. This might be due to the fact that during storage

moisture content of samples decreased and water molecules in protein-starch network was reorganized. The lowest peak intensity of FT-IR spectra at 1040 cm^{-1} was seen in microfluidized hazelnut skin fiber containing biscuit samples. This means that the staling of biscuit samples retarding by using microfluidized hazelnut skin fiber. The results of both FTIR and X-ray analysis were found to be in good agreement in this study.

3.5. Phenolic Content of Raw Materials

Total phenolic content was determined by Folin- Ciocalteu's method. ANOVA results showed that phenolic content was significantly affected with type of raw materials. By considering nutritional value and appetizing character of polyphenols, high total phenolic content in food is desirable (Koeffer, 2008). Figure 3.76 gives quantitative information of phenolic compounds in cacao powder and hazelnut skin samples. Total phenolic content of cacao powder, raw hazelnut skin, ball milled hazelnut skin, and hazelnut skin fiber produced by microfluidization process were 34.23 ± 1.20 mg phenol/L of extract, 147.8 ± 6.66 mg phenol/L of extract, 270.7 ± 3.21 mg phenol/L of extract, and 347.3 ± 6.11 mg phenol/L of extract, respectively. Microfluidization process had a significant effect on total phenolic content. Based on the results, phenolic content of hazelnut skin samples increased with decreasing particle size and increasing surface area. Also, Yüce (2011) observed that phenolic content is higher for all microfluidized orange juices and also, it was stated that phenolic compounds significantly contribute to the antioxidant activity of orange juices. Zieliński et al. (2012) observed that there is positive correlation between total phenolic content and antioxidant activity. Additionally, their studies highlighted that formation of brown melanoidins which are end products of Maillard reaction are enhanced by the increasing antioxidant capacity. Therefore, the expected result was the darkest color of biscuits should be

obtained from biscuits containing 20% microfluidized hazelnut skin fibers. Since, phenolic substances which are known as flavonoids is responsible for the color and flavor of foods (Shahidi & Naczk, 1995), the results of color analysis was supported with this finding. Moreover, according to Zieliński et al. (2012) study higher antioxidant capacity and phenolic acids content in relation for all types of ginger cakes. Therefore, it can be said that the highest antioxidant activity and phenolic content were obtained from hazelnut skin fiber produced by microfluidization process. Also, Aida (2011) study support that phenol enhance the antioxidant power of plant extracts by finding relation between antioxidant activity and total phenol content.

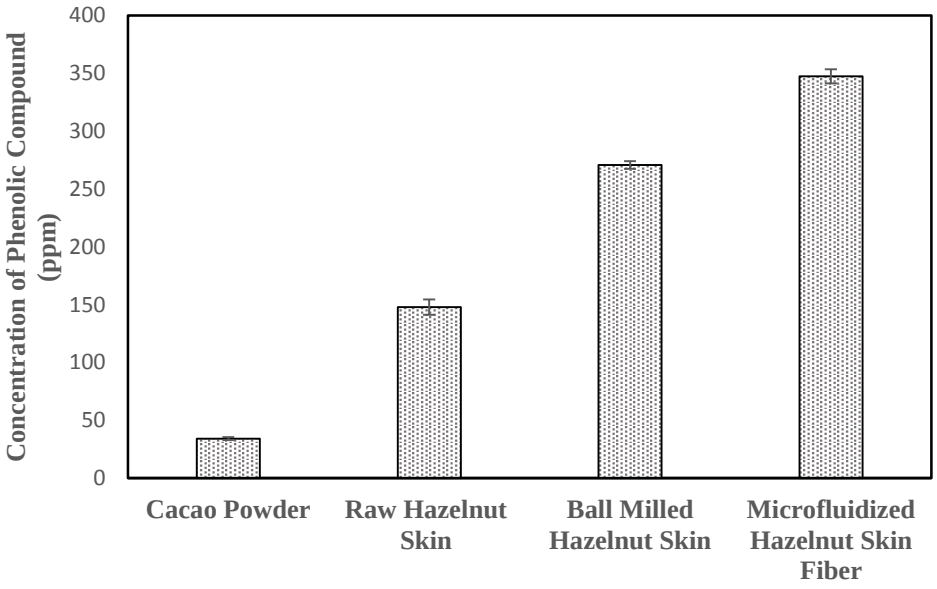

Figure 3. 76 Quantitative information of phenolic compound in cacao powder/hazelnut skin samples. Bars indicate standard deviation of the replicates. Results are expressed as gallic acid equivalents.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In rheological tests, experimental results for all biscuit samples were fitted to power law model and they exhibited shear thinning behavior with different model constants. It was shown that increase percentages of cacao powder, raw hazelnut skin, ball milled hazelnut skin, and microfluidized hazelnut skin fiber led to increases in biaxial extensional viscosity values. The addition of hazelnut skin fiber produced by microfluidization resulted in higher biaxial extensional viscosity than other biscuit samples containing cacao powder, raw hazelnut skin, and ball milled hazelnut skin. The addition of cacao powder and hazelnut skin samples to biscuit dough caused to increases in elastic and viscous moduli. Moreover, increasing percentage of cacao powder and hazelnut skin samples led to increases in elastic and viscous moduli. The highest elastic and viscous moduli was obtained by the replacing flour with 20% microfluidized hazelnut skin fiber. It can be concluded that the rheological properties of biscuit dough strongly depended on addition of fiber and concentration of fiber in biscuit samples as an ingredient.

Cacao powder and hazelnut skin samples microstructure were investigated by using SEM. The structural differences between cacao powder, raw hazelnut skin, ball milled, and microfluidized hazelnut skin fiber were clearly observed. Microfluidization process caused to fibrillation of hazelnut skin leading to increases in water holding capacity and this property resulted in change rheological and textural properties of biscuits. Hazelnut skins' size was reduced by ball milling process, therefore there were differences between raw hazelnut skin and ball milled hazelnut skin SEM images. However, SEM images of

cacao powder and ball milled hazelnut skin were similar to each other because they had relatively same particle size.

When fresh biscuits were investigated in terms of moisture content, size, color, and texture, it was seen that addition of cacao powder and hazelnut skin samples affected these properties. Biscuit containing microfluidized hazelnut skin fiber had higher moisture content due to higher water holding capacity of microfluidized hazelnut skin fiber. As the percentage of flour replacement increased moisture content of biscuit samples also increased. Therefore, the highest moisture content obtained from biscuit samples prepared by replacing flour with 20% microfluidized hazelnut skin fiber. Size of biscuits was also affected from addition of cacao powder or hazelnut skin samples significantly ($p \leq 0.05$). The lowest spreading character of biscuits was obtained from microfluidized hazelnut skin fiber containing samples. As the flour replacement percentages increased, the spreading character of biscuit decreased. The highest diameter value was observed in control biscuits. Addition of microfluidized hazelnut skin fiber affected the color of biscuits significantly ($p \leq 0.05$). Increasing flour replacement percentages (cacao powder/hazelnut skin samples) led to darker color. The darkest color obtained from biscuits containing 20% microfluidized hazelnut skin fiber. Breaking strength of biscuits were also investigated and it was found that as the percentage of cacao powder or hazelnut skin samples increased, breaking strength of biscuits increased.

When staling mechanisms of biscuits were investigated, it was seen that both X-ray and FTIR analysis gave similar results. Also, the other experiment results such as size measurement, moisture content and texture were in agreement with the result of X-ray and FTIR. Retrogradation of starch increased during storage. Starch retrogradation was the smallest in biscuit samples containing microfluidized hazelnut skin fiber. According to total mass crystallinity results,

microfluidized hazelnut skin fiber had the lowest result which means that microfluidized hazelnut skin fiber retarded the staling of biscuits.

When phenolic content of hazelnut skin samples and cacao powder were analyzed, it was seen that the highest phenolic content was observed in microfluidized hazelnut skin fiber containing samples which might be related to the smallest size of microfluidized hazelnut skin fiber.

The results of study showed that biscuits containing microfluidized hazelnut skin fiber rather than cacao powder or other hazelnut skin samples were more acceptable and had longer shelf life.

In the future study, the utilization of microfluidized hazelnut skin samples in biscuit samples can be used to reduce flour need. In addition, microfluidized hazelnut skin fiber can be used in different gluten-free product types.

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APPENDIX A

PICTURES OF CACAO POWDER & HAZELNUT SKIN SAMPLES

Figure A. 1 Picture of cacao powder, raw hazelnut skin, ball milled hazelnut skin, and hazelnut skin fiber processed by microfluidizer

Figure A. 2 Picture of microfluidized hazelnut skin fiber and cacao powder

APPENDIX B

FIGURES OF MATERIAL METHOD

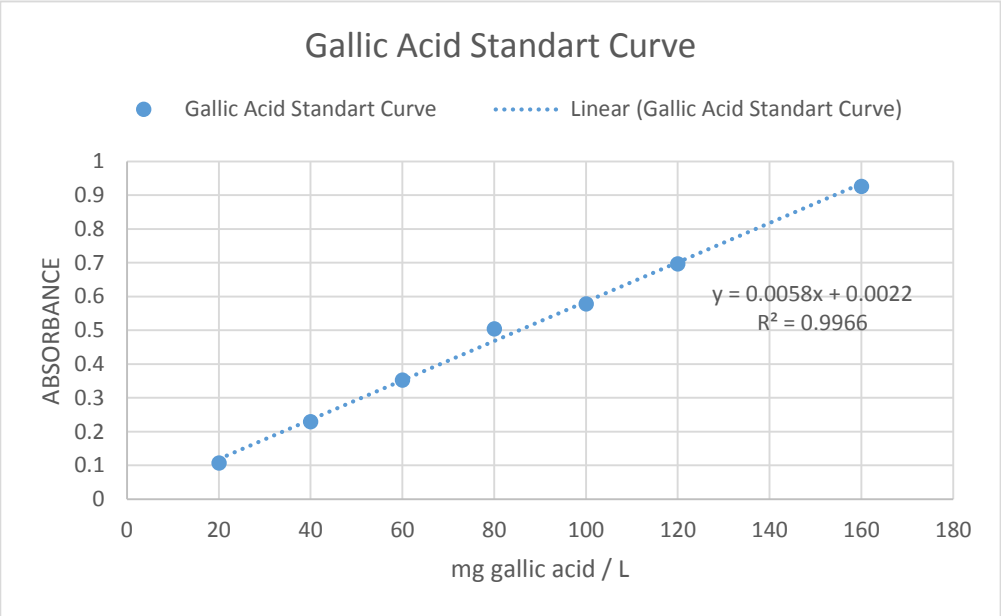

Figure B. 1 Gallic acid standard curve for determination of total phenol content

APPENDIX C

TABLES OF FIGURES

Table C. 1 Moisture content values of biscuit samples

Moisture Content (%)				
Replacement of flour	Cacao Powder	Raw Hazelnut Skin	Ball Milled Hazelnut Skin	Microfluidized Hazelnut Skin Fiber
0%	5.22 ± 0.35^{bc}	5.22 ± 0.35^{bc}	5.22 ± 0.35^{bc}	5.22 ± 0.3^{bc}
10%	4.3 ± 0.68^{d}	4.58 ± 0.65^{c}	4.27 ± 0.99^{c}	6.22 ± 0.82^{bc}
15%	4.66 ± 0.52^{c}	4.85 ± 0.33^{c}	4.69 ± 0.62^{c}	6.33 ± 0.24^{bc}
20%	5.1 ± 0.15^{a}	5.04 ± 0.43^{bc}	5.31 ± 0.90^{bc}	6.64 ± 0.11^{a}

The average of three readings was taken. Formulations with different letters shows statistical different results ($p \leq 0.05$).

Table C. 2 Thickness of biscuit samples

Thickness (cm)				
Replacement of flour	Cacao Powder	Raw Hazelnut Skin	Ball Milled Hazelnut Skin	Microfluidized Hazelnut Skin Fiber
0	0.9 ± 0.06^{e}	0.9 ± 0.06^{e}	0.9 ± 0.06^{e}	0.9 ± 0.06^{e}
10%	1.04 ± 0.01^{d}	1.09 ± 0.03^{d}	1.17 ± 0.03^{abc}	1.17 ± 0.0^{ab}
15%	1.09 ± 0.03^{cd}	1.10 ± 0.02^{bcd}	1.14± 0.02^{abc}	1.14 ± 0.02^{abc}
20%	1.15 ± 0.02^{abc}	1.17 ± 0.0^{ab}	1.16 ± 0.0^{abc}	1.19 ± 0.01^{a}

The average of three readings was taken. Formulations with different letters shows statistical different results ($p \leq 0.05$).

Table C. 3 Diameter of biscuit samples

Diameter (cm)				
Replacement of flour	Cacao Powder	Raw Hazelnut Skin	Ball Milled Hazelnut Skin	Microfluidized Hazelnut Skin Fiber
0	7.21 ± 0.02^{b}	7.21 ± 0.02^{b}	7.21 ± 0.02^{b}	7.21 ± 0.02^{b}
10%	6.80 ± 0.02^{d}	7.39 ± 0.05^{a}	6.98 ± 0.04^{c}	6.38 ± 0.20^{f}
15%	6.55 ± 0.06^{e}	7.24 ± 0.05^{ab}	6.92 ± 0.10^{cd}	6.21 ± 0.06^{f}
20%	6.38 ± 0.03^{f}	7.16 ± 0.02^{b}	6.79 ± 0.08^{d}	6.23 ± 0.04^{f}

The average of three readings was taken. Formulations with different letters shows statistical different results ($p \leq 0.05$).

Table C. 4 Color of biscuit samples

Color (Delta E)				
Replacement of flour	Cacao Powder	Raw Hazelnut Skin	Ball Milled Hazelnut Skin	Microfluidized Hazelnut Skin Fiber
10%	24.9 ± 3.24^{bc}	14.33 ± 3.92^{d}	20.54 ± 1.28^{c}	24.23 ± 1.81^{bc}
15%	27.96 ± 2.16^{ab}	20.78 ± 1.63^{c}	22.52 ± 0.46^{c}	32.2 ± 1.85^{a}
20%	29.16 ± 2.87^{ab}	21.06 ± 3.67^{c}	22.84 ± 3.33^{c}	32.43 ± 2.78^{a}

The average of three readings was taken. Formulations with different letters shows statistical different results ($p \leq 0.05$).

Table C. 5 Breaking strength of biscuit samples

Hardness (g force)				
Replacement of flour	Cacao Powder	Raw Hazelnut Skin	Ball Milled Hazelnut Skin	Microfluidized Hazelnut Skin Fiber
0	1466.46 ± 222.2^{g}	1466.46 ± 222.2^{g}	1466.46 ± 222.2^{g}	1466.46 ± 222.2^{g}
10%	4717.25 ± 222.94^{d}	3543.54 ± 329.15^{ef}	3764.10 ± 329.15^{e}	1969.90 ± 704.15^{g}
15%	6679.83 ± 215.22^{b}	4642.34 ± 77.37^{d}	4477.2 ± 71.32^{d}	2946.19 ± 142.28^{f}
20%	8333.98± 116.66^{a}	4614.85 ± 76.61^{d}	5631.97 ± 85.51^{c}	3511.84 ± 31.9^{ef}

The average of three readings was taken. Formulations with different letters shows statistical different results ($p \leq 0.05$).

Table C. 6 Thickness of biscuit samples during storage

Biscuit sample	Storage Time (day)			
	0	1	3	7
Control	0.90 ± 0.06	0.88 ± 0.03	0.90 ± 0.04	0.93 ± 0.06
CP 10%	1.04 ± 0.01	1.06 ± 0.03	1.07 ± 0.07	1.05 ± 0.02
CP 15%	1.09 ± 0.03	1.09 ± 0.01	1.09 ± 0.01	1.10 ± 0.00
CP 20%	1.15 ± 0.02	1.11 ± 0.05	1.10 ± 0.02	1.11 ± 0.02
RHS 10%	1.09 ± 0.03	1.04 ± 0.05	1.01 ± 0.07	1.06 ± 0.08
RHS 15%	1.10 ± 0.02	1.10 ± 0.04	1.09 ± 0.06	1.09 ± 0.09
RHS 20%	1.17 ± 0.00	1.13 ± 0.06	1.11 ± 0.07	1.11 ± 0.04
BMHS 10%	1.12 ± 0.02	1.06 ± 0.05	1.06 ± 0.04	1.06 ± 0.05
BMHS 15%	1.14 ± 0.02	1.11 ± 0.02	1.09 ±0.05	1.11 ± 0.05
BMHS 20%	1.16 ± 0.03	1.18 ± 0.05	1.15 ± 0.02	1.15 ± 0.05
MFHSF 10%	1.17 ± 0.01	1.16 ± 0.03	1.19 ± 0.05	1.14 ± 0.09
MFHSF 15%	1.14 ± 0.02	1.11 ± 0.05	1.12 ± 0.08	1.13 ± 0.06
MFHSF 20%	1.19 ± 0.01	1.18 ± 0.01	1.18 ± 0.04	1.17 ± 0.01

The average of three readings was taken. (CP): Cacao Powder, (RHS): Raw Hazelnut Skin, (BMHS): Ball Milled Hazelnut Skin, (MFHSF): Microfluidized Hazelnut Skin Fiber

Table C. 7 Diameter of biscuit samples during storage

Biscuit sample	Storage Time (day)			
	0	1	3	7
Control	7.21 ± 0.03	7.23 ± 0.10	7.36 ± 0.28	7.30 ± 0.09
CP 10%	6.80 ± 0.02	6.79 ± 0.08	6.83 ± 0.03	6.90 ± 0.18
CP 15%	6.55 ± 0.06	6.56 ± 0.05	6.60 ± 0.05	6.63 ± 0.09
CP 20%	6.38 ± 0.03	6.35 ± 0.02	6.41 ± 0.10	6.37 ± 0.10
RHS 10%	7.39 ± 0,05	7.23 ± 0.07	7.26 ± 0.05	7.27 ± 0.18
RHS 15%	7.24 ± 0.05	7.26 ± 0.20	7.15 ± 0.41	7.27 ± 0.04
RHS 20%	7.16 ± 0.02	7.14 ± 0.17	7.16 ± 0.01	6.80 ± 0.48
BMHS 10%	6.98 ± 0.04	6.86 ± 0.04	6.90 ± 0.17	6.86 ± 0.05
BMHS 15%	6.92 ± 0.10	6.90 ± 0.10	6.95 ± 0.10	6.85 ± 0.13
BMHS 20%	6.79 ± 0.08	6.78 ± 0.05	6.74 ± 0.09	6.80 ± 0.06
MFHSF 10%	6.38 ± 0.20	6.32 ± 0.07	6.42 ± 0.10	6.43 ± 0.15
MFHSF 15%	6.21 ± 0.06	6.19 ± 0.07	6.27 ± 0.07	6.29 ± 0.05
MFHSF 20%	6.23 ± 0.04	6.19 ± 0.05	6.28 ± 0.12	6.31 ± 0.11

The average of three readings was taken. (CP): Cacao Powder, (RHS): Raw Hazelnut Skin, (BMHS): Ball Milled Hazelnut Skin, (MFHSF): Microfluidized Hazelnut Skin Fiber

Table C. 8 Moisture content of biscuit samples during storage

Biscuit sample	Storage time (day)			
	0	1	3	7
Control	5.22 ± 0.35	6.02 ± 0.78	6.61 ± 0.77	6.26 ± 0.49
CP 10%	4.30 ± 0.68	4.35 ± 0.71	4.86 ± 0.74	4.20 ± 0.13
CP 15%	4.66 ± 0.52	7.54 ± 0.07	7.14 ± 0.57	6.55 ± 0.38
CP 20%	5.10 ± 0.15	7.68 ± 0.18	7.73 ± 0.25	8.36 ± 0.38
RHS 10%	4.58 ± 0.65	4.71 ± 0.67	5.42 ± 0.38	5.56 ± 0.41
RHS 15%	4.85 ± 0.33	5.17 ± 0.69	5.47 ± 0.61	5.54 ± 0.82
RHS 20%	5.04 ± 0.43	5.02 ± 0.85	5.04 ± 0.24	5.20 ± 0.79
BMHS 10%	4.27 ± 0.99	4.36 ± 0.44	5.66 ± 0.51	5.74 ± 0.54
BMHS 15%	4.69 ± 0.62	4.86 ± 0.33	5.54 ± 0.16	5.59 ± 0.04
BMHS 20%	5.31 ± 0.90	5.31 ± 0.69	5.72 ± 0.32	5.79 ± 0.46
MFHSF 10%	6.22 ± 0.83	6.55 ± 0.22	6.71 ± 0.11	6.30 ± 0.68
MFHSF 15%	6.33 ± 0.24	6.17 ± 0.75	6.32 ± 0.54	5.55 ± 0.30
MFHSF 20%	6.64± 0.11	7.24 ± 0.30	6.59 ± 0.12	5.87 ± 0.70

The average of three readings was taken. (CP): Cacao Powder, (RHS): Raw Hazelnut Skin, (BMHS): Ball Milled Hazelnut Skin, (MFHSF): Microfluidized Hazelnut Skin Fiber

Table C. 9 Breaking strength of biscuits during storage

Biscuit sample	Storage time (day)			
	0	1	3	7
Control	1466,46 ± 229,20	4104.67 ± 1045.01	4215.53 ± 1247.79	5366.02 ± 347.24
CP 10%	4717.35 ± 222.93	6860.32 ± 257.52	7510.14 ± 211.40	7646.95 ± 475.81
CP 15%	6679.83 ± 215.23	7023.53 ± 144.45	7707.40 ± 603.93	8266.32 ± 644.89
CP 20%	8333.98 ± 229.20	8446.57 ± 1045.01	9488.46 ± 1247.79	9829.91 ± 347.24
RHS 10%	3543.54 ± 329.15	4721.79 ± 158.49	5862.43 ± 349.81	6723.61 ± 196.78
RHS 15%	4642.35 ± 77.38	5596.10 ± 236.19	5941.30 ± 603.93	7692.71 ± 238.31
RHS 20%	4614.85 ± 116.66	6240.01 ± 291.60	7790.23 ± 145.11	8280.40 ± 74.90
BMHS 10%	3764.10 ± 45.89	5922.96 ± 280.21	7300.18 ± 121.72	8532.03 ± 224.10
BMHS 15%	4477.20 ± 71.33	6713.41 ± 130.84	7687.31 ± 125.78	8710.97 ± 133.33
BMHS 20%	5631.97 ± 85.51	7043.42 ± 119.92	7895.33 ± 366.13	8715.38 ± 238.10
MFHSF 10%	1969.90 ± 704.15	3208.02 ± 135.99	5358.34 ± 372.81	6632.11 ± 64.58
MFHSF 15%	2946.19 ± 142.29	4216.84 ± 134.24	5372.73 ± 92.49	7040.74 ± 32.32
MFHSF 20%	3511.84 ± 31.90	4356.86 ± 122.00	7895.33 ± 88.89	8016.18 ± 110.72

The average of three readings was taken. (CP): Cacao Powder, (RHS): Raw Hazelnut Skin, (BMHS): Ball Milled Hazelnut Skin, (MFHSF): Microfluidized Hazelnut Skin Fiber

Table C. 10 Total phenol content of raw materials

Material Type	Total phenolic content (ppm)
Cacao Powder	34.23 ± 1.20^{a}
Raw Hazelnut Skin	147.8 ± 6.66^{b}
Ball Milled Hazelnut Skin	270.7 ± 3.21^{c}
Microfluidized Hazelnut Skin Fiber	347.3 ± 6.11^{d}

The average of three readings was taken. Formulations with different letters shows statistical different results ($p \leq 0.05$).

APPENDIX D

STATISTICAL ANALYSIS

Table D. 1 Results for Tukey's mean comparison test for moisture content of fresh biscuit samples

General Linear Model: moisture content versus percentage; sample

Factor	Type	Levels	Values
percentage	fixed	4	0; 10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw

Analysis of Variance for moisture content, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
percentage	3	41.6981	41.6981	13.8994	21.44	0.000
sample	3	38.2347	38.2347	12.7449	19.66	0.000
percentage*sample	9	55.1468	55.1468	6.1274	9.45	0.000
Error	128	82.9756	82.9756	0.6482		
Total	143	218.0552				

S = 0.805138 R-Sq = 61.95% R-Sq(adj) = 57.49%

Table D.1 (continued)

Obs	Moisture content	Fit	SE Fit	Residual	St Resid
18	4.89000	2.67778	0.26838	2.21222	2.91 R
30	1.17000	6.22000	0.26838	-5.05000	-6.65 R
44	6.96000	5.31222	0.26838	1.64778	2.17 R
46	8.18000	6.22000	0.26838	1.96000	2.58 R
74	6.35000	4.26889	0.26838	2.08111	2.74 R
142	8.18000	6.22000	0.26838	1.96000	2.58 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

percentage	N	Mean	Grouping
20	36	6.0	A
0	36	5.2	B
15	36	5.1	B
10	36	4,.4	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

sample	N	Mean	Grouping
mf	36	6.1	A
raw	36	4.9	B
ball mill	36	4.9	B
cacao	36	4.8	B

Means that do not share a letter are significantly different.

Table D.1 (continued)

Grouping Information Using Tukey Method and 95,0% Confidence

percentage	sample	N	Mean	Grouping
20	cacao	9	6.8	A
20	mf	9	6.6	A
10	mf	9	6.2	A B
15	mf	9	6.2	A B
20	ball mill	9	5.3	B C
0	ball mill	9	5.2	B C
0	raw	9	5.2	B C
0	cacao	9	5.2	B C
0	mf	9	5.2	B C
20	raw	9	5.0	B C
15	raw	9	4.8	C
15	ball mill	9	4.7	C
15	cacao	9	4.7	C
10	raw	9	4.6	C
10	ball mill	9	4.3	C
10	cacao	9	2.7	D

Means that do not share a letter are significantly different.

Table D. 2 Results for Tukey's mean comparison test for thickness of fresh biscuit samples

General Linear Model: height versus percentage; sample

Factor	Type	Levels	Values
percentage	fixed	4	0; 10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw

Analysis of Variance for height, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
percentage	3	1.54553	1.54553	0.51518	273.07	0.000
sample	3	0.05775	0.05775	0.01925	10.20	0.000
percentage*sample	9	0.04828	0.04828	0.00536	2.84	0.004
Error	128	0.24149	0.24149	0.00189		
Total	143	1.89305				

S = 0.0434354 R-Sq = 87.24% R-Sq(adj) = 85.75%

Unusual Observations for height

Obs	height	Fit	SE Fit	Residual	St Resid
49	1.03333	0.89815	0.01448	0.13519	3.30 R
52	1.06667	1.15481	0.01448	-0.08815	-2.15 R
53	1.03333	0.89815	0.01448	0.13519	3.30 R
57	1.03333	0.89815	0.01448	0.13519	3.30 R
61	1.03333	0.89815	0.01448	0.13519	3.30 R

R denotes an observation with a large standardized residual.

Table D.2 (continued)

Grouping Information Using Tukey Method and 95,0% Confidence

percentage	N	Mean	Grouping
20	36	1.2	A
15	36	1.1	B
10	36	1.1	B
0	36	0.9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

sample	N	Mean	Grouping
mf	36	1.1	A
ball mill	36	1.1	A B
raw	36	1.1	B C
cacao	36	1.0	C

Means that do not share a letter are significantly different.

Table D.2(continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	N	Mean	Grouping
20	mf	9	1.2	A
20	raw	9	1.2	A B
10	mf	9	1.2	A B
20	ball mill	9	1.2	A B C
20	cacao	9	1.2	A B C
15	mf	9	1.1	A B C
15	ball mill	9	1.1	A B C
10	ball mill	9	1.1	A B C
15	raw	9	1.1	B C D
10	raw	9	1.1	C D
15	cacao	9	1.1	C D
10	cacao	9	1.0	D
0	raw	9	0.9	E
0	mf	9	0.9	E
0	cacao	9	0.9	E
0	ball mill	9	0.9	E

Means that do not share a letter are significantly different.

Table D. 3 Results for Tukey's mean comparison test for diameter of fresh biscuit samples

General Linear Model: diameter versus percentage; sample

Factor	Type	Levels	Values
percentage	fixed	4	0; 10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw

Analysis of Variance for diameter. using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
percentage	3	6.7099	6.7099	2.2366	215.89
sample	3	10.9615	10.9615	3.6538	352.69
percentage*sample	9	3.9168	3.9168	0.4352	42.01
Error	128	1.3261	1.3261	0.0104	
Total	143	22.9143			

S = 0.101784 R-Sq = 94.21% R-Sq(adj) = 93.53%

Unusual Observations for diameter

Obs	diameter	Fit	SE Fit	Residual	St Resid
14	6.62500	6.37778	0.03393	0.24722	2.58 R
28	7.05000	6.79389	0.03393	0.25611	2.67 R
31	6.00000	6.21389	0.03393	-0.21389	-2.23 R
66	7.00000	6.80278	0.03393	0.19722	2.06 R
88	7.37500	7.15778	0.03393	0.21722	2.26 R
107	6.62500	6.92389	0.03393	-0.29889	-3.11 R
126	6.00000	6.37778	0.03393	-0.37778	-3.94 R

R denotes an observation with a large standardized residual.

Table D.3 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	N	Mean	Grouping
0	36	7.2	A
10	36	6.9	B
15	36	6.7	C
20	36	6.6	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

sample	N	Mean	Grouping
raw	36	7.2	A
ball mill	36	7.0	B
cacao	36	6.7	C
mf	36	6.5	D

Means that do not share a letter are significantly different.

Table D.3 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	N	Mean	Grouping
10	raw	9	7.4	A
15	raw	9	7.2	A B
0	mf	9	7.2	B
0	raw	9	7.2	B
0	cacao	9	7.2	B
0	ball mill	9	7.2	B
20	raw	9	7.2	B
10	ball mill	9	7.0	C
15	ball mill	9	6.9	C D
10	cacao	9	6.8	D
20	ball mill	9	6.8	D
15	cacao	9	6.5	E
20	cacao	9	6.4	F
10	mf	9	6.4	F
20	mf	9	6.2	F
15	mf	9	6.2	F

Means that do not share a letter are significantly different.

Table D. 4 Results for Tukey's mean comparison test for total color change of fresh biscuit samples

General Linear Model: total color change versus percentage; sample

Factor	Type	Levels	Values
percentage	fixed	3	10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw

Analysis of Variance for total color change. using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
percentage	2	633.16	633.16	316.58	32.09	0.000
sample	3	1999.95	1999.95	666.65	67.58	0.000
percentage*sample	6	134.80	134.80	22.47	2.28	0.042
Error	96	947.01	947.01	9.86		
Total	107	3714.92				

S = 3.14080 R-Sq = 74.51% R-Sq(adj) = 71.59%

Unusual Observations for total color change

Obs	total color change	Fit	SE Fit	Residual	St Resid
6	27.6301	21.0614	1.0469	6.5688	2.22 R
38	20.0900	27.9629	1.0469	-7.8730	-2.66 R
49	18.9775	24.9023	1.0469	-5.9248	-2.00 R
57	16.3446	22.8413	1.0469	-6.4967	-2.19 R
63	20.2054	29.1562	1.0469	-8.9508	-3.02 R
67	11.6126	20.5433	1.0469	-8.9307	-3.02 R
69	16.7935	22.8413	1.0469	-6.0477	-2.04 R

R denotes an observation with a large standardized residual.

Table D.4 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	N	Mean	Grouping
20	36	26.4	A
15	36	25.9	A
10	36	21.0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

sample	N	Mean	Grouping
mf	27	29.6	A
cacao	27	27.3	B
ball mill	27	22.0	C
raw	27	18.7	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	N	Mean	Grouping
20	mf	9	32.4	A
15	mf	9	32.2	A
20	cacao	9	29.2	A B
15	cacao	9	28.0	A B
10	cacao	9	24.9	B C
10	mf	9	24.2	B C
20	ball mill	9	22.8	C
15	ball mill	9	22.5	C
20	raw	9	21.1	C
15	raw	9	20.8	C
10	ball mill	9	20.5	C
10	raw	9	14.3	D

Means that do not share a letter are significantly different.

Table D. 5 Results for Tukey's mean comparison test for total color change in terms of L*a*b scale of fresh biscuit samples

General Linear Model: L versus percentage; sample

Factor	Type	Levels	Values
percentage	fixed	4	0; 10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw

Analysis of Variance for L. using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
percentage	3	107166.2	107166.2	35722.1	2645.83
sample	3	20683.7	20683.7	6894.6	510.66
percentage*sample	9	7826.9	7826.9	869.7	64.41
Error	176	2376.2	2376.2	13.5	
Total	191	138053.0			

S = 3.67441 R-Sq = 98.28% R-Sq(adj) = 98.13%

Table D.5 (continued)

Unusual Observations for L

Obs	L	Fit	SE Fit	Residual	St Resid
3	45.1460	37.8237	1.0607	7.3223	2.08 R
12	28.1630	36.5939	1.0607	-8.4309	-2.40 R
24	48.0450	56.3629	1.0607	-8.3179	-2.36 R
56	48.1260	56.3629	1.0607	-8.2369	-2.34 R
67	30.5170	37.8237	1.0607	-7.3067	-2.08 R
100	41.4000	29.1702	1.0607	12.2298	3.48 R
108	27.4530	36.5939	1.0607	-9.1409	-2.60 R
110	39.6380	31.6160	1.0607	8.0220	2.28 R
124	44.3600	36.5939	1.0607	7.7661	2.21 R
142	23.7860	31.6160	1.0607	-7.8300	-2.23 R
143	29.7850	19.5978	1.0607	10.1872	2.90 R
159	11.7600	19.5978	1.0607	-7.8378	-2.23 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	N	Mean	Grouping
0	48	95.4	A
10	48	51.7	B
15	48	41.2	C
20	48	34.9	D

Means that do not share a letter are significantly different.

Table D.5 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

sample	N	Mean	Grouping
raw	48	70.2	A
ball mill	48	57.7	B
cacao	48	54.2	C
mf	48	41.0	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	N	Mean	Grouping
0	mf	12	95.4	A
0	cacao	12	95.4	A
0	raw	12	95.4	A
0	ball mill	12	95.4	A
10	raw	12	66.6	B
15	raw	12	62.4	B
20	raw	12	56.4	C
10	cacao	12	54.5	C
10	ball mill	12	53.9	C
15	ball mill	12	44.9	D
15	cacao	12	37.8	E
20	ball mill	12	36.6	E F
10	mf	12	31.6	F G
20	cacao	12	29.2	G
15	mf	12	19.6	H
20	mf	12	17.6	H

Means that do not share a letter are significantly different.

Table D.5(continued)

General Linear Model: a versus percentage; sample

Factor	Type	Levels	Values
percentage	fixed	4	0; 10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw

Analysis of Variance for a. using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
percentage	3	19127.9	19127.9	6376.0	236.43	0.000
sample	3	7194.6	7194.6	2398.2	88.93	0.000
percentage*sample	9	4835.5	4835.5	537.3	19.92	0.000
Error	176	4746.3	4746.3	27.0		
Total	191	35904.3				

S = 5.19302 R-Sq = 86.78% R-Sq(adj) = 85.65%

Unusual Observations for a

Obs	a	Fit	SE Fit	Residual	St Resid
2	20.4050	31.0293	1.4991	-10.6243	-2.14 R
8	13.3140	24.4793	1.4991	-11.1653	-2.25 R
35	21.9140	39.1935	1.4991	-17.2795	-3.48 R
51	28.1700	39.1935	1.4991	-11.0235	-2.22 R
66	20.4050	31.0293	1.4991	-10.6243	-2.14 R
74	49.7090	33.8084	1.4991	15.9006	3.20 R
98	46.4330	31.0293	1.4991	15.4037	3.10 R
108	31.1230	20.9568	1.4991	10.1663	2.04 R
130	41.1490	31.0293	1.4991	10.1197	2.04 R
164	47.0800	36.2572	1.4991	10.8228	2.18 R
170	20.9530	33.8084	1.4991	-12.8554	-2.59 R

R denotes an observation with a large standardized residual.

Table D.5 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	N	Mean	Grouping
10	48	26.5	A
15	48	23.7	B
20	48	22.0	B
0	48	1.3	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

sample	N	Mean	Grouping
cacao	48	27.0	A
ball mill	48	19.8	B
raw	48	17.0	C
mf	48	9.9	D

Means that do not share a letter are significantly different.

Table D.5 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	N	Mean	Grouping
15	cacao	12	39.2	A
20	cacao	12	36.3	A B
10	ball mill	12	33.8	A B
10	cacao	12	31.0	B C
10	raw	12	25.9	C D
20	raw	12	24.5	C D
15	ball mill	12	23.1	D E
20	ball mill	12	21.0	D E F
15	mf	12	16.3	E F
15	raw	12	16.2	E F
10	mf	12	15.4	F
20	mf	12	6.4	G
0	mf	12	1.3	G
0	raw	12	1.3	G
0	ball mill	12	1.3	G
0	cacao	12	1.3	G

Means that do not share a letter are significantly different.

General Linear Model: b versus percentage; sample

Factor	Type	Levels	Values
percentage	fixed	4	0; 10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw

Table D.5 (continued)

Analysis of Variance for b. using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
percentage	3	1088.15	1088.15	362.72	23.84
sample	3	8455.75	8455.75	2818.58	185.27
percentage*sample	9	3941.71	3941.71	437.97	28.79
Error	176	2677.56	2677.56	15.21	
Total	191	16163.18			

S = 3.90044 R-Sq = 83.43% R-Sq(adj) = 82.02%

Unusual Observations for b

Obs	b	Fit	SE Fit	Residual	St Resid
6	11.3610	19.6105	1.1260	-8.2495	-2.21 R
38	10.6480	19.6105	1.1260	-8.9625	-2.40 R
51	20.4320	30.1757	1.1260	-9.7437	-2.61 R
68	40.6170	32.2416	1.1260	8.3754	2.24 R
75	17.7460	10.0404	1.1260	7.7056	2.06 R
99	45.8950	30.1757	1.1260	15.7193	4.21 R
100	24.3820	32.2416	1.1260	-7.8596	-2.10 R
114	38.6900	27.8912	1.1260	10.7988	2.89 R
131	22.3400	30.1757	1.1260	-7.8357	-2.10 R
132	40.6170	32.2416	1.1260	8.3754	2.24 R
164	40.3820	32.2416	1.1260	8.1404	2.18 R
175	16.3850	6.2551	1.1260	10.1299	2.71 R
190	15.3140	7.1019	1.1260	8.2121	2.20 R

R denotes an observation with a large standardized residual.

Table D.5 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	N	Mean	Grouping
10	48	18.9	A
20	48	15.4	B
15	48	14.2	B C
0	48	12.4	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

sample	N	Mean	Grouping
cacao	48	25.7	A
raw	48	14.6	B
ball mill	48	13.4	B
mf	48	7.3	C

Means that do not share a letter are significantly different.

Table D.5 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	N	Mean	Grouping
20	cacao	12	32.2	A
15	cacao	12	30.2	A
10	cacao	12	27.9	A
10	ball mill	12	21.1	B
10	raw	12	19.6	B
20	raw	12	15.9	B C
0	mf	12	12.4	C D
0	raw	12	12.4	C D
0	ball mill	12	12.4	C D
0	cacao	12	12.4	C D
15	raw	12	10.5	C D E
15	ball mill	12	10.0	D E
20	ball mill	12	10.0	D E
10	mf	12	7.1	D E F
15	mf	12	6.3	E F
20	mf	12	3.3	F

Means that do not share a letter are significantly different.

Table D. 6 Results for Tukey's mean comparison test for breaking strength of fresh biscuit samples

General Linear Model: hardness versus percentage; sample

Factor	Type	Levels	Values
percentage	fixed	4	0; 10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw

Table D.6 (continued)

Analysis of Variance for hardness. using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
percentage	3	334476678	334476678	111492226	761.81
sample	3	146267649	146267649	48755883	333.14
percentage*sample	9	67261799	67261799	7473533	51.07
Error	128	18733070	18733070	146352	
Total	143	566739196			

S = 382.560 R-Sq = 96.69% R-Sq(adj) = 96.31%

Unusual Observations for hardness

Obs	hardness	Fit	SE Fit	Residual	St Resid
78	2867.65	1969.90	127.52	897.75	2.49 R
82	3886.18	4717.35	127.52	-831.17	-2.30 R
102	4645.60	3543.54	127.52	1102.06	3.06 R
110	2951.83	1969.90	127.52	981.93	2.72 R
114	3886.18	4717.35	127.52	-831.17	-2.30 R
118	2407.32	3543.54	127.52	-1136.22	-3.15 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	N	Mean	Grouping
20	36	5523.2	A
15	36	4686.4	B
10	36	3498.7	C
0	36	1466.5	D

Means that do not share a letter are significantly different.

Table D.6 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

sample	N	Mean	Grouping
cacao	36	5299.4	A
ball mill	36	3834.9	B
raw	36	3566.8	C
mf	36	2473.6	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	N	Mean	Grouping
20	cacao	9	8334.0	A
15	cacao	9	6679.8	B
20	ball mill	9	5632.0	C
10	cacao	9	4717.3	D
15	raw	9	4642.3	D
20	raw	9	4614.9	D
15	ball mill	9	4477.2	D
10	ball mill	9	3764.1	E
10	raw	9	3543.5	E F
20	mf	9	3511.8	E F
15	mf	9	2946.2	F
10	mf	9	1969.9	G
0	mf	9	1466.5	G
0	cacao	9	1466.5	G
0	ball mill	9	1466.5	G
0	raw	9	1466.5	G

Means that do not share a letter are significantly different.

Table D. 7 Results for Tukey's mean comparison test for thickness of biscuit samples during storage

General Linear Model: height versus percentage; sample; day

Factor	Type	Levels	Values
percentage	fixed	4	0; 10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw
day	fixed	4	0; 1; 3; 7

Analysis of Variance for height. using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
percentage	3	4.780882	4.780882	1.593627	567.89
sample	3	0.240585	0.240585	0.080195	28.58
day	3	0.031799	0.031799	0.010600	3.78
percentage*sample	9	0.184165	0.184165	0.020463	7.29
percentage*day	9	0.049407	0.049407	0.005490	1.96
sample*day	9	0.016509	0.016509	0.001834	0.65
percentage*sample*day	27	0.065068	0.065068	0.002410	0.86
Error	512	1.436798	1.436798	0.002806	
Total	575	6.805212			

S = 0.0529740 R-Sq = 78.89% R-Sq(adj) = 76.29%

Unusual Observations for height

Obs	height	Fit	SE Fit	Residual	St Resid
49	1.03333	0.89815	0.01766	0.13519	2.71 R
53	1.03333	0.89815	0.01766	0.13519	2.71 R
57	1.03333	0.89815	0.01766	0.13519	2.71 R
61	1.03333	0.89815	0.01766	0.13519	2.71 R
174	1.05000	1.16296	0.01766	-0.11296	-2.26 R
196	1.00000	1.10519	0.01766	-0.10519	-2.11 R

Table D.7 (continued)

198	1.15000	1.04407	0.01766	0.10593	2.12 R
223	1.00000	1.11037	0.01766	-0.11037	-2.21 R
232	1.28333	1.12630	0.01766	0.15704	3.14 R
262	0.93333	1.04407	0.01766	-0.11074	-2.22 R
276	1.23333	1.10519	0.01766	0.12815	2.57 R
343	1.25000	1.08778	0.01766	0.16222	3.25 R
350	1.30000	1.19000	0.01766	0.11000	2.20 R
354	1.21667	1.07000	0.01766	0.14667	2.94 R
358	1.17333	1.01000	0.01766	0.16333	3.27 R
360	1.23333	1.10963	0.01766	0.12370	2.48 R
378	1.17333	1.06111	0.01766	0.11222	2.25 R
383	0.98333	1.11593	0.01766	-0.13259	-2.65 R
391	0.96667	1.08778	0.01766	-0.12111	-2.42 R
438	0.95667	1.06370	0.01766	-0.10704	-2.14 R
456	1.23333	1.11185	0.01766	0.12148	2.43 R
462	1.01667	1.14296	0.01766	-0.12630	-2.53 R
487	1.28000	1.08815	0.01766	0.19185	3.84 R
494	1.25000	1.14296	0.01766	0.10704	2.14 R
511	1.01667	1.12889	0.01766	-0.11222	-2.25 R
513	1.03333	0.92778	0.01766	0.10556	2.11 R
517	1.03333	0.92778	0.01766	0.10556	2.11 R
520	1.23333	1.11185	0.01766	0.12148	2.43 R
521	1.03333	0.92778	0.01766	0.10556	2.11 R
525	1.03333	0.92778	0.01766	0.10556	2.11 R
527	1.01667	1.12889	0.01766	-0.11222	-2.25 R
534	1.18333	1.06370	0.01766	0.11963	2.40 R

R denotes an observation with a large standardized residual.

Table D.7 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	N	Mean	Grouping
20	144	1.1	A
15	144	1.1	B
10	144	1.1	C
0	144	0.9	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

sample	N	Mean	Grouping
mf	144	1.1	A
ball mill	144	1.1	B
raw	144	1.0	C
cacao	144	1.0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

day	N	Mean	Grouping
0	144	1.1	A
7	144	1.1	A B
1	144	1.1	A B
3	144	1.1	B

Means that do not share a letter are significantly different.

Table D.7 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	N	Mean	Grouping
20	mf	36	1.2	A
10	mf	36	1.2	A B
20	ball mill	36	1.2	A B C
20	raw	36	1.1	B C D
15	mf	36	1.1	B C D
20	cacao	36	1.1	C D E
15	ball mill	36	1.1	D E
15	raw	36	1.1	D E F
15	cacao	36	1.1	D E F G
10	ball mill	36	1.1	E F G
10	cacao	36	1.1	F G
10	raw	36	1.1	G
0	ball mill	36	0.9	H
0	mf	36	0.9	H
0	cacao	36	0.9	H
0	raw	36	0.9	H

Means that do not share a letter are significantly different.

Table D.7 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	day	N	Mean	Grouping
20	0	36	1.2	A
20	1	36	1.1	A B
20	7	36	1.1	A B C
20	3	36	1.1	A B C
15	0	36	1.1	B C D
10	0	36	1.1	B C D
15	7	36	1.1	B C D
15	1	36	1.1	C D
15	3	36	1.1	C D
10	3	36	1.1	D
10	1	36	1.1	D
10	7	36	1.1	D
0	7	36	0.9	E
0	1	36	0.9	E
0	0	36	0.9	E
0	3	36	0.9	E

Means that do not share a letter are significantly different.

Table D.7 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

sample	day	N	Mean	Grouping
mf	0	36	1.1	A
mf	3	36	1.1	A
mf	7	36	1.1	A
mf	1	36	1.1	A
ball mill	0	36	1.1	A B
raw	0	36	1.1	A B C
ball mill	1	36	1.1	A B C
ball mill	7	36	1.1	A B C
ball mill	3	36	1.0	B C
raw	7	36	1.0	B C
cacao	0	36	1.0	B C
raw	1	36	1.0	B C
cacao	7	36	1.0	B C
cacao	1	36	1.0	B C
cacao	3	36	1.0	B C
raw	3	36	1.0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	day	N	Mean	Grouping
20	mf	0	9	1.2	A
10	mf	3	9	1.2	A B
20	mf	1	9	1.2	A B
20	ball mill	1	9	1.2	A B
20	mf	3	9	1.2	A B
10	mf	0	9	1.2	A B C
20	raw	0	9	1.2	A B C

Table D.7 (continued)

20	mf	7	9	1.2	A B C
10	mf	1	9	1.2	A B C D
20	ball mill	0	9	1.2	A B C D E
20	cacao	0	9	1.2	A B C D E
20	ball mill	3	9	1.1	A B C D E
20	ball mill	7	9	1.1	A B C D E
15	mf	0	9	1.1	A B C D E F
10	mf	7	9	1.1	A B C D E F
15	ball mill	0	9	1.1	A B C D E F
15	mf	7	9	1.1	A B C D E F
20	raw	1	9	1.1	A B C D E F
10	ball mill	0	9	1.1	A B C D E F
15	mf	3	9	1.1	A B C D E F
15	ball mill	7	9	1.1	A B C D E F G
20	raw	7	9	1.1	A B C D E F G
15	mf	1	9	1.1	A B C D E F G
20	raw	3	9	1.1	A B C D E F G
20	cacao	7	9	1.1	A B C D E F G
15	ball mill	1	9	1.1	A B C D E F G
20	cacao	1	9	1.1	A B C D E F G
15	raw	1	9	1.1	A B C D E F G
15	raw	0	9	1.1	A B C D E F G
15	cacao	7	9	1.1	A B C D E F G
20	cacao	3	9	1.1	A B C D E F G
15	cacao	3	9	1.1	A B C D E F G
15	cacao	1	9	1.1	A B C D E F G
10	raw	0	9	1.1	A B C D E F G
15	raw	7	9	1.1	B C D E F G
15	cacao	0	9	1.1	B C D E F G
15	raw	3	9	1.1	B C D E F G

Table D.7 (continued)

15	ball mill	3	9	1.1	B C D E F G
10	cacao	3	9	1.1	C D E F G
10	raw	7	9	1.1	D E F G
10	cacao	1	9	1.1	D E F G
10	ball mill	3	9	1.1	D E F G
10	ball mill	1	9	1.1	D E F G
10	ball mill	7	9	1.1	E F G
10	cacao	7	9	1.0	F G
10	cacao	0	9	1.0	F G
10	raw	1	9	1.0	F G
10	raw	3	9	1.0	G H
0	mf	7	9	0.9	H I
0	ball mill	7	9	0.9	H I
0	raw	7	9	0.9	H I
0	cacao	7	9	0.9	H I
0	ball mill	1	9	0.9	H I
0	mf	1	9	0.9	H I
0	cacao	1	9	0.9	H I
0	raw	1	9	0.9	H I
0	cacao	0	9	0.9	I
0	ball mill	0	9	0.9	I
0	mf	0	9	0.9	I
0	raw	0	9	0.9	I
0	Ball mill	3	9	0.9	I
0	raw	3	9	0.9	I
0	cacao	3	9	0.9	I
0	mf	3	9	0.9	I

Means that do not share a letter are significantly different.

Table D. 8 Results for Tukey's mean comparison test for diameter of biscuit samples during storage

General Linear Model: diameter versus percentage; sample; day

Factor	Type	Levels	Values
percentage	fixed	4	0; 10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw
day	fixed	4	0; 1; 3; 7

Analysis of Variance for diameter. using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
percentage	3	35.0331	35.0331	11.6777	461.73
sample	3	35.5953	35.5953	11.8651	469.14
day	3	0.2522	0.2522	0.0841	3.32
percentage*sample	9	13.4800	13.4800	1.4978	59.22
percentage*day	9	0.5716	0.5716	0.0635	2.51
sample*day	9	0.4189	0.4189	0.0465	1.84
percentage*sample*day	27	0.8286	0.8286	0.0307	1.21
Error	512	12.9491	12.9491	0.0253	
Total	575	99.1287			

S = 0.159032 R-Sq = 86.94% R-Sq(adj) = 85.33%

Unusual Observations for diameter

Obs	diameter	Fit	SE Fit	Residual	St Resid
126	6.00000	6.37778	0.05301	-0.37778	-2.52 R
182	7.65000	7.22611	0.05301	0.42389	2.83 R
183	7.75000	7.26389	0.05301	0.48611	3.24 R
231	6.87500	7.26389	0.05301	-0.38889	-2.59 R
295	7.72500	7.15389	0.05301	0.57111	3.81 R
311	7.50000	7.15389	0.05301	0.34611	2.31 R

Table D.8 (continued)

327	7.62500	7.15389	0.05301	0.47111	3.14 R
337	7.75000	7.35833	0.05301	0.39167	2.61 R
341	7.75000	7.35833	0.05301	0.39167	2.61 R
345	7.75000	7.35833	0.05301	0.39167	2.61 R
349	7.75000	7.35833	0.05301	0.39167	2.61 R
353	7.72500	7.35833	0.05301	0.36667	2.45 R
357	7.72500	7.35833	0.05301	0.36667	2.45 R
361	7.72500	7.35833	0.05301	0.36667	2.45 R
365	7.72500	7.35833	0.05301	0.36667	2.45 R
375	6.75000	7.15389	0.05301	-0.40389	-2.69 R
394	7.48500	6.89500	0.05301	0.59000	3.93 R
395	7.67500	6.95278	0.05301	0.72222	4.82 R
444	7.10000	6.79722	0.05301	0.30278	2.02 R
449	7.65000	7.30000	0.05301	0.35000	2.33 R
453	7.65000	7.30000	0.05301	0.35000	2.33 R
457	7.65000	7.30000	0.05301	0.35000	2.33 R
461	7.65000	7.30000	0.05301	0.35000	2.33 R
472	7.10000	6.79611	0.05301	0.30389	2.03 R
488	7.18500	6.79611	0.05301	0.38889	2.59 R
520	7.17500	6.79611	0.05301	0.37889	2.53 R
536	6.23500	6.79611	0.05301	-0.56111	-3.74 R
552	6.28500	6.79611	0.05301	-0.51111	-3.41 R
562	7.40000	6.90000	0.05301	0.50000	3.33 R
566	6.92500	7.27278	0.05301	-0.34778	-2.32 R
568	6.20000	6.79611	0.05301	-0.59611	-3.98 R

R denotes an observation with a large standardized residual.

Table D.8 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	N	Mean	Grouping
0	144	7.3	A
10	144	6.9	B
15	144	6.7	C
20	144	6.6	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

sample	N	Mean	Grouping
raw	144	7.2	A
ball mill	144	7.0	B
cacao	144	6.8	C
mf	144	6.5	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

day	N	Mean	Grouping
3	144	6.9	A
7	144	6.9	A B
0	144	6.9	A B
1	144	6.8	B

Means that do not share a letter are significantly different.

Table D.8 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	N	Mean	Grouping
10	raw	36	7.3	A
0	raw	36	7.3	A
0	cacao	36	7.3	A
0	mf	36	7.3	A
0	ball mill	36	7.3	A
15	raw	36	7.2	A
20	raw	36	7.1	B
15	ball mill	36	6.9	C
10	ball mill	36	6.9	C
10	cacao	36	6.8	C
20	ball mill	36	6.8	C
15	cacao	36	6.6	D
10	mf	36	6.4	E
20	cacao	36	6.4	E F
20	mf	36	6.3	F G
15	mf	36	6.2	G

Means that do not share a letter are significantly different.

Table D.8 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	day	N	Mean	Grouping
0	3	36	7.4	A
0	7	36	7.3	A B
0	1	36	7.2	B
0	0	36	7.2	B
10	0	36	6.9	C
10	7	36	6.9	C D
10	3	36	6.9	C D E
10	1	36	6.8	C D E
15	7	36	6.8	C D E F
15	3	36	6.7	D E F
15	0	36	6.7	E F G
15	1	36	6.7	E F G
20	3	36	6.6	F G H
20	0	36	6.6	F G H
20	1	36	6.6	G H
20	7	36	6.6	H

Means that do not share a letter are significantly different.

Table D.8 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

sample	day	N	Mean	Grouping
raw	0	36	7.2	A
raw	3	36	7.2	A
raw	1	36	7.2	A
raw	7	36	7.2	A
ball mill	3	36	7.0	B
ball mill	0	36	7.0	B
ball mill	7	36	7.0	B
ball mill	1	36	6.9	B
cacao	7	36	6.8	C
cacao	3	36	6.8	C
cacao	0	36	6.7	C
cacao	1	36	6.7	C
mf	7	36	6.6	D
mf	3	36	6.6	D
mf	0	36	6.5	D
mf	1	36	6.5	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	day	N	Mean	Grouping
10	raw	0	9	7.4	A
0	raw	3	9	7.4	A
0	cacao	3	9	7.4	A
0	mf	3	9	7.4	A
0	ball mill	3	9	7.4	A
0	raw	7	9	7.3	A
0	ball mill	7	9	7.3	A

Table D.8 (continued)

0	mf	7	9	7.3	A
0	cacao	7	9	7.3	A
10	raw	7	9	7.3	A B
15	raw	7	9	7.3	A B
15	raw	1	9	7.3	A B
10	raw	3	9	7.3	A B
15	raw	0	9	7.2	A B C
0	raw	1	9	7.2	A B C D
0	cacao	1	9	7.2	A B C D
0	mf	1	9	7.2	A B C D
0	ball mill	1	9	7.2	A B C D
10	raw	1	9	7.2	A B C D
0	raw	0	9	7.2	A B C D
0	cacao	0	9	7.2	A B C D
0	mf	0	9	7.2	A B C D
0	ball mill	0	9	7.2	A B C D
20	raw	3	9	7.2	A B C D E
20	raw	0	9	7.2	A B C D E
15	raw	3	9	7.2	A B C D E
20	raw	1	9	7.1	A B C D E F
10	ball mill	0	9	7.0	B C D E F G
15	ball mill	3	9	7.0	C D E F G
15	ball mill	0	9	6.9	D E F G H
10	cacao	7	9	6.9	E F G H I
15	ball mill	1	9	6.9	E F G H I
10	ball mill	3	9	6.9	E F G H I
10	ball mill	1	9	6.9	E F G H I
10	ball mill	7	9	6.9	E F G H I J
15	ball mill	7	9	6.8	F G H I J K
10	cacao	3	9	6.8	G H I J K

Table D.8 (continued)

10	cacao	0	9	6.8	G H I J K
20	ball mill	7	9	6.8	G H I J K
20	raw	7	9	6.8	G H I J K
20	ball mill	0	9	6.8	G H I J K
10	cacao	1	9	6.8	G H I J K
20	ball mill	1	9	6.8	G H I J K
20	ball mill	3	9	6.7	G H I J K
15	cacao	7	9	6.6	H I J K L
15	cacao	3	9	6.6	I J K L M
15	cacao	1	9	6.6	J K L M N
15	cacao	0	9	6.5	K L M N
10	mf	7	9	6.4	L M N O
10	mf	3	9	6.4	L M N O
20	cacao	3	9	6.4	L M N O
20	cacao	0	9	6.4	L M N O
10	mf	0	9	6.4	L M N O
20	cacao	7	9	6.4	L M N O
20	cacao	1	9	6.4	L M N O
10	mf	1	9	6.3	M N O
20	mf	7	9	6.3	M N O
15	mf	7	9	6.3	N O
20	mf	3	9	6.3	N O
15	mf	3	9	6.3	N O
20	mf	0	9	6.2	O
15	mf	0	9	6.2	O
20	mf	1	9	6.2	O
15	mf	1	9	6.2	O

Means that do not share a letter are significantly different.

Table D. 9 Results for Tukey's mean comparison test for moisture content of biscuit samples during storage

General Linear Model: moisture content versus percentage; sample; day

Factor	Type	Levels	Values
percentage	fixed	4	0; 10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw
day	fixed	4	0; 1; 3;

Analysis of Variance for moisture content. using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
percentage	3	95.8811	95.8811	31.9604	35.44
sample	3	89.0941	89.0941	29.6980	32.94
day	3	78.4704	78.4704	26.1568	29.01
percentage*sample	9	203.3564	203.3564	22.5952	25.06
percentage*day	9	16.0748	16.0748	1.7861	1.98
sample*day	9	41.8035	41.8035	4.6448	5.15
percentage*sample*day	27	27.8789	27.8789	1.0326	1.15
Error	512	461.6760	461.6760	0.9017	
Total	575	1014.2352			

S = 0.949585 R-Sq = 54.48% R-Sq(adj) = 48.88%

Table D.9 (continued)

Unusual Observations for moisture content

Obs	content	Fit	SE Fit	Residual	St Resid
18	4.89000	2.67778	0.31653	2.21222	2.47 R
30	1.17000	6.22000	0.31653	-5.05000	-5.64 R
46	8.18000	6.22000	0.31653	1.96000	2.19 R
74	6.35000	4.26889	0.31653	2.08111	2.32 R
142	8.18000	6.22000	0.31653	1.96000	2.19 R
150	0.32000	4.67556	0.31653	-4.35556	-4.87 R
241	0.29000	6.02111	0.31653	-5.73111	-6.40 R
245	0.29000	6.02111	0.31653	-5.73111	-6.40 R
249	0.29000	6.02111	0.31653	-5.73111	-6.40 R
250	0.25000	4.36444	0.31653	-4.11444	-4.60 R
253	0.29000	6.02111	0.31653	-5.73111	-6.40 R
257	7.87000	6.02111	0.31653	1.84889	2.07 R
261	7.87000	6.02111	0.31653	1.84889	2.07 R
265	7.87000	6.02111	0.31653	1.84889	2.07 R
266	6.44000	4.36444	0.31653	2.07556	2.32 R
269	7.87000	6.02111	0.31653	1.84889	2.07 R
504	0.50000	5.19667	0.31653	-4.69667	-5.25 R
535	7.78000	5.53667	0.31653	2.24333	2.51 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	N	Mean	Grouping
20	144	6.2	A
0	144	6.0	A
15	144	5.7	B
10	144	5.1	C

Means that do not share a letter are significantly different.

Table D.9 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

sample	N	Mean	Grouping
mf	144	6.3	A
cacao	144	6.0	A
ball mill	144	5.4	B
raw	144	5.4	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

day	N	Mean	Grouping
3	144	6.2	A
7	144	6.0	A B
1	144	5.8	B
0	144	5.2	C

Means that do not share a letter are significantly different.

Table D.9 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	N	Mean	Grouping
20	cacao	36	7.6	A
20	mf	36	6.6	B
15	cacao	36	6.5	B
10	mf	36	6.4	B
15	mf	36	6.1	B C
0	mf	36	6.0	B C
0	ball mill	36	6.0	B C
0	raw	36	6.0	B C
0	cacao	36	6.0	B C
20	ball mill	36	5.5	C D
15	raw	36	5.3	D
15	ball mill	36	5.2	D
20	raw	36	5.1	D
10	raw	36	5.1	D
10	ball mill	36	5.0	D
10	cacao	36	4.0	E

Means that do not share a letter are significantly different.

Table D.9 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	day	N	Mean	Grouping
0	3	36	6.6	A
20	1	36	6.3	A B
20	7	36	6.3	A B
20	3	36	6.3	A B
0	7	36	6.3	A B
15	3	36	6.1	A B C
0	1	36	6.0	A B C
20	0	36	6.0	A B C D
15	1	36	5.9	A B C D
15	7	36	5.8	B C D E
10	3	36	5.7	B C D E F
10	7	36	5.5	C D E F
0	0	36	5.2	D E F
15	0	36	5.1	E F G
10	1	36	5.0	F G
10	0	36	4.4	G

Means that do not share a letter are significantly different.

Table D.9 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

sample	day	N	Mean	Grouping
cacao	3	36	6.6	A
mf	3	36	6.6	A
mf	1	36	6.5	A
cacao	1	36	6.4	A B
cacao	7	36	6.3	A B
mf	0	36	6.1	A B
mf	7	36	6.0	A B
ball mill	3	36	5.9	A B C
ball mill	7	36	5.8	A B C
raw	7	36	5.6	B C D
raw	3	36	5.6	B C D
raw	1	36	5.2	C D E
ball mill	1	36	5.1	C D E
raw	0	36	4.9	D E
ball mill	0	36	4.9	D E
cacao	0	36	4.8	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	day	N	Mean	Grouping
20	cacao	7	9	8.4	A
20	cacao	3	9	7.7	A B
20	cacao	1	9	7.7	A B C
15	cacao	1	9	7.5	A B C D
20	mf	1	9	7.2	A B C D E
15	cacao	3	9	7.1	A B C D E F
20	cacao	0	9	6.8	A B C D E F G

Table D.9 (continued)

10	mf	3	9	6.7	A B C D E F G
20	mf	0	9	6.6	A B C D E F G H
0	mf	3	9	6.6	A B C D E F G H
0	ball mill	3	9	6.6	A B C D E F G H
0	raw	3	9	6.6	A B C D E F G H
0	cacao	3	9	6.6	A B C D E F G H
20	mf	3	9	6.6	A B C D E F G H
15	cacao	7	9	6.6	A B C D E F G H
10	mf	1	9	6.5	A B C D E F G H
15	mf	3	9	6.3	B C D E F G H I
10	mf	7	9	6.3	B C D E F G H I
0	mf	7	9	6.3	B C D E F G H I
0	ball mill	7	9	6.3	B C D E F G H I
0	raw	7	9	6.3	B C D E F G H I
0	cacao	7	9	6.3	B C D E F G H I
10	mf	0	9	6.2	B C D E F G H I
15	mf	0	9	6.2	B C D E F G H I J
15	mf	1	9	6.2	B C D E F G H I J
0	mf	1	9	6.0	B C D E F G H I J K
0	cacao	1	9	6.0	B C D E F G H I J K
0	ball mill	1	9	6.0	B C D E F G H I J K
0	raw	1	9	6.0	B C D E F G H I J K
20	mf	7	9	5.9	C D E F G H I J K
20	ball mill	7	9	5.8	D E F G H I J K
10	ball mill	7	9	5.7	D E F G H I J K
20	ball mill	3	9	5.7	D E F G H I J K
10	ball mill	3	9	5.7	E F G H I J K
15	ball mill	7	9	5.6	E F G H I J K
10	raw	7	9	5.6	E F G H I J K
15	mf	7	9	5.5	E F G H I J K

Table D.9 (continued)

15	raw	7	9	5.5	E F G H I J K
15	ball mill	3	9	5.5	E F G H I J K
15	raw	3	9	5.5	E F G H I J K
10	raw	3	9	5.4	E F G H I J K
20	ball mill	0	9	5.3	F G H I J K
20	ball mill	1	9	5.3	F G H I J K
0	ball mill	0	9	5.2	G H I J K
0	mf	0	9	5.2	G H I J K
0	cacao	0	9	5.2	G H I J K
0	raw	0	9	5.2	G H I J K
20	raw	7	9	5.2	G H I J K
15	raw	1	9	5.2	G H I J K
20	raw	0	9	5.0	G H I J K
20	raw	3	9	5.0	G H I J K
20	raw	1	9	5.0	G H I J K
15	ball mill	1	9	4.9	H I J K
10	cacao	3	9	4.9	H I J K
15	raw	0	9	4.8	H I J K
15	ball mill	0	9	4.7	I J K
10	raw	1	9	4.7	I J K
15	cacao	0	9	4.7	I J K
10	raw	0	9	4.6	I J K
10	ball mill	1	9	4.4	J K L
10	cacao	1	9	4.4	J K L
10	ball mill	0	9	4.3	K L
10	cacao	7	9	4.2	K L
10	cacao	0	9	2.7	L

Means that do not share a letter are significantly different.

Table D. 10 Results for Tukey's mean comparison test for breaking strength of biscuit samples during storage

General Linear Model: hardness versus percentage; sample; day

Factor	Type	Levels	Values
percentage	fixed	4	0; 10; 15; 20
sample	fixed	4	ball mill; cacao; mf; raw
day	fixed	4	0; 1; 3; 7

Analysis of Variance for hardness. using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS
percentage	3	802795504	802795504	267598501
sample	3	359508433	359508433	119836144
day	3	1044442899	1044442899	348147633
percentage*sample	9	139347991	139347991	15483110
percentage*day	9	77741955	77741955	8637995
sample*day	9	59410151	59410151	6601128
percentage*sample*day	27	67922855	67922855	2515661
Error	512	237864565	237864565	464579
Total	575	2789034353		

Source	F
percentage	576.00
sample	257.95
day	749.38
percentage*sample	33.33
percentage*day	18.59
sample*day	14.21
percentage*sample*day	5.41
Error	

S = 681.600 R-Sq = 91.47% R-Sq(adj) = 90.42%

Table D.10 (continued)

Unusual Observations for hardness

Obs	hardness	Fit	SE Fit	Residual	St Resid
145	2753.7	4104.7	227.2	-1351.0	-2.10 R
149	2753.7	4104.7	227.2	-1351.0	-2.10 R
153	2753.7	4104.7	227.2	-1351.0	-2.10 R
157	2753.7	4104.7	227.2	-1351.0	-2.10 R
180	10106.4	8446.6	227.2	1659.8	2.58 R
193	2753.7	4104.7	227.2	-1351.0	-2.10 R
194	5492.0	6860.3	227.2	-1368.3	-2.13 R
197	2753.7	4104.7	227.2	-1351.0	-2.10 R
201	2753.7	4104.7	227.2	-1351.0	-2.10 R
205	2753.7	4104.7	227.2	-1351.0	-2.10 R
241	6099.6	4104.7	227.2	1995.0	3.10 R
242	5492.0	6860.3	227.2	-1368.3	-2.13 R
245	6099.6	4104.7	227.2	1995.0	3.10 R
249	6099.6	4104.7	227.2	1995.0	3.10 R
253	6099.6	4104.7	227.2	1995.0	3.10 R
302	4008.5	5358.3	227.2	-1349.8	-2.10 R
306	8940.0	7510.1	227.2	1429.8	2.23 R
353	2656.4	4215.5	227.2	-1559.1	-2.43 R
357	2656.4	4215.5	227.2	-1559.1	-2.43 R
361	2656.4	4215.5	227.2	-1559.1	-2.43 R
365	2656.4	4215.5	227.2	-1559.1	-2.43 R
369	2559.2	4215.5	227.2	-1656.3	-2.58 R
370	6211.7	7510.1	227.2	-1298.4	-2.02 R
373	2559.2	4215.5	227.2	-1656.3	-2.58 R
377	2559.2	4215.5	227.2	-1656.3	-2.58 R
381	2559.2	4215.5	227.2	-1656.3	-2.58 R
391	4375.5	5941.3	227.2	-1565.8	-2.44 R
481	3828.5	5366.0	227.2	-1537.5	-2.39 R

Table D.10 (continued)

485	3828.5	5366.0	227.2	-1537.5	-2.39 R
489	3828.5	5366.0	227.2	-1537.5	-2.39 R
493	3828.5	5366.0	227.2	-1537.5	-2.39 R
502	8080.8	6723.6	227.2	1357.2	2.11 R
513	6697.8	5366.0	227.2	1331.8	2.07 R
514	6311.0	7647.0	227.2	-1335.9	-2.08 R
517	6697.8	5366.0	227.2	1331.8	2.07 R
520	9882.7	8280.4	227.2	1602.3	2.49 R
521	6697.8	5366.0	227.2	1331.8	2.07 R
525	6697.8	5366.0	227.2	1331.8	2.07 R
529	3828.5	5366.0	227.2	-1537.5	-2.39 R
533	3828.5	5366.0	227.2	-1537.5	-2.39 R
537	3828.5	5366.0	227.2	-1537.5	-2.39 R
541	3828.5	5366.0	227.2	-1537.5	-2.39 R
561	6697.8	5366.0	227.2	1331.8	2.07 R
565	6697.8	5366.0	227.2	1331.8	2.07 R
569	6697.8	5366.0	227.2	1331.8	2.07 R
573	6697.8	5366.0	227.2	1331.8	2.07 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	N	Mean	Grouping
20	144	7021.3	A
15	144	6095.3	B
10	144	5433.5	C
0	144	3788.2	D

Means that do not share a letter are significantly different.

Table D.10 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

sample	N	Mean	Grouping
cacao	144	6729.0	A
ball mill	144	5822.0	B
raw	144	5190.3	C
mf	144	4597.1	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

day	N	Mean	Grouping
7	144	7347.0	A
3	144	6284.2	B
1	144	4913.5	C
0	144	3793.7	D

Means that do not share a letter are significantly different.

Table D.10 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	N	Mean	Grouping
20	cacao	36	9024.7	A
15	cacao	36	7419.3	B
20	ball mill	36	7321.5	B
10	cacao	36	6683.7	C
15	ball mill	36	6338.2	C D
20	raw	36	6325.1	C D
10	ball mill	36	5840.1	D E
15	raw	36	5729.7	E
20	mf	36	5413.9	E F
10	raw	36	4918.3	F
15	mf	36	4894.1	F
10	mf	36	4292.1	G
0	raw	36	3788.2	G
0	cacao	36	3788.2	G
0	ball mill	36	3788.2	G
0	mf	36	3788.2	G

Means that do not share a letter are significantly different.

Table D.10 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	day	N	Mean	Grouping
20	7	36	8710.5	A
15	7	36	7927.7	B
20	3	36	7736.2	B
10	7	36	7383.7	B
15	3	36	6677.2	C
10	3	36	6507.8	C D
20	1	36	6115.4	D
20	0	36	5523.2	E
0	7	36	5366.0	E
15	1	36	5090.0	E F
15	0	36	4686.4	F G
10	1	36	4344.0	G H
0	3	36	4215.5	G H
0	1	36	4104.7	H
10	0	36	3498.7	I
0	0	36	1466.5	J

Means that do not share a letter are significantly different.

Table D.10 (continued)

Grouping Information Using Tukey Method and 95.0% Confidence

sample	day	N	Mean	Grouping
ball mill	7	36	7831.1	A
cacao	7	36	7777.3	A B
cacao	3	36	7230.4	B C
raw	7	36	7015.7	C D
ball mill	3	36	6774.6	C D
mf	7	36	6763.8	C D
cacao	1	36	6608.8	D
raw	3	36	5952.4	E
cacao	0	36	5299.4	F
mf	3	36	5179.4	F
ball mill	1	36	4847.3	F
raw	1	36	4226.4	G
mf	1	36	3971.6	G H
ball mill	0	36	3834.9	G H
raw	0	36	3566.8	H
mf	0	36	2473.6	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

percentage	sample	day	N	Mean
20	cacao	7	9	9829.9
20	cacao	3	9	9488.5
20	ball mill	7	9	8715.4
15	ball mill	7	9	8711.0
10	ball mill	7	9	8532.0
20	cacao	1	9	8446.6
20	cacao	0	9	8334.0

Table D.10 (continued)

20	raw	7	9	8280.4
15	cacao	7	9	8266.3
20	mf	7	9	8016.2
20	ball mill	3	9	7895.3
20	raw	3	9	7790.2
15	cacao	3	9	7707.4
15	raw	7	9	7692.7
15	ball mill	3	9	7687.3
10	cacao	7	9	7647.0
10	cacao	3	9	7510.1
10	ball mill	3	9	7300.2
20	ball mill	1	9	7043.4
15	mf	7	9	7040.7
15	cacao	1	9	7023.5
10	cacao	1	9	6860.3
10	raw	7	9	6723.6
15	cacao	0	9	6679.8
10	mf	7	9	6632.1
15	raw	3	9	5941.3
10	raw	3	9	5862.4
20	mf	3	9	5770.9
20	ball mill	0	9	5632.0
15	mf	3	9	5372.7
0	cacao	7	9	5366.0
0	raw	7	9	5366.0
0	ball mill	7	9	5366.0
0	mf	7	9	5366.0
10	mf	3	9	5358.3
10	cacao	0	9	4717.3
15	raw	1	9	4642.3

Table D.10 (continued)

15	raw	0	9	4642.3
20	raw	1	9	4614.9
20	raw	0	9	4614.9
15	ball mill	1	9	4477.2
15	ball mill	0	9	4477.2
20	mf	1	9	4356.9
15	mf	1	9	4216.8
0	raw	3	9	4215.5
0	cacao	3	9	4215.5
0	mf	3	9	4215.5
0	ball mill	3	9	4215.5
0	raw	1	9	4104.7
0	cacao	1	9	4104.7
0	ball mill	1	9	4104.7
0	mf	1	9	4104.7
10	ball mill	1	9	3764.1
10	ball mill	0	9	3764.1
10	raw	1	9	3543.5
10	raw	0	9	3543.5
20	mf	0	9	3511.8
10	mf	1	9	3208.0
15	mf	0	9	2946.2
10	mf	0	9	1969.9
0	raw	0	9	1466.5
0	mf	0	9	1466.5
0	ball mill	0	9	1466.5
0	cacao	0	9	1466.5

Table D.10 (continued)

percentage	sample	day	Grouping
20	cacao	7	A
20	cacao	3	A B
20	ball mill	7	A B C
15	ball mill	7	A B C
10	ball mill	7	A B C D
20	cacao	1	B C D
20	cacao	0	B C D E
20	raw	7	B C D E
15	cacao	7	B C D E
20	mf	7	C D E F
20	ball mill	3	C D E F G
20	raw	3	C D E F G
15	cacao	3	C D E F G
15	raw	7	C D E F G
15	ball mill	3	C D E F G
10	cacao	7	C D E F G
10	cacao	3	C D E F G
10	ball mill	3	D E F G
20	ball mill	1	E F G H
15	mf	7	E F G H
15	cacao	1	E F G H
10	cacao	1	F G H I
10	raw	7	F G H I
15	cacao	0	G H I J
10	mf	7	G H I J K
15	raw	3	H I J K L
10	raw	3	H I J K L M
20	mf	3	H I J K L M N
20	ball mill	0	I J K L M N O

Table D.10 (continued)

15	mf	3	J K L M N O P
0	cacao	7	J K L M N O P
0	raw	7	J K L M N O P
0	ball mill	7	J K L M N O P
0	mf	7	J K L M N O P
10	mf	3	K L M N O P
10	cacao	0	L M N O P Q
15	raw	1	L M N O P Q
15	raw	0	L M N O P Q
20	raw	1	M N O P Q
20	raw	0	M N O P Q
15	ball mill	1	N O P QR
15	ball mill	0	N O P Q R
20	mf	1	O P Q R
15	mf	1	PQRS
0	raw	3	PQRS
0	cacao	3	PQRS
0	mf	3	PQRS
0	ball mill	3	PQRS
0	raw	1	PQRS
0	cacao	1	PQRS
0	ball mill	1	PQRS
0	mf	1	PQRS
10	ball mill	1	QRS
10	ball mill	0	QRS
10	raw	1	QRS
10	raw	0	QRS
20	mf	0	QRS
10	mf	1	RST
15	mf	0	ST

Table D.10 (continued)

10	mf	0	U
0	raw	0	U
0	mf	0	U
0	ball mill	0	U
0	cacao	0	U

Means that do not share a letter are significantly different.

Table D. 11 Results for Tukey's mean comparison test for total phenolic content of raw materials

General Linear Model: Concentration of phenolic(ppm) versus Sample

Factor	Type	Levels	Values
Sample	fixed	4	ball mill; cacao; mf; raw

Analysis of Variance for Concentration of phenolic(ppm). using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sample	3	170703	170703	56901	2435.74	0.000
Error	8	187	187	23		
Total	11	170889				

S = 4.83330 R-Sq = 99.89% R-Sq(adj) = 99.85%

Grouping Information Using Tukey Method and 95.0% Confidence

Sample	N	Mean	Grouping
mf	3	347.3	A
ball mill	3	270.7	B
raw	3	147.8	C

Table D.11 (continued)

cacao	3	34.2	D

Means that do not share a letter are significantly different.