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

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submitted by **SEVİL ÇIKRIKCI** in partial fulfillment of the requirements for the degree of **Master of Science in Food Engineering Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen _______________
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Alev Bayındırlı _______________
Head of Department, **Food Engineering Dept., METU**

Assoc. Prof. Dr. Behiç Mert _______________
Supervisor, **Food Engineering Dept., METU**

Examining Committee Members:

Asst. Prof. Dr. İlkay Şensoy _______________
Food Engineering Dept., METU

Assoc. Prof. Dr. Behiç Mert _______________
Food Engineering Dept., METU

Asst. Prof. Dr. Mecit Öztop _______________
Food Engineering Dept., METU

Asst. Prof. Dr. Aslı İşçi _______________
Food Engineering Dept., Ankara University

Asst. Prof. Dr. Bekir Gökçen Mazı _______________
Food Engineering Dept., Ordu University

Date: 29.08.2013

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.

Name, Last name: Sevil Çıkrıkcı

Signature:

ABSTRACT

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

Çıkrıkcı, Sevil

M.Sc., Department of Food Engineering

Supervisor: Assoc. Prof. Dr. Behiç Mert

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The main objective of this study was to investigate micro and nano hazelnut skin fibers and their effects on quality, texture and staling of cakes. In the first part of the study, microstructural properties of cocoa powder/ hazelnut skin samples and rheological properties of cake batter with different percentages (0%, 10%, 15%, 20%) of cocoa powder/ hazelnut skin were evaluated. Herschel–Bulkley model was found to explain the flow behaviors of dough formulations. The highest elastic (G') and loss (G") module were obtained for dough samples containing 20% replacement of flour with microfluidized hazelnut skin fibers. Additionally, the impact of cocoa powder/ hazelnut skin samples on the quality of cakes (weight loss, color and texture) was determined. The firmest structure was obtained in cakes containing 20% replacement of flour with microfluidized hazelnut skin fiber.

In the second part of the study, staling of cakes was investigated by using X-ray and FT-IR analyses. Cakes prepared with hazelnut skin which was processed by microfluidization showed the lowest starch retrogradation results indicating retardation of cake staling.

Lastly, reduction of flour content in cake formulation was studied for cakes prepared with microfluidized hazelnut skin fibers. Flour reduction decreased firmness results and viscoelastic properties in microfluidized cake samples.

Finally, it was found to be possible to produce cakes containing microfluidized hazelnut skin fiber with the optimization of cake formulation.

Keywords: Hazelnut skin, Micro and nano fiber, Cake, Rheology, Texture, Staling

ÖZ

MİKRO VE NANO FINDIK ZARI LİFLERİNİN ÜRETİMİ VE KEKLERDE KULLANIMI

Çıkrıkcı, Sevil
Yüksek Lisans, Gıda Mühendisliği Bölümü
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Bu çalışmanın ana amacı mikro ve nano fındık zarı liflerinin keklerin kalitesi, tekstürü ve bayatlaması üzerine etkilerini araştırmaktır. Çalışmanın ilk aşamasında kakao tozu ve fındık zarı örneklerinin mikroyapısal görüntüleriyle kek hamurunun reolojik özellikleri incelenmiştir. Keklerde değişik oranlarda (%0, %10, %15, %20) kakao tozu/ fındık zarı örnekleri kullanılmıştır. Kek hamuru örneklerinin kayma ile incelen yapı gösterdiği ve Herschel–Bulkley modeline uyduğu belirlenmiştir. En yüksek G' ve G" değerleri unun %20 oranında mikroakışkanlandırıcıdan geçirilen fındık zarıyla değiştirildiği kek hamurlarında elde edilmiştir. Ayrıca, kakao tozu/fındık zarının kek kalitesi (ağırlık kaybı, renk ve tekstür) üzerindeki etkileri de incelenmiştir. Unun %20'sinin mikro ve nano fındık zarı lifleriyle yer değiştirdiği kek örneklerinde en sert yapı elde edilmiştir.

Çalışmanın ikinci aşamasında, keklerin bayatlamaları X-ray ve FT-IR analizleri ile incelenmiştir. Mikro ve nano fındık zarı lifi içeren kekler en düşük nişasta retrogradasyonunu göstererek bayatlamayı geciktirmişlerdir.

Son olarak, kek formulasyonundaki un miktarı azaltılarak mikro ve nano fındık zarı lifi içeren kek örnekleri hazırlanmıştır. Bu örneklerin hamurlarında daha düşük viskoelastik değerleri elde edilmiş olup, örnek keklerde daha yumuşak yapı elde edilmiştir.

Sonuç olarak, kek formulasyonunun optimize edilmesiyle mikroakışkanlandırıcı yöntemi ile üretilmiş fındık zarı lifi içeren keklerin üretiminin yapılabileceği görülmüştür.

Anahtar kelimeler: Fındık zarı, Mikro ve nano lif, Kek, Reoloji, Tekstür, Bayatlama

To My Beloved Family

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G'	Elastic modulus
G"	Viscous modulus
HS	Hazelnut skin
MF	Microfluidized
BM	Ball milled

CHAPTER 1

INTRODUCTION

1.1 Dietary Fiber

As the interest in food products with high nutritional value increases, different additives are investigated and used in the food industry. Since positive effects of the fibers are seen, its use and consumption are increasing day by day.

In AACC report (2001), it is stated that dietary fiber is the edible part of plant foods or analogous carbohydrates that are resistant to absorption and digestion in human small intestine with complete or partial fermentation in the large intestine (AACC, 2001). It includes some chemical classes such as polysaccharides (like cellulose), lignin (non-carbohydrate complex of polyphenylpropane units), fatty acid derivatives (such as waxes) and other plant substances (such as phytates) (Papathanasopoulos and Camileri, 2010). Constituents of dietary fiber can be seen in Table 1.1 in details.

Dietary fiber is classified into two groups as soluble and insoluble fiber on the basis of water solubility (Kaczmarczyk et al., 2012). While viscous solutions may be formed by soluble dietary fibers, insoluble dietary fibers can increase fecal bulk, softening and laxation (Galisteo et al., 2008). While pectin and gums are some examples to soluble dietary fiber; cellulose, hemicellulose and lignin are among the most important insoluble dietary fibers (Kaczmarczyk et al., 2012). Legume, vegetables, fruits, seeds and oat bran are among the food sources of soluble fibers and whole grain products are among the food sources of insoluble fibers (Bauer and Turler, 2008).

There are some techniques used for modifying the functional properties of fibers such as chemical, enzymatic, mechanical and thermal treatments. Chemical treatments like the use of acidic or basic solutions affect the cell wall and cause the changes of some properties of the fiber such as water holding capacity and oil holding capacity. Enzymatic and thermal processes may modify the soluble and insoluble parts of the fiber by changing their ratios. Mechanical process is another technique and there are some studies showing the effects of grinding or grinding methods on the physicochemical properties of the fiber, especially on hydration properties (Elleuch et al., 2011).

Table 1.1 Constituents of dietary fiber. Derived from AACC Report (2001)

Constituents of dietary fiber
Non Starch Polysaccharides and Resistant Oligosaccharides
Cellulose
Hemicellulose
Arabinogalactans
Arabinoxylans
Polyfructoses
Oligofructans
Inulin
Galactooligosaccharides
Gums
Pectins
Mucilages
Analogous Carbohydrates
Indigestible Dextrins
Resistant Maltodextrins
Resistant Potato Dextrins
Synthesized Carbohydrate Compounds
Polydextrose
Methyl cellulose
Hydroxypropylmethyl Cellulose
Indigestible (Resistant) Starches
Lignin
Substances Associated with Non-Starch Polysaccharide and Lignin Complex in Plants
Waxes
Cutin
Saponins
Tannins
Phytate
Suberin

Dietary fiber has many properties in foods like bulking agent, improving water holding capacity, oil holding capacity, gel forming capacity and improving shelf-life, antioxidant capacity and texturizing and also it is used in foods to decrease health diseases (Sudha et al., 2007; Elleuch et al., 2011; Kim et al., 2012). Epidemiological studies show that some health disorders like obesity, cardiovascular diseases, gastrointestinal diseases and some types of cancer diseases are reduced by consuming fiber (Gómez et al., 2010). High fiber-diet controls blood sugar levels, reduces cholesterol levels, prevents gastrointestinal disorders, reduces calories and protects against colon cancer (Bauer and Turler, 2008). Thus, interest to dietary fiber is increasing day by day.

Water and oil holding capacity are important factors during the selection of food additives because they affect the stability of the products. For instance, to stabilize high fat foods, fiber with high oil holding capacity is chosen. On the other hand; if the prevention of synaeresis is considered, fiber having high water holding capacity can be chosen (Grigelmo-Miguel and Martina-Belloso, 1999).

There are so many natural sources of fiber and some of them are shown in the Table 1.2. Their components, solubility and properties show difference according to their type.

Table 1.2 Dietary fiber content of some products (% dry matter). Derived from Elleuch et al. (2011)

Fiber source	Total dietary fiber content (% dry matter)
Wheat bran	44.46
Corn bran	87.86
Orange dietary fiber concentrate	36.9
Apple pomace	78.2-89.8
Orange peel	64.3
Arame algae	74.6
Nori algae	34.7

Peanut, soy and sunflower hull, apple, sugar beet and carob fiber, wheat, oat, rice and corn bran are among the fibers used in cereal-based products (Pomeranz et al., 1977; Collins et al., 1982; Dreher and Padmanaban, 1983; Babcock, 1987; Chen et al., 1988; Ozkaya, 1997; Park et al., 1997; Glitsø and Bach Knudsen, 1999; Riaz, 2001; Anil, 2002; Anil, 2007). While cellulose, hemicelluloses, lignin are found in cereals; gums, pectin and mucilage are primary found in fruit and vegetables (Normand et al., 1987; Elleuch et al., 2011).

Table 1.3 Major sources[1] of dietary fiber in the U.S. food supply (>1% total)

Sources of fiber	Percent in food supply		
	1995	2000	2005
Grain Products	36.00	36.30	35.50
White flour	16.91	17.14	15.55
Ready-to-eat cereals	4.11	3.62	3.60
Whole wheat flours	1.42	1.26	1.20
Rice	1.20	1.25	1.36
Vegetables	27.50	25.90	24.70
White potatoes	8.57	8.57	8.57
Tomatoes	3.90	3.90	3.90
Deep-yellow vegetables	2.69	2.42	2.03
Dark-green vegetables	1.46	1.80	1.67
Legumes, nuts and soy	14.10	14.00	12.90
Legumes	7.14	6.82	5.54
Nuts	3.69	4.03	4.22
Soy products	3.24	3.11	3.10
Fruits	11.10	11.20	10.00
Bananas, fresh	2.20	2.22	1.95
Citrus fruit	2.21	2.23	2.15
Apples, fresh	2.09	1.87	1.99
Miscellaneous foods[2]	11.30	13.60	16.70

[1]The order in which fiber food sources are listed is based on how much fiber is provided in theU.S. food supply and not as they are consumed.
[2]Spices, cocoa, tea, and coffee.
Source: USDA, The Food Supply and Dietary Fiber: Its Availability and Effect on Health. Nutrition Insight 36. United States Department of Agriculture Center for Nutrition Policy and Promotion, November 2007 (http://www.cnpp.usda.gov/Publications/NutritionInsights/Insight36.pdf. Last visited: August, 2013).

According to the Table 1.3, nuts are among fiber sources and its use has increased between 1995 and 2005, while the use of whole of legumes, nuts and soy as total have declined in 2005.

About the daily fiber requirement, it is recommended that in women younger than 50 can consume 25 g; women older than 50 can consume 21 g; men younger than 50 can consume 38 g and men older than 50 can consume 30 g of dietary fiber per day (Elleuch et al., 2011). To increase fiber consumption, some food products which have higher consumer acceptance are chosen for fiber addition. Some bakery products like cakes, cookies, bread and biscuits are produced with high fiber content to increase the nutritional values (Sreenath et al., 1996). Dairy products, meats, jams and soups can also be produced with fiber content and incorporating fiber into such foods helps stabilizing high fat foods, avoiding synaeresis and improving texture and shelf-life (Elleuch et al., 2011).

There are studies in which different sources of fibers are replaced with flour in bakery products. Figuerola et al. (2005) used apple pomace and citrus peel as a potential fibre source. In the study of Masoodi et al. (2002), wheat cakes prepared with using apple pomace at different percentages (5%, 10% and 15%). Anil (2007) used hazelnut testa in bread making as a source of dietary fiber. Additionally, Kocak (2010) investigated the effects of wheat bran and microfluidized wheat bran on quality of cakes. Also, in the study of Sudha et al. (2007), the acceptable rheological characteristics were obtained from cakes when apple pomace as a source of dietary fiber and polyphenols were used.

Some fibers are also seen as potential antioxidants. Some polysaccharide fractions protect against hydroxyl free and superoxide radical and they reduce power, chelating ferrous ions by resulting high scavenging ability upon hydroxyl radical. The extraction of polysaccharides in rice bran with hot water and precipitation (with 40% ethanol, concentration of 1mg / ml) showed that polysaccharide fractions from rice bran have effective antioxidant property (Zha et al., 2009).

In the study of Saura-Calixto (1998), the definition of antioxidant dietary fiber (ADF) was explained. According to Saura-Calixto, a vegetable material is defined as antioxidant dietary fiber if

- it has dietary fiber content higher than 50% on a dry matter basis.

- one gram of it has a capacity for inhibition of lipid oxidation equivalent to, at least, 200 mg of vitamin E and a free radical scavenging capacity equivalent to, at least, 50 mg of vitamin E.

- antioxidant capacity is an intrinsic characteristic and it is derived from natural component neither by antioxidant addition nor by formation in a enzymatic or chemical way.

1.1.1 New Improvement-Fruit Fibers

Cell walls are the main part of the fruits, vegetables, legumes and cereals containing very complex substances like dietary fiber; cellulose, pectin and lignin are some kinds of them. However, the composition of the cell wall changes according to type of plants. Moreover, the structure and some properties like the water binding capability of the fibers can differ from one type to another. To illustrate, fruit fibers have higher water binding capability than cereal-based fibers and fruit fibers have natural high functionality as an advantage (Cho and Samuel, 2009).

By-products of some fruit processing are mainly fiber rich products. Although there are lots of by-products, most of them are used as animal feed. However, there are some applications of fruit fibers to food products especially to calorie reduced food products.

Some applications are:

- In Low Fat o/w emulsions (mayonnaise-like)
  - Replacement of modified starch
  - Creamy,fat-like consistency even below 10% fat
  - Pseudo-elastic flow behavior
- In Sponge Cake and Muffins
  - Improved succulence
  - Replacement of flour or fat
  - Shape stabilization
- In Liver Sausage and Pate
  - Mimic fat
  - Improved succulence
  - Improved consistency
  - Replacement of cereal-based binders
- In Spreads
  - Stabilization of fat-reduced margarines
  - Replacement of starch or milk powders (Cho and Samuel, 2009).

1.2 Hazelnut

Nuts are seen as nutritive foods including healthful lipids. U.S. FDA has been recognized of them as "heart healthy" foods. Nuts contain dietary fiber, vitamin E, vitamins of B complex, plant sterols, essential minerals, unsaturated fatty acids, plant proteins, micronutrients and phytochemicals (Jakopic et al., 2011). Each of them or combination of them may have positive effects on health (Kabir and Sidhu, 2011). Nuts are good source of bioactive compounds and they may be incorporated into new products to improve health (Delgado et al., 2010). Hazelnut takes place in the most popular nuts and after the production of almond it is ranked as second among tree nuts (Shahidi et al., 2007).

Hazelnuts include high fat content. The major constituents in hazelnut fatty acids are polyunsaturated fatty acids and the existence of them may provide the protection of low density lipoprotein (LDL) from oxidation. Moreover, they can reduce plasma oxidized LDL levels (Orem et al., 2008). Since oxidative stress contributes to all inflammatory diseases, cancer, acquired immunodeficiency syndrome (AIDS), organ transplantation, gastric ulcers, neurological diseases, smoking-related diseases, alcoholism and other diseases; it is meaningful to find decreasing effects of natural products to these phenomena (Delgado et al., 2010).

More than three-quarters of annual worldwide hazelnut production can be obtained from Turkey. Especially, most of the factories and growers take place in Black Sea Region in Turkey. Therefore, hazelnut is important for Turkey's economy (Anil, 2007). While whole nut as raw or unroasted can be consumed, it can also be added to processed foods such as bakery and confectionery products. Hazelnut contains several parts including green leafy cover, shell and skin. Green leafy cover is the byproduct of harvesting process. This means that after harvesting period, the leaves are discarded. Moreover, hazelnut shell is the byproduct of shelling/hulling process. In addition, hazelnut skin is the byproduct of roasting process. As hazelnut hard shell is used for burning purposes, green leafy covers or tree leaves can have function as organic fertilizers (Shahidi et al., 2007). Hazelnut skin can also be used as animal feed since it is low cost waste product. However, hazelnut byproducts should have a commercial value because they can be seen as functional foods due to their potential sources of antioxidants (Shahidi et al., 2007; Contini et al., 2008).

Hazelnut skin is the part of 2.5 % of the total hazelnut kernel (Alasalvar et al., 2009). Although hazelnut skin is generally used as animal feed, some studies show that hazelnut skin is a rich and low-cost source of natural phenolic antioxidants and fibers (Yurttas et al., 2000; Schmitzer et al., 2011). In the study of Montella et al. (2013), it is stated that in Regulation (EC) No. 1924/2006 of the European Parliament and the council of 20 December 2006 on nutrition and health claims made on foods: ''A claim that a food is a source of fibre, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 3 g of fibre per 100 g or at least 1.5 g of fibre per 100 Kcal''.

Table 1.4 Composition of hazelnut skin at dry basis. Derived from Montella et al. (2013)

Hazelnut skin composition (%)	
Total nitrogen	1.30 ± 0.03
Protein	8.01 ± 0.17
Lipid	21.2 ± 0.24
Moisture	7.67 ± 0.76
Ash	4.50 ± 0.78
Total dietary fibre	58.3 ± 1.86
Soluble dietary fibre	3.33 ± 0.66
Insoluble dietary fibre	52.7 ± 1.86

In the study of Montella et al. (2013), it is seen that hazelnut skin is a fibre rich product with the amount of 58.3% of total composition at dry basis (Table 1.4). Moreover, hazelnut skin is rich in terms of phenolic compounds. The phenolic compounds help to reduce some risks of health diseases (Alasalvar et al., 2006). In the study of Yurttas et al. (2000), six phenolic aglycones were identified in Turkish and American hazelnuts: gallic acid, sinapic acid, p-hydroxybenzoic acid, quercetin, caffeic acid and epicatechin. In the study of Shahidi et al. (2007), it has been reported that hazelnut skin had the highest total phenolic content while hazelnut kernel had the lowest total phenolic content. Thus, hazelnut skin phenolic extract might be used as suitable source of natural dietetic flavonoids (Contini et al., 2012). Both flavonoids mainly catechin, quercetin, kaempferol, epicatechin, procyanidin B2 and phenolic acids such as gallic acid, p-hydroxybenzoic and protocatechuic acid are found in hazelnut skin (Rio et al., 2011; Montella et al., 2013). Phenolic content might have an effect on the storage stability, thus it should be considered as significant criteria for hazelnut quality (Yurttas et al., 2000).

Anthocyanin is a natural food color pigment that can be found in some fruit (especially in berries), vegetables, nuts and spices. It gives an intense color which can be red, blue or violet to the food. It may occur as glycosides / aglycosides of agylcone anthocyanidins. Its color changes with respect to its chemical structure (Wu et al., 2006). As the number of methoxyl group increases, redness also increases. On the other hand, the intense of bluish color increases when the number of hydroxyl groups in anthocyanidins increases. Methylated and acylated forms are generally preferred in foods because of their greater stability (Clifford, 2000). Moreover; temperature, sugar level, ascorbic acid, pH and processing influence the destruction rate of the colorant. For example, highly colored ionized anhydro bases are created in slightly alkaline solution which has pH value between 8 and 10. Fully ionized chalcones are hydrolyzed at pH 12 (DeMan, 1999). Since they are seen among various types of flavonoids, anthocyanins pay attention for health benefits (Wu et al., 2006). In the view of hazelnut, it has been stated that hazelnut takes place among foods containing high amount of anthocyanins (Wu et al. 2006). Therefore, the intense of hazelnut skin color and the effect of different processes on the color may be interpreted more accurately.

1.3 Cake and Cake Batter

Cake is one kind of bakery products consumed generally all around the world. Shortened-style cake (yellow cake, pound cake, etc.) and foam-style cake (sponge cake, pound cake, etc.) are two main types of cakes. While shortened-style cakes have a crumb texture due to the effect of batter processing, foam-style cakes may show aerated structure depending on foaming and aerated characteristics of eggs (Conforti, 2006).

Cake batter has a structure containing oil in water emulsion. While dry ingredients are dissolved in liquid phase, the oil phase disperses throughout the liquid phase and it does not become the fraction of the liquid or continuous phase (Painter, 1981). Mixing process provides interaction of ingredients. Baking process provides occurrence of some chemical reactions. Firstly, the production of carbon dioxide gas due to the effects of leavening agents and the rise of water vapor pressure with temperature increase occur which results in the expansion of cake batter. After that, baking continues with some reactions like starch gelatinization and protein coagulation. Finally, process ends up with discoloration of the product.

Flour, egg, sugar, milk, fat and salt are the main ingredients for cake making. Their amounts and ratios in the cake formulation have an importance because it is possible to produce cakes with different quality and sensory attributes by changing the formulations. Thus, it is necessary to know the functions of the ingredients to obtain the products with desired quality.

1.3.1 Cake Ingredients

Ingredients affect both the rheology of the cake batter and the quality of the final product. They have some significant roles in product properties like firmness, springiness, moisture content and shelf life. Thus, it is necessary to have knowledge of the functions of each ingredient for product development.

1.3.1.1 Flour

Flour takes place among the most important ingredients for cake preparation. The type of flour changes with the product it is used in. For instance, the flour used in bread may show differences in some constituents like gluten content than used in cakes. The two flour properties considered as important in cake making are the particle size distribution of flour and post-milling treatment to the flour. Particle size reduction provides an increase in cake quality. Re-grinding flours or applying air-classification techniques are among the methods to reduce particle size of the flour. For post-milling treatment, chlorine gas or heat operation is used. By exposing the flour to chlorine gas at flour mill, “high ratio” cake flour can be obtained. This is the type of flour that it can be blended with sugar and water having higher amount than flour. Moreover, heat treatment has the potential to provide similar properties but flour will have lower final moisture content when it is compared to chlorination. This leads to the need for increase in water amount used in cake formulation and a temperature rise during cake making due to the hydration of heat-treated flour (Cauvain and Young, 2008). Wheat proteins denature due to the heat effect and this brings about a decrease in their water solubility. By this way, viscosity of batter made with heat-treated flour becomes higher than untreated flour (Sahin, 2008).

Generally, flour made from endosperm of soft wheat is chosen as cake flour. Soft wheat flour has low granulation size and low water holding capacity and also low protein content (Sahi, 2008). When compared to cake flour, bread flour has higher gluten content and this gives bread dough high elasticity. High starch and low gluten content gives more tender and fine crumb texture to the product. Therefore, in cakes flour with low gluten are used. Besides protein content, cake flour also contains starch, vitamins, lipids, other nutrients and chlorine treatment affects them.

Moisture content is another important parameter for flour and it is generally about 14% and it should not change more than 1%. Ash content of flour, damaged starch, total alpha amylase and falling number and water absorption are among other measurable characteristics to choose correct flour type in the products (Demirkol, 2007).

1.3.1.2 Shortening

Shortening plays an important role in cake structure and batter properties. Shortening represents fats and oils and it can include other materials like emulsifiers and flavors (Matz, 1992; Kocak, 2010). Aeration capacity and emulsification effects of shortening are significant features in cake making. Creaming fat with sugar breaks down fat and incorporates air into the dispersed particles of fat. Shortening type and sugar size influence these cases. Since breakup of the fat varies with surface area, caster sugar having particle size property of <10% above 425 µm and <22% below 212 µm is generally preferred. Besides aeration function, in cake making, shortening shows stabilizing feature by trapping air bubbles. Moreover, in baking stage, fat crystals provide surface-active proteins adsorbed by them and the proteins make interfacial area to stabilize expanded air bubbles by impeding the release of foam (Sahin, 2008).

There are three main methods which are creaming method, the flour batter method and all-in method to prepare cake batter (Sahin, 2008).

Other than creaming method, all-in method can also be used for cake preparation. In this method, all ingredients are mixed at the same time. Thus, expansion resulting with high volume and fine crumb product occurs with the mechanism of entrapment of air bubbles in baking stage. In both methods, sugar plays an important role to obtain desired cake volume (Sahin, 2008).

1.3.1.3 Sugar

Sucrose is another significant ingredient in formulations of sweet bakery products. It is obtained from sugar beet or sugar cane. If cane sugar is not completely purified, it gives a good taste. On the other hand, beet sugar has an unpleasant taste if it is not completely white. Raw sugar is generally not preferred in bakery industry except for health foods. Some forms of sucrose such as granulated and icing sugar may be used as an ingredient in baked products (Edwards, 2007).

Sucrose provides sweetness to the product and also gives energy. It controls viscosity, heat setting temperature of proteins and gelatinization degree of starch. During baking, gas is produced during gelatinization process. Volume of cakes can be improved due to the effect of sugar on gelatinization temperature providing more time gluten to stretch (Kocak, 2010). Sugar plays also an important role for desired texture by delaying the starch gelatinization. Sugar addition decreases water activity of the solution and prevents gluten formation by this way. Moreover, interaction with starch stabilizes of the amorphous regions of the granule. This mechanisms result in retardation of starch gelatinization (Sahin, 2008). Moreover, in high-ratio cakes, sugar helps air to incorporate in a good way. As a result, more viscous and stable foam are achieved (Kocak, 2010).

Besides structural properties, sugar has lot of functions in bakery products. In cakes, it affects crumb and crust color, shelf-life, flavour and aroma. In biscuits, it has so many properties like surface appearance, browning and spreading during production. In bread, loaf structure, yeast activation, color, proof time and shelf life can be improved with the help of sugar (Demirkol, 2007).

1.3.1.4 Egg and Egg Products

Egg proteins play a significant role for cake batter rheology. Foam stability and coagulation can be developed with egg white proteins. Egg acts as an emulsifier due to the existence of lipoproteins in egg yolk (Sahin, 2008).

Eggs serve lots of functions:

Foam stabilizer: During baking, foams should hold other ingredients together and provide stability of protein matrix.

Coagulation: In coagulation, liquid egg is converted into semi-solid or solid part with the help of heating. Achieving the duplication of this property in other ingredients is difficult.

Toughener: Since egg especially egg white part helps starch gelatinization and gluten development, it acts as a toughener.

Tenderizer: Especially egg yolk part acts as a tenderizer due to fat content.

Binding and thickening agent: Since proteins in egg bind water, it can behave as binding and thickening agent (Kocak, 2010).

Surface active agent: It entraps air bubbles in batter in baking process (Kocak, 2010).

Emulsifying agent: Egg yolk portion serves an emulsifying property. It includes an important emulsifier which is lecithin (Demirkol, 2007).

Color property: Desired color and other organoleptic features can be obtained with the use of egg as an ingredient (Sahin, 2008).

It is possible to consider egg as two parts: egg yolk and egg white. Egg yolk is comprised of mainly lipids (70% of which are triglycerides) and also cholesterol and lecithin. Xanthophyll, a yellow coloring pigment gives the color to the egg yolk. If egg white part is considered, it includes proteins such as ovalbumin, ovotransferrin, conalbumin and lysozyme forming a chemical protection against microorganisms. Egg white has a viscous structure (Demirkol, 2007).

1.3.1.5 Water

Water level is a significant parameter to obtain a desired texture and shelf life in bakery products. Some portion of the required water can be obtained from other ingredients taking place in the formulation.

Water dissolves salt, sugar, nonfat dry milk and other dry ingredients and regulates moisture content in the final product (Sahin, 2008). Water activity is among the most important measurements to determine the shelf life of the cakes and this is another reason to reveal the importance of water. The water hardness also affects the quality of the final product and soft water makes the dough sticky (Kocak, 2010).

1.3.1.6 Leavening Agent

Ammonium bicarbonate, sodium bicarbonate and baking powder are generally preferred as chemical leavening in baked goods. Ammonium bicarbonate is also identified as *vol* indicating volatile salt, since it dissociates completely into carbon dioxide gas, water and ammonia gas. However, it is not seen as an appropriate ingredient in products leaving the oven with more than 5% moisture due to the unpleasant taste. Baking powder includes an alkali, such as sodium bicarbonate and an acid together with starch. With the help of solubility in water, alkali and acid go through a reaction and release carbon dioxide gas which gives a porous and fine grain structure to the product. Single acting and double acting are two kinds of baking powders. While single acting baking powders include only low-temperature acid salts like cream of tartar, double acting baking powders include two acid salts: one of them becomes active at room temperature and another at higher temperature (Sahin, 2008).

In the addition of sodium bicarbonate, two mechanisms take place for decomposition of it. Firstly, carbon dioxide is produced during mix stage due to the reaction with an acid and secondly, carbon dioxide is released in baking process with the help of heat. The thermal decomposition mechanism is the following reaction:

$$2NaHCO_3 + Heat \rightarrow Na_2CO_3 + CO_2 + H_2O$$

The mechanism of acid activated decomposition of sodium bicarbonate also provides carbon dioxide gas production with the following reaction:

$$NaHCO_3 + H^+ \rightarrow Na^+ + CO_2 + H_2O$$ (Bennion and Bamford, 1997).

In terms of mechanical feature, air is incorporated during creaming stage and whipping of eggs. In terms of mechanical means, baking powder and vapor pressure produced by the water lead into the air incorporation and release carbon dioxide gas during baking stage (Sahin, 2008).

1.3.1.7 Nonfat Dry Milk

Nonfat dry milk contains sugar and proteins and they are responsible of crust browning due to its lactose sugar content. Dry milk also contributes to desired textural properties (Sahin, 2008). It affects the pore sizes and stability in cakes. It can contribute to nutritional value of products.

1.3.1.8 Salt

Salt may act as preservative in cake and develop the flavor of other constituents in the product. It affects the solubility of proteins and so solubility of gluten. Moreover, it reduces the caramelization temperature of dough and thus improves crust color (Sahin, 2008).

1.3.1.9 Cocoa Powder

Cocoa (Theobroma cacao L.) contributes to economical increment of some countries like Ivory Coast, Ghana, Nigeria, Malaysia and Indonesia. Malaysia is seen among the main manufacturers of cocoa-based products around the world. Nevertheless, the price of beans of Malaysia is lower than the beans of West African. The reason behind of that is related to weak quality parameters such as low cocoa aroma and bitter taste (Othman et al., 2007).

The production steps of cocoa powder are fermentation, drying, roasting and Dutch process. The generation of flavor precursors occurs during fermentation and drying process. The formation of flavor compounds is achieved during roasting process through maillard browning (Lecumberri et al., 2007; Duman, 2013)

Cocoa powder is an important ingredient for chocolate-coloured or flavoured bakery products like cake, cookie and biscuit since it gives color and flavor to the product. Minimum amount of cocoa solids in product formulation is regulated in the legislation of some countries. Natural and Dutched are two types of cocoa powder. To produce Dutched cocoa powder, alkali treatment is performed after roasting, shelling and nibbing of the beans. Therefore, the colour of cocoa powder changes with respect to the applied processes. Moreover, fat content of cocoa powder may change between 8-32 % (Cauvain and Young, 2008).

To improve rheology of batter, water content in the recipe is increased when cocoa powder is added to the product. Since cocoa powder is dry, it affects rheological properties of dough (more viscous structure can be obtained) and changes in the formulation of product may be needed. Furthermore, normally slightly acidic pH of products is increased with the addition of cocoa powder. When amount of sodium bicarbonate is increased in the cake formulation, alkalinity of product may be improved and this leads to intensification of chocolate colour. Since alkaline pH diminishes mould growth, improvement in the product shelf life may be provided by the alkalinity of product (Cauvain and Young, 2008).

Cocoa-related products like chocolate and cocoa powders are derived from industrially processed seeds of Theobroma cacao L. (Sterculiaceae) and they are rich source of phenolic compounds, mainly procyanidins and flavan-3-ols (Wollgast & Anklam, 2000, Schinella et al., 2010). Monomers catechin and epicatechin, dimer procyanidin B2 are the main flavan-3-ol compounds in cocoa (Lamuela-Raventos et al., 2005; Schinella et al., 2010). The presence of them is significant because molecular size affects bioavailability of cocoa polyphenol. Generally, smaller polyphenols give more benefits. In addition, polyphenolic compounds are generally found in outer parts of plants. That is the reason why shells or skins have poylphenolic compounds (Bravo, 1998; Lecumberri et al., 2007). Moreover, a reduction in pro-inflammatory mediators (Sies et al., 2005) and improvement in endothelial function (Wang-Polagruto et al., 2006) can be achieved by consuming flavanol-rich cocoa (Schinella et al., 2010). Furthermore, risk of cardiovascular disease can be minimized by consuming cocoa or chocolate (Osakabe et al., 1998; Keen, 2001; Othman et al. 2007). Cocoa polyphenols show antimutagenic activity (Yamagishi et al., 2000; Lecumberri et al., 2007) and reduced levels of 8-hydroxy-2'-deoxyguanosine which is a biomarker of oxidative damage to DNA and also they have an impact on cancer disease (Orozco et al., 2003; Lecumberri et al., 2007).

The content of polyphenol in cocoa-derived foods is less than in raw material which is used in their production. Fermentation and high temperature of roasting conditions affect polyphenol content of them, negatively. Similarly, roasting causes a decrease in antioxidant and antiradical properties of cocoa beans and cocoa nibs (Arlorio et al., 2008). After the oxidation of anthocyanidin molecules, (-)-epicatechin and (+)-catechin in aerobic fermentation, polymerization occurs with the presence of polyphenol oxidase (PPO) enzyme. Therefore, preventing the activity of PPO enzyme is important. The methods for processing of cocoa products gains an importance and optimum conditions should be provided to avoid polyphenol degradation. There are some studies for these purposes. For example, in the study of Tomás-Barberán et al. (2007), it was reported that 95 ^{o}C for 5 min was optimum condition for the lowest enzymatic browning of fresh beans.

Besides polyphenols, cocoa and cocoa products are also rich in methylxanthines. Caffeine (1, 3, 7-trimethylxanthine) and caffeine-relatives theobromine (3, 7-dimethylxanthine) exist in cocoa powder (Belscak et al., 2009; Duman, 2013). Additionally, other components like dietary fibre are found in cocoa (Lecumberri et al., 2007; Duman, 2013).

1.3.2 Rheology of Cake Batter

Rheological properties play an important role in the quality of final product. Ingredients affect textural and rheological attributes of cakes and so the change in their amount in the formulation results with different final products having different structure.

Batter viscosity is an important parameter since it influences final cake structure. A rise in viscosity and stability of cake batter provides a porous and noncollapsing structure. Cake volume is affected by the viscosity. As viscosity decreases, the rate of bubble rise increases and then carbon dioxide gas is not held by cake batter. This results with lower volume of cake and firm texture. To obtain desired high cake volume, higher viscosity is needed. Moreover, low viscosity causes more convection flow in batter. In contrast to low viscosity, high value of viscosity diminishes shrinkage because of the prevention of entrapped air from coalescing (Kocak, 2010). Therefore, rheological information is critical in baking industry.

1.3.3 Quality of Cakes

High-quality cakes have several characteristics like uniform crumb structure, high volume, sufficient gelatinization degree, longer shelf life and tolerance to staling. They show varieties in terms of formulation, aeration of cake batters and stability of fluid batters in the thermal setting stage and early baking stage (Gélinas et al., 1999; Gómez et al., 2007). Generally, cakes with low specific volume have a firm texture (Gélinas et al., 1999).

Sensory evaluations and uniaxial compression methods measure texture parameters. For measurement of food acceptability, ranking tests which are hedonic measurements developed for sensory analyses are used. Simple rating scale is used for food acceptance and to supply benchmark number for comparison with products (Kocak, 2010; Resurreccion, 2008).

Firmness of cakes can be determined by compressing the sample and the force necessary to attain a predetermined penetration is measured. Instrumental Texture Profile Analysis (TPA) is used in textural analysis of bakery products. Uniaxial compression is conducted to the sample with specific dimensions in a TPA test and several parameters including springiness, firmness, cohesiveness, gumminess, fracturability and chewiness are determined. Clerici et al. (2009) observed some of the textural characteristics with respect to some texture profile parameter definitions. Hardness is the peak force to attain a given deformation. Springiness (elasticity) is defined as the rate at that a deformed material goes back to its initial condition after the deforming force is removed. Cohesiveness is defined as how well the product resists a second deformation relative to how it behaved under the first deformation. Chewiness is the required energy to crunch a solid food to a state ready to swallow. Gumminess is also defined as the required energy for disintegration a semisolid food to a state ready to swallow. Fracturability is the force with which a material fractures, a product of high degree of hardness and low degree of cohesiveness (Clerici et al. 2009; Koksel, 2009).

1.3.4 Structural Analysis of Foods

In order to interpret physicochemical changes in food products, quantification of structural characteristics using images can be useful way. Cake crumb structure is an important parameter for definition of cake quality due to its effect on volume and texture. Macro and microstructure of cakes give information for product quality (Scanlon and Zghal, 2001).

Image processing systems have an importance for food industry to analyze some properties like color, texture and shape of the products. Image processing system generally includes five steps which are image acquisition, pre-processing, image segmentation, object measurement and classification, respectively (Du and Sun, 2004). There are some studies including the application of image analysis in breads (Scanlon and Zghal, 2001; Datta et al., 2007; Ozkoc et al., 2009a) and in cakes (Sánchez-Pardo et al., 2008; Ashwini et al., 2009; Turabi et al., 2010).

Light microscopy (LM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) are imaging techniques which are usually used in food industry. SEM is an important method to examine varieties during baking stage. It is easier to prepare samples in SEM method than LM method and it produces fewer artifacts due to the no requirement of sectioning. Furthermore, SEM is suitable for wide range of food products. As the electron beam strikes an ultra –fine sample section (100 nm), some part of the incident electrons are transmitted to make an image with the impression of three dimensions (Bozzola and Russell, 1991; Aguilera and Stanley, 1999). It helps three dimensional comparing of starch granules and protein matrix in products. In terms of quantitative analysis, SEM was used to examine morphological changes in maltodextrin particles (Alamilla et al., 2005) and microstructural properties of cakes and breads by practicing Image J software (Turabi et al., 2010; Demirkesen et al., 2013).

1.3.5 Staling of Cakes

Staling is among the factors influencing consumer acceptance in food products. Besides moisture loss and microbial deterioration, staling is also a major process which affects the product shelf life (Gallagher et al., 2003). It includes a complex mechanism and there are still unexplained processes. However, the change in starch components is considered as the major reason for staling.

Starch gluten interactions and changes in amylopectin may cause staling in products (Gallagher et al., 2003). By starch retrogradation, gelatinized starch associates into ordered structure progressively (Ji et al., 2010). Both crumb and crust are subjected to changes. Crumb becomes more firm and crust has a softer texture (Gallagher et al., 2003). Reduction in water absorption capacity, amount of soluble starch and enzyme susceptibility of the starch, flavor loss, rise in starch crystallinity and opacity and the alterations in x-ray diffraction patterns can also be used (Koksel, 2009). In order to reduce staling, some modifications in product formulations and in processes are studied (Sumnu et al., 2010).

Amylopectin retrogradation is considered as the most effective factor for decrease in shelf life and also for undesirable firm texture of starch-based products than amylose. Specific volume, temperature and moisture content of the bakery product have also impacts on the starch retrogradation (Seyhun et al., 2005). These processes cause undesirable mouthfeel and increased firmness in the products. When compared to breads, staling occurs more slowly in cakes. The reason may be due to lower flour and starch level and also higher fat and sugar content in cakes (Lebesi and Tzia, 2011). Thus, lower concentrations of unstable starch may reduce staling rate and native starches can be replaced with cake flour partially (Gélinas et al., 1999).

The methods to study starch retrogradation are divided into two main groups: macroscopic techniques and molecular techniques. Macroscopic techniques are related to changes in physical attributes such as textural or mechanical changes. On the other hand, molecular techniques are related to changes in starch polymer conformation or water mobility in starch gels at molecular levels. As a result, differential scanning calorimetry (DSC), rheological techniques and sensory evaluation of texture can be used as macroscopic techniques. For molecular techniques, nuclear magnetic resonance spectroscopy (NMR), X-ray diffractometry and Fourier transform infra-red (FTIR) spectroscopy may be used (Karim et al., 2000).

X-ray method provides knowledge about regular double helices of molecular structures which is related to three dimensional order of crystallinity in starch but not about irregular packed structures. Crystallinity of starch during staling can be analyzed and it might be compared with the result of rheological analysis. While DSC gives a more sensitive measurement of amylopectin than amylose, X-ray diffraction reflects combination of amylose and amylopectin. Furthermore, X-ray method is considered as less sensitive technique than FTIR (Fourier Transform Infrared Spectroscopy) which could determine even minor manners of recrystallization (Smits et al., 1998; Karim et al., 2000). Retrogradation of amylose and amylopectin may also be monitored by the method of FTIR. FTIR reflects staling mechanism of products which are related to conformational varieties at molecular level and it analyzes degree of short range ordering in system (Karim et al., 2000).

1.4 Microfluidization

Some techniques are used in food industry to develop texture, stability, taste and color of the foods. Microfluidization is one of the novel techniques taking place in food industry and also in other areas. This new techniques give opportunities to produce more uniform samples, to improve texture, color and stability of the products.

Generally, two stage, homogenization is used in industry to obtain higher consistency, glossier and smoother structure in the products by reducing size of the particles. In conventional homogenization, product passes through a microscopic opening in the homogenizing valve. It causes high shear and turbulence; as a result, dispersion and disintegration of the solids occur (Mert, 2012).

Microfluidization is a unique high pressure homogenization technology (Lagoueyte and Paquin, 1998). In Figure 1.1, it is seen that it includes a reaction chamber in that the fluid is divided into two microstreams then colliding with each other at very high speeds (Cook and Lagace, 1987; Lagoueyte and Paquin, 1998; Mert, 2012). Both high shear rate and extreme impact forces causing the formation of fine particle are applied to microstreams (McCrae, 1994; Mert, 2012). Therefore, nano and micro particles are separated from macro particles with the help of high shear rate. Therefore, this mechanism provides smaller particles with smaller size distribution than the mechanism of conventional valve homogenizer (Tunick et al., 2000). There are some studies related to the applications of microfluidization, however there is a lack of studies about its application in foods. It is an optional technology to enhance stability and texture of food products. It reduces particle size and influences rheological and sensory properties of the products (Ciron et al., 2011).

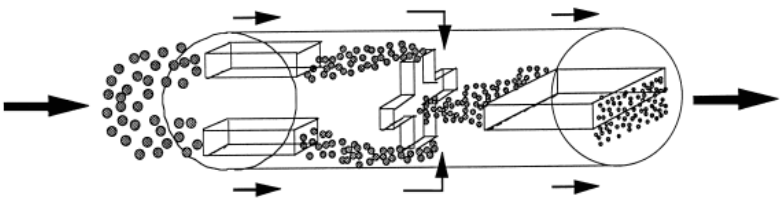

Figure 1.1 Symbolic figure for the reaction chamber of the microfluid equipment (Lagoueyte and Paquin, 1998).

There are some studies about the application of microfluidization including milk (Dalgleish et al., 1996; Hardham et al., 2000), cream liqueurs (Paguin and Giasson, 1989), Xanthan gum (Lagoueyte and Paquin, 1998), ice cream (Olson et al., 2003), mozzerella cheese (Tunick et al., 2000), yoghurt (Ciron et al., 2010), wheat bran

(Wang et al., 2012), high methoxyl pectin (Chen et al., 2012), lentinan (Huang et al., 2012) and ketchup type products (Mert, 2012). It has been reported in the study of Mert (2012) that the increase in the pressure of microfluidization process improved quality parameters of the ketchup samples.

Microfluidizer processor as a laboratory machine uses multiple parallel micro channels in the interaction chamber and provides facility to scale up to pilot volumes (Wang et al., 2012). This mechanism leads to improve consistency of the products with desirable textural properties by reaching to high pressures (Ciron et al., 2011). Process pressure may change in the range of 206-1586 bar (3,000-23,000 psi) and enable to obtain uniform particle, to reduce droplet size, to disrupt high yield cell (http://microfluidicscorp.com. Last visited: August, 2013).

If it is compared to traditional techniques, microfluidization has many advantages:

- Little or no contamination occurs.
- More uniform and smaller particles/droplet sizes are obtained.
- Its processing time is faster (> 2 orders of magnitude in some applications).
- It can be practiced in cGMP manufacturing environments.
- It is easy to clean.
- For both large and small scale production, the principles of the equipment become same.
- It has a property of scalability from small batches to continuous production (Garad et al., 2010).

Grinding mill is among the old traditional methods to reduce particle size. It contains a media like ceramic beads. When mill rotates, the media mills the particles into smaller size. However it has some disadvantages:

- There may be a contamination with grinding media.
- It is not easy to scale up the process.
- Large mills are needed for large production.
- It takes very long time to produce very small particles.
- It is not easy to cleaning and extracting media (http://microfluidicscorp.com. Last visited: August, 2013.).

Ball mill which is kind of grinder requires weeks to obtain similar size of particles as in microfluidizer (Garad et al., 2010). It is filled with sample for grinding and rotates around a horizontal axis. Hammer mill is another type used for milling process. It includes horizontal or vertical rotating shaft or drum and hammers are put on them. It is difficult to reach similar concentration of high energy in conventional grinders and homogenizers other than microfluidizer (http://microfluidicscorp.com. Last visited: August, 2013). When homogenizer valves pushing fluids through a variable geometry spring loaded valve are considered, it is seen that it provides limited reduction of particle size and process pressure. Therefore, results may not satisfy industry (http://microfluidicscorp.com. Last visited: August, 2013).

Microfluidization process has an impact on physicochemical properties of food samples. The value of water holding capacity (WHC), swelling capacity (SC), oil holding capacity (OHC) and bulk density may change with respect to such kind of processes. Hydration properties are important parameters for products and potential use of food products may vary according to changes in hydration properties of food samples. Water holding capacity is the water amount which is retained by a known amount of fiber ingredients under the conditions used (Wang et al., 2012). In the study of Wang et al. (2012), it is stated that particle size distribution is not the only parameter for determination of water holding capacity of insoluble fibers; that's why there are some inconsistences in results of studies. Different methods may affect hydration properties and porosity in a different way. For instance, it was stated in the study of Huang et al. (2010) that fibers ground by ball milling showed an increase in porosity with reduction of bulk density. However, fibers ground and passed through different mesh sieves showed a decrease in porosity as bulk density decreased (Gupta and Premavalli, 2010). Moreover, in the study of Wang et al. (2012), conditions of microfluidization process decreased bulk density of wheat bran samples and caused an increase in the porosity of wheat bran. Moreover, water holding capacity showed an increase as particle size was reduced by leading to increase in surface area. Therefore, raw samples showed lower water holding capacity than wheat bran processed with microfluidizer. On the other hand, there are some studies obtained opposite results which are related to effect of reduction in particle size on some properties like bulk density (Raghavendra et al., 2006; Chau et al., 2007). These explain that not only particle size reduction but also different methods to reduce particle size affect properties of samples.

1.5 Phenolic Compounds

Phenolic compounds are the main classes of secondary metabolites in plants. They provide necessary functions in reproduction and plant growth. Similarly, they show defense mechanism against parasites, pathogens and predators. Phenolics also contribute to the colour of plants (Liu, 2004; Locatelli et al., 2010). The number of molecules detected in plant species is very high. Phenolic compounds have an importance since they enrich foods in terms of sensory and nutritional value (Karakaya, 2004). Moreover, it was reported in some studies that phenolic compounds like flavonoids decrease the risk of heath diseases such as coronary and cardiovascular diseases, some types of cancer and physiological syndromes (Richardson, 1997; Hercberg et al., 1999; Kuntz et al., 1999; Eberhardt et al., 2000, Yurttas et al., 2000; Yardim-Akaydin et al., 2003; Karakaya, 2004; Veeriah et al., 2006; Jakopic et al., 2011).

At low concentration, phenolics may defend foods against oxidative deterioration acting as antioxidant. On the other hand, at high concentration, an interaction of phenolics or their oxidation products may be occur with carbohydrates, proteins or minerals. Phenolic compounds are seen as powerful antioxidant in vitro than vitamin C and E on molar basis (Rice-Evans et al., 1997). Nevertheless, there is a controversial issue about *in vitro* and *in vivo* methods (Karakaya, 2004). Generally, polyphenols have a potential to show free radical-scavenging activity depending on hydrogen or electron-donating agents (Rice-Evans et al., 1997).

There are so many studies related to antioxidant activity and phenolic content of foods. For instance, antioxidant activity and phenolic content of tea were investigated in several studies (Farhoosh et al., 2007; Turkmen et al., 2007; Velioglu, 2007; Kerio et al., 2013). Moreover; cereals, fruit and vegetables were studied for their phenolic and antioxidant properties (Velioglu et al., 1998; Vinson et al., 1998; Paganga et al., 1999; Proteggente et al, 2002; Amin et al., 2004; Soong and Barlow, 2004; Amin et al., 2006; Dykes and Rooney, 2007; Stratil et al., 2007; Vasco et al., 2008;). Phenolic compounds from residual sources such as by-products of wines (Alonso et al., 2002), grape pomace powder (Yi et al., 2009), apple pomace (Sudha et al., 2007), hazelnut kernels (Jakopic et al., 2011), hazelnut skin (Rio et al., 2011), peanut skin (Nepote et al., 2002), cocoa powder, nib and shell (Azizah et al., 1999; Martinez et al., 2012) were also investigated. It is important to have knowledge about the nature of phenolic compounds since it helps investigating the characteristics of phenolics. Phenolics possess an aromatic ring including hydroxyl substituent and a functional residue (Cilek, 2012). Mainly, phenolic compounds are divided into four parts depending on their carbon skeleton: phenolic acids, flavonoids, stilbenes and lignans as shown in Figure 1.2.

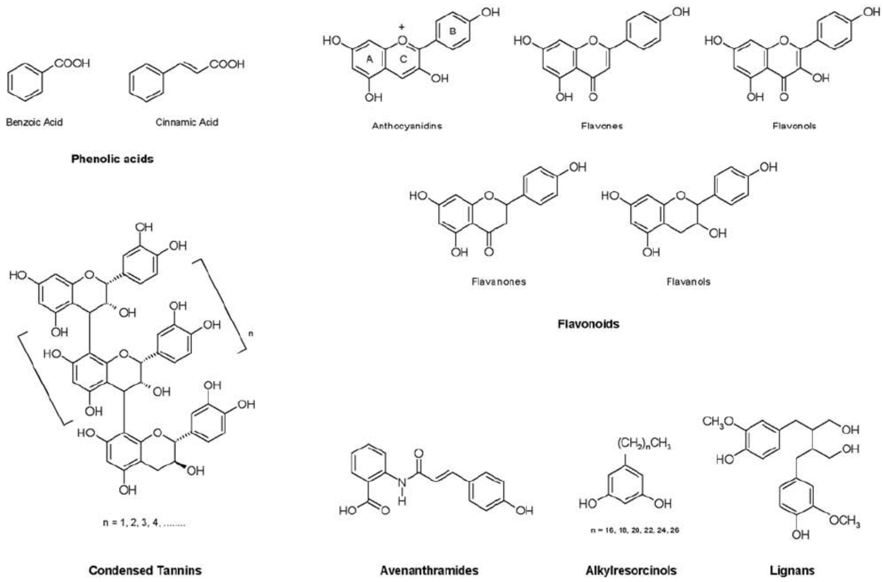

Figure 1.2 Chemical structure of phenolic compounds (Dykes and Rooney, 2007).

1.6 Objectives of The Study

Baked goods such as cakes, cookies, bread and biscuits can be produced in a way to have high fiber content for increased nutritional value. However, the utilization of hazelnut skin micro and nano fibers in cakes has not been investigated. Moreover, cocoa powder is among the mostly used ingredients in bakery products due to its acceptable color and flavor with acceptable viscoelastic and textural properties so it can be a good alternative to make comparison with hazelnut skin. Therefore, the main aim of this study was to produce functional micro and nano scale fibers from hazelnut pellicle (skin) and utilization of these fibers in cake formulations. Microfluidization process was used for micro and nano fiber production from hazelnut skin and besides this application, hazelnut skin was reduced to smaller sizes by using ball mill and hammer mill, respectively. All types of hazelnut skin samples and cocoa powder were compared. Since texture and rheology are important parameters for high quality product, it was aimed to compare textural and rheological properties of cake samples in this study.

Microstructural analysis is also essential for qualitative comparison of different ingredients and cake samples. Thus, other objective of this study was to characterize cocoa powder/ hazelnut skin samples and crumb of cakes, qualitatively. For this purpose, SEM analysis was performed to samples.

Shelf life is also an important parameter for food products and consumer acceptance. Therefore, in this study it was also aimed to investigate staling of different cake samples by using different techniques: X-ray and FTIR.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

For cake preparation, cake flour and cake shortening were obtained from ETI Food Industry Co. Inc. (Eskisehir, Turkey). Sugar, salt, baking powder, non-fat milk powder, egg white powder and cocoa powder were provided from Ulker Biscuit Industry Co. Inc. (Ankara, Turkey). Hazelnut skin was obtained from Sanset Food Tourism Industry Co. Inc. (Ordu, Turkey).

2.2 Methods

2.2.1 Production of Micro and Nano Hazelnut Skin Fibers

For production of micro and nano hazelnut skin fibers from hazelnut skin, Microfluidizer equipment (M-110Y, Microfluidics, USA) was used. Process involved two main stages. Firstly, softening was performed with water. Then, this slurry-like product were processed in microfluidizer pumping it a very high pressure through chamber of 200 µm size and collecting it in output reservoir (Figure 2.1). Again processed sample underwent to a second size reduction step by passing through a chamber of 100 µm size. At second stage, slurry velocity became 800 m/s and shear rate obtained higher than 10^7 1/s. Hazelnut skin particles were broken into fiber during passage through channels.

To compare with microfluidization process, hammer mill (Thomas-WILEY, Laboratory Mill, Model 4, Arthur H. Thomas Company, Philadelphia, PA., USA) and ball mill (Retsch, PM 100, Germany) were used to obtain different size hazelnut skin samples. After hammer mill process, sample was named as raw HS. Also, sample was called as ball milled (BM) HS after ball mill process.

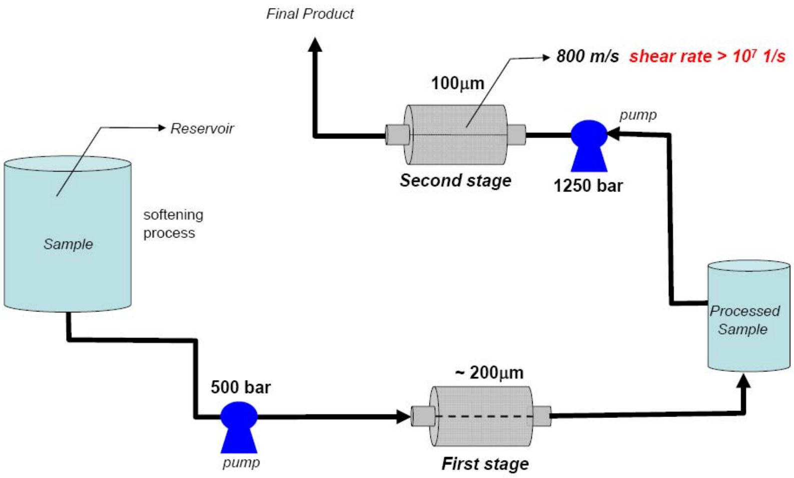

Figure 2.1 Production of fiber (Kocak, 2010)

2.2.2 Cake making Procedure

Cakes were prepared by replacement of flour with different percentages (0%, 10%, 15% and 20%) of raw hazelnut skin (raw HS), ball milled hazelnut skin (ball milled HS), microfluidized hazelnut skin (MF HS fiber) and cocoa powder. As a control, cakes were prepared without any replacement of flour. The formula on a 100 g flour basis was 100% water, 80% sugar, 50% shortening, 12% milk powder, 8% egg white powder, 6% baking powder and 1.5% salt. Other than this formula, cakes with flour reduction were also prepared mainly without changing other ingredients as weight. Cake batter was prepared using a mixer (Krups). To obtain uniform mixing, ingredients were not poured at the same time; baking powder, shortening, and egg white powder were creamed for 1 min at low speed to get a fluffy cream; and then milk powder, sugar, and salt were added. They were whipped together for 2.5 min at low speed, and finally flour, hazelnut skin (HS) or cacao powder and water added simultaneously and mixed; first for 2 min at low speed, then for 1 min at medium speed and finally for 2 min at low speed. After complete mixing, 60 g of cake batter was poured into silicon moulds and baked at 175-180 °C for average of 15 minutes. After baking, cakes were cooled to 25 ˚C for one hour. Some of cake samples were stored at 25 ± 2 °C in vacuum packs for storage analyses.

2.2.3 Rheological Measurements

The rheological measurements were carried out using a TA rheometer (AR 2000ex, Rheometer). All measurements were done at 25 ˚C, using parallel plate geometry (40 mm diameter and 1 mm gap). The dough sample was placed between the plates and the edges were carefully trimmed with a spatula. The flow measurements were conducted where shear stress, τ was measured versus shear rate, γ that changed from 0.1 to 50 l/s. For the relaxation of the residual stresses, the dough was rested at room temperature for 300 s before testing. In dynamic oscillatory experiments, first linear viscoelastic region of the samples were determined. Then frequency sweep test from 0.1 to 10 Hz were conducted at 1% strain rate with a different aliquot of the same samples. Finally, elastic (G') and loss (G") modules value were obtained. All the rheological experiments were performed at least twice.

2.2.4 Weight Loss

The percentage weight loss of cakes was calculated by using the weight of cake batter (W_{dough}) and weight of cake sample just after baking (W_{cake});

$$\mathrm{WL}\ (\%) = \left[\frac{W_{dough} - W_{cake}}{W_{cake}}\right] \times 100 \qquad (2.1)$$

Two dough samples were prepared and the measurements from same dough were done in duplicate.

2.2.5 Scanning of Cake

Cake samples were cut into two halves vertically and then they were sliced. Each slice was placed over the glass of a scanner (CanoScan Lide 110, Tokyo, Japan).

2.2.6 Scanning Electron Microscope (SEM) Analysis

For SEM analysis, cake crumbs which were broken into small pieces (cubes in about 2.5 cm dimension), frozen in liquid nitrogen and then freeze dried (Christ, Alpha 2-4 LD plus, Germany). Freeze-dried samples were sputter coated with gold-palladium to render them electrically conductive by Sputter Coater Device (Polaron Range, East Sussex, England). Samples were then examined and images were recorded with a scanning electron microscope (QUANTA 400F Field Emission SEM, Eindhoven, Holland) at an accelerating voltage of 20 kV. Samples were observed at magnification levels of 100X, 250X, 1000X and 4000X. Additionally, hazelnut skin samples and cocoa powder were also examined. They were done at Central Laboratory of METU (Ankara, Turkey).

2.2.7 Color Analysis

The crumb color of the cake samples was determined using ProImage Program. CIE L*, a*, and b* color scale was used and twelve readings were carried out from different positions of cake crumbs. The a value ranges from −100 (redness) to +100 (greenness), the b value ranges from −100 (blueness) to +100 (yellowness) and L value ranges from 0 (black) to 100 (white). Total color change (ΔE) was calculated from the following equation;

$$\Delta E^* = [(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2]^{1/2} \quad (2.2)$$

Color of control cake was selected as reference point and its L*, a* and b* values were represented as L_0, a_0 and b_0 which were 95.44, 1.19 and 12.41, respectively.

2.2.8 Staling Analysis

For storage analysis, after baking cakes were allowed to cool down for 1 h and then stored in vacuum pack at 25 ± 2 °C for different storage times (3, 6 and 9 days).

Texture measurements were done to all cake samples (at all replacement levels of flour with cocoa powder/ hazelnut skin). On the other hand, cake samples prepared with 10% replacement of flour with cocoa powder/ hazelnut skin were used for X-ray diffraction and FTIR analysis since the best results were obtained from these cakes. Additionally, control cake was analyzed for staling.

For X-ray and FT-IR measurements, cake samples, which were stored at different times, were frozen and then freeze-dried (Christ, Alpha 2-4 LD plus, Germany) for 48 h at a pressure below 1 mbar. Samples were ground in a coffee grinder (Sinbo, SCM-2914, Istanbul, Turkey) and then sieved.

2.2.8.1 Moisture Content

The moisture content of cake crumb samples was determined using Moisture Analyzer (IR-35, Denver Instrument). The temperature was set to 106 ºC. Two replicates were done from same dough and also two replicates were done from same cake sample.

2.2.8.2 Texture Analysis

Crumb texture of cake was evaluated by the Texture Analyzer (The TA.XT*Plus*, England). 1 cm diameter cylindrical probe was used for the compression of cake samples having thickness of 1 cm. The measurements were done at a 1 mm/ s pre-test speed, at 1 mm/s test speed and at 10 mm/s post-test speed with 60 s holding time. The probe compressed sample until it compressed 25% of the sample height. It held at this distance for 60 s and after it put up sample, it returned to its beginning position. After samples were cooled down to 25 ºC, experiment was applied. Four replicates from two different sets of baking were measured and averages of them were taken. Firmness and springiness values were obtained.

2.2.8.3 X-ray Diffraction Analysis

X-ray diffraction analysis was done using CuKa (λ=1.54056) radiation on a Ultima IV X-ray diffractometer (Rigaku, Japan) at 40 kV and 40 mA. The scanning region of the diffraction angle (2θ) was 5°–45° with the scanning speed of 2°/min. The analysis was performed using PeakFit version 4.12 software. After the samples were freeze–dried and ground, they were mounted with 0.5-1 mm thickness on a place of 2x2 cm^2 glass holder. Crystalline peaks were analyzed as pseudo-Voight-form and the amorphous ones as Gaussian-form peaks. The crystallinity levels in the samples were determined by the separation and integration of the areas under the crystalline and amorphous X-ray diffraction peaks. The quantification of relative crystallinity was determined using total mass crystallinity grade (TC) in which the area of the crystalline fraction is divided by the crystalline fraction plus the amorphous fraction, based on the method described by Ribotta et al. (2004), Ozkoc et al. (2009b) and Demirkesen et al. (2013).

$$\mathrm{TC} = \frac{\mathrm{I_c}}{\mathrm{I_c}+\mathrm{I_a}} \qquad (2.3)$$

I_c is the integrated intensity of crystalline phase, and I_a is the integrated intensity of the amorphous phase.

2.2.8.4 FT-IR (Fourier Transform Infrared Spectroscopy) Analysis

FT-IR experiments were performed on a IR Affinity-1 Spectrometer (Shimadzu Corporation, Kyoto, Japan). The analysis was done in the middle-IR region, 600–4,000 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans. Freeze-dried cake samples were put into the crystal surface by providing contact of ATR crystal with the sample. Two replications were done. The analysis was performed using PeakFit version 4.12 software.

2.2.9 Statistical Analysis

Analysis of variance (ANOVA) was performed to determine whether there was a significant difference between cake sample types, the percentages of replacement of flour and storage times ($p \leq 0.05$). If significant difference was obtained, means were compared by the Tukey Single Range test ($p \leq 0.05$) using MINITAB (Version 16) software.

CHAPTER 3

RESULTS AND DISCUSSION

Firstly, in this study SEM analyses of cocoa powder and hazelnut skin samples were done. Then, rheological measurements of dough samples were comprehended and the effects of cocoa powder/ hazelnut skin samples on viscoelastic properties of cake batters were determined.

Quality parameters (weight loss, texture and crumb color) of cakes were evaluated. To determine microscopic structure of cakes, SEM analysis was used.

Finally, the effects of different cake formulations and storage time on staling of cakes were studied. Staling analyses were performed using X-ray diffraction and FT-IR.

3.1 SEM Images of Hazelnut Skin Samples and Cocoa Powder

The characterization of cocoa powder and differently sized hazelnut pellicles by using different methods (hammer mill, ball mill and microfluidizer) was firstly done by Scanning Electron Microscope (SEM). Figs. 3.1-3.4 depict the SEM images of microfluidized hazelnut skin (MF HS) at different magnifications. After the microfluidization process, the production of micro and nano HS fibers was achieved. As illustrated in Figs. 3.1-3.4, MF HS fiber showed larger surface area with branches. HS fiber tends to interwine and make a lattice network providing characteristic rheological and textural properties to its products. With the application of microfluidization, HS was fibrillated into smaller fiber fragments. High pressure homogenization using microfluidizer caused an increase in the dissociation of the HS particles. Similar effect of microfluidization on ketchup samples was also observed in the study of Mert (2012).

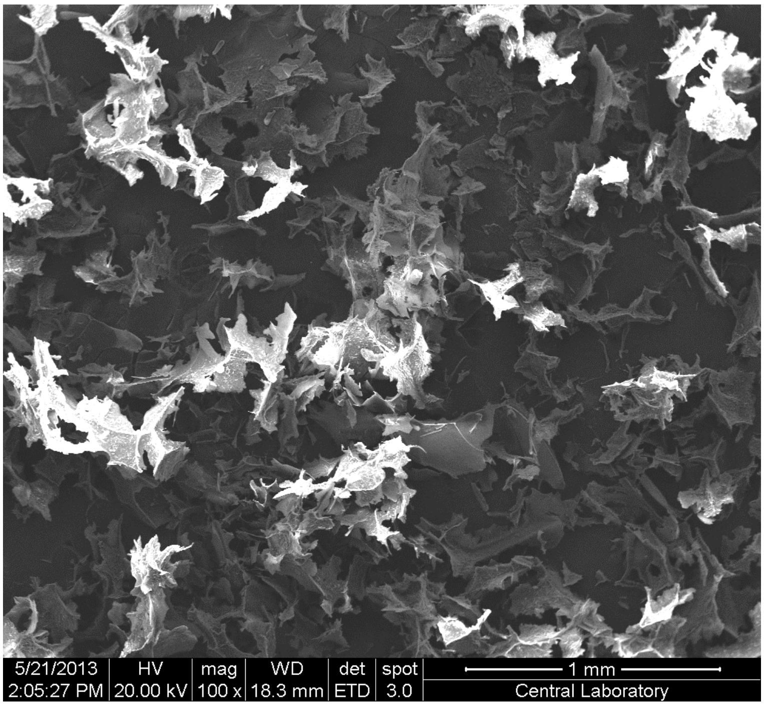

Figure 3.1 SEM image of hazelnut skin (HS) fiber processed by microfluidizer
Magnification: 100x

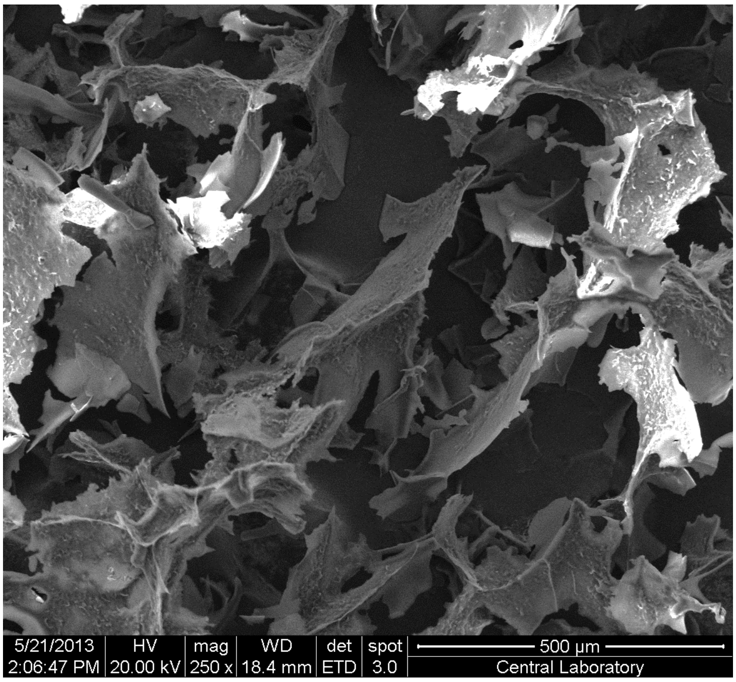

Figure 3.2 SEM image of hazelnut skin (HS) fiber processed by microfluidizer
Magnification: 250x

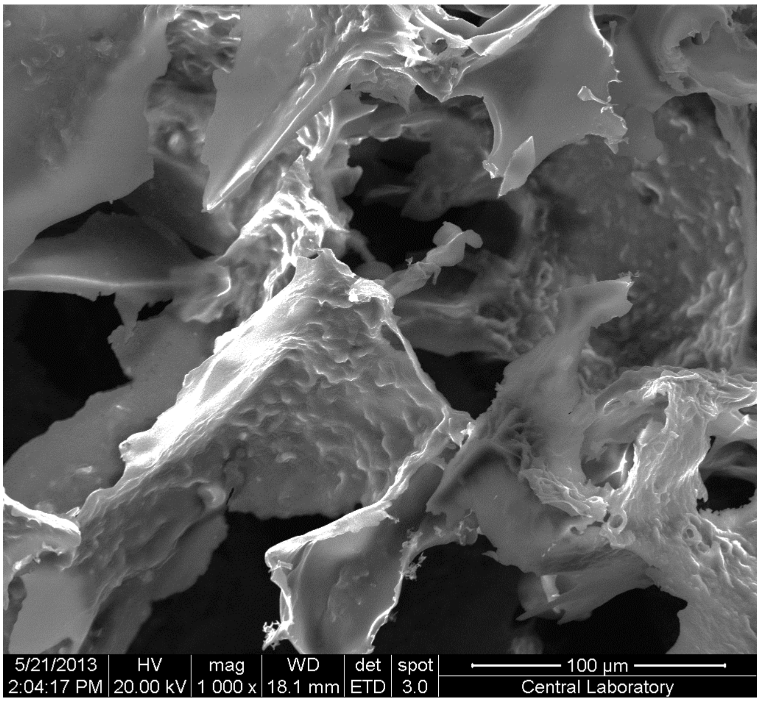

Figure 3.3 SEM image of hazelnut skin (HS) fiber processed by microfluidizer
Magnification: 1000x

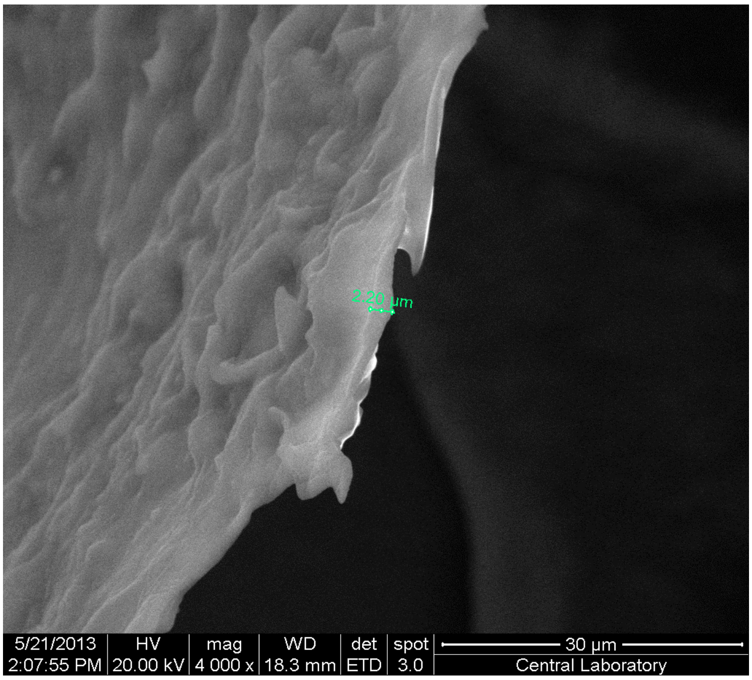

Figure 3.4 SEM image of hazelnut skin (HS) fiber processed by microfluidizer
Magnification: 4000x

When the images were examined, it was seen that larger blocks with different sizes in the picture of raw HS (Figure 3.6) turned into a flaky structure in the image of MF HS fiber. On the other hand, it is possible to see small particles which are more similar to cocoa powder in the images of ball milled HS samples (Figure 3.5). This may be explained by the effect of milling. It can be said that ball mill caused the breakage of blocks into smaller particles in HS samples. In SEM images of cocoa powder, many rounded folds can be seen on the particle surface (Figure 3.7). There is a lack of lattice network in these samples as opposed to microfluidized samples.

SEM images can also help to evaluate the results of other analyses. The flaky structure in HS was obtained by microfluidization process which influenced the physicochemical properties of hazelnut skin. Increased surface area improved water holding capacity of the product leading to changes in textural, rheological properties and in staling mechanism of the product.

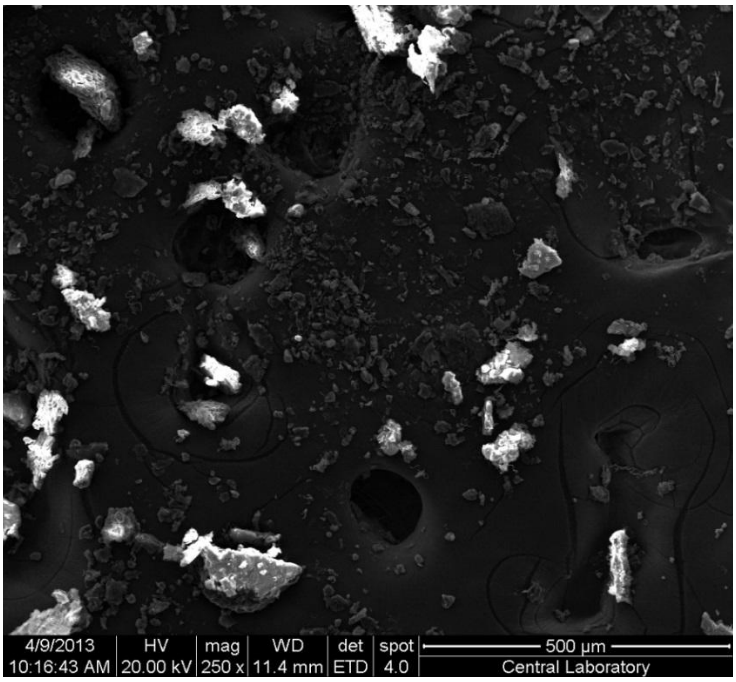

Figure 3.5 SEM image of ball milled HS sample Magnification: 250x

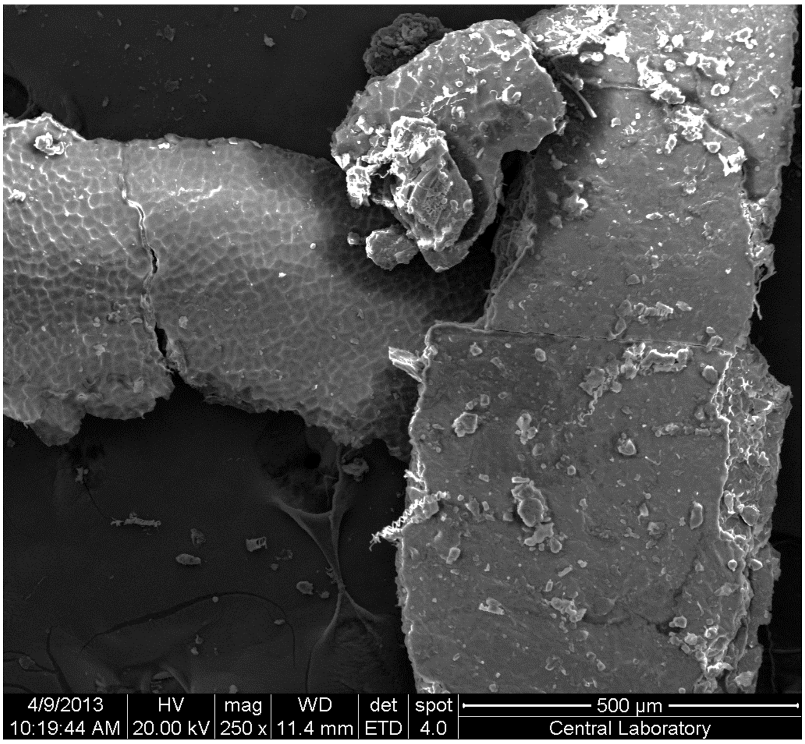

Figure 3.6 SEM image of raw HS sample Magnification: 250x

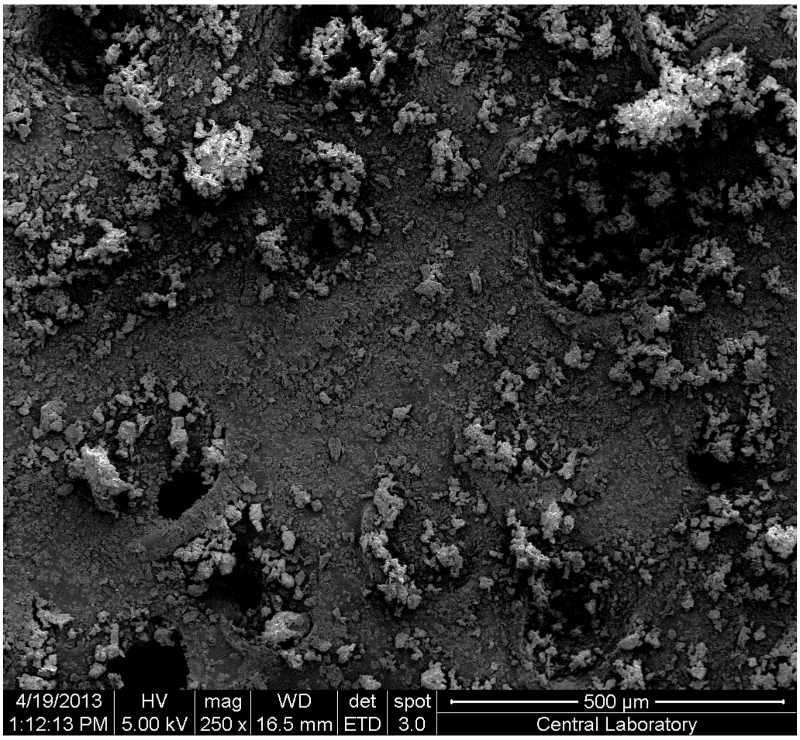

Figure 3.7 SEM image of cocoa powder Magnification: 250x

3.2 Analysis of Fresh Cakes Based on 100g Flour Formulation

3.2.1 Rheological Measurements

3.2.1.1 Flow and Oscillation Measurements of Cakes Based on 100 g flour formulation

The shear stress (τ) versus shear rate (γ) data was fitted well to the Herschel- Bulkley model for all dough formulations at 25 ºC (Eq. (3.1)):

$$\tau = \tau o + K(\dot{\gamma})^{n} \quad (3.2)$$

where τ is the shear stress (Pa), τ_o is the yield stress (Pa), γ is the shear rate (s^{-1}), K is the consistency index (Pas^n) and n is flow behavior index.

Table 3.1 Herschel Bulkley parameters of dough samples at 25 ºC**.**

Formulation	**τ_O (Pa)**	**K ($Pa.s^n$)**	**n**
Control	21.66	24	0.53
Cocoa 10%	71	27.47	0.56
Raw HS 10%	87	34	0.61
BM HS 10%	98	51	0.53
MF HS 10%	214	74	0.49
Cocoa 15%	81	31	0.51
Raw HS 15%	94	51	0.55
BM HS 15%	141	71	0.53
MF HS 15%	291	98	0.49
Cocoa 20%	96	51	0.51
Raw HS 20%	111	89	0.55
BM HS 20%	184	101	0.53
MF HS 20%	446	168	0.46

Table 3.1 shows the Herschel–Bulkley model parameters for the dough samples. For all samples, a shear thinning behavior (pseudoplastic) was observed. Since interactions between components are broken down under the action of shear, viscosity decreases with the increase in shear stress (Demirkesen et al., 2010). The flow behavior index values changed between 0.46 and 0.61. Batter samples with 20% level of microfluidized HS fiber showed the lowest n value as 0.46 which indicated the most complex structure and it differs from Newtonian fluids (n = 1) (Gómez et al., 2010).

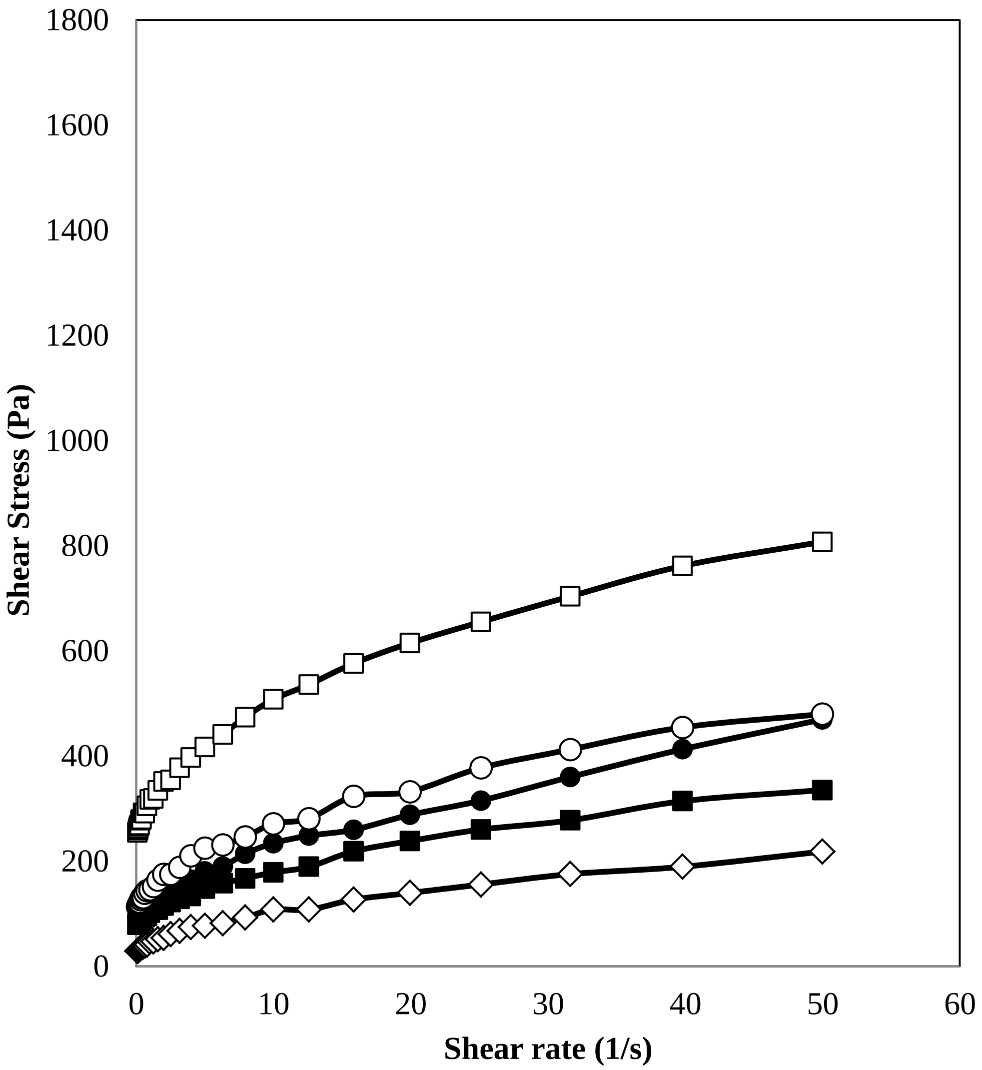

Figure 3.8 Flow curves obtained for dough samples containing 10% different types of hazelnut skin, cacao or none of them. (◇): Control, (■): Cacao 10%, (●): Raw 10%, (○): Ball milled 10%, (□): MF 10%.

The flow curves in Figs. 3.8-3.10 show cake batter samples prepared by the replacement of flour with raw hazelnut skin, ball milled hazelnut skin, microfluidized hazelnut skin and cocoa powder at 0%, 10%, 15% and 20% percentages.

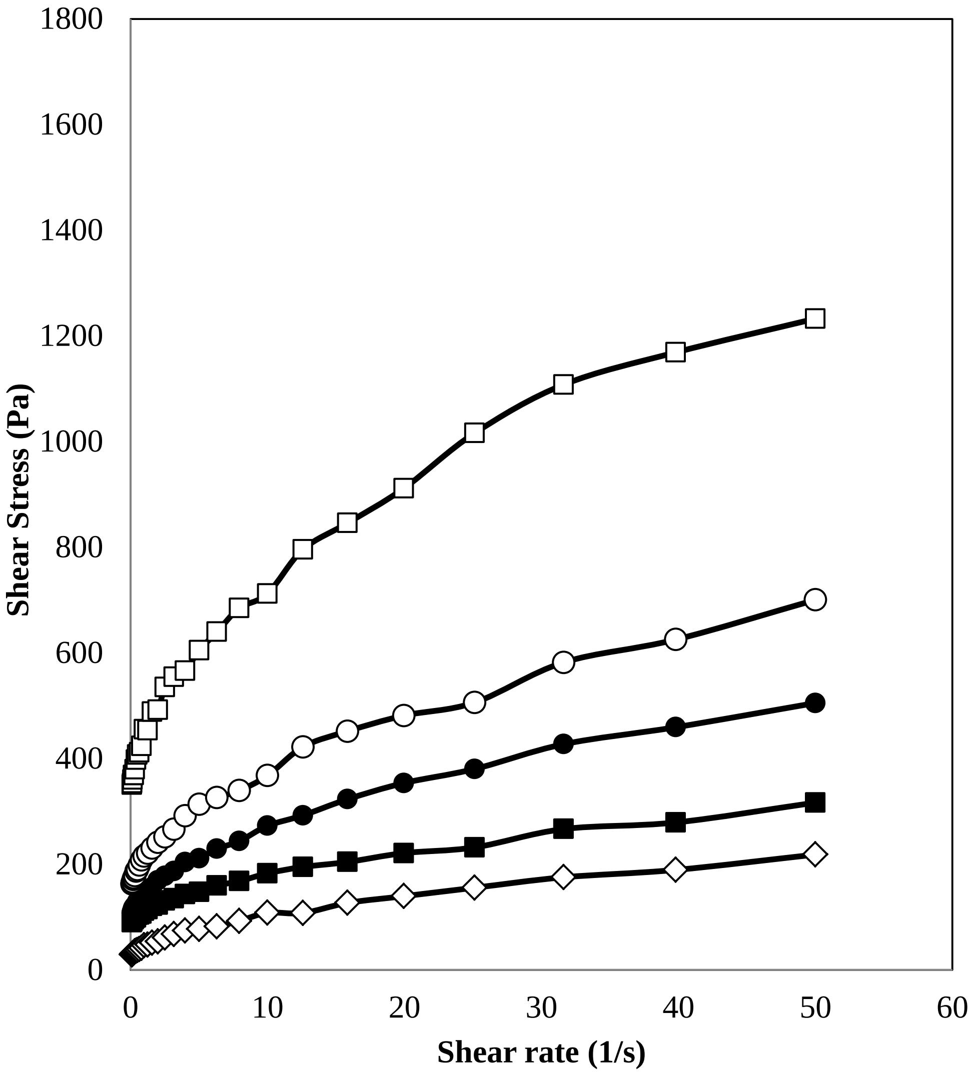

Figure 3.9 Flow curves obtained for dough samples containing 15% different types of hazelnut skin, cacao or none of them. (◇): Control, (■): Cacao 15%, (●): Raw 15%, (○): Ball milled 15%, (□): MF 15%.

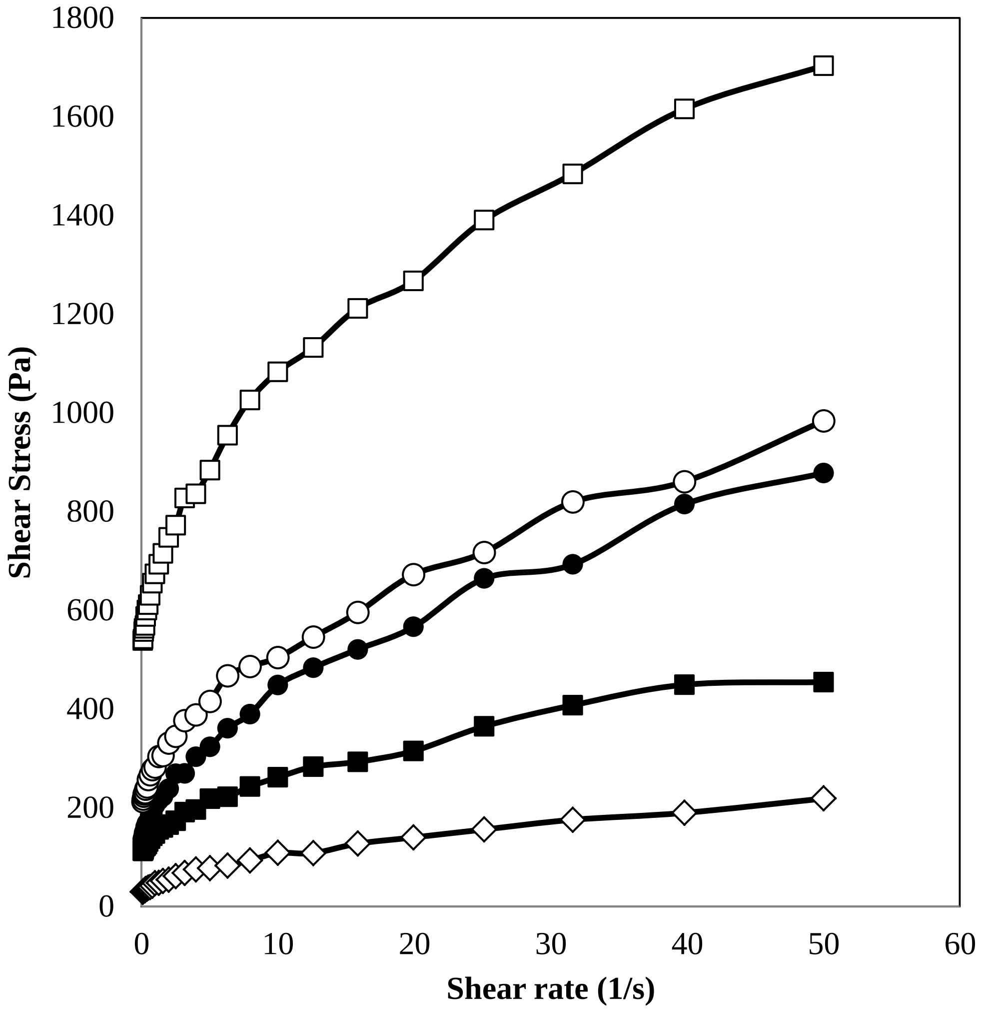

Figure 3.10 Flow curves obtained for dough samples containing 20% different types of hazelnut skin, cacao or none of them. . (◇): Control, (■): Cacao 20%, (●): Raw 20%, (○): Ball milled 20%, (□): MF 20%.

As can be seen in Figs. 3.8-3.10, the shear stress versus shear rate data indicated that higher viscosity values were obtained from cake batter samples prepared with the replacement of flour with MF hazelnut skin. In the study of Masoodi et al., (2002), dough viscosity increased with adding fiber (apple pomace) into cakes and decreasing fiber particle size. Finer samples increased water absorption and so viscosity. Similarly, in this study, finer HS samples were obtained by hammer and ball mill than raw HS samples, respectively. Thereby, they showed higher yield stress and consistency index values than values of cake dough containing raw HS samples. Moreover, MF HS fiber showed branched structure and larger surface area than other samples as shown in Figs. 3.1-3.4. This increased its water holding capacity. Water holding capacity is an important property for fibers. More water can be bound due to the presence of hydroxyl groups in fiber structure and this effect diminishes available water content in the product (Demirkesen et al., 2010). Thus, one reason for the highest yield stress and consistency index values in cake batters prepared with MF hazelnut skin fiber may be higher water holding capacity. MF 20% had the highest yield stress among all of the dough samples.

The lowest yield stress was observed for control batter samples. Yield stress is among the significant rheological parameters to predict product's processing and performance (Kocak, 2010). It is the stress level to initiate flow and it is related to internal structure level in material, which must be destroyed before flow occurs (Tabilo-Munizaga and Barbosa-Canovas, 2005; Kocak, 2010). Generally, at the same replacement of flour, the observed yield stress values were in the following decreasing order; batter sample prepared with the replacement of MF, batter sample prepared with replacement of ball milled, batter sample prepared with raw, batter sample prepared with cacao and batter sample prepared with no replacement. As expected, the increases in the replacement percentages increased the yield stress values of batter samples. Similar to the yield stress values, consistency index also showed an increasing order with the rise in the fiber percentage. The resistance to flow might be caused by entanglement of fibers and this led to increases in apparent viscosity and yield stress. Consistency value of batter samples is a significant parameter because there is a correlation between consistency and the capacity of retaining air (Gómez et al., 2010). When flaky structure was obtained, the greater shear stress was needed for flow of cake batter samples prepared with hazelnut skin. This created a resistance to shearing. It indicates that samples prepared with MF HS fiber produced more consolidated network in the product. More interactions might be achieved due to the greater reduction of particle size (Ciron et al., 2011). In the light of obtained values, it was concluded that higher yield stress and consistency index value of cake batter samples were obtained by reducing sizes of hazelnut skin samples and increasing replacement percentage of flour.

Elastic and viscous moduli values of cake batter samples were shown in Figs. 3.11-3.16. In all samples, both G' and G'' values increased with angular frequency. Elastic modulus (G') was found higher than the viscous modulus (G'') which was indicated a solid like behavior of batter samples in all cake dough. Both elastic (G') and viscous (G'') modulus values increased as amount of cocoa powder/ hazelnut skin samples in the batter increased. Thus, the highest values were obtained in the dough samples containing 20% MF HS fiber (w/w, based on wheat flour used), probably because of their high water holding capacity. The observed moduli values were in the following decreasing order; cakes prepared with MF HS fiber, ball milled HS, raw HS, cocoa powder and control. There was a really sharp increase with the addition of MF HS fiber. The control batter samples showed the lowest (G') and (G'') among all the samples by referring to having the most viscous structure. These results were consistent with the literature. Kocak, (2010) used wheat bran and bran fiber in cake formulation and it was observed that cake batter with the highest percentage of bran fiber (15%, based on wheat flour) had the highest elastic (G') and viscous modulus (G") among all samples. It can be concluded that entanglement of fibers in flour led to high modulus values (Demirkesen et al., 2010). Since microfluidizer creates higher shear rates for longer periods of time, it is more advantageous technique than conventional and so it leads to obtaining higher moduli values. While microfluidizer supplies consistent pressure and uniform particle distribution, conventional valve may cause fluctuation of pressure resulting with some unprocessed samples (Mert, 2012).

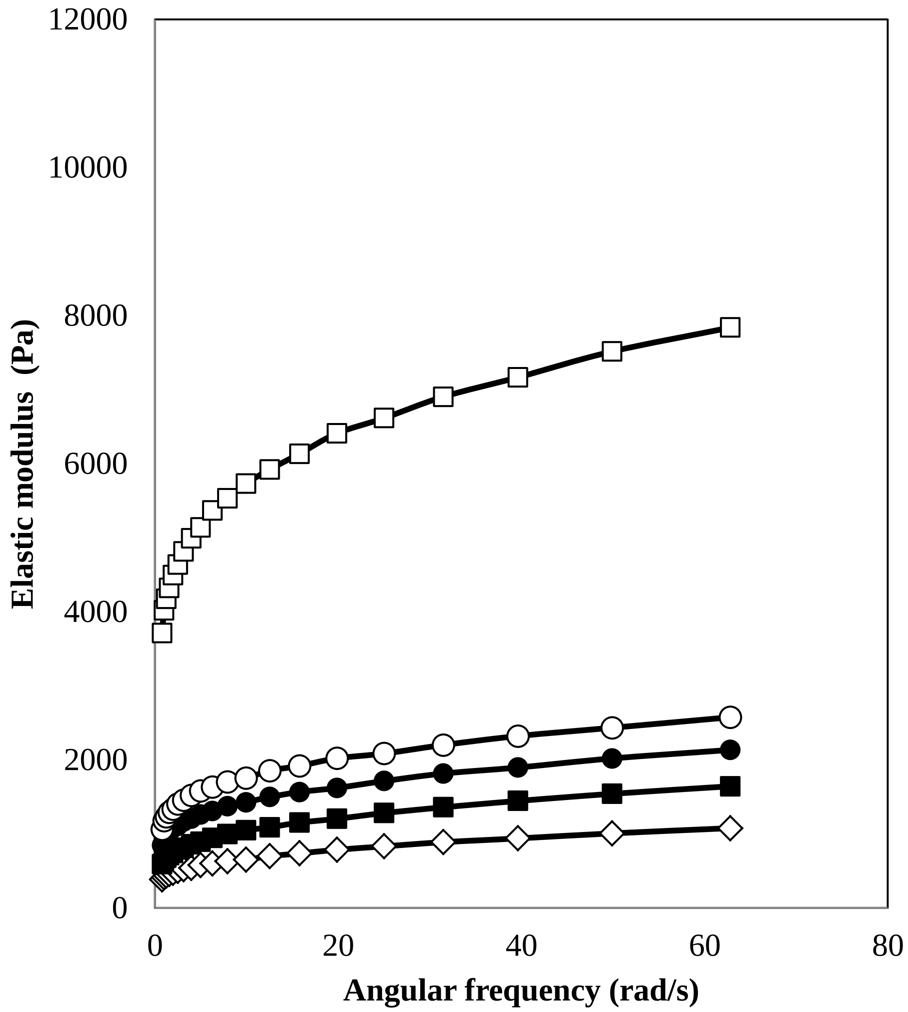

Figure 3.11 Elastic modulus obtained for dough samples containing 10% different types of hazelnut skin, cacao or none of them. (◇): Control, (■): Cacao 10%, (●): Raw 10%, (○): Ball milled 10%, (□): MF 10%.

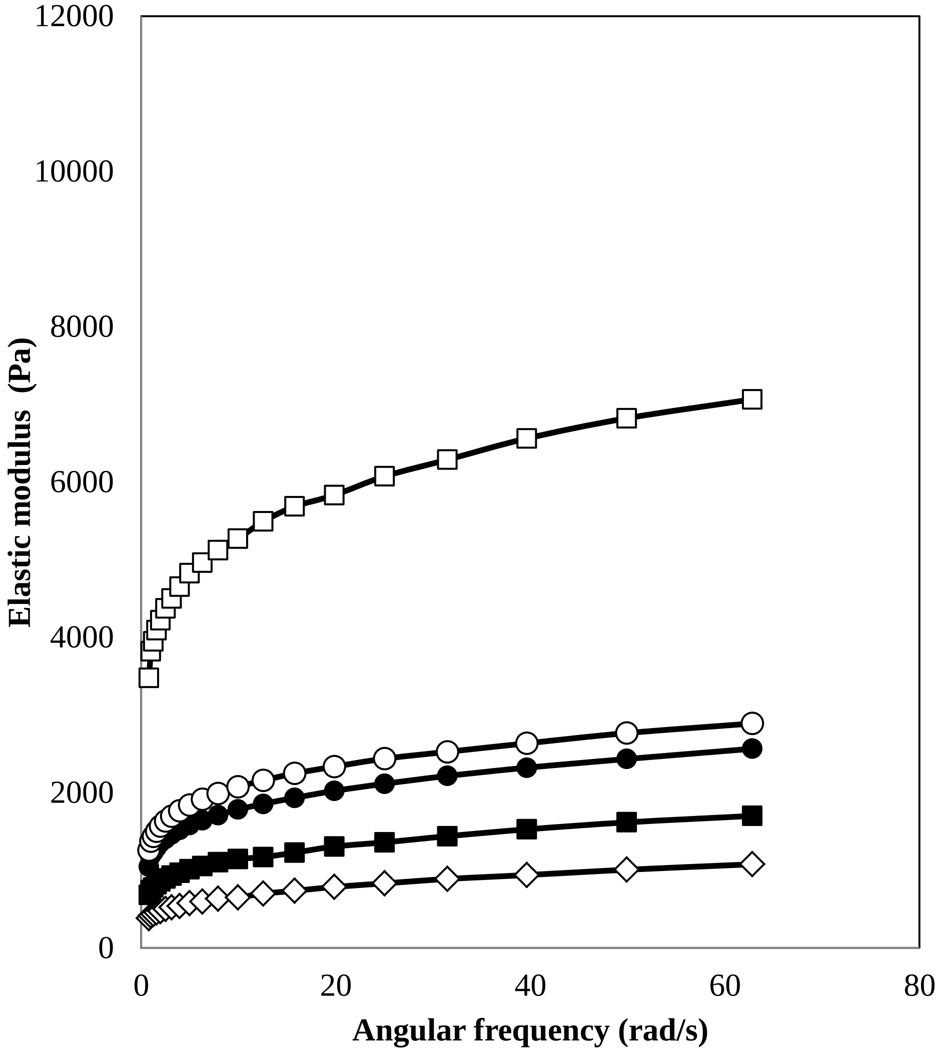

Figure 3.12 Elastic modulus obtained for dough samples containing 15% different types of hazelnut skin, cacao or none of them. (◇): Control, (■): Cacao 15%, (●): Raw 15%, (○): Ball milled 15%, (□): MF 15%.

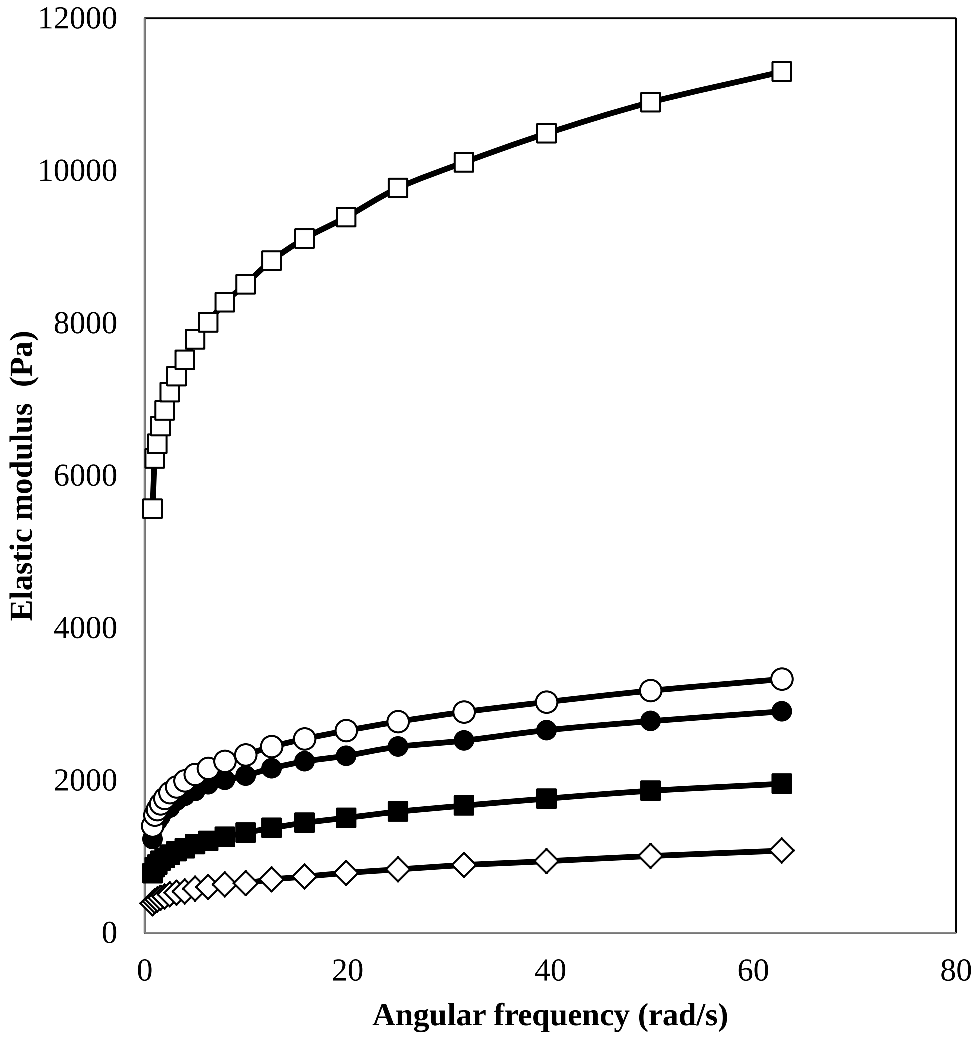

Figure 3.13 Elastic modulus obtained for dough samples containing 20% different types of hazelnut skin, cacao or none of them. (◇): Control, (■): Cacao 20%, (●): Raw 20%, (○): Ball milled 20%, (□): MF 20%.

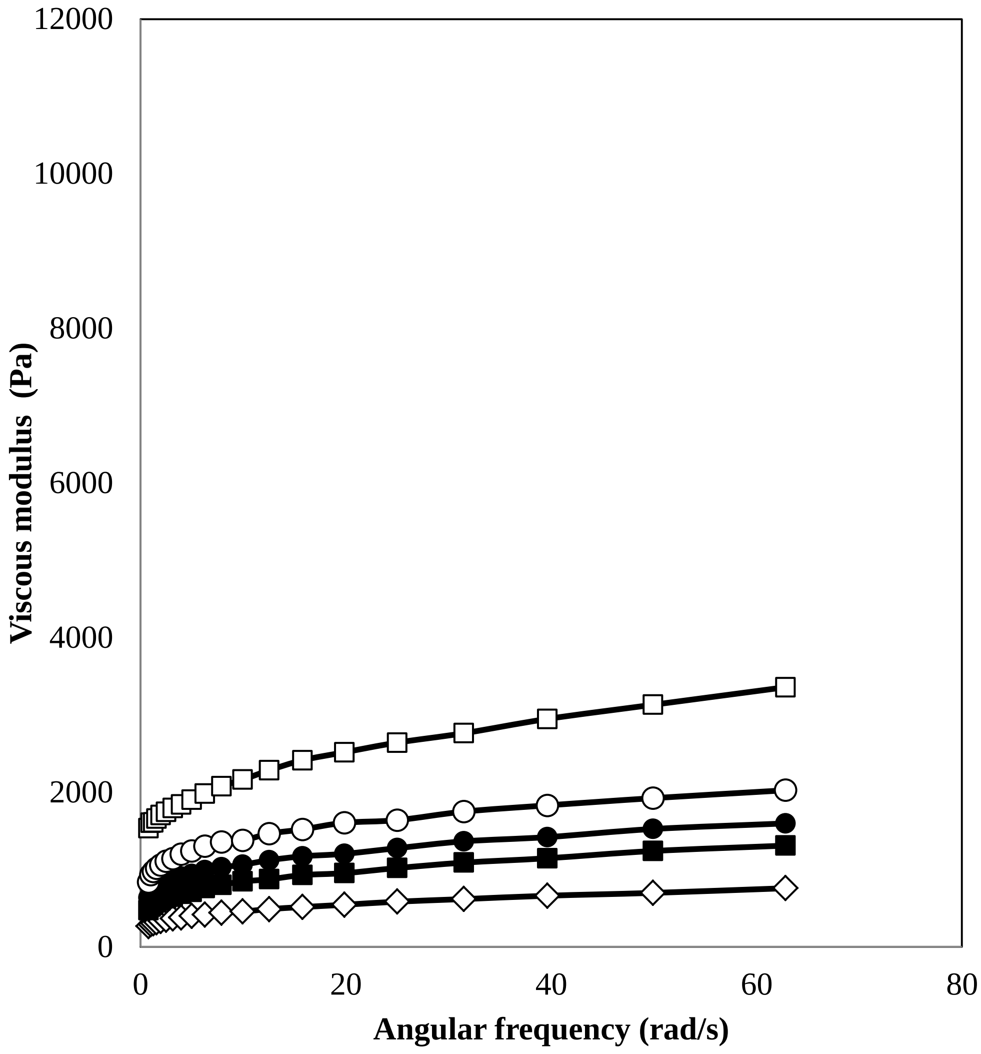

Figure 3.14 Viscous modulus obtained for dough samples containing 10% different types of hazelnut skin, cacao or none of them. (◇): Control, (■): Cacao 10%, (●): Raw 10%, (○): Ball milled 10%, (□): MF 10%.

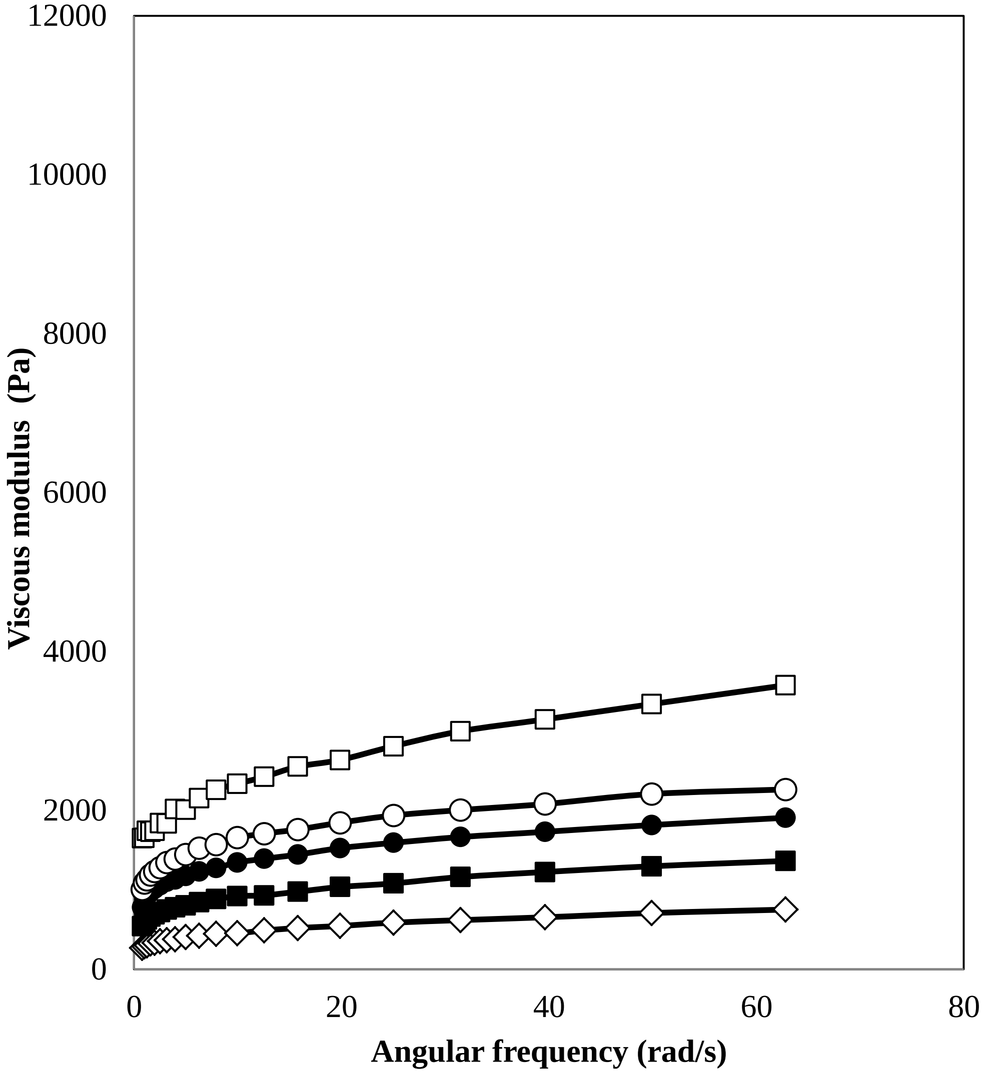

Figure 3.15 Viscous modulus obtained for dough samples containing 15% different types of hazelnut skin, cacao or none of them. (◇): Control, (■): Cacao 15%, (●): Raw 15%, (○): Ball milled 15%, (□): MF 15%.

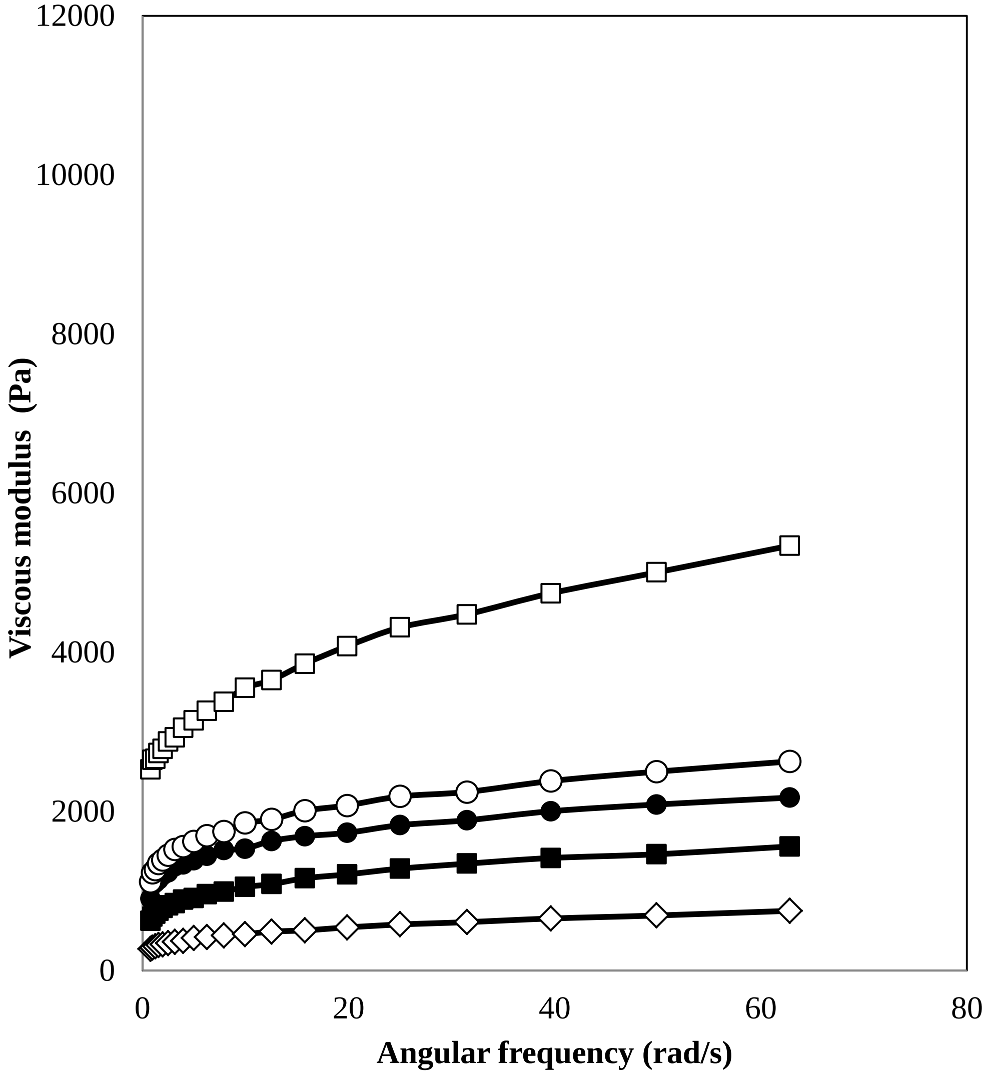

Figure 3.16 Viscous modulus obtained for dough samples containing 20% different types of hazelnut skin, cacao or none of them. (◇): Control, (■): Cacao 20%, (●): Raw 20%, (○): Ball milled 20%, (□): MF 20%.

3.2.2 Weight Loss

The effects of different cake samples prepared with HS samples/cocoa powder and the percentage of flour substitution on weight loss were presented in Table 3.2. According to the results, the addition of MF HS fiber showed a significant reduction in weight loss value when compared with other cake samples ($p \leq 0.05$). The lowest weight loss (%) was obtained in cakes when HS fiber produced by microfluidization at the highest replacement level of flour (20%) was added to cakes. Since hazelnut skin fibers produced by microfluidization process have high water holding capacity, the lowest weight loss value in these samples is an expected result. Moreover, weight loss showed a decrease as the replacement of flour with cocoa powder/ HS samples increased in cake samples. Since weight loss reduces product shelf life, it can be regarded as an important parameter (Duman, 2013). Thus, cake samples prepared with MF HS fiber are expected to have a tendency to retard staling. The results of X-ray and FTIR analyses supported this expectation.

Table 3.2 Weight loss results of cake samples.

Weight loss (%)				
Replacement of flour	Raw HS	Cacao	BM HS	MF HS
0%	9.31 ± 0.15^a	9.31 ± 0.15^a	9.31 ± 0.15^a	9.31 ± 0.15^a
10%	9.23 ± 0.44^a	9.21 ± 0.47^a	9.21 ± 0.13^a	8.25 ± 0.18^b
15%	9.13 ± 0.18^a	9.17 ± 0.18^a	9.08 ± 0.06^a	8.23 ± 0.03^b
20%	9.10 ± 0.27^a	8.98 ± 0.09^a	8.98 ± 0.09^a	8.02 ± 0.32^b

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

3.2.3 SEM Analysis of Cakes

Figure 3.17 and Figure 3.18 represent the scanning electron micrographs of cake samples at 250 and 1000 magnification, respectively. Since the softest texture were obtained in the cake samples with 10% replacement of flour, the percentage of 10% was chosen for SEM analysis of fresh cakes. The examination of structures at specified magnification by using scanning electron microscopy is helpful for qualitative analysis in foods.

In control cake, it was seen that some of the starch granules lost its spherical shape partially or completely due to gelatinization. There were both deformed starch granules and starch granules residues. The existence of starch granules residues is related to incomplete disintegration of starch granules. Starch granules were embedded in protein matrix. Starch has a significant role in the structure of bakery products as filler in matrices formed by gluten and other ingredients (Nandeesh et al., 2011). In cake samples containing cocoa powder/ hazelnut skin, these particles also adhered to starch granules and fibrils of gluten matrix. In cake samples with MF HS fiber, more granular residues were obtained. Fiber content can enhance incomplete disintegration of starch granules (Demirkesen et al., 2012). Absorption of water by the fiber causes a lack of sufficient amount of water and so most of the starch granules in cake dough do not gelatinize. This might be the reason for the increase in residues.

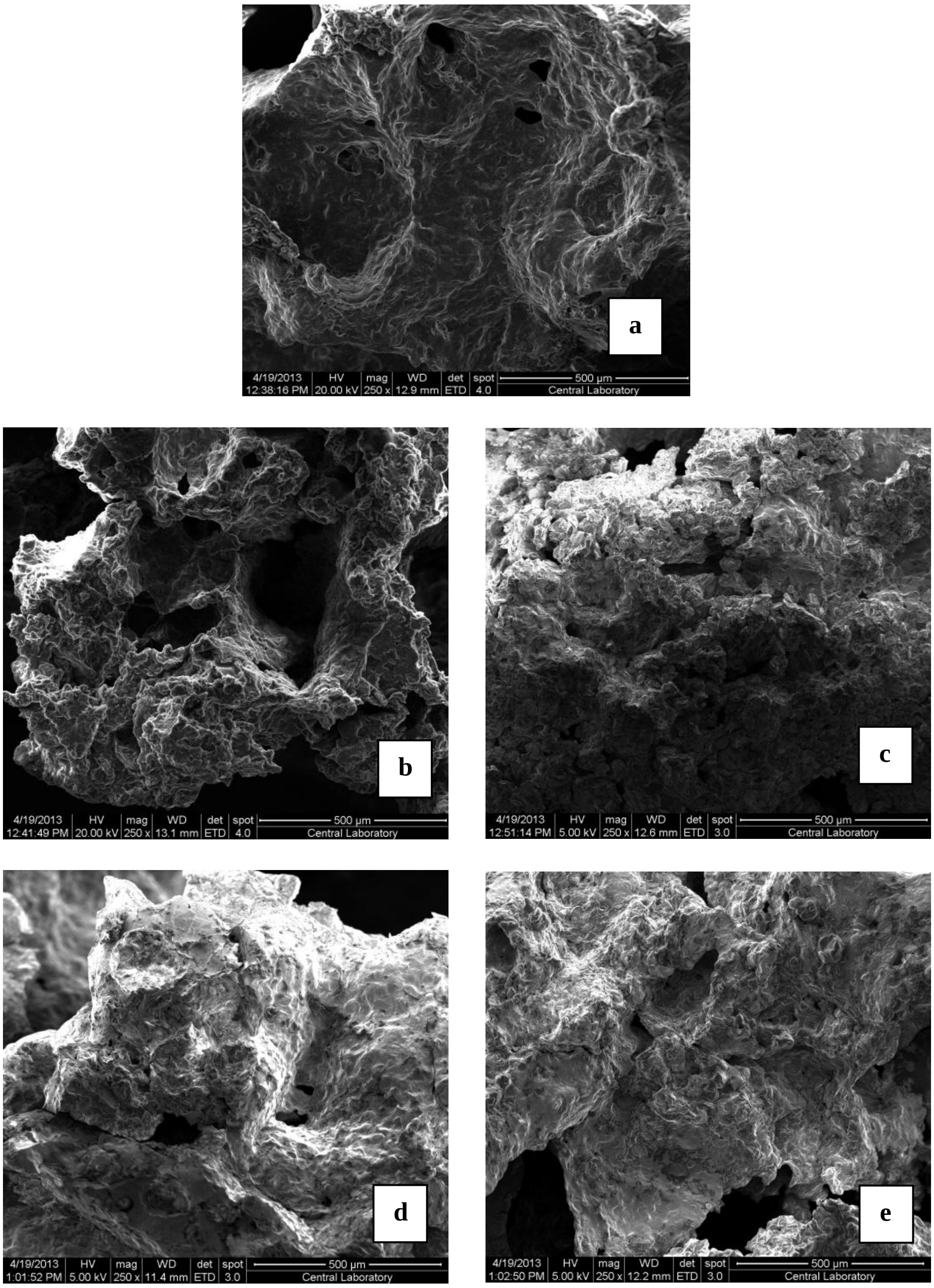

Figure 3.17 SEM images of cake crumbs with 10% replacement of flour. a. control cake. b. cake with cocoa powder. c. cake with MF HS. d. cake with BM HS. e. cake with raw HS. Magnification: 250x

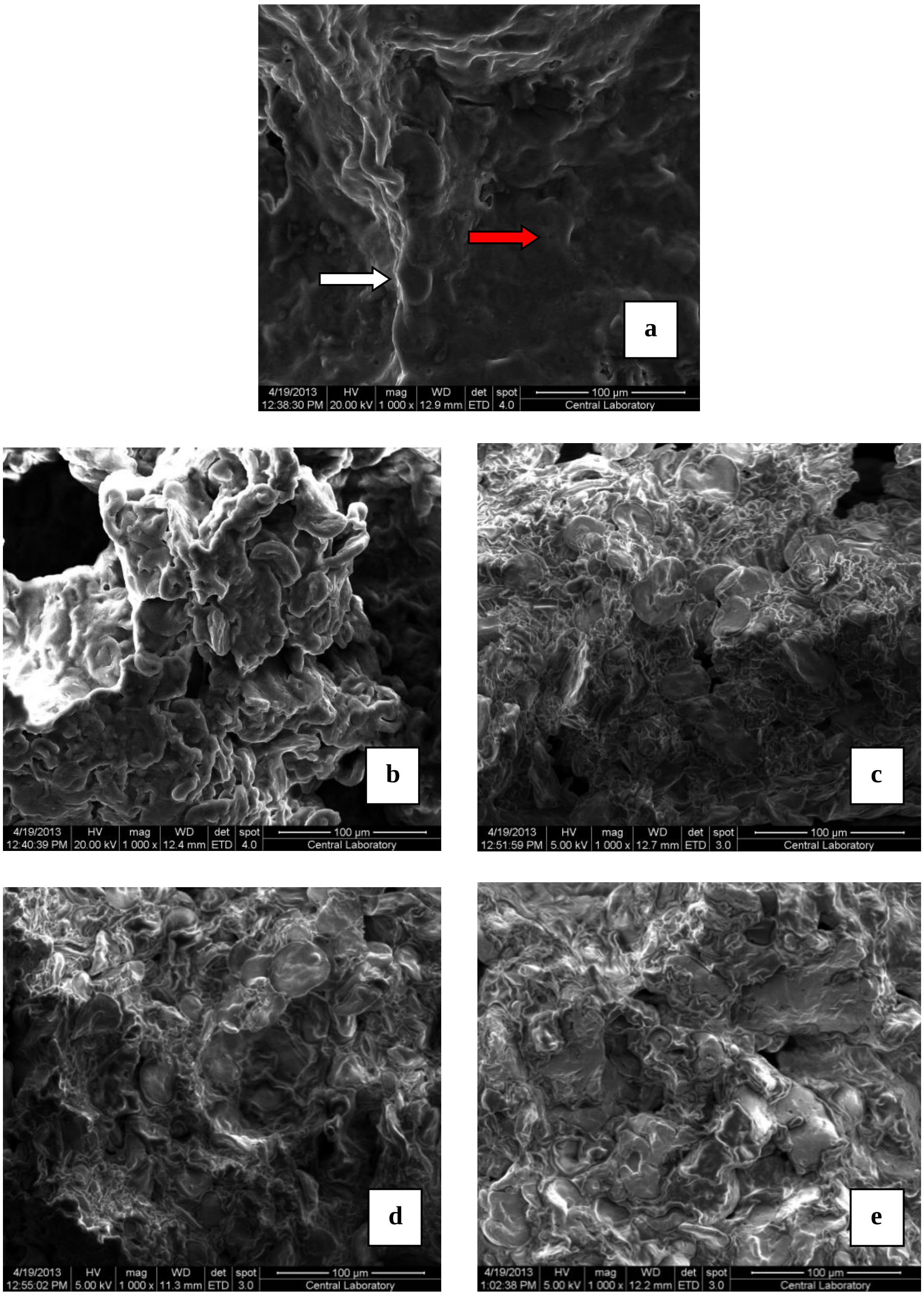

Figure 3.18 SEM images of cake crumbs with 10% replacement of flour. a. control cake. b. cake with cocoa powder. c. cake with MF HS. d. cake with BM HS. e. cake with raw HS. (*White arrow* represents starch granules residues and *red arrow* represents deformed starch granules). Magnification: 1000x

3.2.4 Crumb Color of Cakes

The effect of cocoa powder or hazelnut skin addition on cake crumb color was summarized in Figure 3.19. According to ANOVA results, both cake type and the amount of replacement of flour affected the color results, significantly ($p \leq 0.05$). The addition of raw hazelnut skin, ball milled hazelnut skin, cocoa powder and microfluidized hazelnut skin fiber as an ingredient gave darker crumb color of cake, respectively. The darkest color obtained in cakes containing hazelnut skin processed by microfluidization process (MF HS). ΔE values of the cakes prepared with HS fiber processed by microfluidization were significantly the highest one among the ΔE values of the other cake samples ($p \leq 0.05$). At 10%, 15% and 20% replacement of flour with MF HS, ΔE values were obtained as 65.60, 76.97 and 80.63, respectively. While cakes prepared with raw HS gave ΔE values as 38.66, 39.72, 45.62 with the increase of replacement percentage of flour, cakes containing cocoa powder had ΔE values as 54.35, 70.97 and 76.23 at same percentages. It is related to the original color of the used ingredients. Since the color of the hazelnut skin became darker after the microfluidization process, it gave its original dark color to the cakes. The reason of darker color obtained in HS samples and cakes prepared with them may be due to the differences in the breakage of storage cells of polyphenols during the use of hammer mill, ball mill and microfluidizer and oxidation of free polyphenols. Similarly, Duman (2013) observed that cacao fiber obtained by microfluidization process showed darker color than cacao powder. During baking, high temperature did not influence its intense color property. Since the temperature of cake crumb does not exceed 100 ˚C, the Maillard or caramelization reactions by sugar fail to occur in the crumb of cake. As a result, the color of raw materials and their interactions give the crumb color (Gómez et al., 2008). Moreover, fermentation causes dark color in cocoa beans in which that polyphenols diffuse with cell liquids from their storage cells and exposed to oxidation to condensed high molecular mostly insoluble tannins at the stage of fermentation process of cocoa beans (Wollgast et al., 2000). Polyphenol compounds found in both cocoa powder and hazelnut skin may undergo oxidation reaction and participate to the color value (Lu et al., 2010). As the replacement level of flour with cocoa powder/ hazelnut skin increased, ΔE values of cakes also increased significantly due to the color of hazelnut skin/ cocoa powder ($p \leq 0.05$).

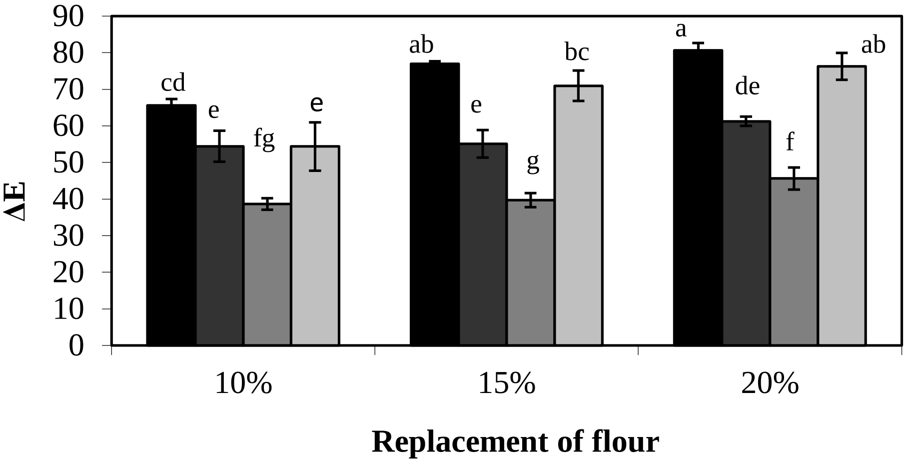

Figure 3.19 ΔE values of cake samples where (black bar): cakes prepared with MF HS fiber, (dark gray bar): cakes prepared with ball milled HS, (gray bar): cakes prepared with raw HS, (light gray bar): cakes prepared with cocoa powder. Bars indicate standard deviation of the replicates.

3.2.5 Texture Profile of Fresh Cakes Based on 100 g Flour Formulation

The impact of percentage of flour replacement and cake type on firmness values of fresh cakes was shown in Figure 3.20. ANOVA results showed that firmness values were significantly affected by cake type and percentage of flour replacement ($p \leq 0.05$). Control cake (the one containing none of HS/cocoa powder) had the lowest firmness result as 85.19 g force. Gómez et al. (2003) determined that fresh bread samples containing dietary fiber such as cocoa, coffee, pea had firm structure but other fresh bread samples including wheat fibers with average fiber length of 250 μm had softer structure than control breads. These results indicate that functional properties of fibers may vary with the source of fiber, its type and also degree of processing (Gómez et al., 2003; Rosell et al., 2009; Rosell et al., 2010). Moreover, in this study, as the fiber content increased in the cake samples, firmer structure was obtained significantly ($p \leq 0.05$). It means that increasing fiber content had a negative effect on firmness of cakes. This is a consistent result with previous several fiber studies. Sudha et al. (2007) also found that the hardness of the cake samples prepared with apple pomace increased when the percentage of fiber in the formulation increased. Rupasinghe et al. (2009) obtained similar results for fruit fibers. The reason may be due to the impeding effect of fiber in intermolecular interaction (Collar et al., 2007). Fiber may restrict available water for gluten development and disrupt starch-gluten matrix. This impedes expansion ability of dough (Ktenioudaki et al., 2012). Thus, a compact structure can be obtained in the final product (Demirkesen et al., 2010). Moreover, thickening of crumb walls surrounding the air bubbles causes an increase in the firmness (Gómez et al., 2003). Among all the samples, cakes prepared with 20% of MF HS fiber showed the hardest texture. Similarly, it may be due to higher water holding capacity of MF HS than other samples. According to ANOVA results, addition of MF HS into cakes as the percentage of 10%, 15% and 20% (based on flour substitution) caused significant difference in firmness values as 246.04 g force, 271.89 g force and 467.35 g force, respectively (p≤ 0.05).

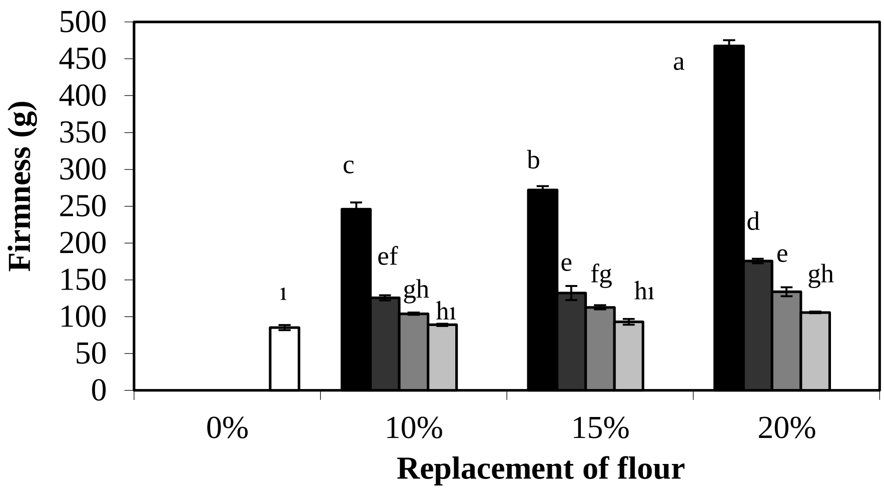

Figure 3.20 Firmness values of fresh cake samples where (white bar): control cake, (black bar): cakes prepared with MF HS fiber, (dark gray bar): cakes prepared with ball milled HS, (gray bar): cakes prepared with raw HS, (light gray bar): cakes prepared with cocoa powder. Bars indicate standard deviation of the replicates.

Hazelnut skin particles were obtained finer by using hammer and ball mill, respectively. At the same percentage of replacement of flour with hazelnut skin (HS), the observed firmness values were in the following increasing order; cake samples prepared with the replacement of raw HS, ball mill HS and MF HS fiber (Figure 3.20). If samples containing raw and ball milled HS are considered, this indicates that smaller particle size increased firmness of the final product. It is consistent with some researches. For example, in some studies, it was seen that reducing particle size reinforced negative effects of fibers in some studies (Zhang and Moore, 1997; Noort et al., 2010). It is suggested that as the particle size becomes finer, relative surface area and total amount of water held by fiber increase in the absence of matrix structure (microcrystalline cellulose) (Sangnark and Noomhorm, 2003). The increase in surface area enhances gluten interaction with active components like ferulic acid, thus it changes function of gluten (Ktenioudaki et al., 2012). Furthermore, water retention capacity might be increased by reducing particle size because of damage of fiber matrix and pores in grinding process (Singh et al., 2013).

The impact of the percentage and cake type on springiness values of fresh cakes was shown in Figure 3.21. ANOVA results showed that springiness values were significantly affected by cake type and percentage of flour replacement ($p \leq 0.05$). Springiness values showed a decrease with the increase of cocoa powder/ HS samples in fresh cakes. High springiness results were observed in the cake samples prepared with MF HS fiber. This indicated that the 10% replacement of flour with MF HS fiber made the final product as the most elastic one with the springiness result as 57.24%. Similar to 10% MF HS, addition of MF HS into cakes at the percentage of 15% (based on flour substitution) had the springiness value as 56.60%. Considering ANOVA results, fresh cakes containing 10% and 15% replacement of flour with MF HS fiber were found to be different than other samples, significantly ($p \leq 0.05$). They showed high springiness value indicating the increase in the strength of the bonds in the three dimensional crumb network (Zahn et al., 2010). The lowest springiness value as 44.90 was obtained in fresh cakes containing cocoa powder with 20% replacement of flour. As a result, cakes containing hazelnut skin fibers processed by microfluidizer have more elastic structure.

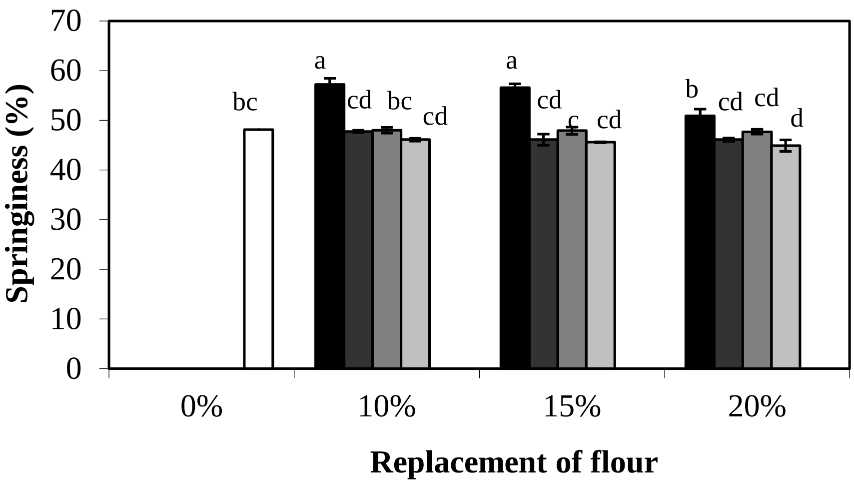

Figure 3.21 Springiness values of fresh cake samples where (white bar): control cake, (black bar): cakes prepared with MF HS fiber, (dark gray bar): cakes prepared with ball milled HS, (gray bar): cakes prepared with raw HS, (light gray bar): cakes prepared with cocoa powder. Bars indicate standard deviation of the replicates.

3.3 Staling Analysis of Cakes Based on 100g Flour Formulation

3.3.1 Texture Profile of Cakes Based on 100g Flour Formulation and Stored for 3 and 6 days

During 6 days of storage, firmness of samples increased with time. The results for "firmness" parameter of the cakes at 3 days and 6 days of storage can be seen in Figure 3.22 and Figure 3.23. According to ANOVA results, it was found that firmness values were dependent on replacement of flour, cake type and storage time ($p \leq 0.05$). Firmness values of all samples increased with increasing storage time. Firmness value of control sample was measured as 168.38 g force at the end of 3^{rd} day and 268.23 g force at the end of 6^{th} day. The firmness results of cakes with cocoa powder at the 10% replacement of flour were increased from 193.76 g force to 273.13 g force during 3 days and 6 days of storage. At the same percentage of replacement, cakes with MF HS fiber gave the highest firmness value as 447.45 g force at the end of 3^{rd} day and 584.65 g force at the end of 6^{th} day of storage.

One reason for the increase in firmness during storage may be due to the decrease in moisture content of samples. Moisture contents of cake samples were given in Table 3.3 and they were comprehensible in terms of storage time. It was seen that moisture content of cakes decreased as storage time increased. According to ANOVA results, in terms of moisture content, it can be noted that cakes made with MF HS fiber showed similar results with other cakes as staling occurs. This means that there are other factors affecting firmness and staling mechanism.

Table 3.3 Moisture content of cake samples at 0, 3 and 6 days of storage. Standard deviations were also indicated.

Cake sample	Storage time (day)		
	0	3	6
Control	31.28 ± 0.33$^{\text{a}}$	25.22 ± 1.55$^{\text{d}}$	22.33 ± 0.46$^{\text{efgh}}$
Cocoa 10%	31.17 ± 0.08$^{\text{a}}$	22.93 ± 0.16$^{\text{ef}}$	21.43 ± 0.14$^{\text{fghıj}}$
Cocoa 15%	31.07 ± 0.38$^{\text{a}}$	22.81 ± 0.13$^{\text{efg}}$	21.40 ± 0.35$^{\text{ghıj}}$
Cocoa 20%	30.97 ± 0.01$^{\text{a}}$	22.78 ± 0.08$^{\text{efg}}$	21.32 ± 0.06$^{\text{ghıj}}$
Raw 10%	31.12 ± 0.05$^{\text{a}}$	22.95 ± 0.10$^{\text{e}}$	21.78 ± 0.05$^{\text{efghıj}}$
Raw 15%	31.03 ± 0.04$^{\text{a}}$	22.80 ± 0.01$^{\text{efg}}$	21.66 ± 0.04$^{\text{efghıj}}$
Raw 20%	30.96 ± 0.55$^{\text{a}}$	22.72 ± 0.47$^{\text{efg}}$	21.62 ± 0.22$^{\text{efghıj}}$
BM 10%	31.06 ± 0.08$^{\text{a}}$	22.70 ± 0.10$^{\text{efg}}$	21.75 ± 0.07$^{\text{efghıj}}$
BM 15%	30.55 ± 0.17$^{\text{ab}}$	22.18 ± 0.41$^{\text{efghıj}}$	21.68 ± 0.12$^{\text{efghıj}}$
BM 20%	30.42 ± 0.03$^{\text{ab}}$	22.12 ± 0.07$^{\text{efghıj}}$	20.76 ± 0.42$^{\text{ıj}}$
MF 10%	30.20 ± 0.19$^{\text{abc}}$	22.26 ± 0.41$^{\text{efghı}}$	21.02 ± 0.17$^{\text{hıj}}$
MF 15%	29.11 ± 0.16$^{\text{bc}}$	21.82 ± 0.24$^{\text{efghıj}}$	21.06 ± 0.02$^{\text{hıj}}$
MF 20%	28.80 ± 0.10$^{\text{c}}$	21.39 ± 1.00$^{\text{ghıj}}$	20.74 ± 0.08$^{\text{j}}$

So many factors may affect firmness. Water, starch and gluten attribute to staling mechanism. Retrogradation of amylopectin and gluten are among the main factors playing an important role in textural property of cakes. It is related to the hydrogen bonding between gelatinized starch granules and gluten network (Ozkoc et al., 2009b). Starch influences staling mechanism due to the roles of amylose and amylopectin. Very fast retrogradation of amylose stabilizes the crumb and the formation of ordered amylose structure in the center of gelatinized granules helps initial firming of cakes (Demirkesen et al., 2013). Moreover, water acts as plasticizer for amorphous regions. When staling of cakes is considered, water cannot behave as plasticizer, it migrates from crumb to crust. Interactions between starch and protein or starch and starch interactions rather than water interaction make the texture harder. As a result, main reasons for firming of cakes during storage may be listed as water migration, starch retrogradation, formation of crystalline regions and double helical structures in cake samples (Arendt et al., 2008; Demirkesen et al., 2013).

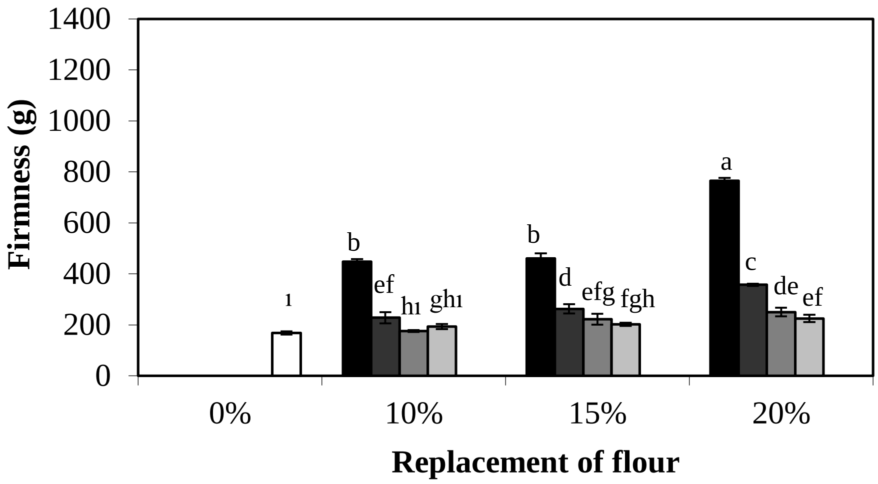

Figure 3.22 Firmness values of cake samples stored for 3 days where (white bar): control cake, (black bar): cakes prepared with MF HS fiber, (dark gray bar): cakes prepared with ball milled HS, (gray bar): cakes prepared with raw HS, (light gray bar): cakes prepared with cocoa powder. Bars indicate standard deviation of the replicates.

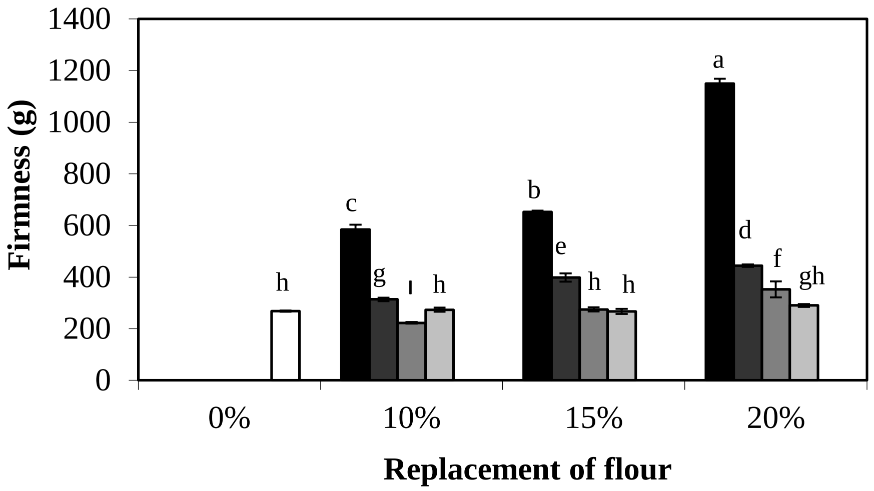

Figure 3.23 Firmness values of cake samples stored for 6 days where (white bar): control cake, (black bar): cakes prepared with MF HS fiber, (dark gray bar): cakes prepared with ball milled HS, (gray bar): cakes prepared with raw HS, (light gray bar): cakes prepared with cocoa powder. Bars indicate standard deviation of the replicates.

During 6 days of storage, springiness of crumb of the cakes changed significantly ($p \leq 0.05$). The results of springiness values of samples at 3 days and 6 days of storage were shown in Figure 3.24 and 3.25. According to ANOVA results, it was found that springiness values were dependent on replacement of flour, cake type and storage time. Control cakes had springiness results as 40.40% and 37.38% in the end of 3rd and 6th days of storage, respectively (48.11 in fresh cake). They showed closer results to cakes prepared with substitution of flour at 10% level BM HS, Raw HS and cocoa powder. Cakes containing MF HS had higher springiness results than other samples. Springiness value of samples prepared with 10% MF HS fiber decreased from 57.24% in fresh sample to 52.95% in the end of 6th day. The results indicated that cakes prepared with MF HS showed high elastic property even during storage time.

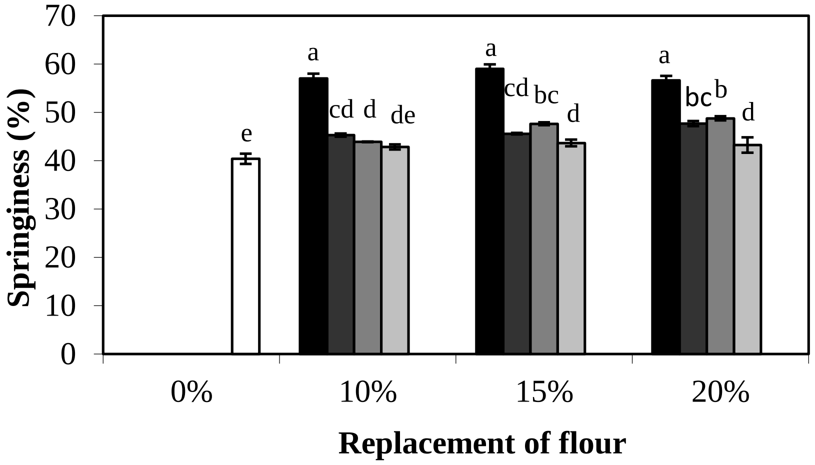

Figure 3.24 Springiness values of cake samples stored for 3 days where (white bar): control cake, (black bar): cakes prepared with MF HS fiber, (dark gray bar): cakes prepared with ball milled HS, (gray bar): cakes prepared with raw HS, (light gray bar): cakes prepared with cocoa powder. Bars indicate standard deviation of the replicates.

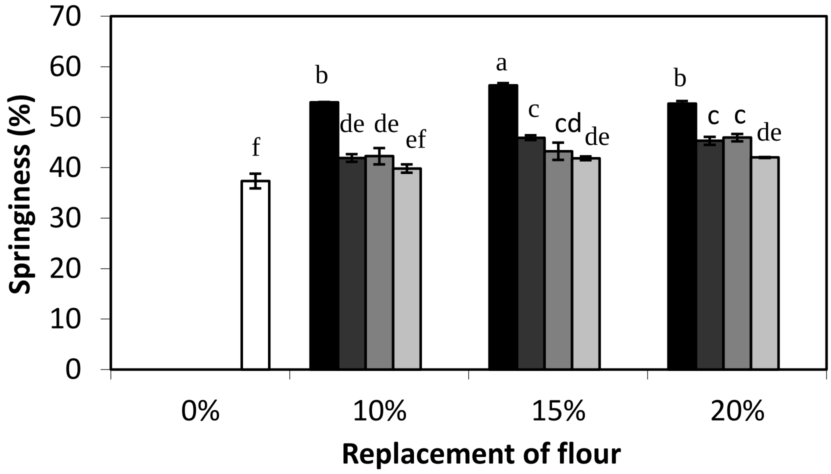

Figure 3.25 Springiness values of cake samples stored for 6 days where (white bar): control cake, (black bar): cakes prepared with MF HS fiber, (dark gray bar): cakes prepared with ball milled HS, (gray bar): cakes prepared with raw HS, (light gray bar): cakes prepared with cocoa powder. Bars indicate standard deviation of the replicates.

3.3.2 X-ray Diffraction

X-ray diffraction analysis has been used to examine cake staling mechanism especially the crystalline structure of starch granules. The diffraction diagrams of fresh (cake crumb 1h after baking) and aged (cake crumb stored for 3, 6 and 9 days) samples were given in Figs. 3.26-3.30. Cake samples with 10% replacement of flour with cocoa powder/ HS samples were chosen for X-ray diffraction analysis since the samples with this percentage showed the softest results than other percentages.

The profiles of the fresh cake samples showed a peak at around 2θ of 20º supporting a typical V-type structure (Figs 3.26a-3.30a). It has been stated in the study of Ribotta et al. (2004) that fresh baked bread samples apparently showed only the V-type structure. It is formed by the helical clathrates between amylose and fatty acids in cake samples (Ribotta et al., 2004; Osella et al., 2005). Starch becomes mostly in the form of amorphous in freshly baked sample but it slowly recrystallizes during aging (Karim et al., 2000). Changes in crystallinity during storage can be seen in the X-ray diffraction patterns.

In this study, it is noticeable that as storage time increased, the peak intensities (i.e. starch crystallinity) also showed an increase. During storage, peak at around 20° remained unchanged. Additionally, peaks at around 17° and 24° were obtained in X-ray diffractograms by indicating B-type structure which caused firming mechanism due to amylopectin retrogradation. It was a consistent result with some other studies. Ribotta et al. (2004) also obtained B-type structure in aged bread samples with peaks at diffraction angles of 15°, 17°, 22.2° and 24°.

Each type of crystals affects water distribution within crumb differently. While A-type crystal includes 8 water molecules, B-type crystal includes 36 water molecules. Therefore, in cakes B-type crystalline regions are formed by recrystallization of amylopectin. Since more water migrates into crystalline region, firmer structure in crumb is formed. This water does not act as a plasticizer anymore for starch-gluten (Ozkoc et al., 2009b). However, this does not determine the initial firmness. In the mechanism of initial firmness; shortly after the cake was baked and cooled, the exuded amylose occurs as retrogradated double helices and it forms juncture points providing gelation within the intergranular space and this contribute the initial loaf firmness (Ribotta et al., 2004). Shortly, amylose retrogradation becomes faster than amylopectin and it ends up until the baked sample cool to room temperature (Sozer et al., 2011). When different cake samples were compared, it can be noted that similar peak intensities were observed in cakes. However, in the figures of cakes prepared with MF HS fiber, the lowest peak intensity was observed by pointing out retardation of staling.

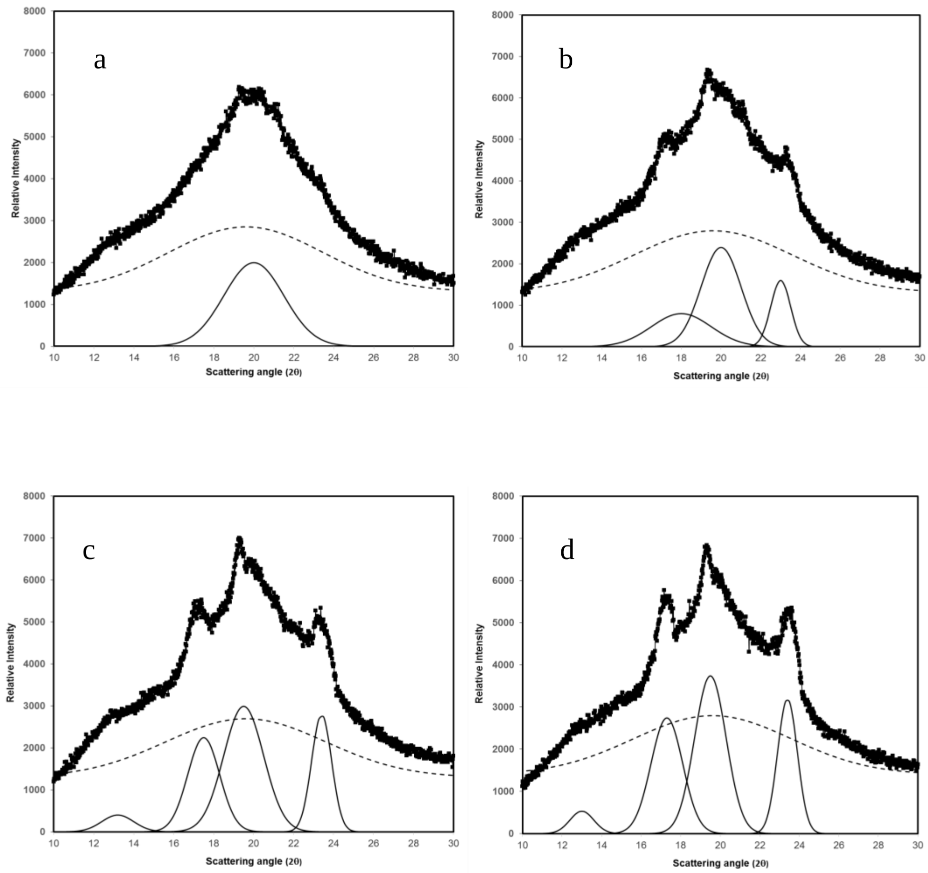

Figure 3.26 X-ray diffraction diagrams of control cake samples stored at different storage times (a. 0 day b. 3 days c. 6 days. d. 9 days)

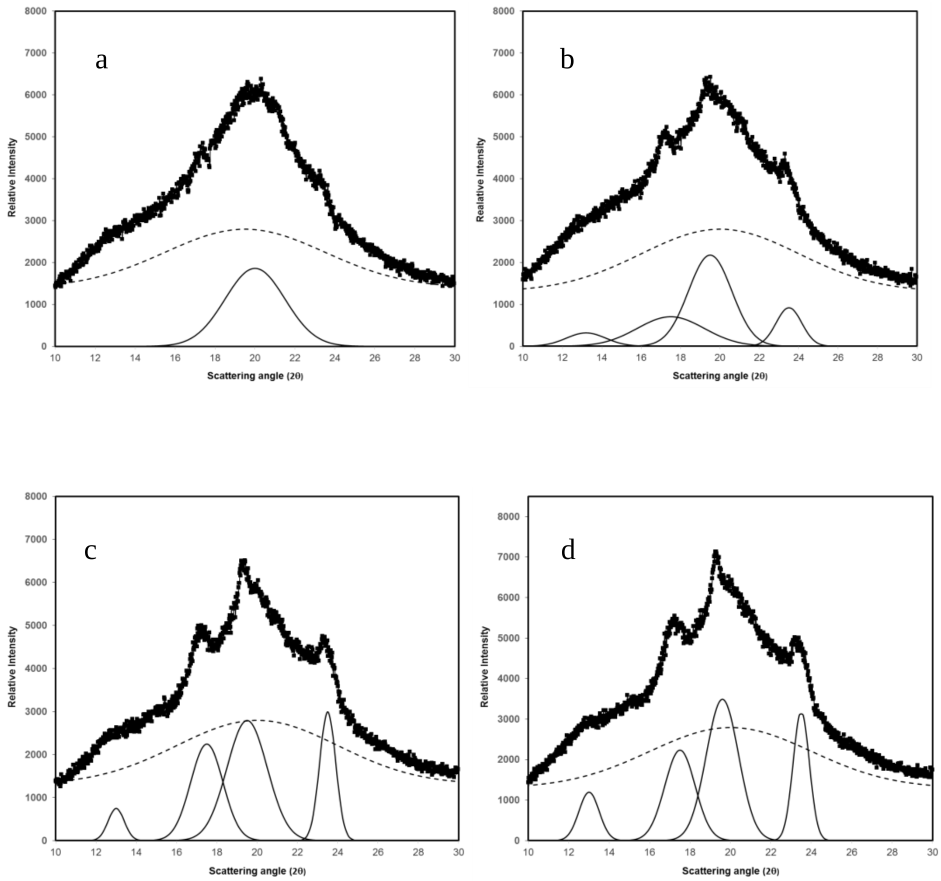

Figure 3.27 X-ray diffraction diagrams of cake samples prepared with 10% replacement of flour with cocoa powder and stored at different storage times (a. 0 day b. 3 days c. 6 days. d. 9 days)

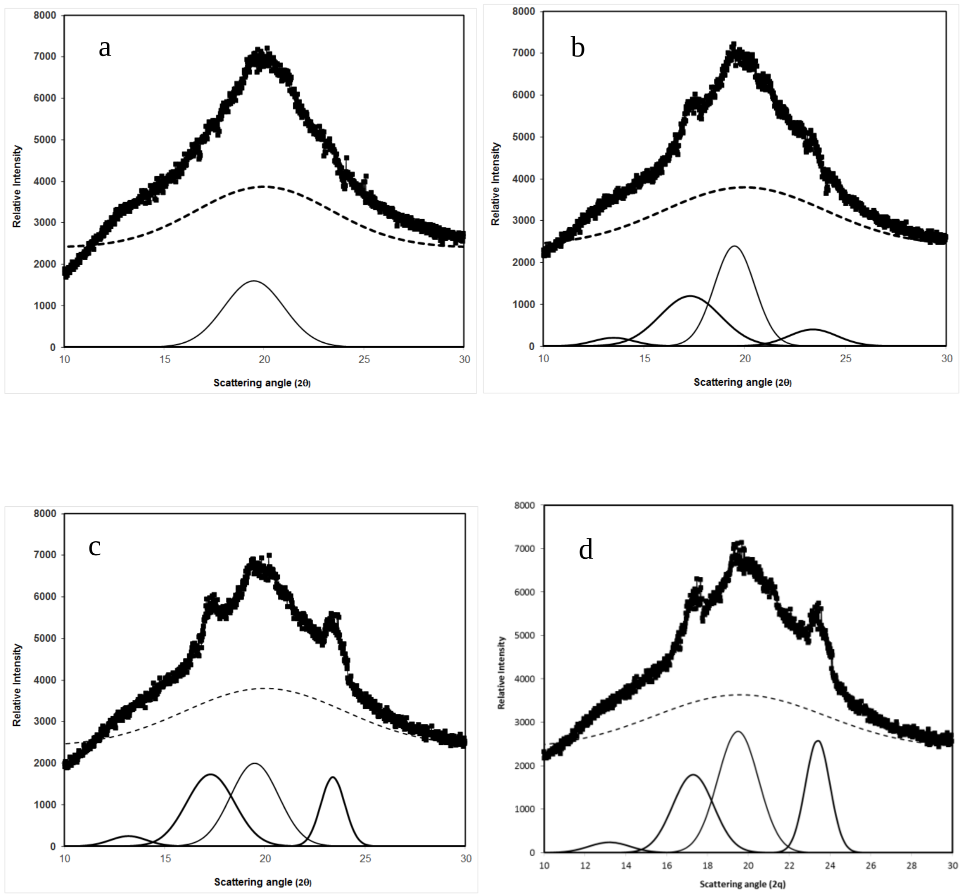

Figure 3.28 X-ray diffraction diagrams of cake samples prepared with 10% replacement of flour with raw HS and stored at different storage times (a. 0 day b. 3 days c. 6 days. d. 9 days)

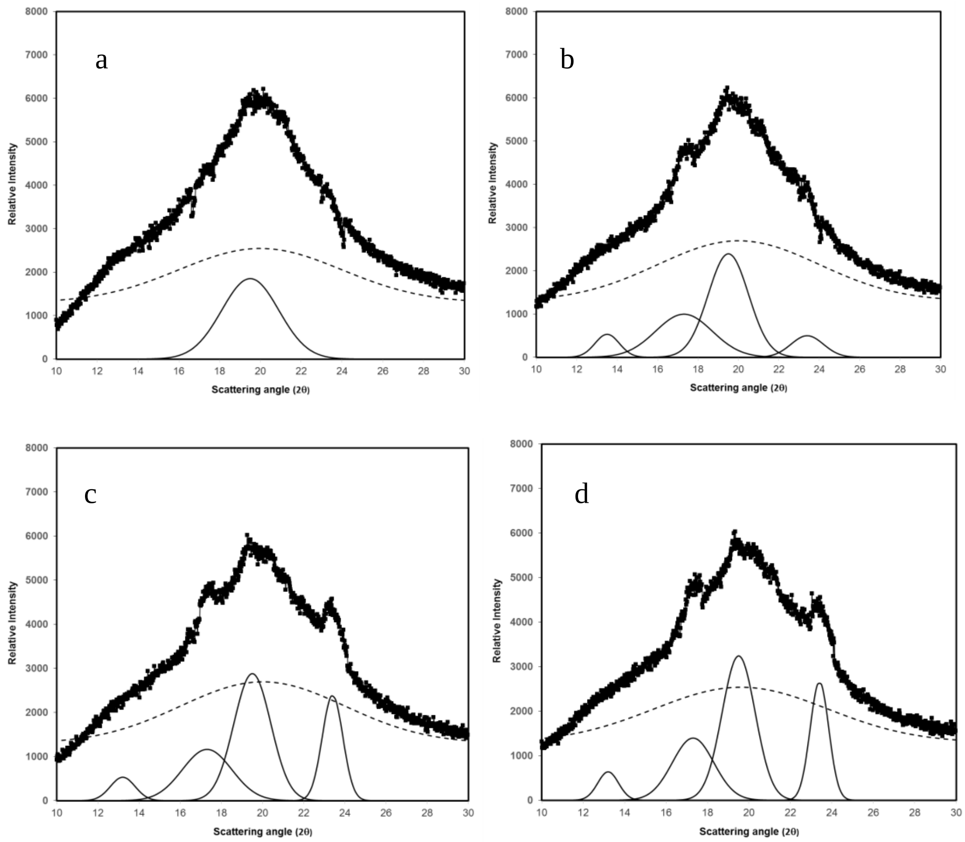

Figure 3.29 X-ray diffraction diagrams of cake samples prepared with 10% replacement of flour with ball milled HS and stored at different storage times (a. 0 day b. 3 days c. 6 days. d. 9 days)

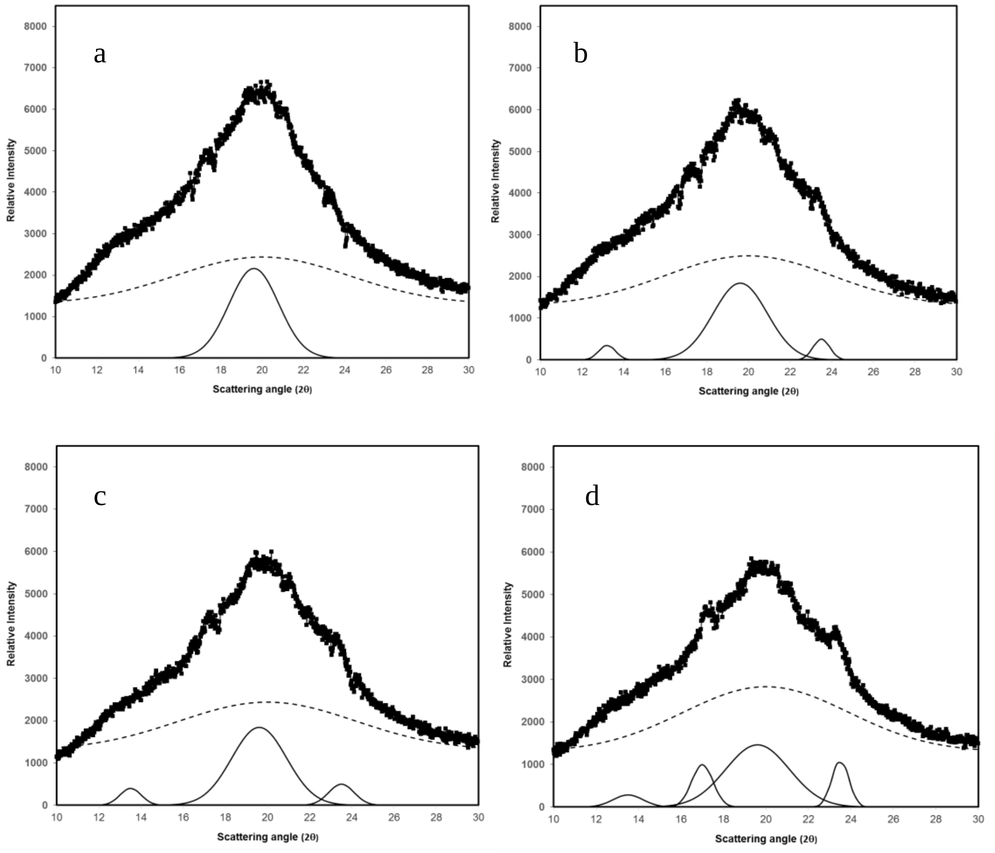

Figure 3.30 X-ray diffraction diagrams of cake samples prepared with 10% replacement of flour with microfluidized HS and stored at different storage times (a. 0 day b. 3 days c. 6 days. d. 9 days)

The total mass crystallinity grades of cake samples can be seen in Figure 3.31. As the storage time increased, the values of crystallinity also increased, so higher crystallinity values were obtained in cake samples stored for 9 days. Since retrogradation commences during aging, more organized starch occurs and crystallinity increases. The highest total crystallinity grades were obtained in control cakes (Figure 3.31). Since control cakes have higher starch content than other cake samples due to the replacement of flour, higher crystallinity results in control cakes are comprehensible. When cakes made with HS types and control cake were compared, it can be said that HS samples showed positive results in terms of crystallization and they can be used in cake preparation. When cocoa powder addition into cakes was considered, it was seen that it gave more positive results for staling than control cakes but it could not show better results like MF HS addition into cakes. As illustrated in Figure 3.31, the lowest crsytallinity results were found in cakes prepared with MF HS fiber. This may be achieved by the ability of MF HS fiber to bind much more water and thus preventing higher water loss during storage. This decreases water content related to starch which is necessary for amylopectin recrystallization. Possible hydrogen binding between starch and fiber prevents interaction of starch and starch and this decreases available organized starch for crystallization (Demirkesen et al., 2013). A complex formation between amylose and HS fiber may prevent crystallization of the amylopectin and retard water distribution, thus retrogradation. The observed decreases in total crystallinity values of cake samples with the addition of MF HS fiber may be due to the flaky structure of fiber affecting the interaction of starch fractions. Consequently, according to the results it can be concluded that cakes prepared with MF HS fiber gained lower staling tendency due to the effect of flaky structure and its water binding property on staling mechanism.

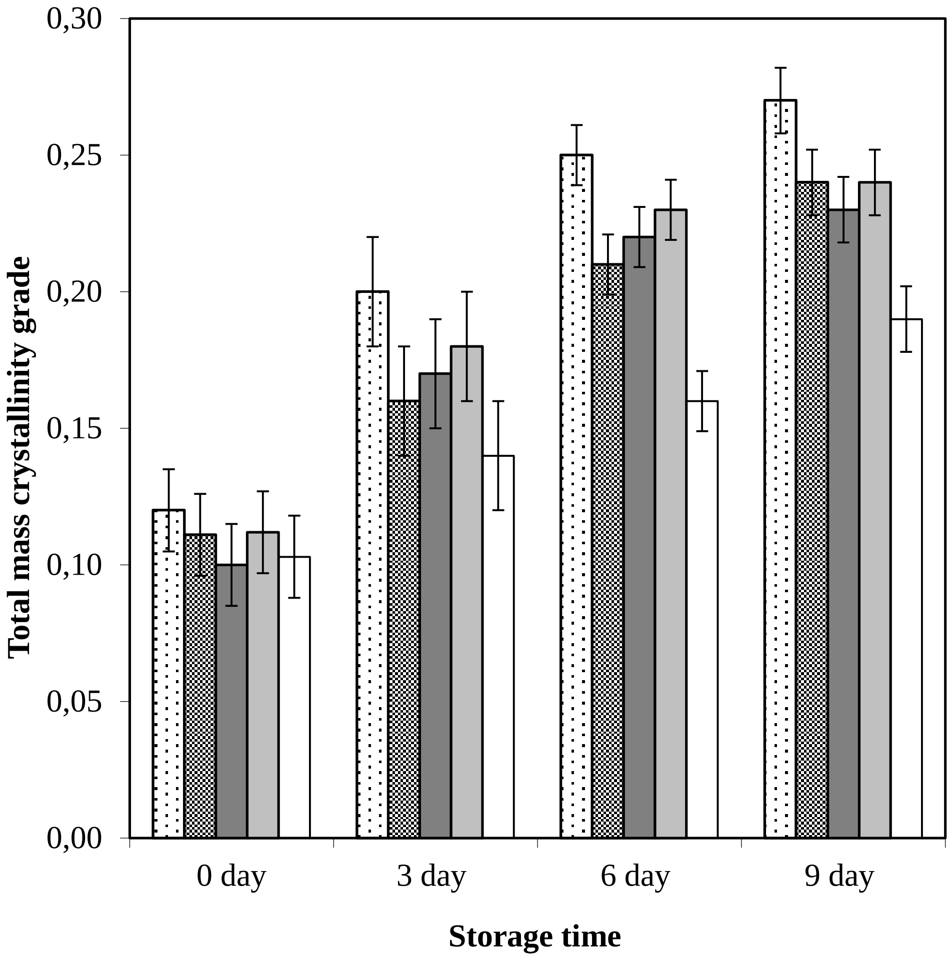

Figure 3.31 Total mass crystallinity grades of different cake samples at different storage times. (dotted bar): control cake, (dashed bar): cake sample prepared with 10% replacement of flour with cocoa powder, (dark gray bar): cake sample prepared with 10% replacement of flour with ball milled HS, (gray bar): cake sample prepared with 10% replacement of flour with raw HS, (white bar): cake sample prepared with 10% replacement of flour with MF HS fiber.

3.3.3 FT-IR (Fourier Transform Infrared Spectroscopy)

The starch retrogradation was also determined using FT-IR spectroscopy. Fourier transform infrared spectroscopy provides to investigate starch structure variations from disorder to order transition. In FT-IR spectra, changes in hydrogen bonding network of the system may be monitored. IR absorbance band between 900 cm^{-1} and 1,200 cm^{-1} can be more sensitive part. Mostly, interesting peaks for starch were obtained at the band around 1,047 cm^{-1}, 1,022 cm^{-1} and 995 cm^{-1}. While the band at 1,047 cm^{-1} is related to ordered or crystalline structure, the band at 1,022 cm^{-1} is related to amorphous starch structure. Moreover, the band at 995 cm^{-1} is associated with water sensitivity (Smits et al., 1998; Ji et al., 2010).

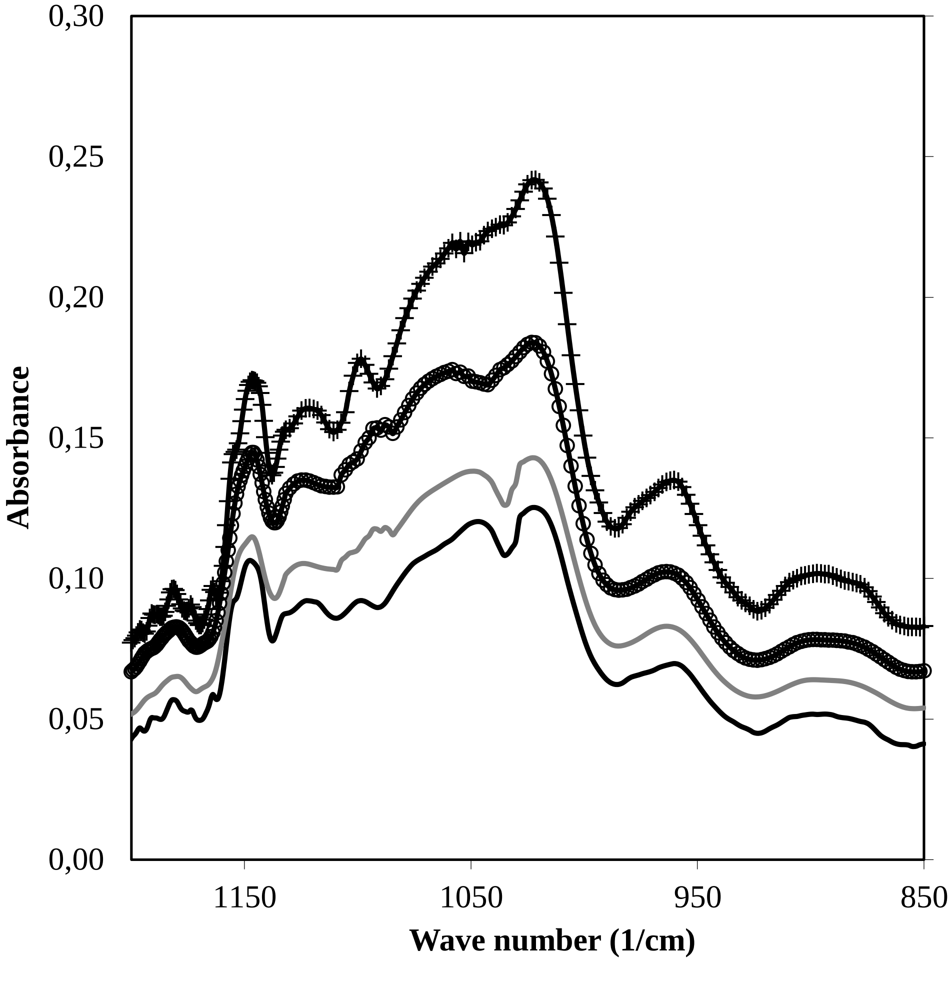

Figure 3.32 FTIR spectrum of control cake samples stored at different storage times (black line): 0 day, (gray line): 3 days, (black line with round shape): 6 days, (black line with dashed line): 9 days

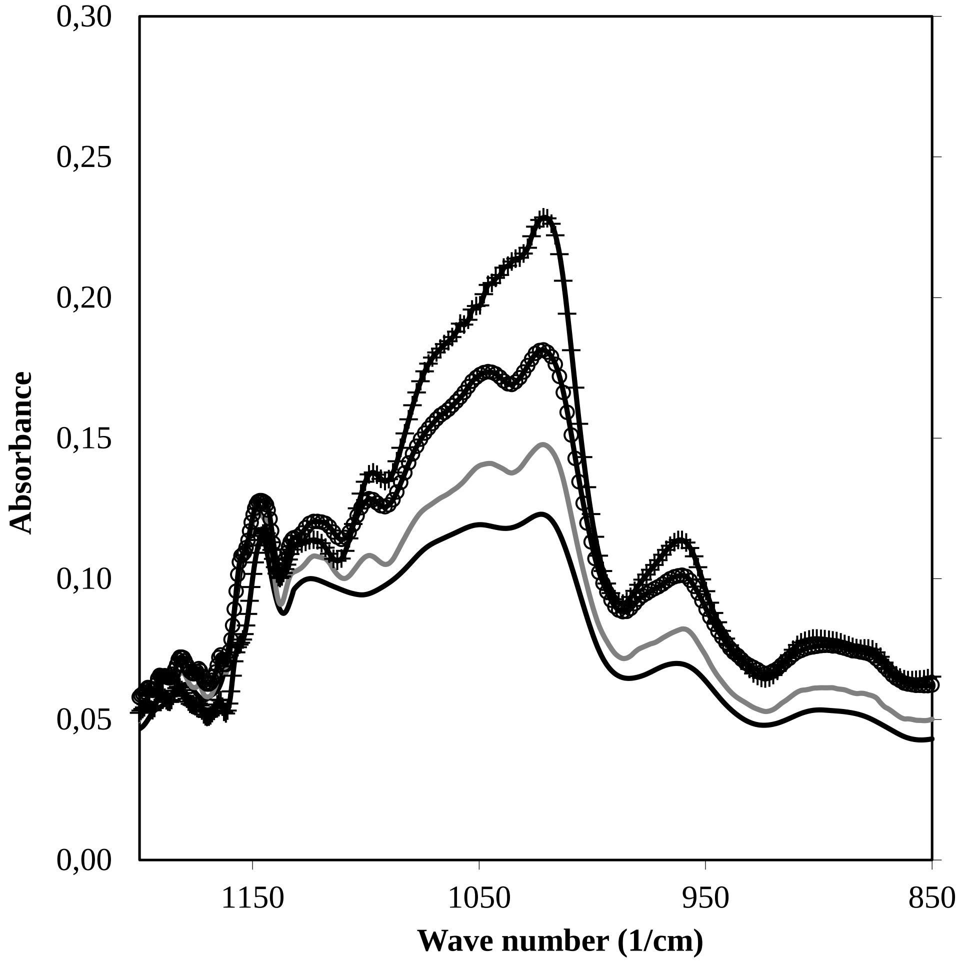

Figure 3.33 FTIR spectrum of cake samples prepared with 10% replacement of flour with cocoa powder and stored at different storage times (black line): 0 day, (gray line): 3 days, (black line with round shape): 6 days, (black line with dashed line): 9 days

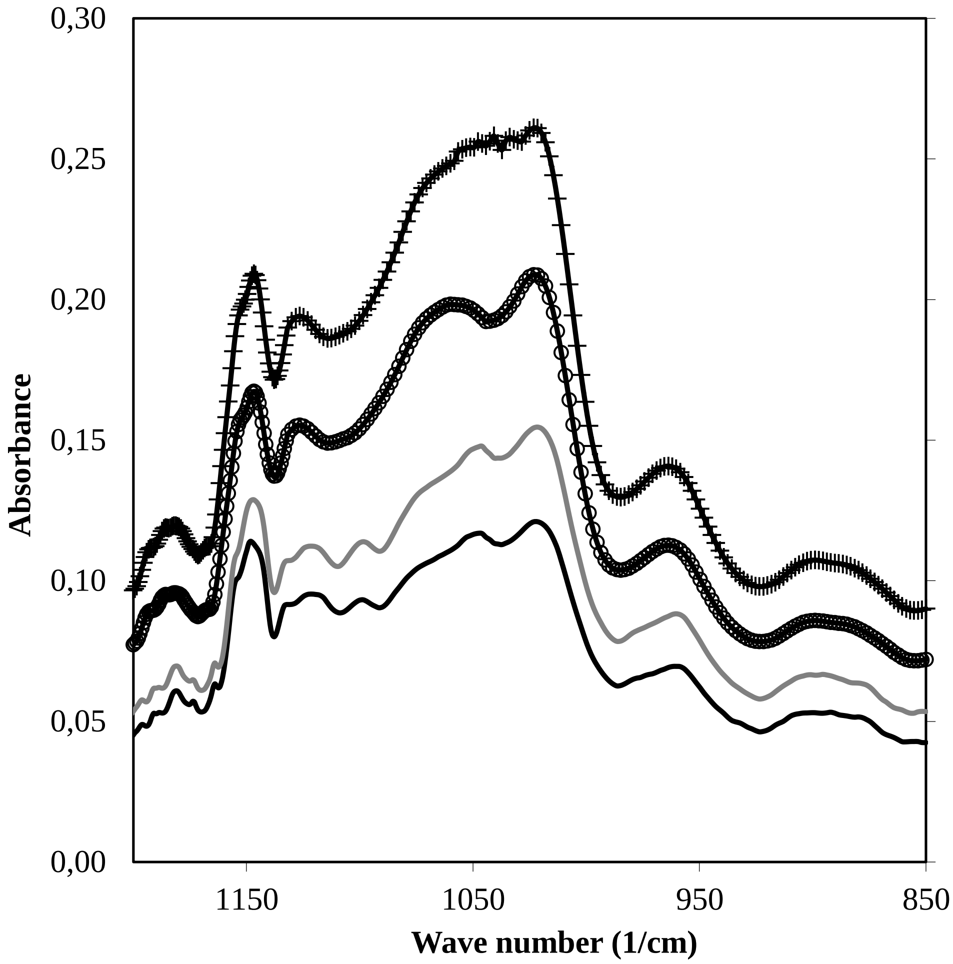

Figure 3.34 FTIR spectrum of cake samples prepared with 10% replacement of flour with raw HS and stored at different storage times (black line): 0 day, (gray line): 3 days, (black line with round shape): 6 days, (black line with dashed line): 9 days

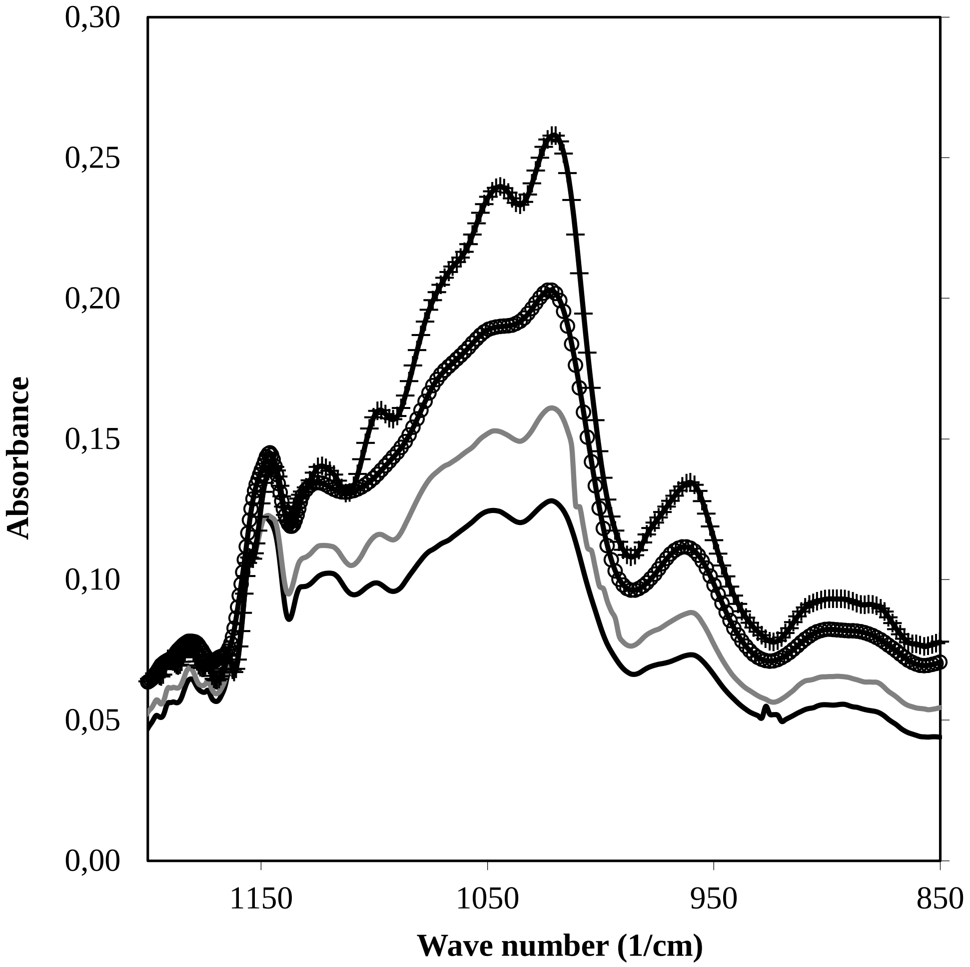

Figure 3.35 FTIR spectrum of cake samples prepared with 10% replacement of flour with ball milled HS (BM HS) and stored at different storage times (black line): 0 day, (gray line): 3 days, (black line with round shape): 6 days, (black line with dashed line): 9 days

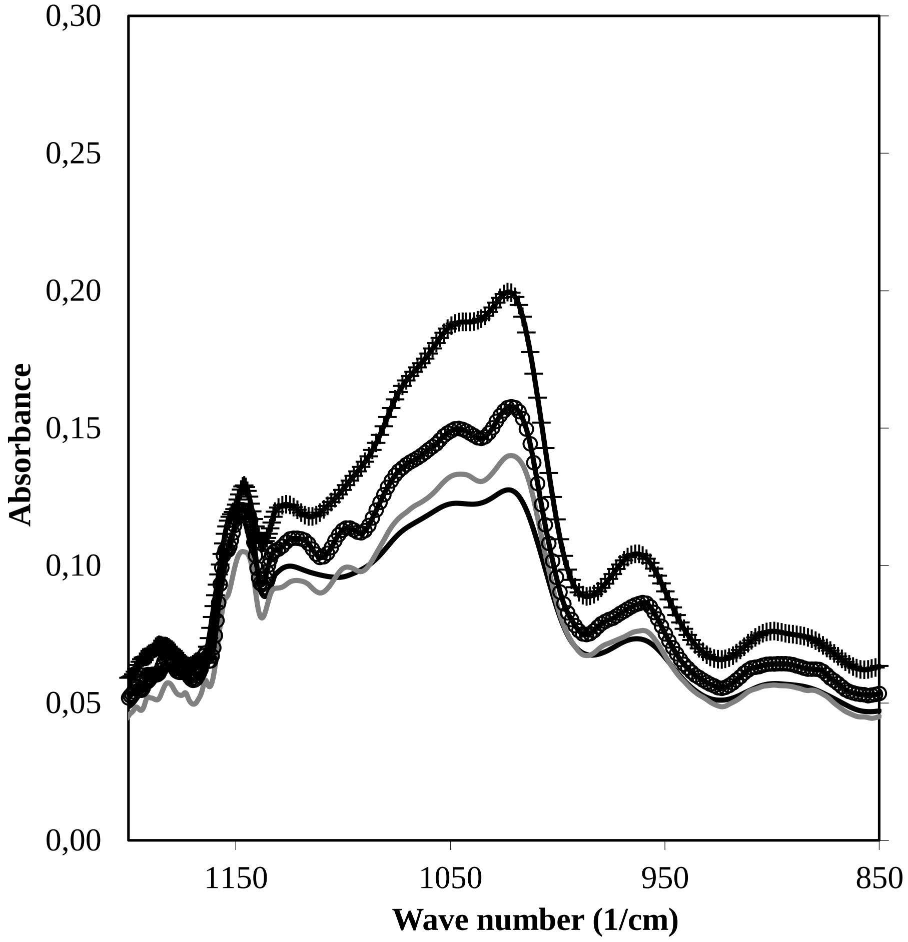

Figure 3.36 FTIR spectrum of cake samples prepared with 10% replacement of flour with MF HS fiber and stored at different storage times (black line): 0 day, (gray line): 3 days, (black line with round shape): 6 days, (black line with dashed line): 9 days

In our FT-IR study, the spectra were dominated by major peaks at around 1,040 cm^{-1} which may be attributed to C-O-H bending and CH_2- related modes and at 1,150 cm^{-1} which may be related to C-O and C-C stretching with COH contributions (Demirkesen et al., 2013). The spectra of different cake samples in the region of 850-1,250 cm^{-1} were depicted in Figs. 3.32-3.36. It was seen that retrogradation of cake samples resulted in increases in the band heights by showing the maximum intensity at 1,150 cm^{-1}. In our study, peaks at 1,040 cm^{-1} may be associated with crystalline regions of starch. The peak at 1,150 cm^{-1} is generally used as an "internal correction standard peak" to achieve measurements independent of uncontrollable factors (Ozkoc et al., 2009b; Demirkesen et al., 2013). As a result, the ratio of peak intensities at 1,040 and 1,150 cm^{-1} was used to analyse starch retrogradation.

The integral area ratios of peaks around 1,041 and 1,150 cm^{-1} have been monitored by other researchers (Ozkoc et al., 2009b; Demirkesen et al., 2013) which were related to the progressive ordering of the amylopectin polymer existing in bread and they have been used to analyse starch retrogradation. The integral area ratios of peaks around 1,040 cm^{-1} (A_1) and 1,150 cm^{-1} (A_2) were given in Table 3.4. As the storage time increased, A_1/A_2 increased indicating cake staling. When results were compared, it was realized that cake samples prepared with MF HS fiber showed low values of A_1/A_2. This also shows that MF HS fiber retards cake staling. The findings from both FTIR and X-ray analysis were in good agreement.

Table 3.4 Ratios of peak areas at around 1040 cm^{-1} (A_1) to at around 1150 cm^{-1} (A_2)

A_1/A_2					
Storage time	Control	Raw HS	Cacao HS	BM HS	MF HS
Day 0	1.2 ± 0.14	1.2 ± 0.15	1.1 ± 0.21	1.4 ± 0.17	1.2 ± 0.18
Day 3	1.8 ± 0.35	2.0 ± 0.21	1.9 ± 0.37	2.1 ± 0.38	1.6 ± 0.39
Day 6	3.1 ± 0.26	3.1 ± 0.27	3.3 ± 0.18	3.5 ± 0.29	2.9 ± 0.30
Day 9	4.6 ± 0.51	4.6 ± 0.61	4.5 ± 0.36	4.6 ± 0.34	3.8 ± 0.24

The ratios of peak intensities at 1,040 and 1,150 cm^{-1} were also reported to monitor retrogradation behaviors of cakes by expressing the amount of ordered starch to amorphous starch (Table 3.5). The ratios of peak intensities generally increased as storage time increased implying a reduced amount of amorphous material, reflecting more organized starch since retrogradation commenced.

Table 3.5 The ratios of peak intensities bands (R) at 1040 cm^{-1} (A_1) and 1150 cm^{-1} (A_2)

R					
Storage time	Control	Raw HS	Cacao	BM HS	MF HS
Day 0	0.93 ± 0.08	0.99 ± 0.07	0.98 ± 0.07	0.98 ± 0.05	0.97 ± 0.03
Day 3	0.96 ± 0.05	0.95 ± 0.04	0.91 ± 0.07	0.97 ± 0.02	0.90 ± 0.02
Day 6	0.97 ± 0.08	0.92 ± 0.05	0.92 ± 0.06	0.95 ± 0.08	0.91 ± 0.05
Day 9	0.87 ± 0.06	0.98 ± 0.03	0.90 ± 0.05	0.91 ± 0.05	0.92 ± 0.06

3.4 Effect of Further Reduction of Flour in Cake Samples Prepared with MF HS Fiber

Although cakes prepared with substitution of flour (in 100g flour basis formulation) with MF HS fiber showed positive results in staling analyses, their textural and rheological results indicated that cake formulation needed some modifications. Thus, as further study, reduction of flour content in cake formulation was also investigated.

In previous experiments, 15g MF HS fiber was used in cakes made with 15% replacement of flour with HS (in 100g flour basis formulation) and it means that formulation includes 15g MF HS fiber and 85g flour. For flour reduction analyses, this quantity (15g) was selected as fixed amount of MF HS fiber in cakes and flour content was decreased gradually. Thus, cake samples were prepared with 15g MF HS fiber with the addition of 65g, 45g and 25g flour, respectively.

3.4.1 Rheological Measurements of Cakes Prepared with MF HS Fiber with Reduction of Flour in Formulation

3.4.1.1 Flow and Oscillation Measurements

Figure 3.37 shows the shear stress versus shear rate graph belonging to cakes prepared with reduction of flour in the formulation. It demonstrated that the flow behavior was again shear thinning. Experimental data again gave good fit to Herschel-Bulkley model. Flow behavior index values (n) were again obtained less than 1. They varied between 0.49 and 0.64. The consistency index values (K) were decreased by reducing flour content in the cake formulation (Table 3.6).

Table 3.6 Herschel Bulkley parameters of dough samples containing MF HS fiber with reduction of flour at 25 ºC**.**

Formulation	**τ_O (Pa)**	**K (Pa.s^n)**	**n**
Control	21.66	24	0.53
85 g flour	291	98	0.49
65 g flour	189.78	54	0.56
45 g flour	113.87	41	0.59
25 g flour	22.77	14	0.64

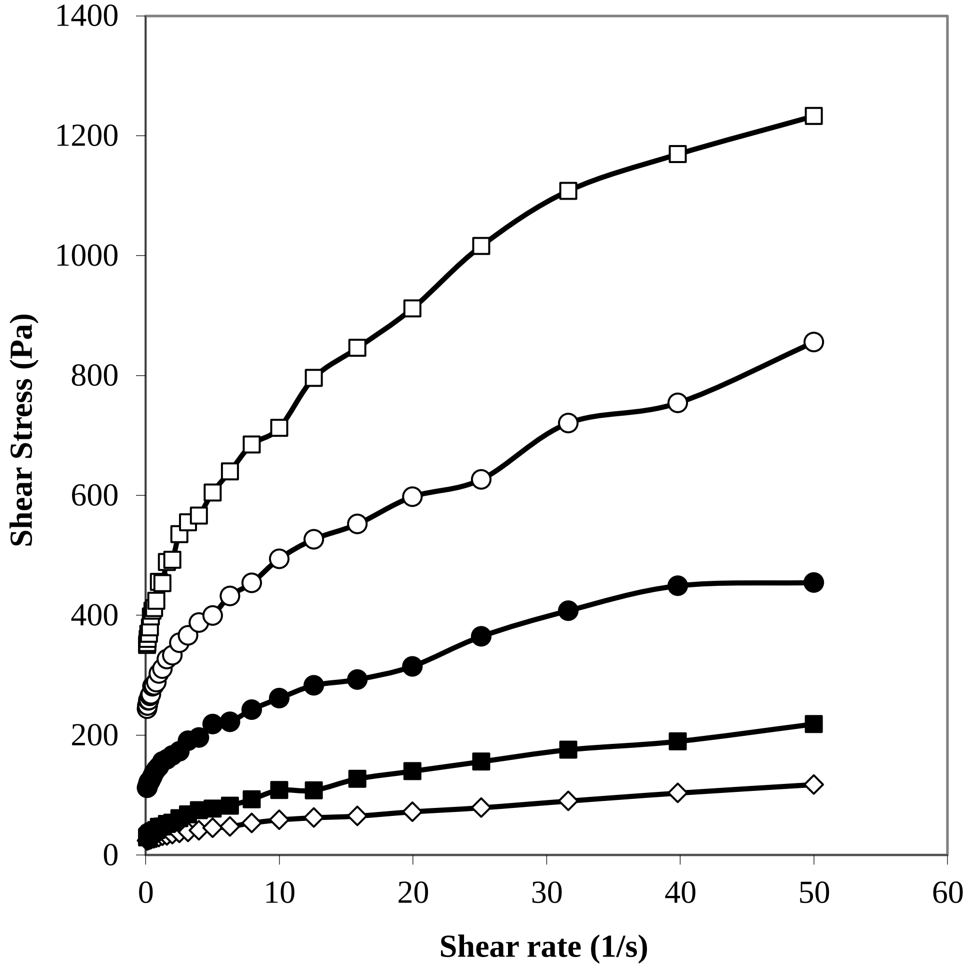

Figure 3.37 Flow curves obtained for control cake dough and also dough samples containing different amount of flour with same amount of MF HS. (■): Control cake, (◇): batter formulation with 25g flour, (●): batter formulation with 45g flour, (○): batter formulation with 65g flour, (□): batter formulation with 85 g flour indicating standard cake with MF 15%.

The graphs of elastic (G') and viscous modulus (G") of dough samples prepared with MF HS fiber by reducing flour content were given in Figure 3.38 and Figure 3.39. As standard MF 15% cake contained 15g MF HS fiber and 85g flour, other cake samples with reduction of flour were prepared by decreasing flour content to 65g, 45g and 25g with the addition of same amount of MF HS fiber (15g). According to the oscillatory measurements, it was seen that dough samples prepared by reducing flour content showed lower elastic (G') and viscous modulus (G") results when compared to standard MF 15% dough. The reason may be related to decrease in dry ingredient content in cake formulation. Similar to cake batters based on 100g flour formulation, all dough samples with flour reduction also showed solid like behavior due to higher G' value than G" value. They were obtained as closer to dough samples containing ball milled, raw HS or cocoa powder (15%). Both G' and G" increased with oscillatory frequency.

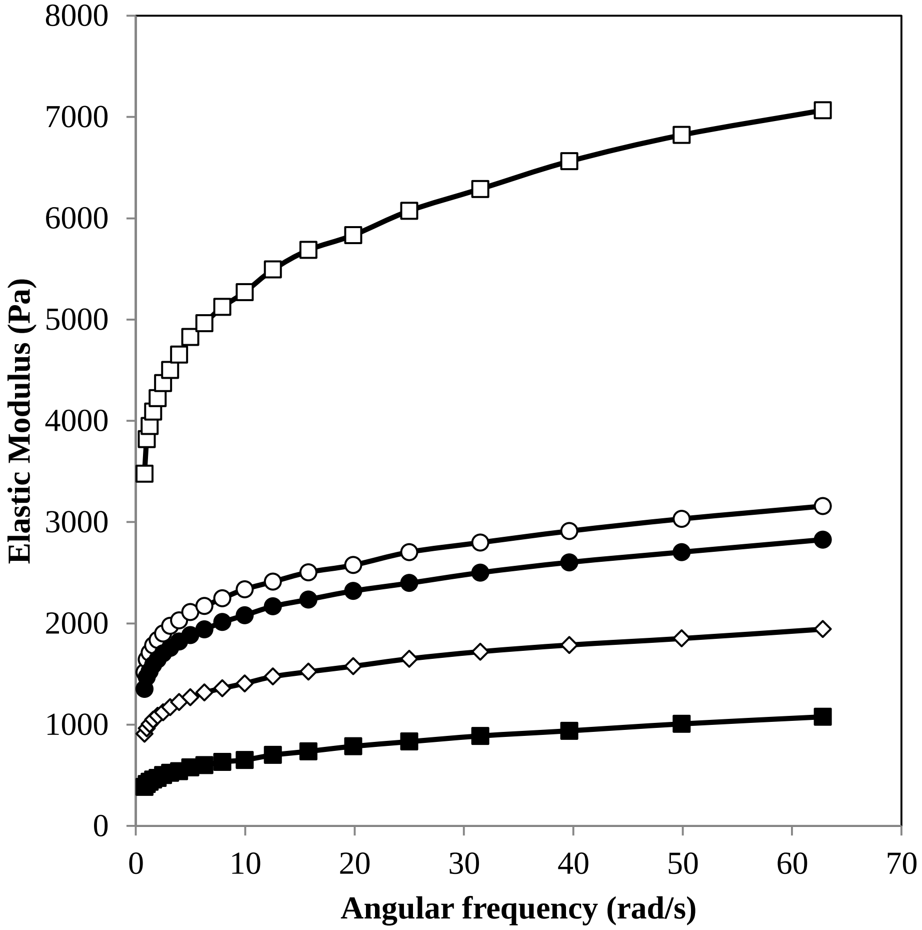

Figure 3.38 Elastic modulus obtained for control cake dough and also dough samples containing different amount of flour with same amount of MF HS. (■): Control cake, (◇): batter formulation with 25g flour, (●): batter formulation with 45g flour, (○): batter formulation with 65g flour, (□): batter formulation with 85 g flour indicating standard cake with MF 15%.

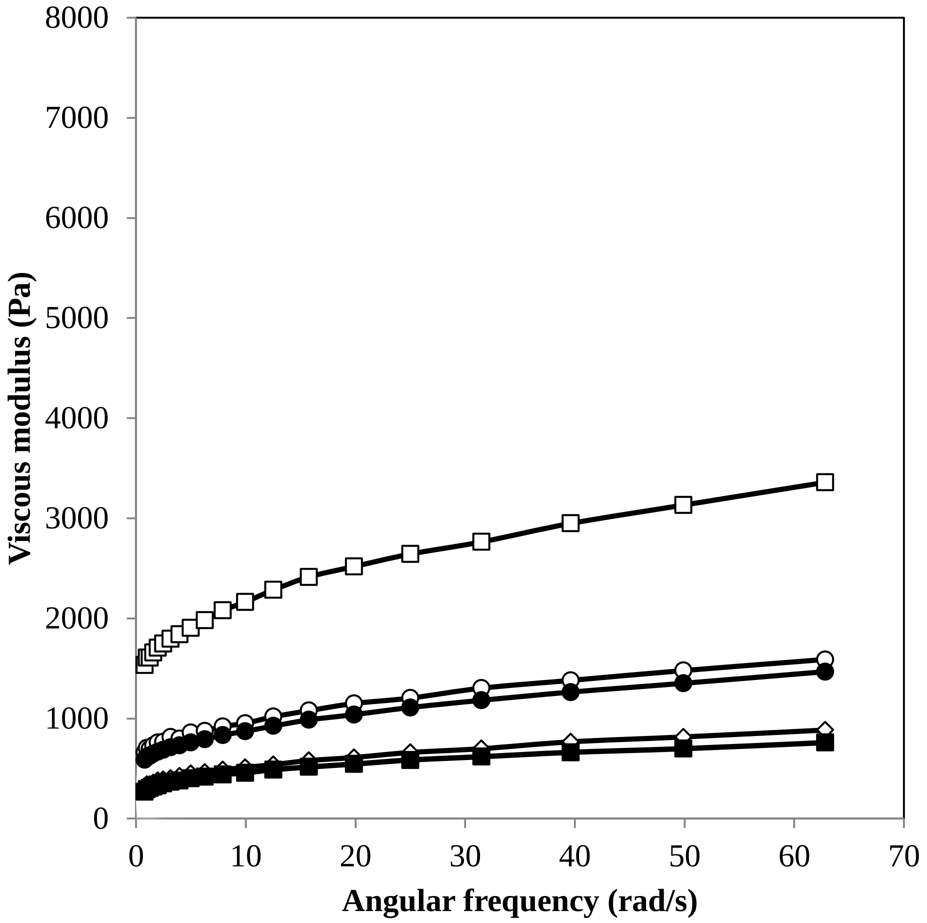

Figure 3.39 Viscous modulus obtained for control cake dough and also dough samples containing different amount of flour with same amount of MF HS. (■): Control cake, (◇): batter formulation with 25g flour, (●): batter formulation with 45g flour, (○): batter formulation with 65g flour, (□): batter formulation with 85 g flour indicating standard cake with MF 15%.

3.4.2 Texture Profile of Cakes Prepared with MF HS Fiber with Reduction of Flour in the Formulation

Firmness and springiness values of cakes prepared with MF HS fiber by reducing flour content in cake formulation were given in Figs. 3.40-3.45 during 0, 3 and 6 days of storage. In both fresh and stored products, flour reduction influenced firmness results, significantly while it did not affect springiness results ($p \leq 0.05$). Cakes prepared with flour reduction showed lower firmness results when compared to standard MF 15% cake containing 85g flour (15% replacement of flour with MF HS fiber based on 100 g flour formulation). Firmness values of fresh cakes prepared with MF HS fiber was decreased from 271.89 g force to 205.18 g force, 160.00 g force and 92.66 g force by reducing flour amount from 85g to 25g in cake formulation (Figure 3.40).

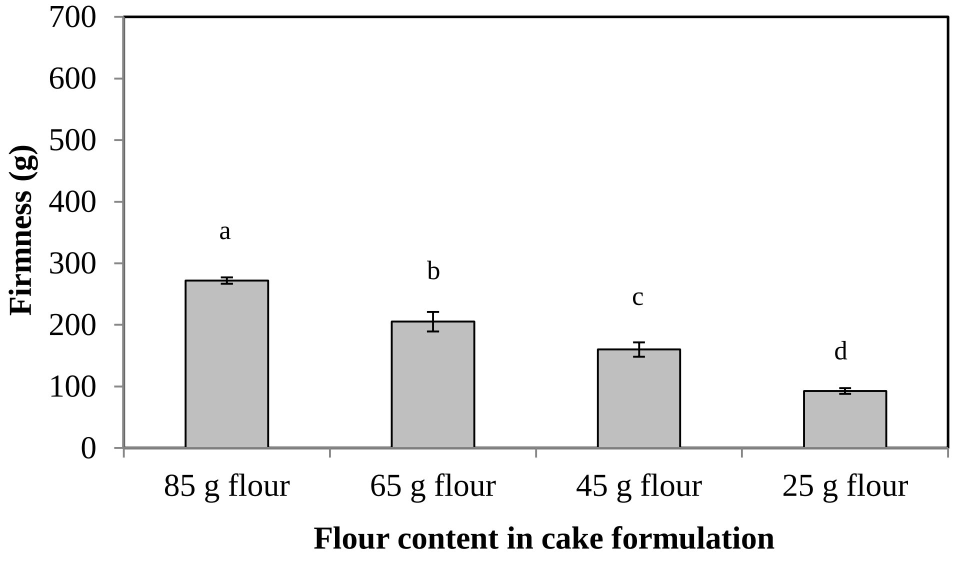

Figure 3.40 Firmness values of fresh cake samples containing MF HS fiber prepared with the reduction of flour content

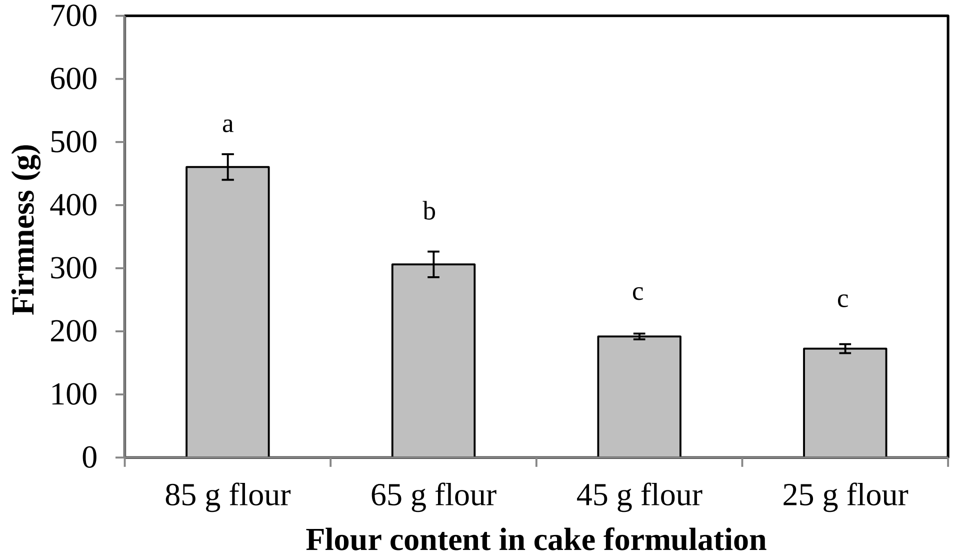

Figure 3.41 Firmness values of cake samples containing MF HS fiber prepared with the reduction of flour content and stored for 3 days

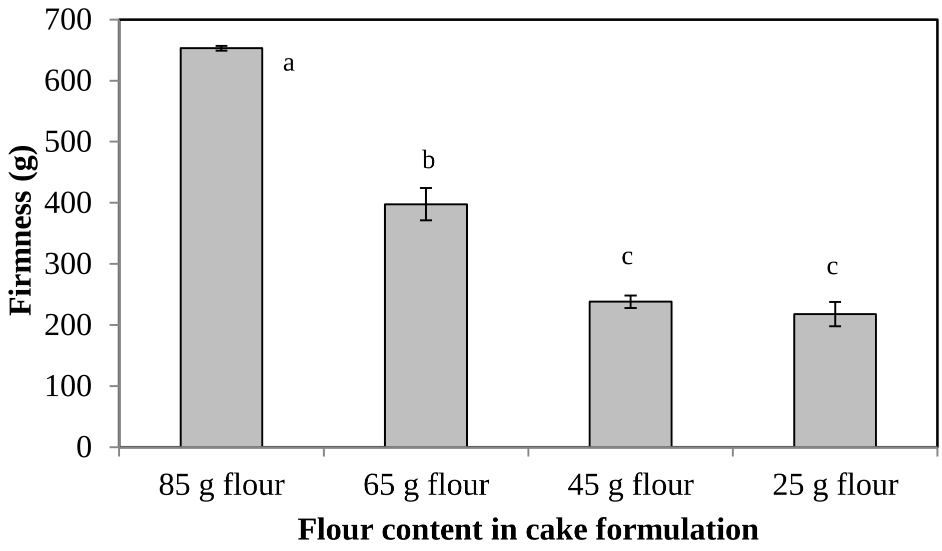

Figure 3.42 Firmness values of cake samples containing MF HS fiber prepared with the reduction of flour content and stored for 6 days

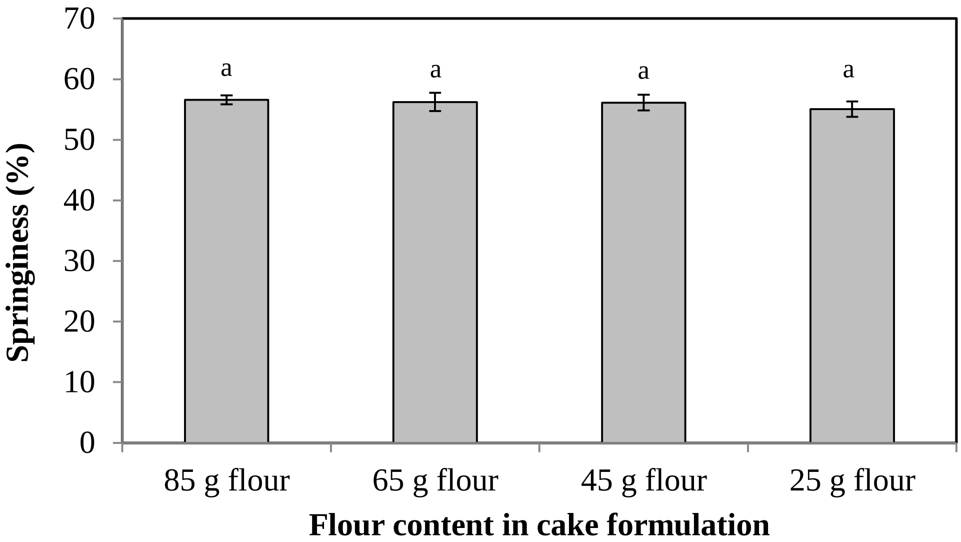

Figure 3.43 Springiness values of fresh cake samples containing MF HS fiber prepared with the reduction of flour content

In springiness values, there was not significant difference between cakes made with flour reduction especially during 0 and 3 days of storage (Figure 3.43 and Figure 3.44). Similar high springiness values were obtained in all samples at around the value of 56%. Although cake made with 25g flour formulation had close firmness results to control cake, it showed very low volume due to the loss of structure provided by gluten.

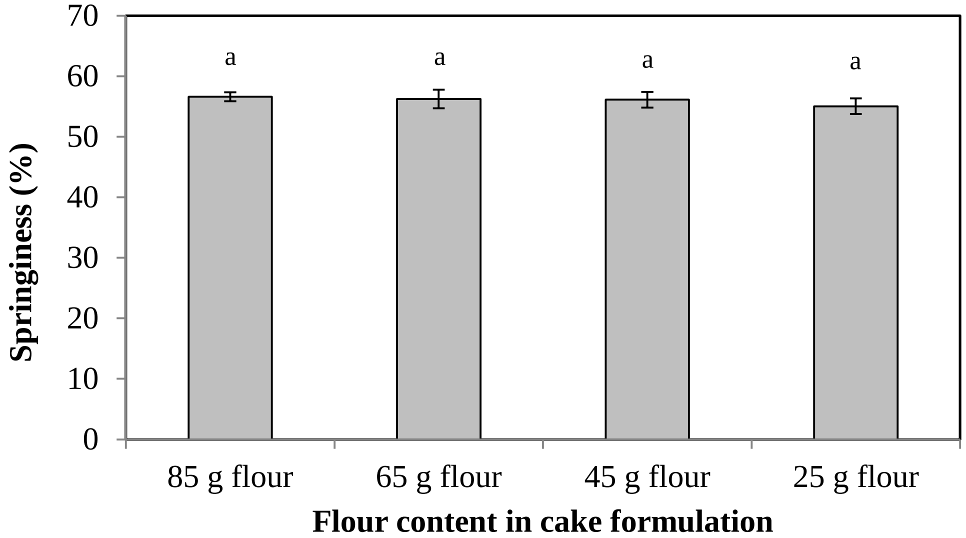

Figure 3.44 Springiness values of cake samples containing MF HS fiber prepared with the reduction of flour content and stored for 3 days

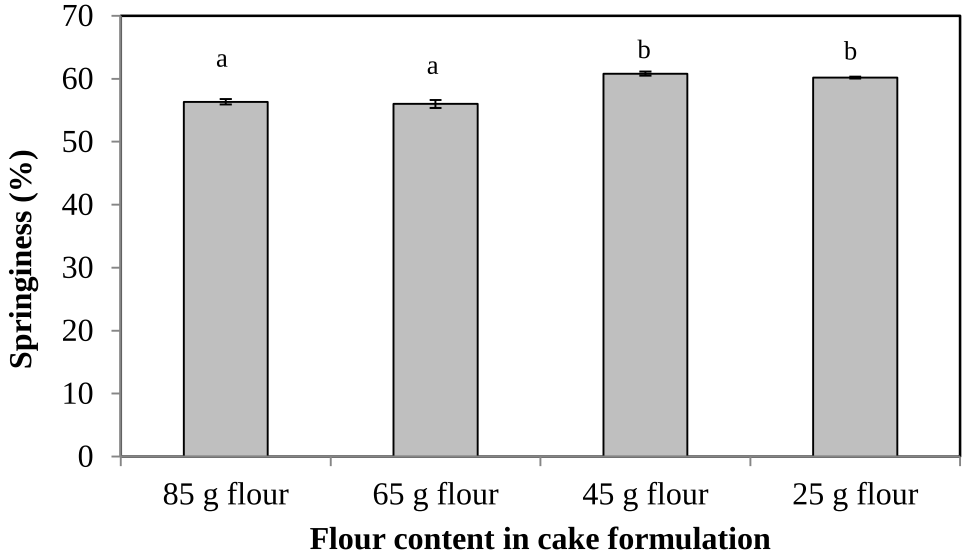

Figure 3.45 Springiness values of cake samples containing MF HS fiber prepared with the reduction of flour content and stored for 6 days

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

All cake batter samples exhibited shear thinning behavior with different model constants. Experimental results showed that they were fitted to Herschel–Bulkley model. The addition of MF HS fiber into the cakes increased elastic (G') and viscous moduli (G") values of dough samples. As percentage of cocoa powder/ hazelnut skin samples increased, rheological results also showed an increase. Moreover, in all dough samples elastic (G') modules was higher than viscous (G") modules indicating solid like behavior. It can be concluded that production of micro and nano fibers from hazelnut skin changed rheological properties of cakes when they were added into cake formulation as ingredient.

In the microstructure analyses of cocoa powder and hazelnut skin samples, the effect of microfluidization on hazelnut skin was clearly seen and structural differences between raw HS, ball milled HS, MF HS fiber and cocoa powder were clearly observed. Hazelnut skin was fibrillated into smaller fiber fragments in microfluidizer and it caused changes in both rheological and textural properties of cakes due to the increase in water holding capacity of hazelnut skin. With the use of ball mill, large blocks of raw HS were broken into smaller particles which are closer to cocoa powder image.

When staling of cakes was investigated, it was seen that both X-ray and FT-IR analyses gave similar results. They showed that starch retrogradation in cake samples increased during storage of cakes ($p \leq 0.05$). Total mass crystallinity results of cakes pointed out that it should be possible the use of MF HS fiber instead of cocoa powder since cakes prepared with MF HS fiber had the lowest results by indicating the increase in shelf life.

When the texture profiles of cakes were investigated in terms of firmness and springiness, it was seen that as the concentration of cocoa powder/ hazelnut skin samples increased, the firmness values increased in both fresh and stored cake samples. Moreover, higher springiness values were obtained in cakes containing MF HS fiber indicating more elastic structure. Furthermore, crumb colors of these cakes were observed as darker. The highest firmness was obtained in cakes prepared with 20% replacement of flour with MF HS fiber. It was an expected result because MF HS fiber due to its flaky structure absorbed more water and it leaded to more firm cake. Additionally, by decreasing flour in cake formulations, firmness values of cakes with MF HS fiber were also decreased.

It can be concluded that using MF HS fiber may provide healthier cakes with reduced amount of flour. With the optimization of fiber percentage and firmness value, acceptable cakes with MF HS fiber can be obtained with longer shelf life. Moreover, it can be an alternative to cocoa powder because of its intense color when the cocoa flavor is added to cake product. Therefore, MF HS fiber may be recommended to be used in cake formulations. Photographs of all cakes can be seen in Figs.A.2 and A.3.

As future study, production of gluten-free cakes with hazelnut skin samples may be investigated in details.

REFERENCES

Ajila, C. M., Leelavathi, K., Prasada Rao, U. J. S., 2008. Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. Journal of Cereal Science, 48, 319-326.

Aguilera, J. M. and Stanley, D. W., 1999. Examining Food Microstructure. In: Microstructural Principles of Food Processing and Engineering. (2nd eds.), Aspen Publishers, Inc., Gaithersburg, Maryland, USA, pp. 1-65.

Aherne, S. A. and O'Brien, N. M., 2002. Dietary flavonols: Food content and metabolism. Nutrition, 18, 75–81.

Alamilla, B. L., Chanona, P. J. J., Jiménez, A. A. R., Gutiérrez, L. G. F., 2005. Description of morphological changes of particles along spray drying. Journal of Food Engineering, 67, 179-184.

Alasalvar, C., Karamać, M., Amarowicz, R., Shahidi, F., 2006. Antioxidant and antiradical activity in extracts of hazelnut kernel (Corylus avellana L.) and in hazelnut green leafy cover. Journal of Agricultural and Food Chemistry, 54, 4826–4832.

Alasalvar, C., Karamać, M., Kosińska, A., Rybarczyk, Anna., Shahidi, F., Amarowicz, R., 2009. Antioxidant activity of hazelnut skin phenolics. Journal of Agricultural and Food Chemistry, 57: 4645-4650.

AACC., 2001. American Association of Cereal Chemists. The definition of dietary fiber. A report. Cereal Foods World, 46(3), 112–129.

Amin, I., Norazaidah, Y., Hainida, K. I. E., 2006. Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. Food Chemistry, 94(1), 47-52.

Amin, I., Marjan, Z. M., Foong, C. W., 2004. Total antioxidant activity and phenolic content in selected vegetables. Food Chemistry, 87(4), 581-586.

Anil, M., 2002. Usage of flaxseed as a source of dietary fiber in breadmaking, PhD Thesis, Ondokuz Mayis University, Samsun.

Anil, M., 2007. Using of hazelnut testa as a source of dietary fiber in breadmaking. Journal of Food Engineering, 80, 61-67.

Arendt, E. K., Morrissey, A., Moore, M. M., Dal Bello, F., 2008. Gluten-free breads. In: Gluten-free Cereal Products and Beverages, (Eds.) Arendt, E. K. and Dal Bello F., Academic Press, Oxford, UK, pp. 289–319.

Arlorio, M., Locatelli, M., Travaglia, F., Coïsson, J-D., Del Grosso, E., Minassi, A., Appendino, G., Martelli, A., 2008. Roasting impact on the contents of clovamide (*N*-caffeoyl-L-DOPA) and the antioxidant activity of cocoa beans (*Theobroma cacao* L.). Food Chemistry, 106, 967-975.

Asami, D.K., Hong, Y. J., Barrett, D. M., Mitchell, A. E., 2003. Comparison of total phenolic and ascorbic acid content of freeze dried and air dried marionberry, strawberry and corn grown using conventional, organic and sustainable agricultural practices. Journal of Agricultural and Food Chemistry, 51, 1237–1241.

Ashwini, A., Jyotsna, R., Indrani, D., 2009. Effect of hydrocolloids and emulsifiers on the rheological, microstructural and quality characteristics of eggless cake. Food Hydrocolloids 23, 700–707.

Azizah, A. H., Nik Ruslawati, N. M., Swee Tee, T., 1999. Extraction and characterization of antioxidant from cocoa by-products. Food Chemistry, 64(2), 199-202.

Babcock, D., 1987. Rice bran as a source of dietary fiber. Cereal Foods World, 32(8), 538–539.

Belščak, A., Komes, D., Horžić, D., Ganić, K., Karlović, K. D., 2009. Comparative study of commercially available cocoa products in terms of their bioactive composition. Food Research International, 42, 707-716.

Bennion, E. B. and Bamford, G. S. T., 1997. The Technology of Cake Making, (Eds.) Bent, A. J. (6th eds.), Blackie Academic and Professional, London, UK.

Boeckner, L., Schledewitz, K., NebGuide, from http://www.ianrpubs.unl.edu/epublic/pages/publicationD.jsp?publicationId=447 last visited: August, 2013.

Bozzola, J. J. and Russell, L. D., 1991. Electron microscopy: Principles and Techniques for Biologists, Jones and Bartlett Publishers Inc., Mississauga, Canada, pp. 14-62.

Bravo, L., 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews, 56, 317-333.

Cauvain, S., Young, L., 2008. Effects of Water on Product Textural Properties and Their Changes During Storage. In: Bakery food manufacture and quality, water control and effects, (2nd Ed.), Wiley-Blackwell Publishing, UK, pp. 143-170.

Chau, C. F., Wang, Y. T., Wen, Y. L., 2007. Different micronization methods significantly improve the functionality of carrot insoluble fibre. Food Chemistry, 100, 1402–1408.

Chen, H., Rubenthaler, G. L., Schanus, E. G., 1988. Effect of apple fiber and cellulose on the physical properties of wheat flour. Journal of Food Science, 53(1), 304-305.

Chen, J., Liang, R-H., Liu, W., Liu, C-M., Li, T., Tu, Z-C., Wan, J., 2012. Degradation of high-methoxyl pectin by dynamic high pressure microfluidization and its mechanism. Food Hydrocolloids, 28, 121-129.

Cho, S. S., Samuel, P., 2009. Fiber Ingredients: Food Applications and Health Benefits, CRC Press Taylor and Francis Group, USA, pp. 431-441.

Cilek B., 2012. Microencapsulation of phenolic compounds extracted from sour cherry (*Prunus cerasus* L.) pomace, MS Thesis, METU, Ankara.

Ciron, C. I. E., Gee, V. L., Kelly, A. L., Auty, M. A. E., 2010. Comparison of the effects of high-pressure microfluidization and conventional homogenization of milk on particle size, water retention and texture of non-fat and low-fat yoghurts. International Dairy Journal, 20 (5), 314-320.

Ciron, C. I. E., Gee, V. L., Kelly, A. L., Auty, M. A. E., 2011. Effect of microfluidization of heat-treated milk on rheology and sensory properties of reduced fat yoghurt. Food Hydrocolloids, 25, 1470-1476.

Clerici, M. T. P. S., Airoldi, C., El-Dash, A. A., 2009. Production of acidic extruded rice flour and its influence on the qualities of gluten-free bread. LTW, Food Science and Technology, 42 (2), 618-623.

Clifford, M. N., 2000. Anthocyanins – nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture, 80, 1063-1072.

Collar, C., Santos, E., Rosell, C. M., 2007. Assessment of the rheological profile of fibre-enriched bread doughs by response surface methodology. Journal of Food Engineering 78, 820–826.

Collins, J. L., Kalantari, S. M., Post, A. R., 1982. Peanut hull flour as dietary fiber in wheat bread. Journal of Food Science, 47, 1899–1902.

Conforti, F. D., 2006. Bakery products: Science and Technology, (Eds.), Conforti, F. D. and Hui, Y. H (1st ed.), Blackwell publishing, Iowa, USA, pp. 393-411.

Contini, M., Baccelloni, S., Frangipane, M. T., Merendino, N., Massantini, R., 2012. Increasing espresso coffee brew antioxidant capacity using phenolic extract recovered from hazelnut skin waste. Journal of Functional Foods, 4, I37-I46.

Contini, M., Baccelloni, S., Massantini, R., Anelli, G., 2008. Extraction of natural antioxidants from hazelnut (Corylus avellana L.) shell and skin wastes by long maceration at room temperature. Food Chemistry. 110, 659-669.

Cook, E. J., and Lagace, A. P., 1987. Forming a microemulsion – comprises impinging streams of mixtures containing components of micro-emulsion into each other at high pressure in low pressure zone, US Patent, US4908154-A.

Dalgleish, D. G., Tosh, S. M., West, S., 1996. Beyond homogenization : the formation of very small emulsion droplets during the processing of milk by a microfluidizer. Netherlands Milk and Dairy Journal, 50, 135-148.

Datta, A. K., Sahin, S., Sumnu, G., Keskin, S. O., 2007. Porous media characterization of breads baked using novel heating modes. Journal of Food Engineering, 79, 106-116.

Delgado, T., Malheiro, R., Pereira, J. A., Ramalhosa, E., 2010. Hazelnut (Corylus avellana L.) kernels as a source of antioxidants and their potential in relation to other nuts. Industrial Crops and Products 32, 621–626.

DeMan, J. M., 1999. Principles of Food Chemistry, Aspen Publishers, Inc. Gaithersburg, Maryland.

Demirkesen, I., Campanella, O. H., Sumnu, G., Sahin, S., Hamaker, B. R., 2013. A study on staling characteristics of gluten-free breads prepared with chestnut and rice flours. Food Bioprocess Technology, doi: 10.1007/s11947-013-1099-3.

Demirkesen, I., Mert, B., Sumnu, G., Sahin, S. 2010. Rheological properties of gluten-free bread formulations. Journal of Food Engineering, 96, 295-303.

Demirkol, O. S., 2007. Investigation of physical properties of different cake formulations during baking with microwave and infrared-microwave combination, PhD Thesis, METU, Ankara.

Dreher, M. L., and Padmanaban, G., 1983. Sunflower hull flour as a potential dietary fiber supplement. Journal of Food Science, 48, 1463–1465.

Du, C.-J., and Sun, D.-W., 2004. Recent developments in the applications of image processing techniques for food quality evaluation. Trends in Food Science and Technology, 15, 230-249.

Duman, B., 2013. Production of cacao micro and nano fibers and utilization in cakes, MS Thesis, METU, Ankara.

Dykes, L. and Rooney, L. W., 2007. Phenolic compounds in cereal grains and their health benefits. Cereals Food World, 52, pp. 105-111.

Eberhardt, M. V., Lee, C. Y., Liu, R.H., 2000. Antioxidant activity of fresh apples. Nature, 405 (6789): 903-904.

Edwards, W. P., 2007. Products Other Than Bread. In: The science of bakery products, The Royal Society of Chemistry, Cambridge, UK, pp. 208-232.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., Attia, H., 2011. Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. Food Chemistry, 124, 411-421.

Farhoosh, R., Golmovahhed, G. A., Khodaparast, M. H. H., 2007. Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.). Food Chemistry, 100, (1), 231-236.

Figuerola, F., Hurtado, M. L., Estévez A. M., Chiffelle, I., Asenjo, F., 2005. Fibre concentrates from apple pomace and citrus peel as potential source for food enrichment. Food Chemistry, 91, 395-401.

Galisteo, M., Duarte, J., Zarzuelo, A., 2008. Effects of dietary fibers on disturbances clustered in the metabolic syndrome. The Journal of Nutritional Biochemistry, 19, 71–84.

Gallagher, E., Kunkel, A., Gormley, T. R., Arendt, E. K., 2003. The effect of dairy and rice powder addition on loaf and crumb characteristics, and on shelf life (intermediate and long-term) of gluten-free breads stored in a modified atmosphere. European Food Research and Technology, 218, 44–48.

Garad, S., Wang, J., Joshi, Y., Panicucci., 2010. Pharmaceutical Suspensions: From Formulation Development to Manufacturing, (Eds.), Kulshreshtha, A. K.., Singh, O. N., Wall, G. M., AAPS Press, USA, pp. 127-177.

Gélinas, P., Roy, G., Guillet, M., 1999. Relative effects of ingredients on cake staling based on an accelerated shelf-life test. Journal of Food Science, 64(5), 937-940.

Glitsø, L. V. and Bach Knudsen, K. E., 1999. Milling of whole grain rye to obtain fractions with different dietary fibre characteristics. Journal of Cereal Science, 29, 89-97.

Gómez, M., Moraleja, A., Oliete, B., Ruiz, E., Caballero, P.A., 2010. Effect of fiber size on the quality of fibre-enriched layer cakes. Food Science and Technology, 43, 33-38.

Gómez, M., Oliete, B., Rosell, C. M., Pando, V., Fernández, E., 2008. Studies on cake quality made of wheat-chickpea flour blends. LWT - Food Science and Technology, 41, 1701-1709.

Gómez, M., Ronda, F., Blanco, C. A., Caballero, P. A., Apesteguía, A., 2003. Effect of dietary fibre on dough rheology and bread quality. Eur Food Res Technol, 216, 51-56.

Gómez, M., Ronda, F., Caballero, P. A., Blanco, C. A., Rosell, C. M., 2007. Functionality of different hydrocolloids on the quality and shelf-life of yellow layer cakes. Food Hydrocolloids, 21 (2), 167-173.

Grigelmo-Miguel, N. and Martina-Belloso, O., 1999. Characterization of dietary fibre from orange juice extraction. Food Research International, 131, 355–361.

Gupta, P. and Premavalli, K. S., 2010. Effect of particle size reduction on physicochemical properties of ashgourd (Benincasa hispida) and radish (Raphanus sativus) fibres. International Journal of Food Sciences and Nutrition, 61, 18–28.

Hardham, J. F., Imison, B. W., French, H. M., 2000. Effect of homogenisation and microfluidisation on the extent of fat separation during storage of UHT milk. Australian Journal of Dairy Technology , 55 (1), 16-22.

Hercberg, S., Preziosi, P., Galan, P., Faure, H., Arnaud, J., N. Duport, N., Malvy, D., Roussel, A. M., S. Briançon, & S., Favier A. 1999. "The su.vi.max study": a primary prevention trial using nutritional doses of antioxidant vitamins and minerals in cardiovascular diseases and cancers. Supplementation on Vitamines et Minéraux AntioXydants. Food and Chemical Toxicology, 37 (9-10), 925-930.

Huang, C. C., Chen, Y. F., Wang, C. C. R., 2010. Effects of micronization on the physico-chemical properties of peels of three root and tuber crops. Journal of the Science of Food and Agriculture, 90, 759–763.

Huang, X., Tu, Z., Jiang, Y., Xiao, H., Zhang, Q., Wang, H., 2012. Dynamic high pressure microfluidization –assisted extraction and antioxidant activities of lentinan. International Journal of Biological Macromolecules, 51, 926-932.

Jakopic, J., Petkovsek, M. M., Likozar, A., Solar, A., Stampar, F., Veberic, R. 2011. HPLC–MS identification of phenols in hazelnut (Corylus avellana L.) kernels. Food Chemsitry, 124, 1100-1106.

Ji, Y., Zhu, K. X., Zhou, H. M., Qian, H. F., 2010. Study of the retrogradation behaviour of rice cake using rapid visco analyser, Fourier transform infrared spectroscopy and X-ray analysis. International Journal of Food Science and Technology, 45, 871–876.

Kaczmarczyk, M. M., Miller, M. J., Freund, G. G., 2012. The health benefits of dietary fiber: Beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. Metabolism, 61, 1058-1066.

Karakaya, S., 2004. Bioavailability of phenolic compounds. Critical Reviews in Food Science and Nutrition, 44, 453-464.

Karim, A. A., Norziah, M. H., Seow, C. C., 2000. Methods for study of starch retrogradation. Food Chemistry, 71, 9-36.

Keen, C. L., 2001. Chocolate: Food as Medicine/ Medicine as food. Journal of the American College of Nutrition, 20, 436S–439S.

Kerio, L. C., Wachira, F. N., Wanyoko, J. K., Rotich, M. K., 2013. Total polyphenols, catechin profiles and antioxidant activity of tea products from purple leaf coloured tea cultivars. Food Chemistry, 136 (3-4): 1405-1413.

Kim, J. H., Lee, H. J., Lee, H., Lim, E., Imm, J., Suh, H. J., 2012. Physical and sensory characteristics of fibre-enriched sponge cakes made with Opuntia humifusa. LWT-Food Science and Technology, 47, 478-484.

Kocak, G., 2010. Producing micro and nano fibers having high water holding capacity from tomato and wheat waste products and using them in model foods, MS Thesis, METU, Ankara.

Koksel, H. F., 2009. Effects of xanthan and guar gums on quality and staling of gluten free cakes baked in microwave-infrared combination oven, MS Thesis, METU, Ankara.

Ktenioudaki, A., Gallagher, E., 2012. Recent advances in the development of high-fibre baked products. Trends in Food Science & Technology, 28, 4-14.

Kuntz, S., Wenzel, U., Daniel, H., 1999. Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity, and apoptosis in human colon cancer cell lines. European Journal of Nutrition, 38 (3), 133-142.

Lagoueyte, N., Paquin, P., 1998. Effects of microfluidization on the functional properties of xanthan gum. Food Hydrocolloids, 12 (3), 365-371.

Lamuela-Raventós, R. M., Romero-Pérez, A. I., Andrés-Lacueva, C., & Tornero, A., 2005. Review: Health effects of cocoa flavonoids. Food Science and Technology International, 11, 159–176.

Lebesi, DM., Tzia, C., 2011. Staling of cereal bran enriched cakes and the effect of an endoxylanase enzyme on the physicochemical and sensorial characteristics. Journal of Food Science, 76 (6), 380-387.

Lecumberri, E., Mateos, R., Izquierdo-Pulido, M., Rupérez, P., Goya, L., Bravo, L., 2007. Dietary fibre composition, antioxidant capacity and physic-chemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L). Food Chemistry, 104, 948-954.

Liu, R. H., 2004. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. Journal of Nutrition, 134 (12), 3479S-3485S.

Locatelli, M., Travaglia, F., Coisson, J. D., Martelli, A., Stevigny, C., Arlorio, M., 2010. Total antioxidant activity of hazelnut skin (Nocciola Piemonte PGI): Impact of different roasting conditions. Food Chemistry. 119, 1647-1655.

Lu, T-M., Lee, C-C., Mau, J-J., Lin, S-D., 2010. Quality and antioxidant property of green tea sponge cake. Food Chemistry, 119, 1090-1095.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L., 2004. Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition, 79, 727-747.

Martinez, R., Torres, P., Meneses, M. A., Figueroa, J. G., Perez-Alvarez, J. A., Viuda-Martos, M., 2012. Chemical, technological and in vitro antioxidant properties of cocoa (Theobroma cacao L.) co-products. Food Research International, 49(1), 39-45.

Masoodi, F. A., Bhavana, S., Chauhan, G. S., 2002. Use of apple pomace as a source of dietary fiber in cakes. Plant Foods for Human Nutrition, 57, 121–128.

Matz, S. A., 1992. Bakery technology and engineering, (3rd ed.), Book News, Inc., USA.

McCrae, C. H., 1994. Homogenization of milk emulsions – use of microfluidizer. Journal of the Society of Dairy Technology, 47, 28–31.

Mert, B., 2012. Using high pressure microfluidization to improve physical properties and lycopene content of ketchup type products. Journal of Food Engineering, 109, 579-587.

Microfluidics, M-110Y Microfluidizer® Materials Processor, from http://microfluidicscorp.com/images/stories/pdf/m-110y.pdf last visited: August, 2013.

Montella, R., Coïsson, J. D., Travaglia, F., Locatelli, M., Malfa, P., Martelli, A., Arlorio, M., 2013. Bioactive compounds from hazelnut skin (Corylus avellana L.): effects on Lactobacillus plantarum P17630 and Lactobacillus crispatus P17631. Journal of Functional Foods, 5, 306-315.

Morton, L. W., Caccetta, R. A., Puddey, I. B., Croft, K. D., 2000. Chemistry and biological effects of dietary phenolic compounds: Relevance to cardiovascular disease. Clin. Exp. Pharmacol. & Physiol., 27, 152–159.

Nandeesh, K., Jyotsna, R., Venkateswara Rao, G., 2011. Effect of differently treated wheat bran on rheology, microstructure and quality characteristics of soft dough biscuits. Journal of Food Processing and Preservation, 35, 179-200.

Nepote, V., Grosso, N. R., Guzmán, C. A., 2002. Extraction of antioxidant components from peanut skins. Grasas y Aceites, 53(4), 391-395.

Bauer, W. and Turler, S., Dietary Fibre and its various health benefits, Nestle Professional Nutrition Magazine 8/08, from http://www.nestleprofessional.com/united states/en/Documents/NUTRIPRO/2808_nutripro_6_dietary_fibres.pdf last visited August, 2013.

Noort, M. W. J., Van Haaster, D., Hemery, Y., Schols, H.A., Hamer, R. J., 2010.The effect of particle size of wheat bran fractions on bread quality - Evidence for fibre-protein interactions. Journal of Cereal Science, 52, 59-64.

Normand, F. L., Ory, R. L., Mod, R. R., 1987. Binding of bile acids and trace minerals by soluble hemicelluloses of rice. Food Technology, 41, 86–99.

Olson, D. W., White, C. H., Watson, C. E., 2003. Properties of frozen dairy desserts processed by microfluidization of their mixes. Journal of Dairy Science, 86, 1157-1162.

Orem, A., Balaban, F., Kural, B. V., Orem, C., Turhan, I., 2008. Hazelnut consumption protect low density lipoprotein (LDL) against oxidation and decrease plasma oxidazed LDL level. In: Proceedings of the 77th Congress of the European Atherosclerosis Society, Istanbul, Turkey, pp. 215.

Orozco, T. J., Wang, J. F., Keen, C. L., 2003. Chronic consumption of a flavonols- and procyanidin-rich diet is associated with reduced levels of 8-hydroxy-20 -deoxyguanosine in rat testes. Journal of Nutritional Biochemistry, 14, 104–110.

Osakabe, N., Yamagishi, M., Sanbogi, C., Natsume, M., Takizawa, T., Osawa, T., 1998. The antioxidative substances in cacao liquor. Journal of Nutrition Science Vitaminol, 53, 4290–4302.

Osella, C. A., Sánchez, H. D., Carrara, C. R., Torre, M. A. D. L., Buera, M. P., 2005. Water Redistribution and Structural Changes of Starch During Storage of a Gluten-free Bread. Starch/Stärke, 57, 208-216.

Othman, A., Ismail, A., Ghani, N. A., Adenan, I., 2007. Antioxidant capacity and phenolic content of cocoa beans. Food Chemistry, 100, 1523-1530.

Ozkaya, B., 1997. Effects of sugar beet dietary fiber of different particle size on rheological properties of dough and quality of bread. Gıda Teknolojisi, 2(9), 57-64.

Ozkoc, S. O., Sumnu, G., Sahin S., 2009a. The effects of gums on macro and micro-structure of breads baked in different ovens. Food Hydrocolloids, 23(8), 2182-2189.

Ozkoc, S. O., Sumnu, G., Sahin, S., Turabi, E., 2009b. Investigation of physicochemical properties of breads baked in microwave and infrared-microwave combination ovens during storage. European Food Research and Technology, 228 (6), 883-893.

Paganga, G., Miller, N., Rice-Evans, C. A., 1999. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute?. Free Radical Research, 30(2), 153-162.

Painter, K. A., 1981. Functions and requirements of fats and emulsifiers in prepared cake mixes. Journal of the American Society of Oil Chemists, 58, 92-95.

Papathanasopoulos, A., Camilleri, M., 2010. Dietary fiber supplements: effects in obesity and metabolic syndrome and relationship to gastrointestinal functions. Gastroenterology,138, 65-72.e2.

Paquin, P., Giasson, J., 1989. Microfluidization as an homogenization process for cream liqueur. Lait, 69, 491-498.

Park, H., Seib, P. A., Chung, O. K., 1997. Fortifying bread with a mixture of wheat fiber and psyllium husk fiber plus three antioxidants. Cereal Chemistry, 74(3), 207–211.

Pereira, D. M., Valentáo, P., Pereira, J. A., Andrade, P. B., 2009. Phenolics: From Chemistry to Biology. Molecules, 14, 2202-2211.

Pomeranz, Y., Shogren, M. D., Finney, K. F., Bechtel, D. B., 1977. Fiber in breadmaking—effects on functional properties. Cereal Chemistry, 54(1), 25–41.

Proteggente, A. R., Pannala, A. S., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S.,Van de Put, F., Dacombe, C., Rice-Evans, C. A., 2002. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C consumption. Free Radical Research, 36(2), 217-233.

Raghavendra, S. N., Ramachandra Swamy, S. R., Rastogi, N. K., Raghavarao, K. S. M. S., Kumar, S., Tharanathan, R. N., 2006. Grinding characteristics and hydration properties of coconut residue: A source of dietary fiber. Journal of Food Engineering, 72, 281–286.

Resurreccion, A. V. A., 2008. Consumer Sensory Testing for Food Product Development. In: Developing New Food Products for a Changing Marketplace, (2nd ed.), (Eds.) Aaron L. Brody and John B. Lord, CRC Press Taylor and Francis Group, Florida, USA.

Riaz, M. N., 2001. Uses and benefits of soy fiber. Cereal Foods World, 46(3), 98–100.

Ribotta, P. D., Cuffini, S., León, A. E., 2004. The staling of bread: X-ray diffraction study. European Food and Research Technology, 218, 219-223.

Rice-Evans, C. A., Miller, N. J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology & Medicine, 20, (7), 933-956.

Rice-Evans, C. A., Miller, N. J., Paganga, G., 1997. Antioxidant properties of phenolic compounds. Trends in Plant Science, 2(4), 152-159.

Richardson, D. G., 1997. The health benefits of eating hazelnuts: Implications for blood lipid profiles, coronary heart disease, and cancer risks. Acta Horticulturae (ISHS), 445, 295–300.

Rio, D. D., Calani, L., Dall'Asta, M., Brighenti, F., 2011. Polyphenolic composition of hazelnut skin. Journal of Agricultural and Food Chemistry, 59(18), 9935-9941.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., and Glover, W., 1999. Phenolic compounds and their role in oxidative processes in fruits. Food Chemistry, 66, 401–436.

Rosell, C. M., Santos, E., 2010. Impact of fibers on physical characteristics of fresh and staled bake off bread. Journal of Food Engineering, 98, 273-281.

Rosell, C. M., Santos, E., Collar, C., 2009. Physico-chemical properties of dietary fibers from different sources: a comparative approach. Food Research International, 42, 176-184.

Roy, M. K., Takenaka, M., Isobe, S., Tsushida, T., 2007. Antioxidant potential, anti-proliferative activities, and phenolic content in water-soluble fractions of some commonly consumed vegetables: effect of thermal treatment. Food Chemistry 103, 106-114.

Rupasinghe, H. P. V., Wang, L. X., Pitts, N. L., & Astatkie, T., 2009. Baking and sensory characteristics of muffins incorporated with apple skin powder. Journal of Food Quality, 32, 685-694.

Sánchez-Pardo, M. E., Ortiz-Moreno, A., Mora-Escobedo, R., Chanona-Peréz, J. J., Necoechea-Mondragón, H., 2008. Comparison of crumb microstructure from pound cakes baked in a microwave or conventional oven. LWT- Food Science and Technology, 41, 620-627.

Sangnark, A., Noomhorm, A., 2003. Effect of particle sizes on functional properties of dietary fibre prepared from sugarcane bagasse. Food Chemistry, 80, 221-229.

Saura-Calixto, F., 1998. Antioxidant dietary fiber product: a new concept and a potential food ingredient. J. Agric. Food Chem, 46, 4303-4306.

Scalbert, A. and Williamson, G., 2000. Dietary intake and bioavailability of polyphenols. Journal of Nutrition, 130(8), 2073S–2085S.

Scanlon, M. G., Zghal, M. C., 2001. Bread properties and crumb structure. Food Research International 34, 841–864.

Schinella, G., Mosca, S., Cienfuegos-Jovellanos, E., Pasamar, M. A., Muguerza, B., Ramón, D., Ríos, J. L., 2010. Antioxidant properties of polyphenol-rich cocoa products industrially processed. Food Research International, 43, 1614-1623.

Schmitzer, V., Slatnar, A., Veberic, R., Stampar, F., Solar, A., 2011. Roasting affects phenolic composition and antioxidative activity of hazelnuts (Corylus avellana L.). Journal of Food Science, 76(1), 14-19.

Seyhun, N., Sumnu, G., Sahin, S., 2005. Effects of different starch types on retardation of staling of microwave-baked cakes, Foods and Bioproducts Processing, 83(1), 1-5.

Shahidi, F., Alasalvar, C., Liyana-Pathirana, C. M., 2007. Antioxidant Phytochemicals in Hazelnut Kernel (Corylus avellana L.) and hazelnut byproducts.Journal of Agricultural and Food Chemistry, 55, 1212−1220.

Shahidi, F., Naczk, M., 1995. Food phenolics, sources, chemistry, effects, applications. Lancaster, PA: Technomic Publishing Co Inc.

Kabir, Y. and Sidhu, J. S., 2011. Antioxidant Functional Factors in Nuts. In: Functional Foods of the East, (Eds.) Shi, J., Ho, C-T., Shahidi, F., CRC Press, Taylor and Francis Group, FL, USA, pp. 343-399.

Sahi, S. S., 2008. Cake Emulsions. In: Food Engineering Aspects of Baking Sweet Goods, (Eds.) Sumnu, G. and Sahin, S., CRC Press Taylor and Francis Group, FL, USA, pp. 81-99.

Sahin, S., 2008. Cake Batter Rheology. In: Food Engineering Aspects of Baking Sweet Goods, (Eds.) Sumnu, G. and Sahin, S., CRC Press Taylor and Francis Group, FL, USA, pp. 99-121.

Sies, H., Schewe, T., Heiss, C., Kelm, M., 2005. Cocoa polyphenols and inflammatory meditors.American Journal of Clinical Nutrition, 81, S304–S312.

Singh, M., Liu, S. X., Vaughn, S. F., 2013. Effect of corn bran particle size on rheology and pasting characteristics of flour gels. Biocatalysis and Agricultural Biotechnology, 2(2), 138-142.

Sivam, A. S., Sun-Waterhouse, D., Quek, SY., Perera, C. O., 2010. Properties of bread dough with added fiber polysaccharides and phenolic antioxidants: A Review. Institute of Food Technologists, 75(8), R163-R174.

Smits, A. L. M., Ruhnau, F. C., Vliegenthart, J. F. G., Van Soest, J. J. G., 1998. Ageing of starch based systems as observed with FT-IR and solid state NMR spectroscopy. Starch/StaÈrke, 50, 478-483.

Soong, Y-Y., Barlow, P.J., 2004. Antioxidant activity and phenolic content of selected fruit seeds. Food Chemistry, 88(3), 411-417.

Sozer, N., Bruins, R., Dietzel, C., Franke, W., Kokini, J. L., 2011. Improvement of shelf life stability of cakes. Journal of Food Quality, 34, 151-162.

Sreenath, H. K., Sudarshanakrishna, K. R., Prasad, N. N., Santhanam, K., 1996. Characteristics of some fiber incorporated cake preparations and their dietary fiber content. Starch, 48(2), 72-76.

Stratil, P., Klejdus, B., Kubán, V., 2007. Determination of phenolic compounds and their antioxidant activity in fruits and cereals. Talanta, 71(4), 1741-1751.

Sudha, M. L., Baskaran, V., Leelavathi, K., 2007. Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. Food Chemistry, 104, 686-692.

Sumnu, G., Koksel, F., Sahin, S., Basman, A., Meda, V., 2010. The effects of xanthan and guar gums on staling of gluten-free rice cakes baked in differet ovens. International Journal of Food Science and Technology 45(1), 87-93.

Tabilo-Munizaga, G. and Barbosa-Canovas, G. V., 2005. Rheology for the food industry. Journal of Food Engineering, 67, 147–156.

Tomás-Barberán, F. A., Cienfuegos-Jovellanos, E., Marín, A., Muguerza, B., GilIzquierdo, A., Cerdá, B., et al., 2007. A new process to develop a cocoa powder with higher flavonoid monomer content and enhanced bioavailability in healthy humans. Journal of Agricultural and Food Chemistry, 55, 3926–3935.

Tunick, M. H., Van Hekken, D. L., Cooke, P. H., Smith, P. W., Malin, E. L., 2000. Effect of high pressure microfluidization on microstructure of mozzarella cheese. Lebensmittel-Wissenschaft und-Technologie: Food Science and Technology, 33, 538-544.

Turabi, E., 2010. Design of gluten free rice cake formulations for baking in infrared microwave combination oven, MS Thesis, METU, Ankara.

Turabi, E., Sumnu, G., Sahin, S. 2010. Quantitative analysis of macro and micro-structure of gluten-free rice cakes containing different types of gums baked in different ovens. Food Hydrocolloids, 24, 755-762.

Turkmen, N., Sari, F., Velioglu, Y. S., 2005. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chemistry 93(4), 713-718.

Turkmen, N., Velioglu, Y. S., Sari, F., Polat, G., 2007. Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. Molecules, 12(3), 484-496.

USDA, The Food Supply and Dietary Fiber: Its Availability and Effect on Health, Nutrition Insight 36, from http://www.cnpp.usda.gov/Publications/NutritionInsights/Insight36.pdf last visited: August, 2013.

Vasco, C., Ruales, J., Kamal-Eldin, A., 2008. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. Food Chemistry, 111, 816-823.

Veeriah, S., Kautenburger, T., Habermann, N., Sauer, J., Dietrich, H., Will, F., Pool-Zobel, B. L., 2006. Apple flavonoids inhibit growth of HT29 human colon cancer cells and modulate expression of genes involved in the biotransformation of xenobiotics. Molecular Carcinogenesis, 45, 164-174.

Velioglu, S., 2007. Farklı çay ekstraktlarının antioksidan, antibakteriyal etkileri ve fenolik madde dagılımının HPLC ile belirlenmesi, T.C. Ankara Üniversitesi Bilimsel Araştırma Projesi Kesin Raporu.

Velioglu, Y. S., Mazza, G., Gao, L., Oomah, B.D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry, 46, 4113–4117.

Vinson, J. A., Hap, Y., Su, X., Zubik, L., 1998. Phenolic antioxidant quantity and quality in foods: vegetables. Journal of Agricultural and Food Chemistry, 46, 3630–3634.

Wang-Polagruto, J. F., Villablanca, A. C., Polagruto, J. A., Lee, L., Holt, R. R., Schrader, H. R., Ensunsa, J. L., Steinberg, F. M., Schmitz, H. H., Keen, C. L., 2006. Chronic consumption of flavanol-rich cocoa improves endothelial function and decreases vascular cell adhesion molecule in hypercholesterolemic postmenopausal women. Journal of Cardiovascular Pharmacology, 47, Suppl. 2, S177-S186; discussion S206–S179.

Wang, T., Sun, X., Zhou, Z., Chen, G., 2012. Effects of microfluidization process on physicochemical properties of wheat bran. Food Research International 48(2), 742–747.

Wollgast, J. and Anklam, E., 2000. Review on polyphenols in *Theobroma cacao*: changes in composition during the manufacture of chocolate and methodology for identification and quantification. Food Research International, 33(6), 423-447.

Wollgast, J. and Anklam, E., 2000. Polyphenols in chocolate: Is there a contribution to human health?, Food Research International, 33, 449-459.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D.B., Gebhardt, S.E., Prior, R.L., 2006. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. Journal of Agricultural and Food Chemistry, 54, 4069-4075.

Yamagishi, M., Natsume, M., Magaki, A., Adachi, T., Osakabe, N., Takizawa, T., et al., 2000. Antimutagenic activity of cacao: inhibitory effect of cacao liquor polyphenols on the mutagenic action of heterocyclic amines. Journal of Agricultural and Food Chemistry, 48, 5074–5078.

Yardim-Akaydin, S., Ozkan, Y., Ozkan, E., Torun, M., Simsek, B., 2003. The role of plasma thiol compounds and antioxidant vitamins in patients with cardiovascular diseases. Clinica Chimica Acta, 338, 99-105.

Yurttas, H. C., Schafer, H. W., Warthesen, J.J., 2000. Antioxidant activity of nontocopherol hazelnut (*Corylus spp*.) phenolics. Journal of Food Science, 65(2), 276–280.

Zahn, S., Pepke, F., Rohm, H., 2010. Effect of inulin as a fat replacer on texture and sensory properties of muffins. International Journal of Food Science and Technology, 45, 2531-2537.

Zha, X-Q., Wang, J-H., Yang, X-F., Liang, H., Zhao, L-L., Bao, S-H., Luo, J-P., Xu, Y-Y., Zhou, BB., 2009. Antioxidant properties of polysaccharide fraction with different molecular mass extracted with hot-water from rice bran. Carbohydrate Polymers, 78, 570–575.

Zhang, D. and Moore, W. R., 1997. Effect of wheat bran particle size on dough rheological properties. Journal of the Science of Food and Agriculture, 74, 490-496.

Zhu, KX., Huang, S., Peng, W., Qian, HF., Zhou, HM., 2010. Effect of ultrafine grinding on hydration and antioxidant properties of wheat bran dietary fiber. Food Research International, 43(4), 943-948.

APPENDIX A

PICTURES OF SAMPLES

Figure A.4 Picture of cocoa powder and hazelnut skin processed by microfluidizer

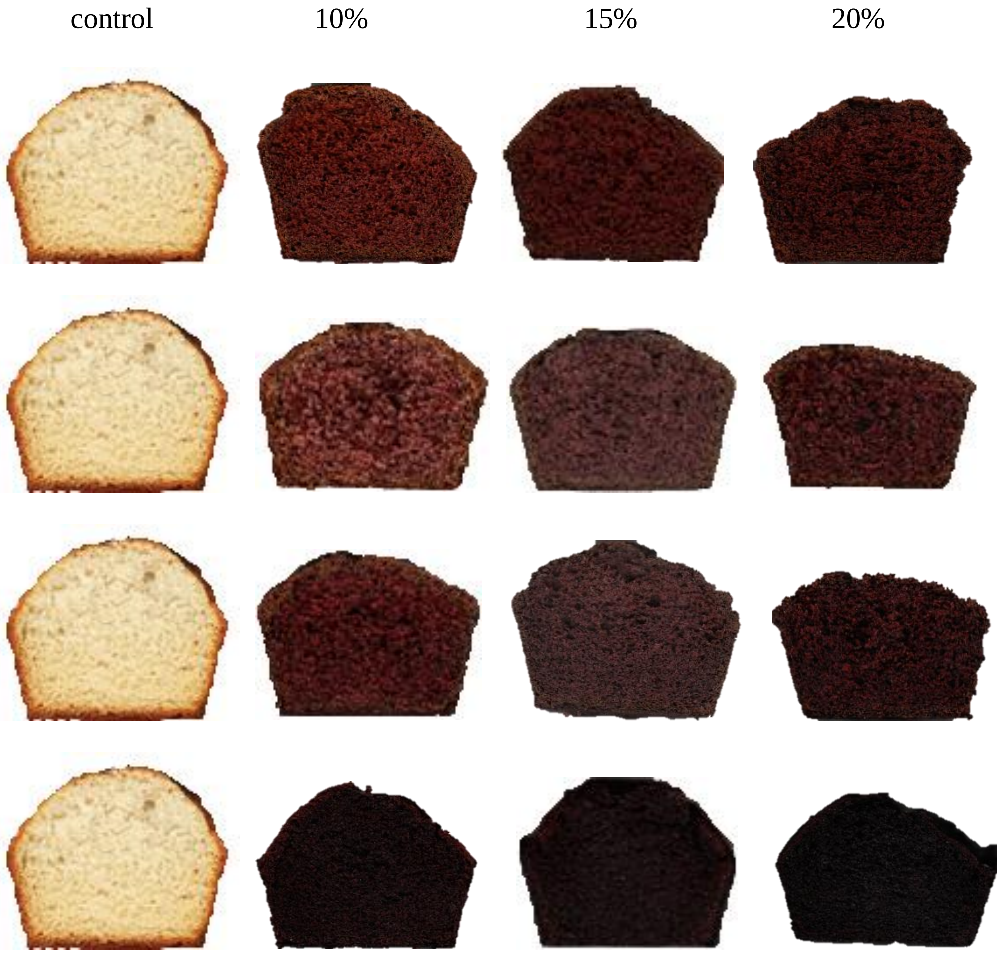

Figure A.2 Photographs of cake samples based on 100g flour formulation. From top to bottom pictures represent cake samples prepared with cocoa powder, raw HS, ball milled HS and MF HS fiber, respectively.

Figure A.3 Photographs of cake samples containing MF HS fiber with flour reduction. From left to right pictures represent cake samples prepared with 85g flour (standard MF 15% cake), 65g flour, 45g flour and 25g flour, respectively.

APPENDIX B

STATISTICAL ANALYSES

Table B.1 Results for Tukey's mean comparison test for weight loss of cake samples

General Linear Model: weight loss 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 weight loss, using Adjusted SS for Tests

Source             DF    Seq SS   Adj SS   Adj MS      F      P
percentage          3   2,56337  2,56337  0,85446  15,94  0,000
sample              3   6,15712  6,15712  2,05237  38,29  0,000
percentage*sample   9   2,10243  2,10243  0,23360   4,36  0,000
Error              48   2,57292  2,57292  0,05360
Total              63  13,39583


S = 0,231522   R-Sq = 80,79%   R-Sq(adj) = 74,79%


Unusual Observations for weight loss

Obs  weight loss      Fit   SE Fit  Residual  St Resid
 22      9,66667  9,22917  0,11576   0,43750      2,18 R
 43      9,50000  9,08333  0,11576   0,41667      2,08 R
 54      8,66667  9,22917  0,11576  -0,56250     -2,81 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

percentage   N  Mean  Grouping
 0          16   9,3  A
10          16   9,0    B
15          16   8,9    B
20          16   8,8    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

sample      N  Mean  Grouping
raw        16   9,2  A
cacao      16   9,2  A
ball mill  16   9,1  A
mf         16   8,5    B

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          raw        4   9,3  A
 0          mf         4   9,3  A
 0          cacao      4   9,3  A
 0          ball mill  4   9,3  A
10          raw        4   9,2  A
10          cacao      4   9,2  A
10          ball mill  4   9,2  A
15          cacao      4   9,2  A
15          raw        4   9,1  A
20          raw        4   9,1  A
15          ball mill  4   9,1  A
20          cacao      4   9,0  A
20          ball mill  4   9,0  A
10          mf         4   8,2    B
15          mf         4   8,2    B
20          mf         4   8,0    B

Means that do not share a letter are significantly different.
```

Table B.2 Results for Tukey's mean comparison test for total color change in cake 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    3805,8   3805,8  1902,9   69,75  0,000
sample               3   23727,0  23727,0  7909,0  289,90  0,000
percentage*sample    6    1633,5   1633,5   272,3    9,98  0,000
Error              132    3601,2   3601,2    27,3
Total              143   32767,5


S = 5,22318   R-Sq = 89,01%   R-Sq(adj) = 88,09%


Unusual Observations for total color change

     total color
Obs       change      Fit  SE Fit  Residual  St Resid
 49      65,4696  55,2997  1,5078   10,1700      2,03 R
 67      68,3514  54,8714  1,5078   13,4800      2,70 R
 75      64,7898  76,7121  1,5078  -11,9222     -2,38 R
 81      74,2852  61,7021  1,5078   12,5831      2,52 R
107      66,3994  77,2847  1,5078  -10,8854     -2,18 R
```

```
R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

percentage   N  Mean  Grouping
20          48  66,3  A
15          48  60,3    B
10          48  53,8      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  74,6  A
cacao      36  67,9    B
ball mill  36  57,3      C
raw        36  40,6        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          mf         12  80,8  A
15          mf         12  77,3  A B
20          cacao      12  76,7  A B
15          cacao      12  71,7    B C
10          mf         12  65,8      C D
20          ball mill  12  61,7        D E
15          ball mill  12  55,5          E
10          cacao      12  55,3          E
10          ball mill  12  54,9          E
20          raw        12  46,2            F
10          raw        12  39,1            F G
15          raw        12  36,6              G

Means that do not share a letter are significantly different.
```

Table B.3 Results for Tukey's mean comparison test for firmness values of fresh cake samples based on 100g flour formulation

General Linear Model: firmness 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 firmness, using Adjusted SS for Tests

Source              DF   Seq SS  Adj SS  Adj MS        F      P
percentage           3   297018  297018   99006   992,79  0,000
```

```
sample               3   612826  612826  204275  2048,38  0,000
percentage*sample    9   336904  336904   37434   375,37  0,000
Error              112    11169   11169     100
Total              127  1257917


S = 9,98624   R-Sq = 99,11%   R-Sq(adj) = 98,99%


Unusual Observations for firmness

Obs  firmness      Fit  SE Fit  Residual  St Resid
 31   296,989  271,891   3,531    25,098      2,69 R
 47   231,772  271,891   3,531   -40,119     -4,29 R
 62   220,473  246,042   3,531   -25,569     -2,74 R
 78   271,830  246,042   3,531    25,788      2,76 R
 94   282,231  246,042   3,531    36,189      3,87 R
112   498,190  467,348   3,531    30,842      3,30 R
126   220,473  246,042   3,531   -25,569     -2,74 R
128   436,417  467,348   3,531   -30,931     -3,31 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

percentage   N   Mean  Grouping
20          32  220,7  A
15          32  152,5    B
10          32  141,2      C
 0          32   85,2        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         32  267,6  A
ball mill  32  129,7    B
raw        32  109,0      C
cacao      32   93,3        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         8  467,3  A
15          mf         8  271,9    B
10          mf         8  246,0      C
20          ball mill  8  175,6        D
20          raw        8  134,0          E
15          ball mill  8  132,2          E
10          ball mill  8  125,6          E F
15          raw        8  112,7            F G
20          cacao      8  105,8              G H
10          raw        8  104,1              G H
15          cacao      8   93,0                H I
10          cacao      8   89,0                H I
 0          mf         8   85,2                  I
```

```
 0          raw        8   85,2                  I
 0          ball mill  8   85,2                  I
 0          cacao      8   85,2                  I

Means that do not share a letter are significantly different.
```

Table B.4 Results for Tukey's mean comparison test for springiness values of fresh cake samples based on 100g flour formulation

General Linear Model: springiness 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 springiness, using Adjusted SS for Tests

Source             DF   Seq SS  Adj SS  Adj MS       F      P
percentage          3   105,30  105,30   35,10   12,52  0,000
sample              3   962,07  962,07  320,69  114,35  0,000
percentage*sample   9   442,11  442,11   49,12   17,52  0,000
Error             112   314,11  314,11    2,80
Total             127  1823,58


S = 1,67468   R-Sq = 82,78%   R-Sq(adj) = 80,47%


Unusual Observations for springiness

Obs  springiness      Fit  SE Fit  Residual  St Resid
 14      52,9640  57,2420  0,5921   -4,2780     -2,73 R
 30      60,4180  57,2420  0,5921    3,1760      2,03 R
 46      63,9710  57,2420  0,5921    6,7290      4,30 R
 56      44,3510  47,6936  0,5921   -3,3426     -2,13 R
 75      49,6940  46,1096  0,5921    3,5844      2,29 R
112      44,7900  50,8835  0,5921   -6,0935     -3,89 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

percentage   N  Mean  Grouping
10          32  49,8  A
15          32  49,1  A B
 0          32  48,1    B C
20          32  47,4      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         32  53,2  A
```

```
raw        32  47,9    B
ball mill  32  47,0    B C
cacao      32  46,2      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

percentage  sample     N  Mean  Grouping
10          mf         8  57,2  A
15          mf         8  56,6  A
20          mf         8  50,9    B
 0          mf         8  48,1    B C
 0          raw        8  48,1    B C
 0          cacao      8  48,1    B C
 0          ball mill  8  48,1    B C
10          raw        8  48,0    B C
15          raw        8  47,9      C
10          ball mill  8  47,8      C  D
20          raw        8  47,7      C  D
15          ball mill  8  46,1      C  D
20          ball mill  8  46,1      C  D
10          cacao      8  46,1      C  D
15          cacao      8  45,6      C  D
20          cacao      8  44,9         D

Means that do not share a letter are significantly different.
```

Table B.5 Results for Tukey's mean comparison test for firmness values of cakes based on 100g flour formulation and stored for 3 days

General Linear Model: firmness 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 firmness, using Adjusted SS for Tests

Source              DF   Seq SS   Adj SS  Adj MS        F      P
percentage           3   857030   857030  285677  1002,61  0,000
sample               3  1470972  1470972  490324  1720,85  0,000
percentage*sample    9   764836   764836   84982   298,25  0,000
Error              112    31912    31912     285
Total              127  3124749


S = 16,8799   R-Sq = 98,98%   R-Sq(adj) = 98,84%


Unusual Observations for firmness

Obs  firmness      Fit  SE Fit  Residual  St Resid
 23   255,614  222,278   5,968    33,336      2,11 R
```

31	496,978	460,351	5,968	36,627	2,32	R
39	255,192	222,278	5,968	32,914	2,08	R
40	278,526	244,398	5,968	34,128	2,16	R
55	187,300	222,278	5,968	-34,978	-2,22	R
59	297,181	262,907	5,968	34,274	2,17	R
64	804,751	765,173	5,968	39,578	2,51	R
72	196,233	244,398	5,968	-48,165	-3,05	R
79	492,480	460,351	5,968	32,129	2,03	R
128	800,113	765,173	5,968	34,940	2,21	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

percentage	N	Mean	Grouping
20	32	398,0	A
15	32	286,9	B
10	32	261,4	C
0	32	168,4	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	32	460,3	A
ball mill	32	254,3	B
raw	32	202,7	C
cacao	32	197,4	C

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	8	765,2	A
15	mf	8	460,4	B
10	mf	8	447,5	B
20	ball mill	8	357,2	C
15	ball mill	8	262,9	D
20	raw	8	244,4	D E
10	ball mill	8	228,5	E F
20	cacao	8	225,4	E F
15	raw	8	222,3	E F G
15	cacao	8	202,2	F G H
10	cacao	8	193,8	G H I
10	raw	8	175,8	H I
0	mf	8	168,4	I
0	raw	8	168,4	I
0	ball mill	8	168,4	I
0	cacao	8	168,4	I

Means that do not share a letter are significantly different.

Table B.6 Results for Tukey‘s mean comparison test for firmness values of cakes based on 100g flour formulation and stored for 6 days

General Linear Model: firmness 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 firmness, using Adjusted SS for Tests

Source              DF   Seq SS   Adj SS   Adj MS        F      P
percentage           3  1443836  1443836   481279  1151,41  0,000
sample               3  3250677  3250677  1083559  2592,30  0,000
percentage*sample    9  1968651  1968651   218739   523,31  0,000
Error              112    46815    46815      418
Total              127  6709979


S = 20,4448   R-Sq = 99,30%   R-Sq(adj) = 99,21%


Unusual Observations for firmness

Obs  firmness      Fit  SE Fit  Residual  St Resid
 39    539,92   584,65    7,23    -44,73     -2,34 R
 48    236,34   274,74    7,23    -38,40     -2,01 R
 57   1197,92  1149,17    7,23     48,75      2,55 R
 69    405,37   444,38    7,23    -39,02     -2,04 R
 73   1057,00  1149,17    7,23    -92,17     -4,82 R
 89   1195,53  1149,17    7,23     46,36      2,42 R
117    488,02   444,38    7,23     43,63      2,28 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

percentage   N   Mean  Grouping
20          32  559,0  A
15          32  398,1    B
10          32  348,5      C
 0          32  268,2        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         32  663,8  A
ball mill  32  356,1    B
raw        32  279,5      C
cacao      32  274,5      C

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          8  1149,2  A
15          mf          8   653,0    B
10          mf          8   584,6      C
20          ball mill   8   444,4        D
15          ball mill   8   398,2          E
20          raw         8   352,4            F
10          ball mill   8   313,6              G
20          cacao       8   290,0              G H
15          raw         8   274,7                H
10          cacao       8   273,1                H
 0          mf          8   268,2                H
 0          raw         8   268,2                H
 0          ball mill   8   268,2                H
 0          cacao       8   268,2                H
15          cacao       8   266,6                H
10          raw         8   222,8                  I

Means that do not share a letter are significantly different.
```

Table B.7 Results for Tukey's mean comparison test for springiness values of cakes based on 100g flour formulation and stored for 3 days

General Linear Model: springiness 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 springiness, using Adjusted SS for Tests

Source              DF   Seq SS   Adj SS  Adj MS       F      P
percentage           3  1624,52  1624,52  541,51  212,04  0,000
sample               3  2119,05  2119,05  706,35  276,59  0,000
percentage*sample    9   799,34   799,34   88,82   34,78  0,000
Error              112   286,02   286,02    2,55
Total              127  4828,93


S = 1,59804   R-Sq = 94,08%   R-Sq(adj) = 93,28%


Unusual Observations for springiness

Obs  springiness      Fit  SE Fit  Residual  St Resid
 14      60,5660  57,0326  0,5650    3,5334      2,36 R
 80      52,7640  56,6318  0,5650   -3,8678     -2,59 R
 92      44,4440  47,7024  0,5650   -3,2584     -2,18 R
 95      62,3260  59,0463  0,5650    3,2797      2,19 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

percentage   N  Mean  Grouping
```

20	32	49,1	A
15	32	49,0	A
10	32	47,3	B
0	32	40,4	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	32	53,3	A
raw	32	45,2	B
ball mill	32	44,8	B
cacao	32	42,6	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

percentage	sample	N	Mean	Grouping
15	mf	8	59,0	A
10	mf	8	57,0	A
20	mf	8	56,6	A
20	raw	8	48,8	B
20	ball mill	8	47,7	B C
15	raw	8	47,7	B C
15	ball mill	8	45,6	C D
10	ball mill	8	45,3	C D
10	raw	8	43,9	D
15	cacao	8	43,7	D
20	cacao	8	43,3	D
10	cacao	8	42,9	D E
0	raw	8	40,4	E
0	mf	8	40,4	E
0	ball mill	8	40,4	E
0	cacao	8	40,4	E

Means that do not share a letter are significantly different.

Table B.8 Results for Tukey‘s mean comparison test for springiness values of cakes based on 100g flour formulation and stored for 6 days

General Linear Model: springiness 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 springiness, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
percentage	3	1855,72	1855,72	618,57	228,29	0,000
sample	3	1693,77	1693,77	564,59	208,37	0,000
percentage*sample	9	659,35	659,35	73,26	27,04	0,000
Error	112	303,47	303,47	2,71		

```
Total              127  4512,32


S = 1,64607   R-Sq = 93,27%   R-Sq(adj) = 92,37%


Unusual Observations for springiness

Obs  springiness      Fit  SE Fit  Residual  St Resid
  4      45,2530  42,0222  0,5820    3,2308      2,10 R
 27      41,5110  45,9361  0,5820   -4,4251     -2,87 R
106      45,8250  41,9323  0,5820    3,8927      2,53 R
120      49,0980  45,9465  0,5820    3,1515      2,05 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

percentage   N  Mean  Grouping
15          32  46,8  A
20          32  46,5  A
10          32  44,2    B
 0          32  37,4      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         32  49,9  A
ball mill  32  42,6    B
raw        32  42,2    B
cacao      32  40,3      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

percentage  sample     N  Mean  Grouping
15          mf         8  56,4  A
10          mf         8  52,9    B
20          mf         8  52,7    B
20          raw        8  45,9      C
15          ball mill  8  45,9      C
20          ball mill  8  45,3      C
15          raw        8  43,2      C D
10          raw        8  42,3        D E
20          cacao      8  42,0        D E
10          ball mill  8  41,9        D E
15          cacao      8  41,9        D E
10          cacao      8  39,8          E F
 0          raw        8  37,4            F
 0          mf         8  37,4            F
 0          ball mill  8  37,4            F
 0          cacao      8  37,4            F

Means that do not share a letter are significantly different.
```

Table B.9 Results for Tukey‘s mean comparison test for moisture content of cake samples based on 100g flour formulation

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	3	0; 3; 6

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

Source	DF	Seq SS	Adj SS	Adj MS	F	P
percentage	3	86,382	86,382	28,794	105,08	0,000
sample	3	21,681	21,681	7,227	26,37	0,000
day	2	3053,435	3053,435	1526,718	5571,69	0,000
percentage*sample	9	9,677	9,677	1,075	3,92	0,000
percentage*day	6	30,115	30,115	5,019	18,32	0,000
sample*day	6	4,374	4,374	0,729	2,66	0,018
percentage*sample*day	18	3,406	3,406	0,189	0,69	0,816
Error	144	39,458	39,458	0,274		
Total	191	3248,528				

S = 0,523463   R-Sq = 98,79%   R-Sq(adj) = 98,39%

Unusual Observations for moisture content

Obs	moisture content	Fit	SE Fit	Residual	St Resid	
8	32,1100	30,9625	0,2617	1,1475	2,53	R
65	26,2900	25,2225	0,2617	1,0675	2,35	R
69	26,2900	25,2225	0,2617	1,0675	2,35	R
73	26,2900	25,2225	0,2617	1,0675	2,35	R
77	26,2900	25,2225	0,2617	1,0675	2,35	R
81	26,3500	25,2225	0,2617	1,1275	2,49	R
85	26,3500	25,2225	0,2617	1,1275	2,49	R
89	26,3500	25,2225	0,2617	1,1275	2,49	R
93	26,3500	25,2225	0,2617	1,1275	2,49	R
97	24,1400	25,2225	0,2617	-1,0825	-2,39	R
101	24,1400	25,2225	0,2617	-1,0825	-2,39	R
105	24,1400	25,2225	0,2617	-1,0825	-2,39	R
109	24,1400	25,2225	0,2617	-1,0825	-2,39	R
112	20,3500	21,3900	0,2617	-1,0400	-2,29	R
113	24,1100	25,2225	0,2617	-1,1125	-2,45	R
117	24,1100	25,2225	0,2617	-1,1125	-2,45	R
120	23,6300	22,7150	0,2617	0,9150	2,02	R
121	24,1100	25,2225	0,2617	-1,1125	-2,45	R

125	24,1100	25,2225	0,2617	-1,1125	-2,45 R
132	22,2900	21,3200	0,2617	0,9700	2,14 R
164	22,2700	21,3200	0,2617	0,9500	2,10 R
180	20,2800	21,3200	0,2617	-1,0400	-2,29 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	26,3	A
10	48	25,0	B
15	48	24,8	B C
20	48	24,5	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

sample	N	Mean	Grouping
raw	48	25,5	A
cacao	48	25,4	A B
ball mill	48	25,2	B
mf	48	24,6	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	64	30,7	A
3	64	23,1	B
6	64	21,6	C

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	raw	12	26,3	A
0	ball mill	12	26,3	A
0	mf	12	26,3	A
0	cacao	12	26,3	A
10	raw	12	25,3	B
10	cacao	12	25,2	B C
10	ball mill	12	25,2	B C
15	raw	12	25,2	B C
20	raw	12	25,1	B C
15	cacao	12	25,1	B C
20	cacao	12	25,0	B C
15	ball mill	12	24,8	B C
10	mf	12	24,5	C D
20	ball mill	12	24,4	C D
15	mf	12	24,0	D E
20	mf	12	23,6	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

percentage	day	N	Mean	Grouping
0	0	16	31,3	A
10	0	16	30,9	A B
15	0	16	30,4	B
20	0	16	30,3	B
0	3	16	25,2	C
10	3	16	22,7	D
15	3	16	22,4	D
0	6	16	22,3	D
20	3	16	22,3	D
10	6	16	21,5	E
15	6	16	21,4	E
20	6	16	21,1	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

sample	day	N	Mean	Grouping
cacao	0	16	31,1	A
raw	0	16	31,1	A
ball mill	0	16	30,8	A
mf	0	16	29,8	B
cacao	3	16	23,4	C
raw	3	16	23,4	C
ball mill	3	16	23,1	C D
mf	3	16	22,7	D
raw	6	16	21,8	E
ball mill	6	16	21,6	E
cacao	6	16	21,6	E
mf	6	16	21,3	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
0	raw	0	4	31,3	A
0	mf	0	4	31,3	A
0	ball mill	0	4	31,3	A
0	cacao	0	4	31,3	A
10	cacao	0	4	31,2	A
10	raw	0	4	31,1	A
15	cacao	0	4	31,1	A
10	ball mill	0	4	31,1	A
15	raw	0	4	31,0	A
20	cacao	0	4	31,0	A
20	raw	0	4	31,0	A
15	ball mill	0	4	30,5	A B
20	ball mill	0	4	30,4	A B
10	mf	0	4	30,2	A B C
15	mf	0	4	29,1	B C
20	mf	0	4	28,8	C
0	raw	3	4	25,2	D
0	ball mill	3	4	25,2	D
0	mf	3	4	25,2	D
0	cacao	3	4	25,2	D

10	raw	3	4	22,9	E
10	cacao	3	4	22,9	E F
15	cacao	3	4	22,8	E F G
15	raw	3	4	22,8	E F G
20	cacao	3	4	22,8	E F G
20	raw	3	4	22,7	E F G
10	ball mill	3	4	22,7	E F G
0	ball mill	6	4	22,3	E F G H
0	cacao	6	4	22,3	E F G H
0	raw	6	4	22,3	E F G H
0	mf	6	4	22,3	E F G H
10	mf	3	4	22,3	E F G H I
15	ball mill	3	4	22,2	E F G H I J
20	ball mill	3	4	22,1	E F G H I J
15	mf	3	4	21,8	E F G H I J
10	raw	6	4	21,8	E F G H I J
10	ball mill	6	4	21,7	E F G H I J
15	ball mill	6	4	21,7	E F G H I J
15	raw	6	4	21,7	E F G H I J
20	raw	6	4	21,6	E F G H I J
10	cacao	6	4	21,4	F G H I J
15	cacao	6	4	21,4	G H I J
20	mf	3	4	21,4	G H I J
20	cacao	6	4	21,3	G H I J
15	mf	6	4	21,1	H I J
10	mf	6	4	21,0	H I J
20	ball mill	6	4	20,8	I J
20	mf	6	4	20,7	J

Means that do not share a letter are significantly different.

Table B.10 Results for Tukey's mean comparison test for firmness values of fresh cake samples with flour reduction

One-way ANOVA: firmness versus mf samples

Source	DF	SS	MS	F	P
mf samples	3	136662	45554	83,71	0,000
Error	28	15237	544		
Total	31	151899			

S = 23,33   R-Sq = 89,97%   R-Sq(adj) = 88,89%

```
                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level        N    Mean  StDev   -------+---------+---------+---------+-
-
25 g flour  8   92,66  12,21   (-*--)
45 g flour  8  160,00  32,03              (--*-)
65 g flour  8  205,18  24,23                     (--*--)
85 g flour  8  271,89  20,37                                (--*--)
                                -------+---------+---------+---------+-
-
                                     120       180       240       300

Pooled StDev = 23,33
```

```
Grouping Information Using Tukey Method

mf samples  N    Mean  Grouping
85 g flour  8  271,89  A
65 g flour  8  205,18    B
45 g flour  8  160,00      C
25 g flour  8   92,66        D

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of mf samples

Individual confidence level = 98,92%


mf samples = 25 g flour subtracted from:

mf samples   Lower  Center   Upper  ----+---------+---------+--------
-+-----
45 g flour   35,51   67,34   99,18                  (--*--)
65 g flour   80,69  112,52  144,36                      (--*--)
85 g flour  147,40  179,23  211,07                             (--
*--)
                                    ----+---------+---------+--------
-+-----
                                     -100         0       100
200


mf samples = 45 g flour subtracted from:

mf samples  Lower  Center   Upper  ----+---------+---------+---------
+-----
65 g flour  13,34   45,18   77,02              (---*--)
85 g flour  80,05  111,89  143,73                     (--*--)
                                   ----+---------+---------+---------
+-----
                                    -100         0       100
200


mf samples = 65 g flour subtracted from:

mf samples  Lower  Center  Upper  ----+---------+---------+---------
+-----
85 g flour  34,87   66,71  98,54                  (---*--)
                                  ----+---------+---------+---------
+-----
                                   -100         0       100       200
```

Table B.11 Results for Tukey's mean comparison test for firmness values of cake samples with flour reduction during 3 days of storage

One-way ANOVA: firmness versus mf samples

```
Source      DF      SS      MS      F      P
mf samples   3  420404  140135  79,05  0,000
Error       28   49636    1773
Total       31  470041

S = 42,10   R-Sq = 89,44%   R-Sq(adj) = 88,31%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level       N    Mean  StDev  ------+---------+---------+---------+--
-
25 g flour  8  172,30  23,31  (--*--)
45 g flour  8  191,81  45,66    (--*--)
65 g flour  8  305,99  62,26                (--*--)
85 g flour  8  460,35  24,21                               (--*--)
                              ------+---------+---------+---------+--
-
                                  200       300       400       500

Pooled StDev = 42,10


Grouping Information Using Tukey Method

mf samples  N    Mean  Grouping
85 g flour  8  460,35  A
65 g flour  8  305,99    B
45 g flour  8  191,81      C
25 g flour  8  172,30      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of mf samples

Individual confidence level = 98,92%


mf samples = 25 g flour subtracted from:

mf samples   Lower  Center   Upper  ------+---------+---------+------
---+---
45 g flour  -37,95   19,51   76,96              (--*--)
65 g flour   76,23  133,69  191,15                    (--*--)
85 g flour  230,59  288,05  345,51                            (-*--
)
                                    ------+---------+---------+------
---+---
                                      -200         0       200
400


mf samples = 45 g flour subtracted from:
```

```
mf samples   Lower  Center   Upper  ------+---------+---------+------
---+---
65 g flour   56,72  114,18  171,64                     (--*--)
85 g flour  211,08  268,54  326,00                             (-*--)
                                    ------+---------+---------+------
---+---
                                       -200         0       200
400


mf samples = 65 g flour subtracted from:

mf samples  Lower  Center   Upper  ------+---------+---------+-------
--+---
85 g flour  96,90  154,36  211,82                        (--*--)
                                   ------+---------+---------+-------
--+---
                                      -200         0       200
400
```

Table B.12 Results for Tukey's mean comparison test for firmness values of cake samples with flour reduction during 6 days of storage

One-way ANOVA: firmness versus mf samples

```
Source      DF       SS      MS       F      P
mf samples   3   969209  323070  176,52  0,000
Error       28    51247    1830
Total       31  1020456

S = 42,78   R-Sq = 94,98%   R-Sq(adj) = 94,44%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level       N    Mean  StDev  --------+---------+---------+---------
+-
25 g flour  8  217,97  38,45  (--*-)
45 g flour  8  238,15  15,05    (-*-)
65 g flour  8  397,64  73,83            (--*-)
85 g flour  8  652,96  12,85                             (--*-)
                              --------+---------+---------+---------
+-
                                    300       450       600       750

Pooled StDev = 42,78


Grouping Information Using Tukey Method

mf samples  N    Mean  Grouping
85 g flour  8  652,96  A
65 g flour  8  397,64    B
45 g flour  8  238,15      C
25 g flour  8  217,97      C

Means that do not share a letter are significantly different.
```

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of mf samples

Individual confidence level = 98,92%


mf samples = 25 g flour subtracted from:

mf samples   Lower  Center   Upper  ---------+---------+---------+---
------+
45 g flour  -38,21   20,17   78,56                   (--*-)
65 g flour  121,28  179,67  238,05                          (-*--)
85 g flour  376,60  434,99  493,37
(-*--)
                                    ---------+---------+---------+---
------+
                                          -250         0       250
500


mf samples = 45 g flour subtracted from:

mf samples   Lower  Center   Upper  ---------+---------+---------+---
------+
65 g flour  101,11  159,50  217,88                         (-*--)
85 g flour  356,43  414,82  473,20
(--*-)
                                    ---------+---------+---------+---
------+
                                          -250         0       250
500


mf samples = 65 g flour subtracted from:

mf samples   Lower  Center   Upper  ---------+---------+---------+---
------+
85 g flour  196,94  255,32  313,71                             (-*--)
                                    ---------+---------+---------+---
------+
                                          -250         0       250
500
```

Table B.13 Results for Tukey's mean comparison test for springiness values of fresh cake samples with flour reduction

One-way ANOVA: springiness versus mf samples

```
Source      DF      SS    MS     F      P
mf samples   3   10,65  3,55  0,86  0,475
Error       28  116,22  4,15
Total       31  126,87

S = 2,037   R-Sq = 8,40%   R-Sq(adj) = 0,00%
```

```
                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level       N    Mean  StDev  ----+---------+---------+---------+----
-
25 g flour  8  55,052  2,439  (------------*-----------)
45 g flour  8  56,128  1,947           (------------*-----------)
65 g flour  8  56,237  1,398            (------------*-----------)
85 g flour  8  56,600  2,216               (------------*-----------)
                              ----+---------+---------+---------+----

-
                               54,0      55,2      56,4      57,6

Pooled StDev = 2,037


Grouping Information Using Tukey Method

mf samples  N    Mean  Grouping
85 g flour  8  56,600  A
65 g flour  8  56,237  A
45 g flour  8  56,128  A
25 g flour  8  55,052  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of mf samples

Individual confidence level = 98,92%


mf samples = 25 g flour subtracted from:

mf samples   Lower  Center  Upper  ------+---------+---------+-------
--+---
45 g flour  -1,704   1,076  3,856         (-------------*------------
-)
65 g flour  -1,595   1,185  3,966          (-------------*-----------
--)
85 g flour  -1,232   1,548  4,329            (-------------*---------
----)
                                   ------+---------+---------+-------
--+---
                                      -2,0       0,0       2,0
4,0


mf samples = 45 g flour subtracted from:

mf samples   Lower  Center  Upper  ------+---------+---------+-------
--+---
65 g flour  -2,671   0,109  2,890     (-------------*------------)
85 g flour  -2,308   0,472  3,253      (-------------*-------------)
                                   ------+---------+---------+-------
--+---
                                      -2,0       0,0       2,0
4,0
```

```
mf samples = 65 g flour subtracted from:

mf samples   Lower  Center  Upper  ------+---------+---------+-------
--+---
85 g flour  -2,417   0,363  3,143      (-------------*-------------)
                                   ------+---------+---------+-------
--+---
```

Table B.14 Results for Tukey's mean comparison test for springiness values of cake samples with flour reduction during 3 days of storage

One-way ANOVA: springiness versus mf samples

```
Source      DF      SS    MS     F      P
mf samples   3   17,54  5,85  1,73  0,184
Error       28   94,86  3,39
Total       31  112,40

S = 1,841   R-Sq = 15,61%   R-Sq(adj) = 6,56%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level       N    Mean  StDev  ---+---------+---------+---------+-----
-
25 g flour  8  59,367  1,302                   (----------*----------
)
45 g flour  8  57,420  2,374  (----------*-----------)
65 g flour  8  58,481  0,622           (----------*----------)
85 g flour  8  59,046  2,415                (----------*----------)
                              ---+---------+---------+---------+-----
-
                              56,4      57,6      58,8      60,0

Pooled StDev = 1,841


Grouping Information Using Tukey Method

mf samples  N    Mean  Grouping
25 g flour  8  59,367  A
85 g flour  8  59,046  A
65 g flour  8  58,481  A
45 g flour  8  57,420  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of mf samples

Individual confidence level = 98,92%


mf samples = 25 g flour subtracted from:
```

```
mf samples   Lower  Center  Upper  --------+---------+---------+-----
----+-
45 g flour  -4,459  -1,947  0,565  (---------*---------)
65 g flour  -3,398  -0,886  1,625      (---------*----------)
85 g flour  -2,833  -0,321  2,191         (---------*---------)
                                   --------+---------+---------+-----
----+-
                                        -2,5       0,0       2,5
5,0


mf samples = 45 g flour subtracted from:

mf samples   Lower  Center  Upper  --------+---------+---------+-----
----+-
65 g flour  -1,451   1,061  3,573              (---------*---------)
85 g flour  -0,886   1,626  4,138                (----------*--------
-)
                                   --------+---------+---------+-----
----+-
                                        -2,5       0,0       2,5
5,0


mf samples = 65 g flour subtracted from:

mf samples   Lower  Center  Upper  --------+---------+---------+-----
----+-
85 g flour  -1,946   0,566  3,078            (---------*---------)
                                   --------+---------+---------+-----
----+-
                                        -2,5       0,0       2,5
5,0
```

Table B.15 Results for Tukey's mean comparison test for springiness values of cake samples with flour reduction during 6 days of storage

One-way ANOVA: springiness versus mf samples

```
Source      DF      SS     MS      F      P
mf samples   3  151,70  50,57  15,51  0,000
Error       28   91,31   3,26
Total       31  243,01

S = 1,806   R-Sq = 62,43%   R-Sq(adj) = 58,40%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level       N    Mean  StDev  -------+---------+---------+---------+-
-
25 g flour  8  60,186  1,574                     (------*-----)
45 g flour  8  60,819  1,707                         (-----*------)
65 g flour  8  56,002  2,478  (------*------)
85 g flour  8  56,354  1,229    (------*-----)
                              -------+---------+---------+---------+-
-
                                  56,0      58,0      60,0      62,0
```

```
Pooled StDev = 1,806


Grouping Information Using Tukey Method

mf samples  N    Mean  Grouping
45 g flour  8  60,819  A
25 g flour  8  60,186  A
85 g flour  8  56,354    B
65 g flour  8  56,002    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of mf samples

Individual confidence level = 98,92%


mf samples = 25 g flour subtracted from:

mf samples   Lower  Center   Upper  --------+---------+---------+----
-----+-
45 g flour  -1,832   0,633   3,097              (------*-----)
65 g flour  -6,649  -4,184  -1,720   (------*-----)
85 g flour  -6,296  -3,832  -1,368    (-----*------)
                                    --------+---------+---------+----
-----+-
                                         -4,0       0,0       4,0
8,0


mf samples = 45 g flour subtracted from:

mf samples   Lower  Center   Upper  --------+---------+---------+----
-----+-
65 g flour  -7,281  -4,817  -2,352  (-----*-----)
85 g flour  -6,929  -4,465  -2,000   (-----*-----)
                                    --------+---------+---------+----
-----+-
                                         -4,0       0,0       4,0
8,0


mf samples = 65 g flour subtracted from:

mf samples   Lower  Center  Upper  --------+---------+---------+-----
----+-
85 g flour  -2,112   0,352  2,817              (-----*-----)
                                   --------+---------+---------+-----
----+-
                                        -4,0       0,0       4,0
8,0
```