PRODUCTION AND CHARACTERIZATION OF MICROPARTICULATED CORN ZEIN, AND ITS APPLICATIONS ON EMULSIONS AND BREAD-MAKING

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ABSTRACT

THE PRODUCTION AND CHARACTERIZATION OF MICROPARTICULATED CORN ZEIN, AND ITS APPLICATIONS ON EMULSIONS AND BREAD-MAKING

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The main objective of this study was to investigate the potential use of microfluidization as a milling process for corn zein, and its effects on emulsifying properties of zein emulsions, and on rheological and textural properties of gluten-free bread formulations. Also, the effects of microfluidization were tried to improved with alkaline treatment. In the first part of the study, microstructural properties of zein slurries were evaluated by SEM analysis.

In the second part of the study, rheological properties of zein emulsions and their stabilities were determined. Herschel-Bulkley model was found to explain the flow behaviors of zein emulsions. Also, emulsions were examined in terms of their viscoelastic properties, and the highest elastic (G') and viscous (G") moduli values were obtained for emulsions containing 50% oil and 15% zein concentrations. The stability of the emulsions were measured, and emulsion containing 10% of zein concentration and 30% of oil concentration (10:30:60) gave the best result in terms of emulsion stability according to our study.

Lastly, the rheological properties of different gluten-free dough formulations were investigated. For this purpose, the effects of microfluidized corn zein and different

hydrocolloids at different pH values on the rheological properties of bread dough and the final quality parameters (texture, specific volume, color and storage) of gluten-free bread samples were measured. In dough rheology experiments, firstly, the linear viscoelastic region was determined as approximately a strain of lower than 0.3% for all formulations. Then, the viscoelastic behavior of dough in viscoelastic region was examined, and the highest moduli values were obtained from samples with hpmc, containing 80g starch content and treated by microfluidization. In the end, the effects of different formulations and storage time on staling were studied, and texture profile of the bread samples were measured. Volume of the bread samples were improved especially with the addition of hpmc and guar.

Keywords: Zein, microfluidization, emulsion, gluten-free bread, hydrocolloid, rheology, texture

ÖZ

MİKRO TANECİKLİ MISIR ZEİNİNİN ÜRETİMİ VE KARAKTERİZE EDİLMESİ; VE EMÜLSİYONLARDA VE EKMEK YAPIMINDA UYGULAMALARI

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Bu çalışmanın ana amacı, mikro akışkanlaştırmayı mısır zeini için bir öğütme işlemi olarak kullanma potansiyelini ve bu yöntemin zein emülsiyonlarının emülsiyon özellikleri ve glutensiz ekmek formulasyonlarının reolojik ve tekstürel özellikleri üzerindeki etkilerini araştırmaktır. Ayrıca, mikro akışkanlaştırmanın etkileri bazik işlemlerle geliştirilmeye çalışılmıştır. Çalışmanın ilk bölümünde, sıvılaştırılmış zeinin mikroyapısal özellikleri taramalı elektron mikroskobuyla incelenmiştir.

Çalışmanın ikinci bölümünde, zein emülsiyonlarının reolojik özellikleri ve bu emülsiyonların kararlılıkları belirlenmiştir. Zein emülsiyonlarının akış davranışlarının Herschel-Bulkley modeliyle açıklanabildiği bulunmuştur. Ayrıca, emülsiyonlar ağdalı esneklikleri yönünden incelenmiş ve en yüksek esnek (G') ve akmaz (G'') katsayı değerleri %50 yağ ve %15 zein konsantrasyonları içeren emülsiyonlarda elde edilmiştir. Emülsiyonların kararlılıkları ölçülmüş ve bizim çalışmamıza göre %10 zein ve %30 yağ konsantrasyonu (10:30:60) içeren emülsiyon emülsiyon kararlılığı açısından en iyi sonucu vermiştir.

Son olarak, farklı glutensiz ekmek formulasyonlarının reolojik özellikleri incelenmiştir. Bu amaçla mikro akışkanlaştırılmış mısır zeinin ve farklı

hidrokoloidlerin farklı pH değerlerinde ekmek hamurunun reolojik özellikleri ve glutensiz ekmek örneklerinin son kalite değişkenleri (tekstür, özgül hacim, renk ve bekletme) üzerindeki etkileri ölçülmüştür. Hamur reolojisi deneylerinde, ilk olarak, doğrusal ağdalı esneklik bölgeleri bütün formulasyonlar için baskı yaklaşık %0.3'ten küçük olacak şekilde belirlenmiştir. Bundan sonra, hamurun ağdalı esneklik bölgesindeki davranışları incelenmiş ve en yüksek katsayı değerleri hpmc ve 80g nişasta içeren, ve mikro akışkanlaştırmayla işlenen örneklerden elde edilmiştir. En sonunda, farklı formulasyonların ve bekletme sürelerinin bayatlama üzerindeki etkileri çalışılmış ve ekmek örneklerinin tekstür profilleri ölçülmüştür. Ekmek örneklerinin özgül hacimleri özellikle hpmc ve guarla geliştirilmiştir.

Anahtar kelimeler: Zein, mikro akışkanlaştırma, glutensiz ekmek, hidrokoloid, reoloji, tekstür

To all my beloved…

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INTRODUCTION

1.1 Corn

Corn or -with the known name of it in the United States- maize is the most adaptable and abundant cereal grain all over the world (Györi, 2010; Shukla & Cheryan, 2001). It is known as *Zea mays Linnaeus* among botanists. As being a member of green plants, corn can use sun energy, carbon dioxide and water and as a result of that one of the most important grain in the world come into existence (Inglett, 1970).

According to the archeological findings, the oldest corn grains were found in the valley of Tehuacán in Mexico and because of that reason Western Hemisphere is thought to be the native area of the corn (Matz, 1969). Some findings show that corn grain was spread to the north and south of the America continent, from Canada to Argentina, as a first step. After the discovery of America, corn was transported to Europe and to other continents through European merchants. Due to its versatility and easy adaptation, nowadays it can be grown on most of the agricultural areas of all continents (Györi, 2010).

Figure 1.1 *Zea mays Linnaeus*

Corn or maize is a member of the Gramineae family which generally has fibrous roots, successive leaves with two-ranked parallel veins, split leaf cases, cylindrical stems and flowers in cobs (Matz, 1969). The distinctive feature of them is that they show monoecious and cross-pollinated structure with having female and male flowers in different inflorescences of the same stalk. Each of the stalk consists of a few cobs and each of these cobs contain 300 to 1000 kernels with a systematic order (Inglett, 1970).

There are many types of corn depending on their color, shape, hardness and structure such as pod corn, dent corn, sweet corn, flint corn, popcorn, waxy corn and flour corn (Watson & Ramstad, 1991). The genetics of all these types of corn are the same with having 20 chromosome, but the differences between them come from a single gene (Matz, 1969). The most primitive type of corn is pod corn which possesses fibrous husks to envelope each kernel (Magelsdorf, 1947). The major characteristics of dent corn is that the kernels of it have a concave shape as a result of endosperm shrinkage (Matz, 1969). The difference of sweet corn is its sweetness and tenderness. Waxy corn contains large amounts of amylopectin in its structure which is the result of its waxy nature (Liu et al., 2013). As understood from its name, the kernels of flint corn are very hard. Popcorn can be differentiated by its kernel expansion amount. The characteristics of flour corn are that the kernels are large and soft, and that leads to easy crumbling which provides an easy grinding process of kernels to obtain flour (Matz, 1969).

After the cultivation of corn, it has become one of the basic food necessity for humans all over the world due to its grain size, easy cultivation, storage durability and, decent and plentiful yield (Inglett, 1970). Corn grain is mainly used as animal feed. In addition to that, it is a main raw material in food, starch, fermentation and chemical industries, and also a by-product for cellulose, energy and chemical industries (Godon & Willm, 1994). According to the report of FAO in 2007, almost 85% of the produced corn is used in animal feeding and bioethanol processing (Serna-Saldivar, 2010). Also, Maisadour Semences - the seed producer in France which has an experience of control breeding, production and marketing of corn for about 60 years - stated that 72% of whole corn is used for animal feedstuff, 18%

for starch production, 8% for coarse meal and remaining 2% for other uses in Europe (http://www.maisadour-semences.fr/global/, Last visited: July, 2014). An important characteristic of corn food products is that they own a unique flavor that does not resemble to any other food grain.

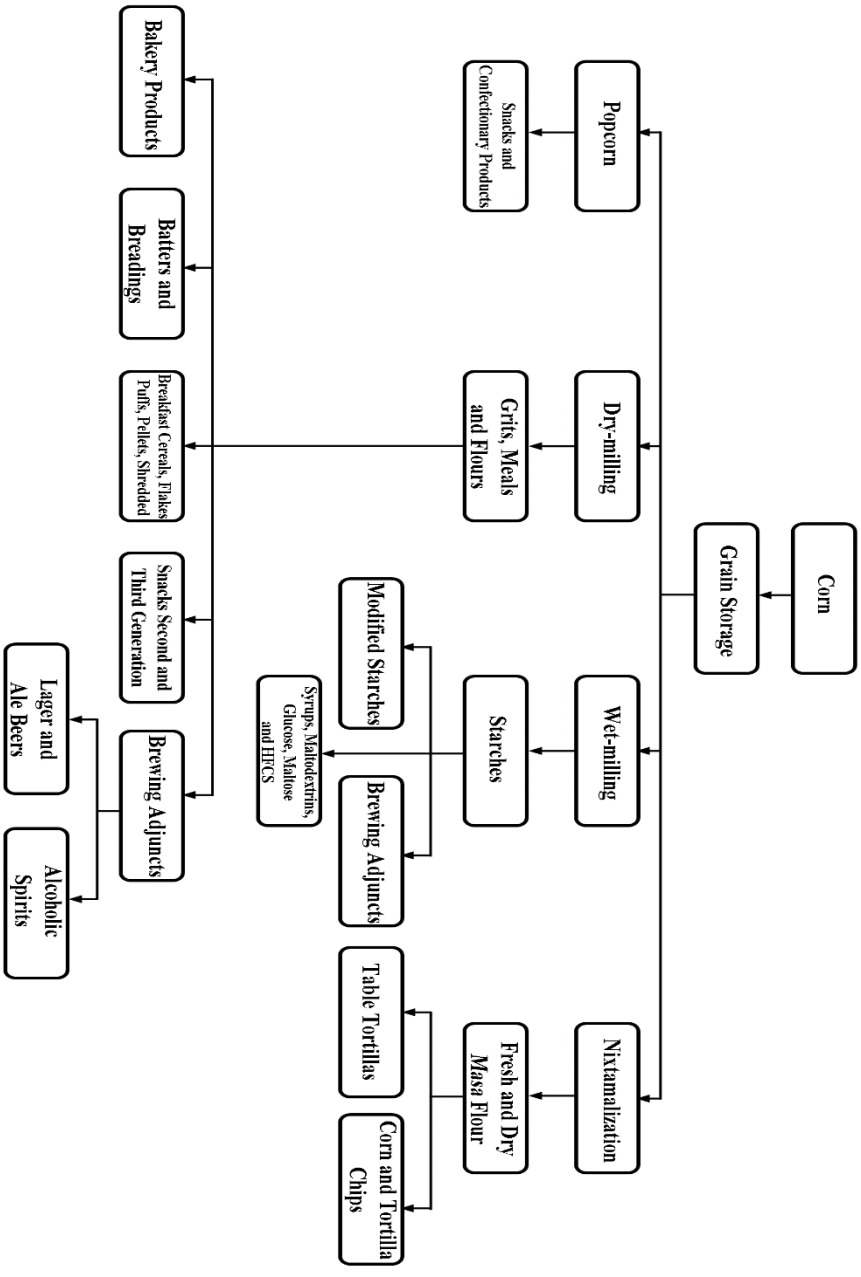

Figure 1.2 Flow chart of the main food uses of corn

Although the use of corn in the food processing is limited, in recent years direct and industrial uses of corn are rapidly increasing and as a result of that many different types of corn products are produced as can be seen from Fig. 1.2. Dry milling, wet milling and nixtamalization are the main processes to treat corn. The obtained flour from dry milling is used in the production of bakery products, batters, breakfast cereals, snacks and as brewing adjuncts. On the other hand, starch content of the corn can be separated by wet milling process and which can be used for syrups such as high fructose corn syrup (HFCS), and also as brewing adjuncts and modified starches (Serna-Saldivar, 2010). Fresh and dry masa flour can be acquired with the help of nixtamalization process to make tortillas and chips. Direct use of corn is also preferred with the boiling of whole cob and flavoring of it with salt and butter (Györi, 2010). The final and maybe the most attractive food product that is made from corn is popcorn and its usage in some snacks and confectionary products (Serna-Saldivar, 2010).

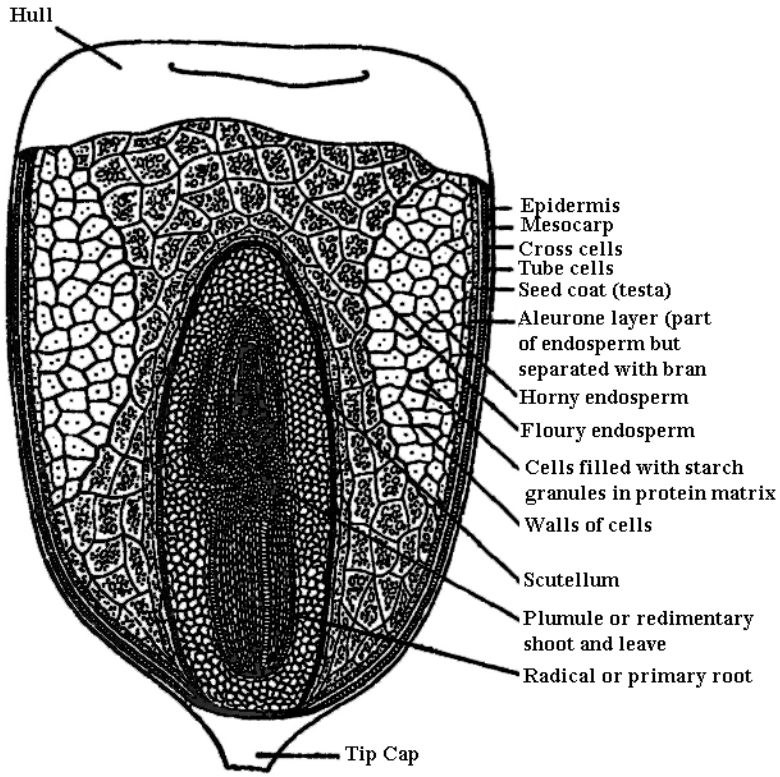

Figure 1.3 Longitudinal section of corn

Corn grain includes three main parts, which are the pericarp (hull), the endosperm and the germ as can be seen from the Fig. 1.3 (Courtesy of wheat flour Institute). The composition of the constituents varies among corn types and even kernels, but major constituents are water, carbohydrates, proteins, lipids, vitamins, minerals and compounds which are responsible from the aroma, flavor and color. In overall, corn kernels have high concentration of starch, noteworthy amount of protein and low fat content. Most of the lipids and major amount of sugar and ash are found in the germ. On the other hand, approximately 75% of the protein and almost the whole starch content present in the endosperm (Györi, 2010). The distribution of the main components among different parts of corn kernel is indicated below in Table 1.1 (Earle et al., 1946; Györi & Györine, 1999).

Table 1.1 The distribution of the main components among different parts of corn kernel (%)

Botanical Parts	**Starch**	**Fat**	**Protein**	**Ash**	**Sugar**
Endosperm	97.8-98.7	13.3-17.4	69.5-78.9	12.6-23.3	23-37.3
Germ	0.7-1.7	80.9-85	18.4-27.8	72.4-83.3	60.8-75.1
Hull	0.4-0.7	0.8-1.7	1.4-2.6	0.9-3.6	0.7-1.7

Note: Proportion of the parts to whole kernel: endosperm-82%, germ-12%, hull-6%

As present in all living organisms, corn also includes water in its structure. In general, water can be found in two forms: free water and bound water, and both form is present in corn (Györi, 2010).

Starch, cellulose, non-cellulosic polysaccharides and sugar are the forms which are seen in corn kernel. Sugar content is too low with an amount of 1-3% (Mertz, 1970). On the other hand, starch is the superior component which changes between 60 to 80% according to origin of the corn (Matz, 1969). Amylose and amylopectin are the two components of the starch and their ratio in the corn structure is about 1:3. Generally, that amylose to amylopectin ratio causes structure differences between corn types (Györi, 2010).

Corn kernel consists of 6-12% protein content on dry basis and albumins, globumins, glutelins and prolamins are the four major classes of protein in corn (Shukla & Cheryan, 2001). Proteins have two tasks in the structure of corn: functional and storage proteins. Most of them are found in the endosperm layer of the corn. There is almost no protein in the hull (Matz, 1969).

As stated before, germ is rich in terms of lipids, on the other hand the endosperm and the hull are low. Main fatty acid of corn is linoleic acid (18:2) with 56% which is followed by oleic acid (18:1) with 30% which are unsaturated fatty acids. Because of its unsaturated structure, corn plays an important role in the diet (Beadle et al., 1965; Matz, 1969).

1.2 Proteins

Proteins are one of the main constituents -and probably the most worthy ones- of all parts of cells of all living organisms (Whitford, 2005). The diversity of the proteins is in a great extent with lots of different types, which are the combination of thousands or millions of amino acids, even for a single cell. As stated above related to amino acids, from the most primitive living organisms to most developed ones, all proteins comprises of 20 amino acids, which are linked by different

sequences and combinations. Therefore, proteins can be stated as polymers of amino acids which are connected to each other with a specific covalent bond. The most outstanding point of the proteins is that their functional properties and activities in the body are remarkably important (Nelson & Cox, 2008). The biological functions of the proteins vary from production of biological metabolites and DNA replication to some transportation of molecules and forming of body tissues. The variety of biological functions of proteins are endless which is only the tip of the iceberg. Some of the biological functions of proteins are stated with the examples in Table 1.2 (Whitford, 2005).

Table 1.2 Some functional roles of proteins

Functions	**Examples**
Enzymes or catalytic proteins	DNA polymerases, Trypsin
Contractile proteins	Myosin, tubulin
Structural or cytoskeletal proteins	Keratin, tropocollagen
Transport proteins	Myoglobin, serum albumin
Effector proteins	Insulin, thyroid stimulating hormone
Defence proteins	Immunoglobulins, ricin
Electron transfer proteins	Ferredoxin, plastocyanin
Storage proteins	Ferritin, gliadin

The primary structure, which is the sequence of amino acids, indicates the specific protein. The conformation of the amino acids is defined by secondary structure,

which gives information about the formations of hydrogen bonds. The most known one is α- helix or β- sheets conformation. Finally, tertiary structure is the result of different intramolecular secondary structures (Rand, 1976).

1.2.1 Corn Protein (Zein)

Proteins play significant roles in germination, maturation and storage processes of the corn. As known, digestibility, nutritional value and some other properties of foods are directly related with proteins (Györi, 2010).

Table 1.3 Distribution of protein fractions (%) in corn

Protein	Solubility	Whole kernel	Endosperm	Germ
Albumins	Water	8	4	30
Globulins	Salt	9	4	30
Glutelins	Alkali	40	39	25
Prolamins	Alcohol	39	47	5

The most recognized classification technique of cereal grain proteins is Osborne fractionation, which took its name from its inventor. According to Osborne fractionation, albumins, globulins, prolamins and glutelins are the four categories of cereal grain proteins (Györi, 2010). The distribution of proteins found in corn are given in Table 1.3 (Shukla & Cheryan, 2001). Albumins, which are water soluble, and globulins, which are soluble in salt solutions, are stated as simple proteins in

cereal grains and present in little amounts. The most abundant albumin and globulin are leucosin and mayzin in corn, respectively. On the other hand, prolamin and glutelin content of cereals are generally higher. Glutelins are insoluble in water, even in alcohol and salt solutions, but soluble in weak acids and alkali solutions. Zeanin is the glutelin protein of the corn. Prolamins cannot be dissolved in water and salt solutions, but can be dissolved in solutions with alcohol higher than 70% and the prolamin in corn is called as zein (Györi, 2010). Zein is the main protein fraction of the corn and because of that reason, zein is called as corn protein (Hamaker et al., 1995).

Zein is a class of prolamine -a class of alcohol soluble storage protein which is found in grains- protein found in corn, which does not contain gluten (Fevzioglu et al., 2012; Osborne, 1924; Zhong & Ikeda, 2012). The deficiency of zein in terms of some essential amino acids such as lysine and tryptophan is the reason why zein has a poor quality (Shukla & Cheryan, 2001). It is the most abundant group in corn endosperm which consists of approximately 62-74% of the endosperm that changes according to the separation method and variety of the corn (Hoseney, 1994; Larkins, 1981). α, β, γ and δ are the classes of zein which are identified according to their solubilities, amino acid sequences, molecular weights and immunological responses (Esen, 1986; McKinney, 1958). α-zein is the major part of zein with approximately 75-85%. Also, Z19 and Z22 are two subtypes of α-zein whose names are given with respect to their molecular weights (Hamaker et al., 1995) (Li et al., 2012). γ-zein follows α-zein with a percentage of approximately 20% (Tsai, 1980) and β is about 5%. Also, as stated above, some subclasses like δ-zein are present in the structure with fragment amounts (Lawton & Wilson, 2003). As α-zein has some subtypes, the other kinds of the zein possess subtypes which are again named with their molecular weights. Z14 and Z16, Z28, Z10 are the subtypes of β, γ and δ-zeins, respectively (Shewry & Tatham, 1990). Central region of protein body host α-zein while periphery keeps β-zein and γ-zein (Lending & Larkins, 1989). Because zein includes high amount of hydrophobic amino acids especially aliphatic amino acids (leucine, proline, alanine and phenylalanine) in its amino acid composition, it also shows high surface hydrophobicity and thus it cannot be dissolved in water,

but can be dissolved in alcoholic solutions (Cabra et al., 2007; Fevzioglu et al., 2012; Gianazza et al., 1977). The amount of polar charged amino acids is low in the structure of zein and that leads to aggregation tendency (Cabra et al., 2007).

Various studies have been conducted to understand the structure of the zein and different models have been proposed by researchers. Argos et al. (1982) described a model in which nine homologous and contiguous helices, that are anti-parallel to each other, are linked by hydrogen bonds and the resulting protein molecule shows asymmetry. That model offered a compact conformation. Conversely, some other studies such as Matsushima et al. (1997) and Tatham et al. (1993) reported that α-zein has an extended conformation.

Zein, which is the natural storage protein in corn kernel, has been very popular lately and numerous scientific studies related with zein have been published with the increasing demand of environmentally friendly industrial materials in recent years (Li et al., 2012). Insolubility in water, resistance to grease and microbial attack, glossy appearance and large availability are some of the important characteristics of its popularity and it has been used in several fields such as foods, pharmaceuticals (Batterman-Azcona et al., 1998), neutraceuticals, fibers, plastics (Holding & Larkins, 2009), films and coatings (Shi et al., 2009), and specific delivery systems (Mukhidinov et al., 2011). Because of its deficiency in terms of some essential amino acids such as lysine and tryptophan, the nutritional quality of the zein can be stated as poor. Moreover, its insolubility in water puts some limitations to its usage in food industry and generally it is used as animal feed. There have been several studies which struggle to develop an effective process for zein (Shukla & Cheryan, 2001).

1.3 Homogenization Techniques

Homogenization methods are crucial to obtain a product that has appropriate properties, for the following analysis. Although there are several homogenization

techniques, colloidal mill and microfluidization are the most frequently used ones in food industry and emulsion preparation (McClements, 2005).

1.3.1 Colloidal Mill

For the homogenization of liquid slurries that have high or medium viscosity, colloidal mills are commonly used in the food industry (Loncin & Merson, 1979). The operation system of the colloidal mill is very simple. It comprises two disks, one of them is static and the other is rotating. The liquid slurry that has to be homogenized is fed to the colloidal mill. When it passes through between the disks, rotor generates a shear stress with its rapid rotation and the particles or droplets of the feed are broken down, and smaller particles are obtained. The applied shear stress can be arranged by changing the gap between the disks, altering rotation speed of the rotor and using different surfaces which can be smooth or rough according to process (McClements, 2005; Schubert, 1997).

1.3.2 Microfluidization

The other homogenization technique used in food industry and emulsion preparation to improve the stability, taste, color and textural properties, is microfluidization. That technique is known with the capability of generating emulsions whose droplet sizes are extremely small (McClements, 2005).

Microfluidization is a unique technique, which applies high pressure homogenization to product streams (Lagoueyte & Paquin, 1998). There are combined forces in that high pressure technology such as high velocity impact, ultra high pressure, intense shear rate, cavitation, high frequency vibration and instantaneous pressure drop (Feijoo et al., 1997; Liu et al., 2009). Both shear rate and extreme impact forces are applied to micro channels for the development of

fine particles (McCrae, 1994; Mert, 2012). In this technique, product is pressurized and passed through two geometrically same micro channels at high velocity and then colliding with each other at very high velocity which results in deformation and breakage of structures coming from product stream (Iordache & Jelen, 2003; Lagoueyte & Paquin, 1998; Mert, 2012; USA Patent No. US4908154-A, 1987). As a result of this technique, micro and nano particles are obtained.

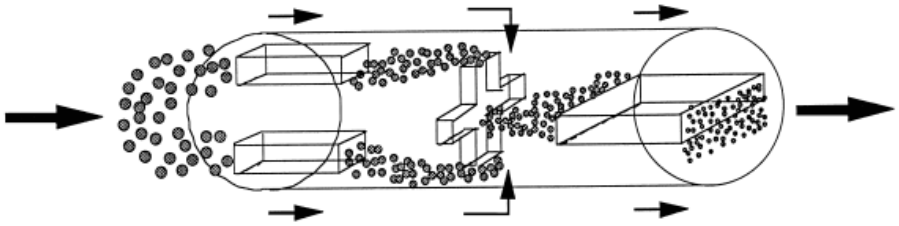

Figure 1.4 Symbolic representation of the microfluidization process (Lagoueyte & Paquin, 1998)

In the study of Tunick et al. (2000), it was showed that this technique provides smaller particles than conventional homogenizer. The particle sizes of the product that is exposed to microfluidization technique are extremely reduced due to mechanical forces (Dissanayake & Vasiljevic, 2009; Iordache & Jelen, 2003). High pressure microfluidization can be stated as a physical modification method of enzymes (Liu et al., 2009), proteins (Zhang et al., 2009) and dietary fibers (Wan et al., 2009). Microfluidization leads to conformational changes in the structure of the product which are important because they are directly related with the physical functionalities and the functional properties of the product (Dissanayake & Vasiljevic, 2009; Johnston et al., 1992). De la Fuente et al. (2002) indicated that different physicochemical and functional properties can be seen with variations in the protein structure. Ternary or quaternary structures of the proteins are affected as a result of mechanical forces that are implemented during microfluidization, and

conformational changings induce different mechanisms which provide functional properties to proteins (Iordache & Jelen, 2003).

Many studies have been reported to investigate the effects and the efficiency of microfluidization by comparing it with other techniques. Most of the researchers agree on that microfluidization gives smaller particles than others (Abismail et al., 1999; Jafari et al., 2006; Maa & Hsu, 1999). On the other hand, some researchers stated that microfluidization is not a practical application for the industry because of its high equipment cost (Tadros et al., 2004).

Physicochemical properties of various foods have been modified by using different techniques such as micronization and microfluidization (Chau et al., 2006). Also, Wang et al. (2012)'s study showed that not only particle sizes of the samples change, but also bulk density and hydration properties, such as swelling capacity, water-holding capacity and oil-holding capacity, vary with microfluidization technique.

In general, homogenization technique, which has two stages as stated above, is applied to foods to manufacture glossier and smoother products with higher consistency. This process develops the quality of the product by breakage of the large structures and reducing the particle sizes (Mert, 2012; Thakur et al., 1995). In conventional homogenization, the feed is forced to pass through a microscopic opening and that leads to high turbulence and high shear with some combined forces such as pressure drop and cavitation. As a result of that effect, dispersion and disintegration of product take place (Mert, 2012).

Microfluidization has several advantages on traditional techniques like conventional homogenization. First of all, contamination risk in the microfluidization process is almost zero and the equipment cleaning is too easy. Also, operation duration of microfluidization is faster than the others. Moreover, as stated before, more uniform and smaller particles are produced with this technique. Besides, application of microfluidization to large scale production is easier than other applications. The final advantage of microfluidization is that it is more suitable for continuous production (Garad et al., 2010).

Microfluidization technique has been applied to various foods including milk (Cobos et al., 1995; Dalgleish et al., 1996; Hardham et al., 2000; McCrae, 1994; Strawbridge et al., 1995; Whiteley & Muir, 1996), cheese (Lebeuf et al., 1998; Lemay et al., 1994; Tunick et al., 2000), cream liqueurs (Paquin & Giasson, 1989), ice cream (Olson et al., 2003), yoghurt (Ciron et al., 2010), ketchup type products (Mert, 2012), whey protein (Iordache & Jelen, 2003), lentinan (Huang et al., 2012), xanthan gum (Lagoueyte & Paquin, 1998) and high methoxyl pectin (Chen et al., 2012). In a very recent study of Mert et al. (2014), microfluidization was employed to produce highly branched fibrous structure form of wheat bran and it has been suggested that microfluidization can be used as a novel milling technology to produce fibrous products with enhanced physical properties.

1.4 Emulsions

There has been substantial number of food products and related studies, that comprise emulsions or have an emulsified state during their manufacture, such as milk, fruit beverages, soups, cake batters, sauces, cream, coffee whitener, butter, margarine, salad dressings and mayonnaise (Dickinson, 1992; Dickinson & Stainsby, 1982; Krog et al., 1983; Stauffer, 1999). The reason behind the variety of sensory and physicochemical characteristics of emulsion based foods is that they are constituted from different types of ingredients and by different processing conditions which reflect their characteristics to emulsion (McClements, 2005). The types and concentrations of raw materials such as water, oil, flavors, emulsifiers and also the processing method such as freezing or sterilization are the important parameters that provide the main quality attributes to emulsions (Sloan, 2003).

An emulsion includes two immiscible liquids such as oil and water, and one of the liquids flows through the other one as small droplets (Dickinson, 2001; Lynch & Griffin, 1974). Also, Becker (1965) defined emulsion as the macroscopic dispersion of two liquids in which one of them forms the continuous part and the other one is discontinuous or dispersed part. The forming process of an emulsion from two

immiscible liquids or the reduction of particle size of the components can be stated as homogenization (McClements, 2005) and the main methods for foods are stated in the previous part. Milk, dressings, soups and cream are the examples of oil-in-water (O/W) emulsions in which oil droplets are dispersed around water. Vice versa is called as water-in-oil (W/O) emulsions such as margarine and butter (Becker, 1957; McClements, 2005).

The important point for emulsions is sticking on to stability. Because emulsion systems' interfacial surface area is large, emulsions tend to disperse (Dean, 1948). Being one of the ingredients of food applications, emulsifying properties of proteins are very crucial in food industry and these properties are generally discoursed around emulsion stability and emulsifying activity (Pearce & Kinsella, 1978). Emulsion stability is described as the ability of an emulsion to resist changes in its properties over time by McClements (2005). Some physical and chemical processes can lead to instability. Examples of physical instabilities are sedimentation, creaming, flocculation, coalescence, Ostwald ripening and phase inversion (Walstra, 1996) as can be seen in Fig. 1.5. On the other hand hydrolysis and oxidation are given as the examples of chemical instability by McClements & Decker (2000). Sedimentation and creaming are the mechanisms in which gravitational separation is in charge. Sedimentation occurs because of the higher density of droplets than surrounding liquid, while the mechanism of the creaming is the opposite. On the other hand, flocculation and coalescence happen due to aggregation. Flocculation arises when droplets come together but not unite, whereas when coalescence occurs droplets come together to unite and form a single huge droplet (McClements, 2005). Phase inversion has completely different mechanism in which emulsion type changes to another one because of compositional and environmental factors. For example, O/W emulsion transforms to W/O emulsion (Campbell et al., 1996). Finally, Ostwald ripening is the mechanism in which large droplets become larger with shrinkage of small droplets (Kabalnov & Shchukin, 1992).

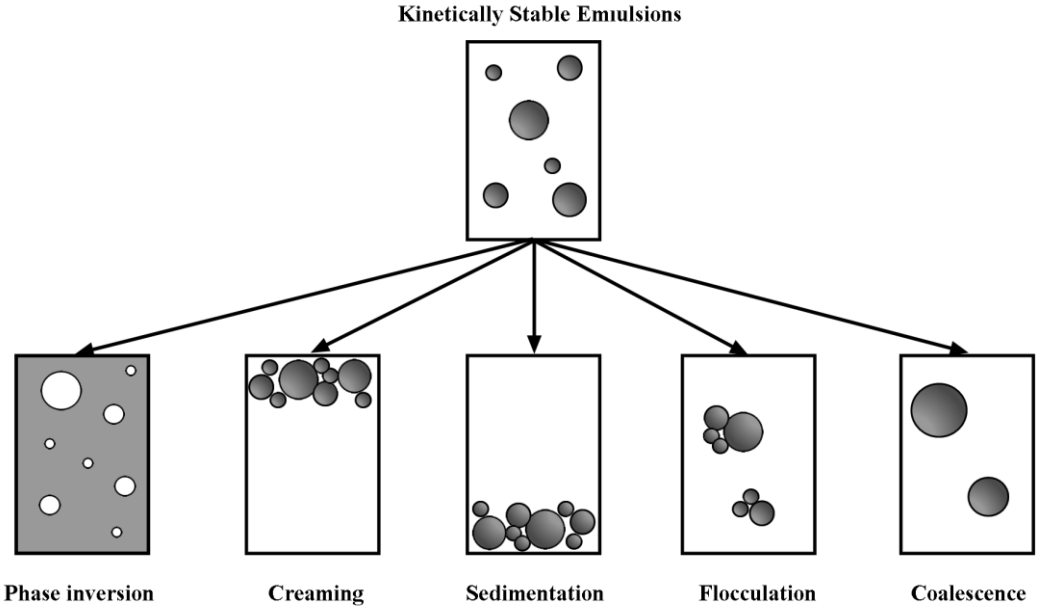

Figure 1.5 Mechanisms of emulsion instability

Proteins ease the formation of emulsions, especially oil-in-water (O/W) emulsions and play an important role with their ability to stabilize emulsions (Dickinson, 2001; Mine et al., 1991). Because of the ability of forming visco-elastic interfacial layers of proteins, they act as barriers against coalescence and present steric and electrostatic repulsions against flocculation (Ma et al., 2011). Several studies have been conducted in different food products to understand the stabilization ability of various proteins such as casein, whey protein (Dickinson, 1998), egg protein (Horn, 1980), soybean protein and muscle protein (Mine et al., 1991).

Argos et al. (1982) proposed that zein shows a helical wheel structure which is slightly asymmetric. Poon et al. (2001) showed that proteins that have α-helices in their structure have better emulsifying properties. Due to its α-helices structure, functional properties especially emulsifying properties of α-zein can be enhanced by some modifications (Cabra et al., 2007). Physical, chemical and enzymatic treatments can be stated as those modifications and they are performed to change

and modify the structure and conformation of proteins which therefore, changes the physiochemical and functional properties of proteins (Kinsella, 1976).

1.5 Gluten and Its Role in Bread-making

Bread has been one of the oldest and dominant food component of human life from ancient times, and wheat is by far the most useful and suitable cereal grain for bread-making procedure with a composition of 70-75% starch, 14% water, 10-12% proteins, 2-3% non-starch polysaccharides, 2% lipids and little amount of arabinoxylans (Goesaert et al., 2005). Gluten and non-gluten proteins are the two main types of wheat grain proteins. Albumins and globulins, which are non-gluten proteins, consist of 15-20% of whole protein and almost the effect of them on bread is in negligible level (Osborne, 1924). On the other hand, gluten proteins include water insoluble part with having 80-85% of total wheat grain protein (Veraverbeke & Delcour, 2002) and that gluten part is the cause of strong, cohesive and viscoelastic network of bread dough (Goesaert et al., 2005).

The quantity and the composition of proteins found in wheat are very critical for the bread quality. As a result of that, bread-making procedure is directly related with protein content (Finney & Barmore, 1948). The gluten proteins, which allow the formation of appropriate bread dough, can be stated as the main quality factor of bread-making. Moreover, the properties of those proteins are unique and any other cereal grain such as barley and rye, which are in the same taxonomy with wheat, cannot imitate these properties (Goesaert et al., 2005).

Gluten is a complex mixture of proteins that is responsible for obtaining the desired volume, texture and storage life in bread-making process of wheat dough. It also plays an important role on viscoelasticity of dough by contributing strong protein network, which gives gas retention ability to dough. The majority of gluten includes glutenin and prolamin- in the form of gliadin in wheat- which are responsible for different mechanisms in dough structure (Demirkesen et al., 2010b). Furthermore,

the linkages of glutenin and gliadin proteins are different from each other and those linkage types give them different properties. Glutenins are linked by intermolecular disulfide bonds, which provide network structure to dough; however the linkage of gliadin is intramolecular disulfide bonds, which results in a globular conformation (Tronsmo et al., 2002). Glutenin provides elasticity and cohesiveness, whereas prolamin provides viscosity and extensibility to dough (Gujral & Rosell, 2004; Lindsay & Skeritt, 1999; Pomeranz, 1988). The lack of gluten in dough structure makes it very difficult to obtain an acceptable product because of the absence of a proper network necessary to hold the carbon dioxide resulting in lower gas retention and poor structural quality which is already seen in gluten-free breads as lower volume, dry crumbly texture, rapid staling and bland aroma (Blanco et al., 2011; Hager & Arendt, 2013).

1.6 Celiac Disease

Celiac disease, also known as celiac sprue and gluten sensitive enteropathy, can be stated as a specific type of food intolerance. The reason behind that intolerance is the gliadin fraction of gluten found in food (Blanco et al., 2011; Jahar & Jahar, 2001). Certain types of cereals such as wheat, barley, rye, kamut and spelt, and some of hybrids like triticale and semolina contain gluten as protein, that cannot be consumed by celiac people (O'Brien, 2007; Sciarini et al., 2012). When celiac patients consume gluten containing foods, it can lead to destructive effects on the patients metabolism (Feighery, 1999; O'Brien, 2007). The body responds to it and a series of events happen which leads to the destruction of villous structure of the small intestine. Thus, celiac patients' small intestine cannot absorb nutrients and obviously this situation affects all systems of the body adversely. Before 2000s, celiac disease is thought as an uncommon disorder problem, which is seen one in thousand (Schober et al., 2003). However, the latest studies show that the prevalence of celiac disease is more frequent among population. According to latest screenings, approximately 0.9-1.2% of the Western population shows the

symptoms of the disease and struggle with it (Pruska-Kedzior et al., 2008). Because celiac is a life lasting health problem, the only way to treat this disease is to adhere to a strict gluten-free diet, which is the complete elimination of gluten containing foods even medications, throughout the patient's lifetime that may improve villous structure of the small intestine (Carlo & Alessio, 2008; Demirkesen et al., 2010a; Green et al., 2001; Sciarini et al., 2012). There have been numerous studies that show strict gluten-free diet is enough for the recovery of the body metabolism (Bode et al., 1991). On the other hand, some researchers have been indicated that although complete gluten-free diet is sustained, full intestinal recovery takes time (Bode et al., 1991). A good gluten-free diet must forbid all products and byproducts that include gluten in its structure (Pruska-Kedzior et al., 2008).

Greater awareness against celiac disease and increasing number of the cases related with celiac make this topic more attractive recently. For those reasons, a niche product necessity was pointed out in the market for celiac people (Hager & Arendt, 2013; Schober et al., 2008). Corn, sorghum, teff, buckwheat and rice can be counted as safer cereals for celiac people but the production of baked goods from those cereals is a challenge because their structure lacks viscoelasticity gluten network (Hager & Arendt, 2013; Moore et al., 2007).

1.7 Gluten-free Bread and Bread Dough

The oldest processed food of the world is possibly bread and it has become a part of mankind diet all over the world (Cauvain, 2000). Since the production of the first bread first, lots of different types have been produced depending on traditions, needs and technologies of the human (Narvhus & Sorhaug, 2006).

Since celiac disease become widespread among humans, there has been lots of studies conducted by researchers to overcome it. Also, the studies about this health problem display a niche market and opportunities to new products (Fasano et al., 2003; Gallagher et al., 2004).

As understood from its name, gluten-free bread means gluten content of the product is almost zero. The upper limit of the gluten content of a food is determined as 20 ppm by Codex Alimentarius of WHO/FAO to refer it as gluten-free (Pruska-Kedzior et al., 2008). In the preparation of gluten-free breads, flours from gluten-free cereals such as corn, sorghum, millet and rice are used (Schober et al., 2008). In the literature, there are a few studies on investigating the production of corn bread. Olatunji et al. (1992) produced corn bread after their previous study on sorghum. Also, Edema et al. (2005) and Sanni et al. (1998) studied on corn bread production. Those studies indicated that obtained corn breads has low specific volume, which is one of the major problematic side of the gluten-free breads.

Several studies have been published to formulate gluten-free breads from different sources, even those studies have been improved by some innovations and interactions (Kadan et al., 2001). However, although innovations have been made, gluten-free dough remains soft. As a result of that softness, they are close to easy collapsing which results in huge holes and denser areas in the bread structure (Cauvain, 1998; Schober et al., 2005).

As stated before, gluten is responsible from the desired volume, texture and storage life in bread-making properties of wheat dough (Demirkesen et al., 2010b). Because of the absence of gluten in gluten-free dough, it shows some differences on rheology, process and quality of products. General differences can be counted as follows: The gluten-free dough shows less cohesiveness and elasticity than wheat dough. They exhibit a paste-like structure and are stickier. Because of those reasons, they are difficult to handle (Cauvain, 1998). As a result of its handling problem, the gluten-free dough is called as batter (Schober et al., 2005).

In recent years, zein has gained a popularity for the production of gluten-free bread (Schober et al., 2008). This idea is the result of the similarities between zein and wheat doughs. However, those similarities can be seen only and only when the zein and starch mixtures are mixed above the glass transition temperature of zein which is approximately 28 ˚C (Lawton, 1992). Above that temperature, zein is able to constitute a viscoelastic dough. Nevertheless, when the applied stress is removed,

whatever the temperature is, viscoelastic properties are lost. That means viscoelastic structure of the zein dough is not stable (Lawton, 1992; Mejia et al., 2007).

1.7.1 Gluten-free Bread Ingredients

The basic ingredients used in the production of gluten-free bread are flour or starch, water, salt, sugar and yeast. To produce high quality products, to improve shelf life and to meet customer expectations, some other ingredients such as preservatives, emulsifiers and yeast food can be added (Stauffer, 1990; Sultan, 1983). Because ingredients are very effective on the quality and rheological properties of final product, it is important to have knowledge about the functions of the ingredients in the process (Cauvain, 2000).

1.7.1.1 Flour or Starch

This ingredient comprises the majority of the product. Since it affects the process parameters, functionality and properties of the product directly, it is seen as the most significant constituent of bread-making procedure. The amounts of other ingredients are dictated by the flour amount. Dough development time, water absorption and stability tolerance are the remarkable characteristics of flour because these are the most effective ones on bread quality (Serna-Saldivar, 2010). As a general knowledge, if flour contains high amount of protein in its structure, water absorption and dough development time have to be prolonged to reach optimum dough stability (Serna-Saldivar, 2010). Also, when it has high amount of protein, the entrapped amount of carbon dioxide increases which results in larger bread volume. Besides, in addition to amount of protein, protein quality of the flour is critical (Cauvain, 1998).

1.7.1.2 Water

Water is a keystone ingredient for all bakery products because it is the medium where all other ingredients are solubilized, and thus activated. For instance, water is required for the network formation which is provided by zein in corn bread above glass transition temperature. Also, it activates yeasts and enzymes, dissolves sugar, salt and other dry components, and hydrates starch molecules for volume expansion (Sahin, 2008; Serna-Saldivar, 2010). Being an important factor in every food product, water activity also plays a significant role in bread because it specifies shelf life and the desired textural properties (Kocak, 2010). Moreover, the hardness of the water affects the quality of the product because the amount of minerals changes with the hardness which can affect the dough structure (Serna-Saldivar, 2010).

1.7.1.3 Salt

Salt is another important parameter of bread-making procedure. First of all, it strengths the network structure of dough with some modifications on proteins. Also, it expands the dough mixing duration and improves the flavor of the product. Moreover, it stabilizes the fermentation rate (Miller & Hoseney, 2008; Strong, 1969; Williams & Pullen, 1998). Salt is also known to increase the sweetness of sugar and mask the metallic and bitter flavors. It decreases the gas production rate by controlling fermentation, and because of that reason it acts as a stabilizer (Serna-Saldivar, 2010). Lastly, it diminishes the water activity which improves shelf life of the product, so it acts as a preservative (Sahin, 2008; Serna-Saldivar, 2010).

1.7.1.4 Sugar

In bread-making procedure, various kinds of sweeteners such as sugar and fructose syrups can be used. The fundamental functionalities of those ingredients are gathered into three main topics by Dubois (1984). These are: giving flavor and color, enhancing the shelf life and controlling the yeast activity. Sugar breakdown during fermentation gives the flavor profile. Also, sugars are very effective on the color of the crust by causing Maillard reactions and non-enzymatic browning reactions which come up with high oven temperature. Finaly, as stated above, sugars enhance shelf life because organic acids are revealed with the reactions of sugars which lower pH and water activity (Eliasson & Larsson, 1993; Pyler, 1988; Sluimer, 2005; Stauffer, 1990; Sultan, 1983).

1.7.1.5 Yeast

Saccharomyces cereviceae is a species of yeasts which is used as a biological fermenting agent in bread-making procedures. It decomposes saccharides to many different compounds which are responsible from different properties. By decomposition, organic acids are formed which lower pH. On the other hand, aldehydes and ketones are constituted which influence aroma and flavor directly. Finally, carbon dioxide, which is entrapped in the network structure and gives the volume of the bread, is formed (Reed & Peppler, 1973; Serna-Saldivar, 2010).

1.7.2 Ingredients Used in Gluten-free Baked Products to Improve Quality

The usage of additives in the food industry has gained popularity after the effects of them were seen on rheological properties of dough and textural properties of

bread. To improve the general quality of products, many substances have been tried on (Haros et al., 2002). Because gluten-free bread dough cannot imitate viscoelastic properties of gluten, the usage of additives became an important starting point and gained importance for gluten-free bread formulations (Kohajdová et al., 2009). Especially, hydrocolloids are the most used group in the food industry, which are known with their effect on bread volume, crumb texture, freshness, and viscoelastic properties of dough (Bárcenas et al., 2003; Twillman & White, 1988). Guar gum, xanthan gum, locust bean gum, cellulose and cellulose derivatives like hydroxypropyl methylcellulose (HPMC) and carboxymethylcellulose (CMC) are the most abundant hydrocolloids in the food industry (Kohajdová et al., 2009). Emulsifiers has been employed to gluten-free bread formulations to enhance dough handling and quality as well. Diacetyl tartaric acid esters of monodiglycerides (DATEM) is the most common used emulsifier in the food industry (Demirkesen et al., 2010b). Fibers are another additive that can affect the quality of product directly. Citrus fiber and corn fiber are the examples of fibers, which are used to assist and improve the properties of dough ingredients.

1.7.2.1 Hydrocolloids

Hydrocolloids, commonly known as gums, are the combination of many polysaccharides and proteins, which originate from botanical, algal, microbial and animal sources as stated in Table 1.4 (Williams & Phillips, 2000).

There are lots of functions of hydrocolloids that are the reasons why they are used extensively in the food industry. Stabilizing effect of them on emulsions, foams and dispersions, thickening and gelling structure on aqueous solutions, controlling flavor release and, inhibition of ice and sugar crystal formation are the major functions of hydrocolloids (Arendt & Dal Bello, 2008; Williams & Phillips, 2000). Hydrocolloids provide viscoelastic properties of gluten to gluten-free breads which enhance shelf-life, acceptability and structure of gluten-free breads (Blanco et al., 2011).

Table 1.4 Sources of hydrocolloids

Main Source	Sub-source	Examples
Botanical	trees	cellulose
	tree gum exudates	gum arabic, gum kataya, gum ghatti, gum tragacanth
	plants	starch, pectin, cellulose
	seeds	guar gum, locust bean gum, tara gum, taramind gum
	tubers	konjac mannan
Algal	red seaweeds	agar, carrageenan
	brown seaweeds	alginate
Microbial		xanthan gum, curdlan, dextran, gellan gum, cellulose
Animal		gelatin, caseinate, whey protein, chitosan

Moreover, hydrocolloids give additional water binding capacity to gluten-free breads, which results in softer bread structure. Also, they improve bread volume by providing more efficient gas retention, which is more difficult because of the lack of gluten network in the gluten-free bread formulations (Gallagher et al., 2003; Schober et al., 2007). Furthermore, they are directly related with swelling and gelatinization of dough, and retrogradation of starch (Arendt & Moore, 2006). Hydrocolloids work with water in the system, and prevent the diffusion of water and provide stability to the system (Houben et al., 2012). Nonetheless, the selection of the appropriate hydrocolloid to improve a specific property of a specific product is an important task because many factors affect the functions of hydrocolloids such

as type of hydrocolloid, process temperature and pH, particle size, and concentrations of other molecules (Demirkesen, 2013).

Hydrocolloids have been used in gluten-free bread formulations to observe the effects of them on the quality of gluten-free bread by many researchers (Andersson et al., 2011; Blanco et al., 2011; Crockett et al., 2011; Demirkesen et al., 2010b; Ericksen et al., 2012; Hager & Arendt, 2013; Sabanis & Tzia, 2010; Schober et al., 2007; Schober et al., 2008; Sciarini et al., 2012).

1.7.2.1.1 Hydroxypropyl methylcellulose (HPMC)

Hydroxypropyl methylcellulose (HPMC) is a kind of cellulose derivative. It is formed by a chemical modification of cellulose, which is probably the most generous organic material found in nature. As can be understood from its name and seen from Fig. 1.6, HPMC contains hydroxypropyl and methoxy groups in its structure (Hoefler, 2004).

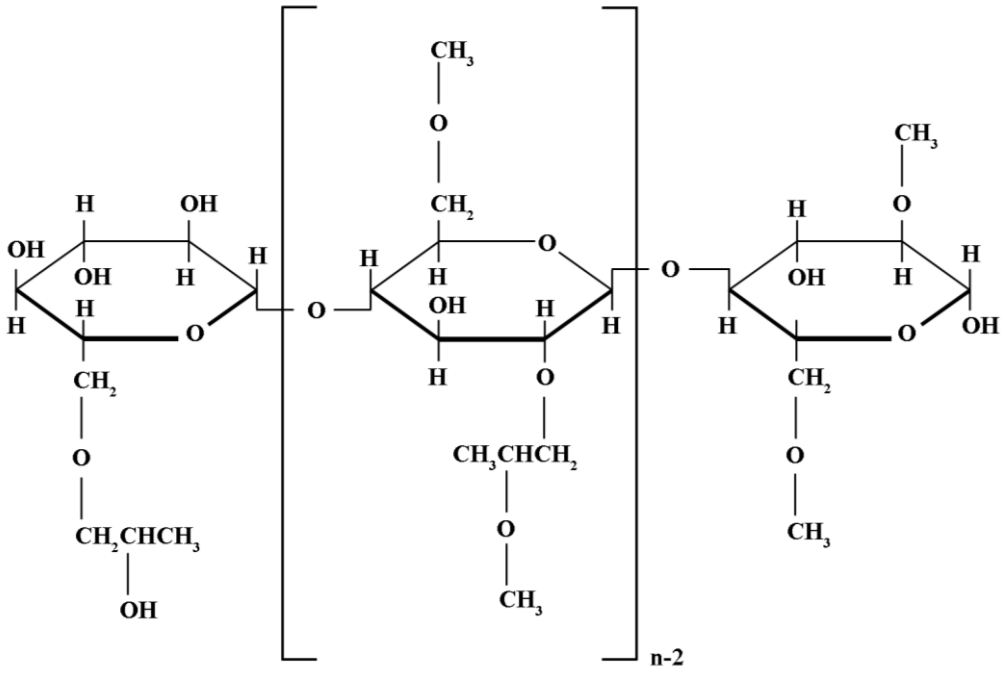

Figure 1.6 Primary structure of HPMC

HPMC shows different properties because of its hydroxypropyl groups and methoxy groups, which are hydrophilic and hydrophobic, respectively. Water solubility of HPMC is increased by chemical groups in its structure and that provides uniformity in the dough structure and also preserves emulsion stability during breadmaking procedure. It provides viscosity to solutions by binding water to its structure, that lowers the possibility of complex formations (Selomulyo & Zhou, 2007). Also, this hydrocolloid constitutes interfacial films which provide stability against gas expansion and some other changes (Bell, 1990). Like interfacial films, gels are formed by HPMC which creates transient network, and by that way strength of dough is increased and also volume loss is prevented. These gels also create a barrier against the removal of water (Sarkar & Walker, 1995; Selomulyo & Zhou, 2007). The presence of ether groups in the structure of HPMC provides easy emulsion and foam stabilization. Also, as stated above with some properties, it is commonly used in gluten-free bakery products to enhance bread volume, crumb structure and moisture, staling properties, starch retrogradation and sensory properties (Demirkesen, 2013; Guarda et al., 2004).

1.7.2.1.2 Guar Gum

Guar gum, which is known as *Cyamopsis tetragonoloba,* is obtained from guar plant seeds, which belongs to Luguminosae family. The chain structure of the guar is showed in Fig. 1.7, which consists of mannopyranosyl and galactopyranosyl units (Hoefler, 2004). Guar gum includes 75-85% polysaccharide, 5-6% protein and 8-14% water. Because of neutral polysaccharides, pH and ions affect guar gum solubility directly (BeMiller, 2008).

Guar gum has a structure which enables dissolving in cold water. As particle size of the guar gum decreases and temperature increases, dissolution rate of guar gum accelerates (Hoefler, 2004). It is the strongest hydrocolloid in terms of producing high viscosity with minimum concentration. Because of that property of guar gum, it has been widely preferred in the food industry as food stabilizer (BeMiller, 2008).

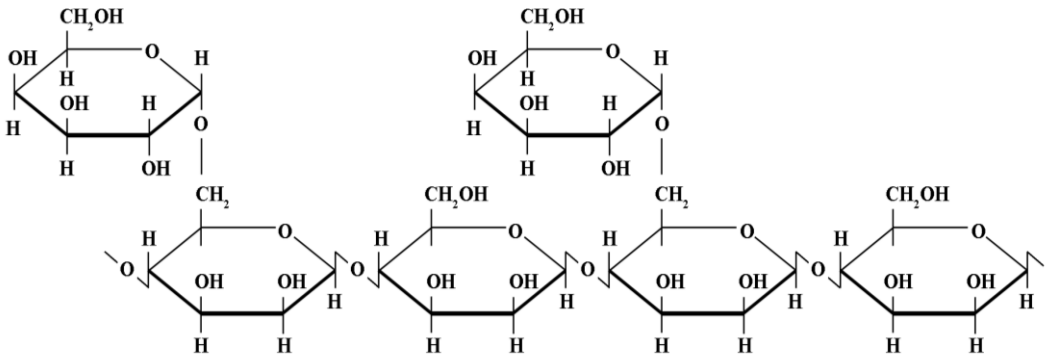

Figure 1.7 Primary structure of guar gum

Also, it became an appropriate additive for some products such as bakery products, ice cream mixes and salad dressings because of its hydrophilic nature, which avoids water release and aggregation of polymers (Demirkesen, 2013). Furthermore, Maier et al. (1993) stated that guar gum advances shelf life of products by providing moisture retention. On the other hand, it prevents starch retrogradation due to its binding properties to starches and amylopectins. As a result of that, guar gum plays an important role in controlling staling of bread (Demirkesen, 2013).

1.7.2.1.3 Xanthan Gum

Xanthan gum is hydrocolloid which has a microbial origin, and which is a secretion of a microorganism- *Xanthomonas campestris*. That microorganism produces that gum for the purpose of protective coating (Hoefler, 2004). The chain structure of xanthan gum is showed in Fig. 1.8, which consists of glucose backbone, trisaccharide side chains, glucuronic acid residues and mannose units (Hoefler, 2004; Sworn, 2000).

Xanthan gum can be dissolved in both cold and hot water, and on the contrary to most of the other hydrocolloids, ionic concentrations, pH and temperature variations do not affect its solubility. It increases the viscosity of the solutions and enhances stability through the system (BeMiller, 2008).

The studies of Collar et al. (1999) and Rosell et al. (2001) showed that the capability of water absorption and gas retention of xanthan gum improves specific bread volume and crumb moisture of the product. However, those effects are only seen in a range of xanthan gum concentration. The study of Mandala (2005) pointed out that bread volume has been decreased when used amount of xanthan gum increases. The concentration has a key role not only in xanthan gum but also in other hydrocolloids.

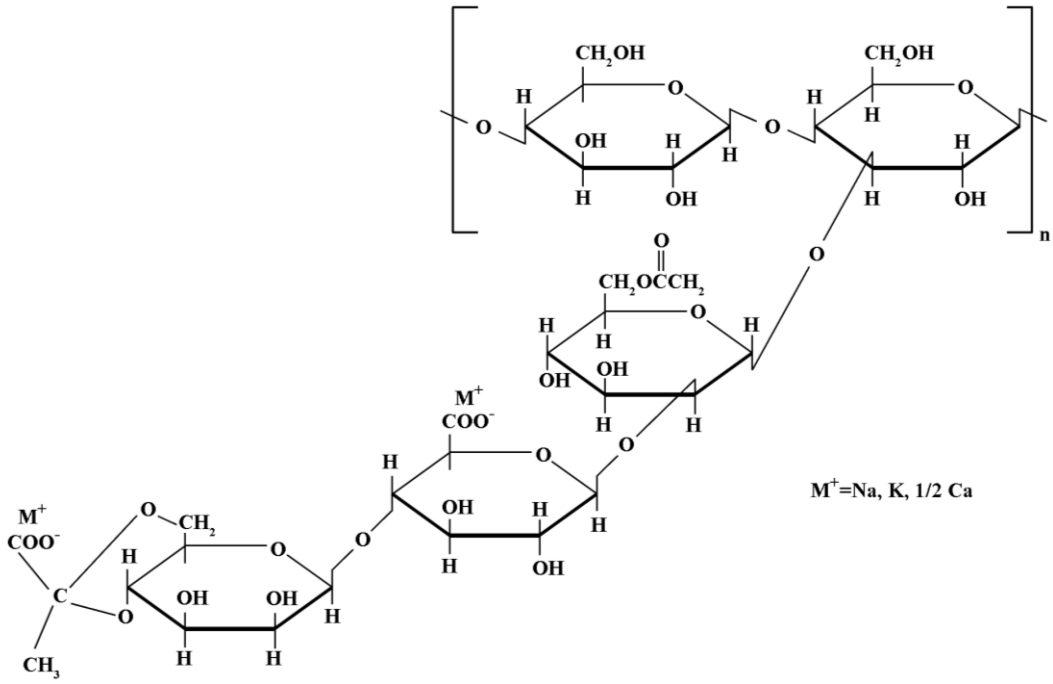

Figure 1.8 Primary structure of xanthan gum

1.7.2.2 Emulsifiers

The other commonly used additives in the food industry are emulsifiers. They have many effects on gluten-free bread processing such as improving the stiffness of the dough, which is a problem of gluten-free dough formulations, and enhancing the shelf life by decreasing staling speed (Nunes et al., 2009). Moreover, they delay starch retrogradation and prevent the migration of water molecules (Stauffer, 2000). Also, Hoseney (1984) indicated that emulsifiers decrease gas bubble surface tension which provides higher gas absorption of dough structure. Besides, bread volume enlargement, dough handling ability and lower crumb drying rate are the other characteristics provided by emulsifiers (Defloor et al., 1991). They contribute tolerance to fermentation and resting processes. Also, they provide uniformity through structure in dough and bread, and higher gas retention ability, which provides using of lower yeast amount (Stampfli & Nersten, 1995). Also, Arendt & Moore (2006) stated that strenght of both the dough and the resulted bread can be enhanced by emulsifier-protein interaction.

Diacetyl tartaric acid esters of monodiglycerides (DATEM), monodiglycerides (MDG), sodium stearoyl-2-lactylate (SSL), lecithin and epoxylated monoglycerides (EMG) are the major types of emulsifiers used in bakery products (Orthoefer, 2008). As stated in the hydrocolloid topic, functions of emulsifiers depend on some factors. As being one of the most important factors, type of the emulsifier is significant, however the dosage of the emulsifier is as important as its type (Schober, 2009).

1.7.3 Rheology of Gluten-free Bread Dough

Rheological properties of dough play a crucial role since dough structure is directly related with the baking procedure and, consequently with, the quality of the final product. Also, these properties are important because they specify the acceptability,

stability and textural properties of the products (Dobraszczyk et al., 2001). Also, the ingredients used in the formulation and their amounts are completely determinative for the textural and rheological properties of breads (Çıkrıkcı, 2013). Dobraszczyk et al. (2001) indicated that bread volume and texture of crumb, which are seen as final quality of breads, are directly correlated with dough handling ability. Moreover, they emphasized that in wheat dough, that correlation is observable. However, it is not easy to say that there is a correlation between gluten-free bread dough and bread.

Rheology is the science which investigates the relationship between deformation and flow. Deformation behaviour of a material is measured according to its response and named as elastic and viscous. G' represents elastic modulus and gives information about the elastic behaviour of the material, while G'' represents viscous modulus and related with viscous behaviour (Mezger, 2011). Wheat dough shows nonlinear shear thinning viscoelastic behaviour. That means it flows with low shear, but shows elastic behaviour with high shear. That kind of properties are not observed in gluten-free dough structure because of its high viscous modulus, which results in flow with low shear, but direct deformation with high shear (Crockett, 2009; Dobraszczyk et al., 2001).

As a result of that problem, researchers have been focused on rheological properties of gluten-free doughs. Especially, the effects of hydrocolloids on gluten-free bread formulations have been investigated by many researchers to overcome that problem. Andersson et al. (2011), Blanco et al. (2011), Crockett et al. (2011), Demirkesen et al. (2010b), Lazaridou et al. (2007), Sabanis & Tzia (2010), Schober et al. (2007) and Turabi et al. (2008) are only some of these researches, who have tried to find a relationship between rheological properties of gluten-free dough and gluten-free bread in recent years.

1.7.4 Structural Analysis of Bakery Products

Appearance, bread volume, texture and sensory are the major properties to determine the quality of a bakery product (Zghal et al., 1999). Structure of the foods affects these properties in every aspect. Because of that reason, both macro and micro structures of foods have an important place in analysis. In spite of its importance, microstructure examination of food products is too difficult, because the fundamental structure shows complexity and has smaller particle sizes (Aguilera, 2005). Various new techniques, especially scanning and spectrometric techniques, have been applied to envision of structures at different levels without any deformation (Falcone et al., 2006).

To analyze basic properties such as shape, color and appearance, image processing systems are used in the food industry. Several studies has been conducted to determine the size, number and distribution of cells in the bread structure by many researchers such as Datta et al. (2007), Ozkoc et al. (2009a) and Scanlon & Zghal (2001).

In order to understand the changes in the structure and in the properties, quantification of structural properties has to be done. As stated above, that quantification can be done by some imaging techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and light microscopy (LM). These imaging techniques allow to analyze micro and macro structural characteristics of food products from basically color and shape to morphological changes. SEM has been used for different food products including maltodextrin particles (Alamilla et al., 2005), cakes (Turabi et al., 2010) and breads (Demirkesen et al., 2013) in recent years.

1.7.5 Texture Profile Analysis (TPA)

Texture Profile Analysis (TPA) is used to analyze the textural properties of bakery products. To obtain the texture profile for a food product, texture analyzers are used which compresses the food with an appropriate probe. In TPA, uniaxial compression is carried on to food sample twice as done in chewing action. As a result of that analysis, some sensory properties such as cohesiveness, gumminess and chewiness are measured (Sahin & Sumnu, 2006).

Force vs time curve is drawn and that curve is known as texture profile of the food sample which is indicated in Fig. 1.9.

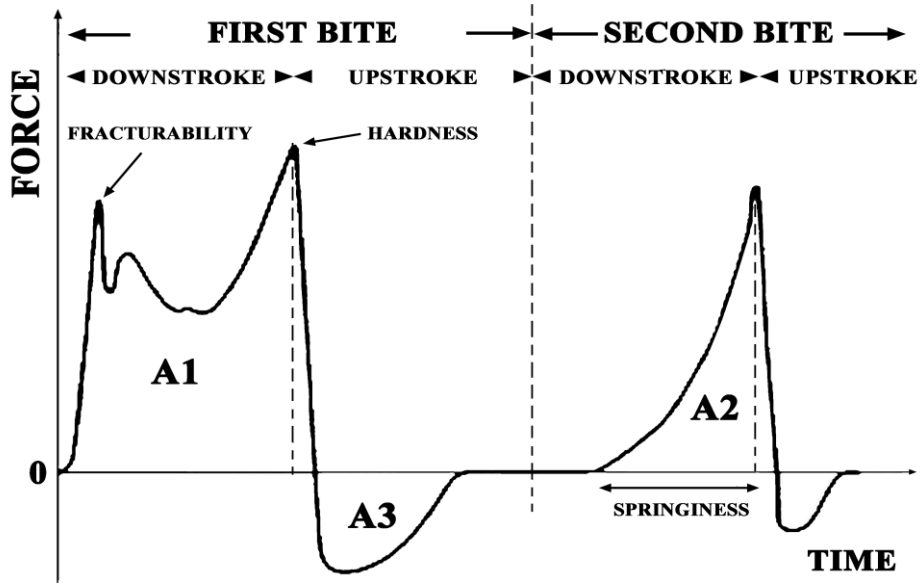

Figure 1.9 Generalize Texture Profile

Since texture analyzer compresses the sample twice with a waiting period between compressions as stated above, curve owns positive and negative areas. Also, forces when the compressions reach their peak values and the areas under the curves are measured by that analysis. As a result of those values, several parameters, which

are directly related with the textural and sensory properties, including hardness, fracturability, cohesiveness, springiness, gumminess and chewiness were determined (Sahin & Sumnu, 2006). The definitions of these parameters are stated below according to Fig. 1.9.

Hardness is the peak force during the first compression cycle to reach the desired deformation. Fracturability is the force, which results in crumbling or cracking of the sample, at the first significant break in the first positive area. Generally food samples, which have high hardness and low cohesiveness, show fracturability. Cohesiveness is related to the resistance of the food sample to second deformation force after the first one. It is mathematically defined as the ratio of second positive bite area to first positive bite area. Springiness (elasticity) is the rate, which is required for food sample to go back to its initial condition. It is defined as the length or distance between the end of the first bite and the start of the second bite. Gumminess is the required energy for disintegration of a semisolid food and get ready to swallow. Its definition is the multiplication of cohesiveness and hardness. Chewiness is the required energy to break apart a solid food and get ready to swallow. Its definition is the multiplication of gumminess and springiness (Sahin & Sumnu, 2006).

1.7.6 Staling of Breads

Staling is resulted by physicochemical reactions, which lead to physical, chemical and sensory changes, in bakery products. Generally, it is defined as crust and crumb aging (Pomeranz, 1987). It is one of the main factors that affects shelf life of the products directly with microbial deterioration and moisture loss (Gallagher et al., 2003). Bread dough consists of several ingredients in its structure and every one of them plays different roles in bread-making procedure, but again every one of them changes during and after the process. Because of the complex structure and reactions, staling is still remains as an unexplained process completely (Gray & BeMiller, 2003).

Starch retrogradation or crystallization is considered as the dominant reason for staling, but not the only one. Bloksma & Bushuk (1988) also stated that starch retrogradation, which is the change in starch structure, is the major reason of bread staling. Some factors such as moisture content and temperature trigger and accelerates the effect of starch retrogradation on bakery products (Seyhun et al., 2005). Besides starch retrogradation, moisture diffusion through the bread and from environment to the bread, fractions of crumb and crust are important reasons behind staling (Ozkoc et al., 2009b). As stated before, starch retrogradation is seen as the main factor, but some studies show that gluten interactions also play an important role in the staling process (Martin & Hoseney, 1991). However, Morgan et al. (1997) showed that starch retrogradation can lead to staling alone without gluten interactions. During staling process, reactions occur and these reactions cause redistribution of water in the structure of bread, which occurs commonly from crumb to crust, change in flavor and mouth feel, and bread crust hardening and crumbling (Pomeranz, 1987). To overcome staling or at least to slow down its effect, some modifications are employed in product procedures (Sumnu et al., 2010).

To characterize and understand the staling process, different studies have been conducted and various techniques have been used. Those techniques can be classified into two main groups, which are macroscopic and molecular techniques. As can be understood from its name, macroscopic techniques focus on physical attributes. Similarly, molecular ones focuses on the molecular levels of the product. The major methods for macroscopic techniques are rheological measurements, sensory evaluations and differential scanning calorimetry (DSC). On the other hand, Fourier transform infrared spectroscopy (FTIR), X-ray diffractometry and nuclear magnetic resonance are included in the molecular techniques (Karim et al., 2000).

1.8 Objectives of the Study

Microfluidization is a unique technique, which includes several forces in its processing. Various studies indicated that microfluidization leads to conformational changes in the structure of the product, which are important because they are directly related with physical functionalities and functional properties of the product. As a result of this technique, nano and micro particles are obtained. Some studies showed that this technique provides smaller particles than conventional homogenizer. Thus, one of main objective of this study was to compare the microfluidizer and colloidal mill, and display the effect of microfluidization on zein slurries at different pH levels by SEM analysis.

Moreover, microfluidization has been implemented to various foods and numbers of studies showed that this unique technique adds some extraordinary structural properties to emulsions and suspensions, and that leads to change in physical properties. In the literature, there is no study on investigating the effect of microfluidization on the emulsifying properties of corn zein. Therefore, another objective of this study was to evaluate the potential usage of microfluidization as a milling process for corn zein and investigate the effects of microfluidization on the emulsifying properties of corn zein.

Foods that contain gluten in its structure cannot be disintegrated by people suffering from celiac disease. Because of that reason, many studies have been conducted to find alternatives to provide the nutritional needs of celiac patients. Most of the corn, which does not contain gluten in its structure, is used as animal feed. Because of its gluten-free structure, it draws attention for gluten-free bread formulations. However, viscoelastic properties are important for a valuable dough formation and gluten is the responsible protein fraction for that purpose. To obtain a valuable dough without gluten, it is necessary to use additional ingredients in gluten-free bread formulations to attain those viscoelastic properties. Therefore, another objective of this study was to evaluate the potential usage of microfluidization as a milling process for corn zein, and investigate the effects of microfluidized corn zein

and different hydrocolloids at different pH levels on the rheological properties of bread dough and final quality parameters (texture, specific volume, color, storage and sensory characteristics) of gluten-free bread samples to develop a product with improved dough stability, better baking characteristics and higher consumer acceptance.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

For the whole experiments, zein having 62% protein, which is the core material used in the emulsion preparation and the corn bread-making process, was obtained from PNS Pendik Nişasta San. A. Ş. (Pendik, İstanbul, Turkey).

For the preparation of emulsions, corn oil of Yudum Gıda San. ve Tic. A. Ş. (Küçükköy, Balıkesir, Turkey) was bought from local markets. Also, distilled water was used in emulsion rheology and stability experiments to obtain required proportions.

Hydrochloric acid (HCl) was supplied from Merck KGaA (Darmstadt, Germany) and sodium hydroxide (NaOH pellets, extra pure) of Honeywell Riedel-de Haën was obtained from Sigma-Aldrich Laborchemikalien GmbH (Steinheim, Germany).

For the bread-making experiments, several ingredients were needed. Sugar and salt were from Ülker Biscuit Industry Co. Inc. (Ankara, Turkey). Corn starch was also taken from PNS Pendik Nişasta San. A. Ş. (Pendik, İstanbul, Turkey). Instant yeast containing natural dough yeast (*Saccharomyces cereviceae*) of Dr. Oetker (Torbalı, İzmir, Turkey) were bought from local markets.

HPMC (Methocel K4M Food Grade) was taken from Dow Chemical Co. (Midland, MI, USA). Xanthan gum and guar gum were obtained from Sigma-Aldrich Chemical Co. (Steinheim, Germany). Citrus fiber (Citri-Fi I00FG) was supplied from Fiberstar, Inc. (Willmar, MN, USA).

2.2 Methods

2.2.1 Preparation of Zein Slurry (Microfluidization)

A colloidal mill (Magic Lab, IKA, Staufen, Germany) and a microfluidizer equipment (M-110Y, Microfluidics, USA) were used for the production of micro and nano zein particles. This process included two main phases which were performed by different equipments as stated above. Firstly, softening was performed by pre-mixing of zein with water in proportion of 1:3 by weight to constitute a homogenous structure. However, because of the hydrophobicity of the zein, the mixing procedure was not effective for a long time. That mixing was just to prepare zein slurry for effective milling. Because of that reason, obtained mixture was passed through colloidal mill whose set point was 20000 rev.min^{-1} at 21°C. Secondly, obtained slurry-like product passed through microfluidizer which had two stages. In the first stage of the microfluidizer, obtained product from colloidal mill was pumped through a chamber of 200 µm size with 500 bar pressure force. In the second stage of the process, the size of the chamber is smaller and the pressure force is greater than the first stage of the process, which are 100 µm and 1250 bar, respectively. The velocity of the product increased to 800 m/s with a higher shear rate than 10^7 1/s in the second stage of the microfluidizer (Fig. 2.1).

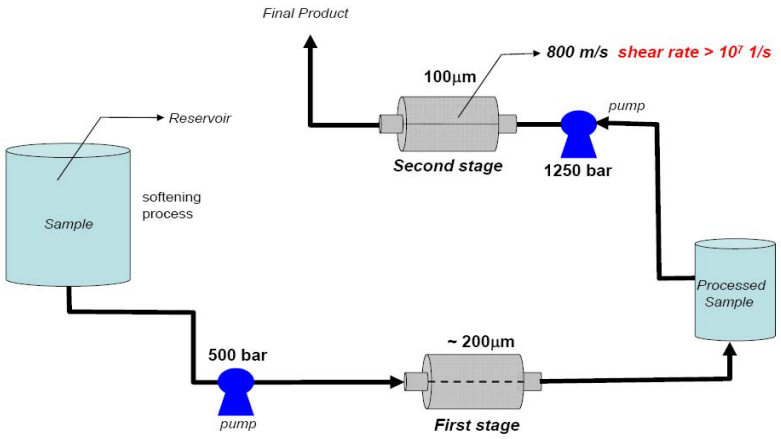

Figure 2.1 Production of micro and nano zein particles

Moreover, this procedure was applied to both control zein (pH=3.86) and zein whose pH was arranged to 6, 8 and 10 to see the effect of acid-base interactivity on zein.

Furthermore, this process was repeated many times to obtain smaller particle sized zein particles. Through this process homogenous zein slurry, which was used in emulsion and bread-making experiments, was obtained by breakage of zein particles.

2.2.2 Analysis on Zein Slurry

2.2.2.1 Scanning Electron Microscope (SEM) Analysis on Zein Slurries

To observe and compare the effect of colloidal mill (Magic Lab, IKA, Staufen, Germany) and microfuidizer (M-110Y, Microfluidics, USA) on zein particles, SEM analysis was performed.

First of all, the preparation of zein slurries was done in two different procedures to observe the effect of colloidal mill and microfluidizer. In one of them, colloidal mill was performed only which cause a skin deep effect on zein particles. In the second one, both colloidal mill and microfluidizer were applied to zein slurry serially which was the main procedure for our emulsion and bread-making experiments.

Moreover, these processes were performed to both control zein (pH=3.86) and zein whose pH was arranged to 6, 8 and 10. Also, these processes were repeated many times to see the effect of application quantity. Finally, for each of the samples, SEM analysis was carried out.

In SEM analysis, mainly three different sample sets were analyzed which were zein passed through colloidal mill, microparticulated zein and also microparticulated

zein whose pH was arranged. For SEM analysis, all samples were dried in freeze-drier (Christ, Alpha 2-4 LD plus, Germany) for 48 hours.

Central Laboratory of METU (Ankara, Turkey) carried out the remaining part of the Scanning Electron Microscope (SEM) Analysis. Firstly, gold-palladium material was used as coating material which was required to render the freeze-dried samples electrically conductive by Sputter Coater Device (Polaron Range, East Sussex, England). Afterward, samples were examined with the helping of scanning electron microscope (QUANTA 400F Field Emission SEM, Eindhoven, Holland) at Central Laboratory of METU which has an accelerating voltage of 20 kV. Finally, images of the freeze-dried samples were recorded at 200X and 500X magnification levels.

2.2.3 Emulsion Preparation

For the preparation process of emulsions, zein, corn oil and distilled water were used in different proportions to investigate the effects on emulsifying properties of these ingredients with varying amounts. First of all, 5 g corn zein was mixed with 15 ml corn oil and 80 ml distilled water and 5:15:80 (w/v/v) proportion was obtained. Similarly, some other proportions were prepared such as 5:30:65, 5:50:45, 10:15:75, 10:30:60, 10:50:40, 15:15:70, 15:30:55 and 15:50:35, which were the combinations of 5-10-15 g zein, 15-30-50 ml corn oil and the remaining part was distilled water. After the mixing part, homogenization was done at 20000 rpm at 21°C in the colloidal mill (Magic Lab, IKA, Staufen, Germany) and that homogenization process was repeated for 3 times to apply homogenization to all structure from biggest molecules to smallest ones.

2.2.4 Analyses on Emulsions

2.2.4.1 Rheological Measurements on Emulsions

TA rheometer (AR 2000ex Rheometer, England) was used for all rheological measurements of emulsions. The parallel plate geometry, that has 20 mm diameter and 1 mm gap, was applied to emulsions at room temperature (25˚C) to obtain the required measurements. Through these measurements, different emulsions, which were stated above with different proportions, were analyzed. First of all, the emulsion was placed between the plates and cleaning was done for accurate measurement. Two different measurements were applied to emulsions, which were flow measurement and viscoelastic measurement. In the flow measurements, shear stress (τ) was calculated according to the shear rate (γ), which changes from 0.1 to 30 Hz with 0.1% strain rate as controlled variable. On the other hand, frequency sweep test was carried out as dynamic oscillatory experiment. Frequency sweep test was performed at 25˚C with an equilibration duration of 2 minutes. Elastic moduli (G') and viscous moduli (G") values of emulsion samples were measured according to angular frequency, which varies from 3.142 to 150 rad/s with 0.1% strain rate as controlled variable.

2.2.4.2 Emulsion Stability Analysis

The emulsion stability of the prepared emulsion samples were measured by using of Dispersion Analyzer (LUMiSizer, LUM, Berlin, Germany). The prepared samples were placed to reservoirs of analyzer in small tubes. The analyzer basically resembles to a centrifuge, but it has multi-wavelength light source and sensors to analyze the passing light as could be seen from Fig. 2.2.

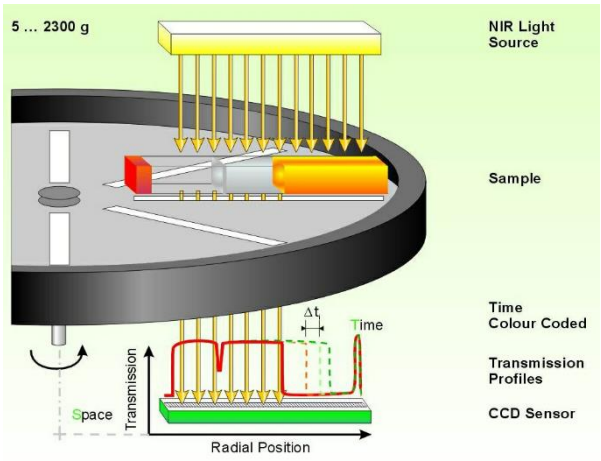

Figure 2.2 Schematic of dispersion analyzer

The samples were rotated with a frequency of 3000 rpm for 3600 seconds and intensity of the light was measured through this method. Due to high frequency, emulsions showed instability and began to disperse through time, which resulted in easy penetration of light. According to penetration of light, transmission percentages were calculated, which was defined as emulsion stability.

2.2.5 Bread-making Procedure

The basic gluten-free bread-making procedure was applied based on the study of Schober et al. (2008). The main bread-making procedure involved pre-mixing of microparticulated zein-water slurry obtained after microfluidization (20 g zein, 60 g water) with additional water (15 g) and gum which was planned to use in that experiment (5 g HPMC, xanthan, guar or citrus fiber) by a mixer (Krups, Groupe SEB, Germany) with standard-shaped agitator for 5 minutes to mix gum better. Then, corn starch -60, 70 or 80 g depending on experiment- 5 g sugar, 2 g table salt and 1 g dry yeast were added to obtained mixture and the whole of the ingredients

were mixed by the mixer which has a spiral-shaped agitator that provides quicker mixing because of less agitator friction. All these processes were done about 40°C, which is higher than the glass-transition temperature of zein to obtain a better dough. The covered dough was allowed to rest for 1 hour at 40°C for leavening process. Then, it was mixed again with spiral-shaped agitator until the homogenous and smooth structure of the dough was seen which generally lasted for 3 minutes. Afterwards, it was allowed to rest again for 30 minutes at 40°C. After that, the dough was poured into silicon molds and baked in conventional oven (Guangzhou Hongbang Western Kitchen Equipment Co. Ltd., China) at 190°C for average of 12 minutes. After baking, breads were cooled to room temperature for an hour to get ready for the analyses. For some staling analyses, some of the bread samples were kept at room temperature in vacuum packs.

Also, non-processed zein was used in bread-making procedure to observe the effect of microparticulation. As stated before, different gum types such as guar, xanthan gum and citrus fiber were used instead of HPMC; in others, the effect of the presence of DATEM was observed. 180, 200 and 210°C were also tried as baking temperature. Water amount was changed in a range from 60 to 90 g. Finally, mixing procedure was done at lower temperatures to observe the effect of glass-transition temperature. To see the effects of microparticulation, hydrocolloids, temperature change in leavening, water amount and mechanical treatment below glass-transition temperature, formulation and procedure could be modified.

2.2.6 Analyses on Bread Dough and Bread

2.2.6.1 Rheological Measurements on Bread Dough

All rheological measurements were carried out by using of TA rheometer (AR 2000ex Rheometer, England) as done in the emulsion rheology analysis. The required measurements were done at room temperature (25˚C) by using parallel

plate geometry, which is steel and has 20 mm diameter and 1 mm gap. In these measurements, the dough samples from different formulas were placed between the plates. After that, to obtain a good result and to prevent exterior effects, the edges of dough sample between the plates were carefully cleaned with a spatula. Also, the dough samples were rested at room temperature for 5 minutes before testing to discard residual stresses. In dynamic oscillatory experiments, frequency sweep and strain sweep tests were carried out. Strain sweep test was performed at same conditions as frequency sweep test to identify the linear viscoelastic region of the dough. Strain rate altered between 0.01% and 10% with angular frequency of 6.283 rad/s. According to these, elastic moduli (G') and viscous moduli (G") values were measured. Frequency sweep test was performed at 25˚C with an equilibration duration of 2 minutes. During the measurement, frequency changed from 0.1 to 100 Hz with 0.1% strain rate as controlled variable. Again, elastic moduli (G') and viscous moduli (G") values were obtained from the measurements.

2.2.6.2 Moisture Analysis

The moisture content of the bread samples were calculated by using of the weight of fresh bread samples (W_{fresh}) and the weight of bread samples which were waited in incubator (Memmert, Germany) at 103 ˚C for 48 hours (W_{bread}).

$$\%\ \text{Moisture Content (MC)} = \frac{W_{fresh} - W_{bread}}{W_{bread}} \times 100 \qquad (2.1)$$

All moisture content analysis were carried out as duplicates.

2.2.6.3 Scanning of Breads

Bread samples were cut vertically to see the inside structure of the breads and each slice was placed over the scanner (CanoScan Lide 110, Tokyo, Japan) and images were taken.

2.2.6.4 Color Analysis

ProImage Program was used to determine the color of the bread samples. CIE L*, a*, and b* color scale was applied to samples by that program in this pat of the study. Twelve readings were performed from different points of the breads to obtain L*, a*, and b* values. According to CIE L*, a*, and b* color scale, the L* value ranges from 0 (black) to 100 (white), the a* value ranges from −100 (redness) to +100 (greenness) and the b* value ranges from −100 (blueness) to +100 (yellowness), respectively. Total color change (ΔE) was calculated from the equation below;

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

Reference point (L_0, a_0 and b_0) was selected as the L*, a*, and b* values of the white sheet, which were 100, 0 and 0, respectively.

2.2.6.5 Texture Analysis

Texture of bread samples were evaluated by the Texture Analyzer (The TA.XT*Plus*, England). This experiment was done when bread samples were cooled down to room temperature, 25°C. The probe, which was used to compress bread samples in that experiment, has 1 cm diameter. Before testing, weight calibration was done with 2 kg load and height was calibrated to 11 mm which was the thickness of the

bread samples. After the calibration procedure, texture profile analysis (TPA) was performed at a 1 mm/s pre-test speed, 1 mm/s test speed and 1.7 mm/s post-test speed with 30 s holding time between the compressions. Firstly, the bread sample was compressed by the probe up to 50% of its height. Then, it returned to its beginning position for 30 s as stated before. Afterward, it again compressed the sample by same strain, 50%. As the result of that analysis, forces and elapsed time during the compressions and also areas beneath the compression forces, which were the product of forces and durations, were measured and recorded. Hardness, cohesiveness, springiness and chewiness values were calculated from these values. Six readings were carried out from different bread samples of same set in this experiment.

2.2.7 Statistical Analysis

To determine whether there was a significant difference between different bread sample types which were prepared by different compositions, hydrocolloids and also stored for staling, analysis of variance (ANOVA) was carried out by using MINITAB (Version 16) software. If significant difference was seen, Tukey Single Range Test was applied to samples and means of them were compared with each other.

CHAPTER 3

RESULTS AND DISCUSSION

Firstly, micro structure of zein slurries, which obtained by different treatments, were analyzed in this study. With the help of this analysis, the effects of microfluidization and acid-base interactions were researched.

Then, emulsion rheology in terms of flow measurement and viscoelastic measurement, and emulsion stability analyses were conducted to investigate the emulsifying properties of zein slurries.

After the emulsion analyses, zein was used as a bread-making ingredient to overcome the counter effects of gluten on celiac people. During bread-making procedure, bread dough and bread were analyzed in terms of their rheological and textural properties. Rheological measurements were carried out and viscoelastic properties of corn bread dough were determined. After that, some quality parameters such as moisture content, crumb color and texture of gluten-free corn bread were measured. The textural analyses were done for four days to examine storage duration on staling of corn bread.

3.1 Micro Structure of Zein Slurries

Before micro structure of the samples were examined, the difference between untreated and microfluidized zein was tried to point out by Fig. 3.1. As could be seen from figure, the effect of microfluidization on zein slurry was easily noticeable. The Fig. 3.1A displays the hydrophobicity of zein, which resulted in quick phase separation through the system. On the other hand, zein slurry treated

by microfluidization provided more homogenous structure through the system as could be seen from Fig. 3.1B. After displaying that fact, the understanding the effect of microfluidization on micro structure and also on other analyses could be simpler.

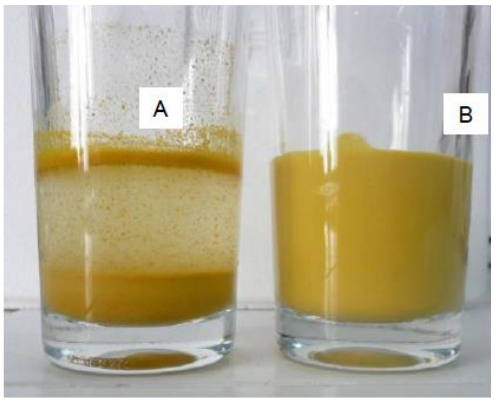

Figure 3.1 The images of untreated (A) and microfluidized (B) zein

Scanning Electron Microscope (SEM) analysis was carried out to investigate the effect of colloidal mill, microfluidizer and acid-base interaction on the micro structure, and to see the effect of these treatments on size, distribution and structure of corn zein in this study. For this purpose, zein slurry without treatment, zein slurry passed only from colloidal mill, zein slurry passed serially from colloidal mill and microfluidizer at different pH values (4=control, 6, 8 and 10) were used. The images were recorded at 200x and 500x magnification levels.

The SEM images of the samples prepared by different treatments with 200x and 500x magnification levels are demonstrated from Fig. 3.2 to Fig. 3.13. 200x magnification level provided only to see the rough structure of the samples. On the other hand, 500x magnification level gave us more information about the surface structure of the sample. However, as could be seen easily from figures, differences between treatment types were noticeable.

The SEM images of zein slurries without treatment are illustrated in Fig. 3.2 and Fig. 3.3 at two different magnification levels. Zein slurry without treatment with 200x magnification (Fig. 3.2) showed aggregation through some part of the sample. Moreover, distribution was not uniform and that could be the reason of hydrophobicity of zein. Because, proteins tend to diminish their energy by folding and aggregating in the structure. Also, not to encounter with aqueous surroundings, hydrophobic amino acids aggregate in the hidden part of the proteins (Cabra et al., 2007). Because of that reason, untreated zein tended to aggregate. Also, as could be seen from Fig. 3.3, surface of the sample was completely closed and that could lead to decreasing water holding capacity of sample which is an important parameter for bread-making procedure.

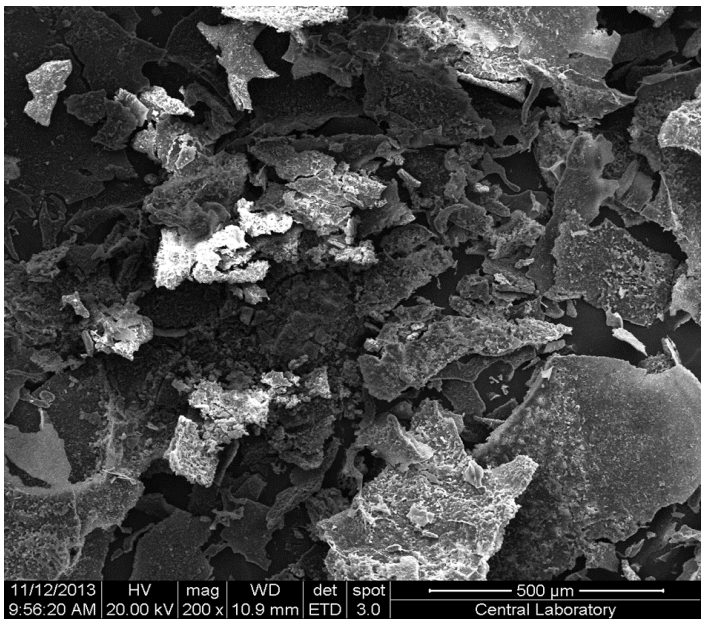

Figure 3.2 SEM image of zein slurry without treatment (×200 magnification)

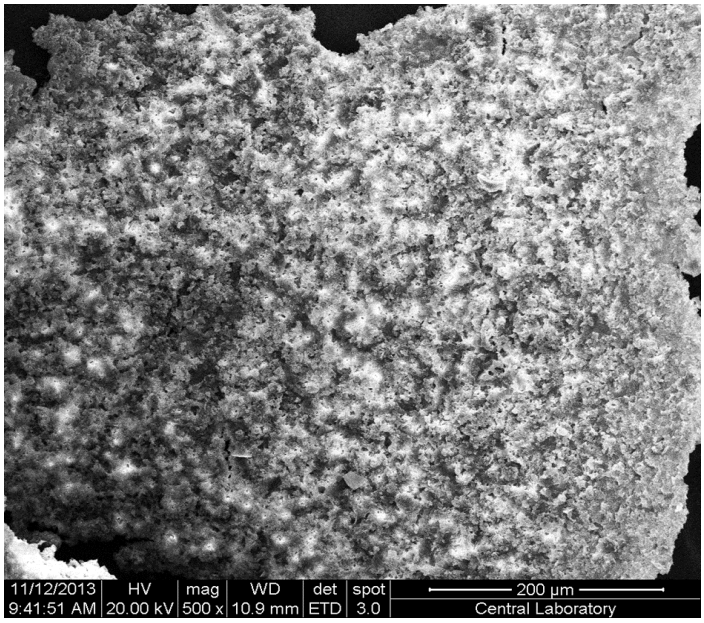

Figure 3.3 SEM image of zein slurry without treatment (×500 magnification)

The images of zein slurries processed only by colloidal mill are represented in Fig. 3.4 and Fig. 3.5. As could be seen from figures, although colloidal mill did not provide a perfect uniformity, it roughly affected the structure and provided more complexity than the untreated one. As comparing figures of untreated one with treated by colloidal mill, the uniformity difference could be easily distinguishable. As could be seen from Fig. 3.5, colloidal mill provided more fragmented structure by decreasing the particle size of the sample. The closed structure of zein, which could be seen in Fig. 3.3, was ruptured and more open structure was obtained. Also, zein molecules distributed through the structure and aggregation tendency diminished by breaking of zein molecules, which resulted in decreasing hydrophobicity of zein. Nevertheless, colloidal mill was not a good solution to overcome the hydrophobicity of zein. The study of Çıkrıkcı (2013) on hazelnut skin supported our findings. They used hammer and ball mills as conventional methods in their study, and the effect of them found lower than microfluidization.

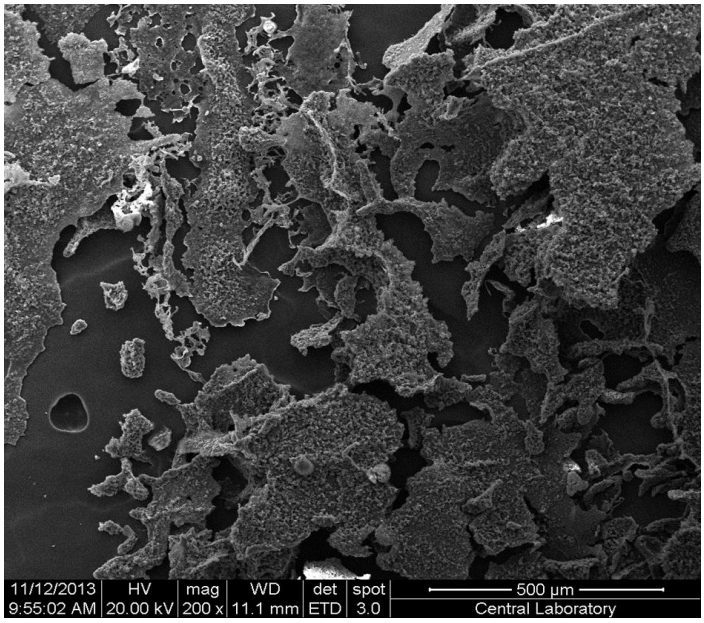

Figure 3.4 SEM image of zein slurry processed by colloidal mill (×200 magnification)

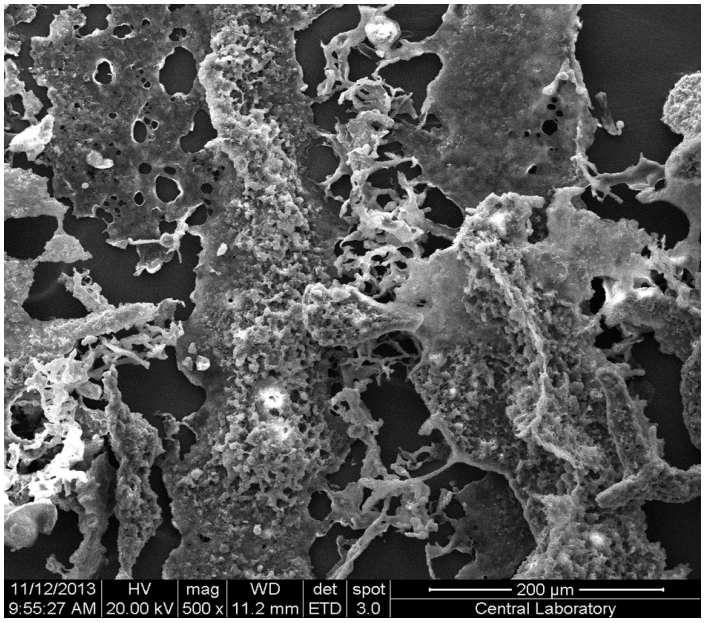

Figure 3.5 SEM image of zein slurry processed by colloidal mill (×500 magnification)

The picture completely changed with microfluidization process, which led to production of micro and nano particles with high pressure. The images related to microfluidization treatment are demonstrated in Fig. 3.6 and Fig. 3.7. As could be seen from Fig. 3.6, massive blocks in Fig. 3.2 and 3.4 were destroyed with microfluidization and pressure resulted in the formation of smaller particles from aggregated ones that could easily lead to formation of homogenous structure. Dissanayake and Vasiljevic (2009) indicated that due to the mechanical forces in microfluidization, particle size of the products became extremely smaller. Also, the shapes of the particles were changed by microfluidization. The smooth surface seen in Fig. 3.2 and Fig. 3.4 were altered to branchy network by this treatment. Additionally, pores and tissues appeared through the system. Tu et al. (2013) stated similar findings on maize amylose. Also, Brookman (1975) indicated that micropores and cavities were obtained by high pressure treatment. Also, Liu et al. (2011) stated that after microfluidization, bigger globular proteins became smaller and even difficult to find in the structure. Moreover, microfluidization process increased the surface area of the sample with providing dispersion through the surface and forming micropores, as could be noticable from Fig. 3.6. The study of Wang et al. (2013) supported our findings on surface area. Also, Chau et al. (2006) stated that larger area resulted in more water binding sites, which provided high hydration properties. The particles became integrated and formed a lattice network by the effect of microfluidization. On the contrary, conventional methods, which was colloidal mill in our case, caused less compression and shear forces. Because of that reason, it provided less homogenous structure with bigger particle sizes. Therefore, the hydration properties of the products obtained from colloidal mill were lower than the microfluidized products. The study of Mert (2012) showed the similar findings about microfluidization on ketchup type products. The studies of Tunick et al. (2000) on cheese and Çıkrıkcı (2013) on hazelnut skin expressed that microfluidization was more effective than the conventional methods, and it provided more desired products as being in our study.

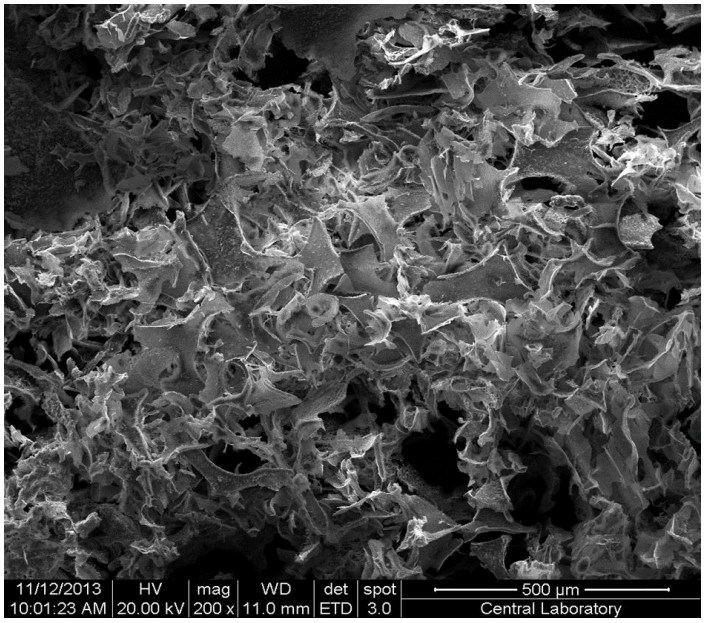

Figure 3.6 SEM image of zein slurry processed by microfluidizer (×200 magnification)

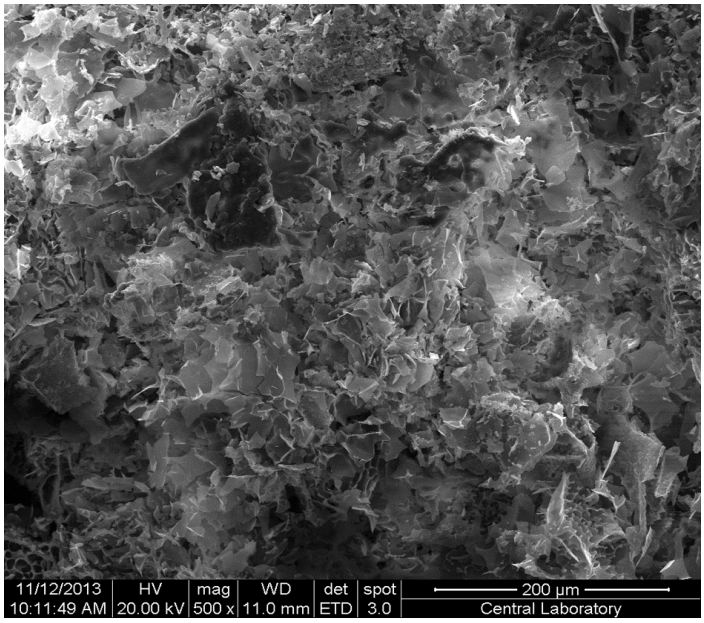

Figure 3.7 SEM image of zein slurry processed by microfluidizer (×500 magnification)

As the final concept of that analysis, acid-base interactions were evaluated to see the effects of alkalinity on the micro structure. As could be seen from Fig. 3.8 to 3.13, the structure of the samples opened and dispersed with increasing alkalinity in addition to microfluidization. That could be the reason of protein modification resulted from alkalinity, which provided smaller particles. Proteins were forced to breakdown and open by this treatment (Cabra et al., 2007). Also, opened structure due to protein modification conduced to a branchy appearance. That appearance became more obvious at 500x magnification levels of pH=6 and pH=8 (Fig. 3.8 and Fig. 3.10). However, there was an important point about alkalinity according to our study. Although, protein modification by alkalinity gave positive results in terms of surface area and particle size, after some point it completely changed the structure of the protein and led to deformation of the samples as could be seen from Fig. 3.12 and Fig. 3.13. Because of that reason, we concluded that pH=8 with microfluidization gave the best result on micro structure of the samples.

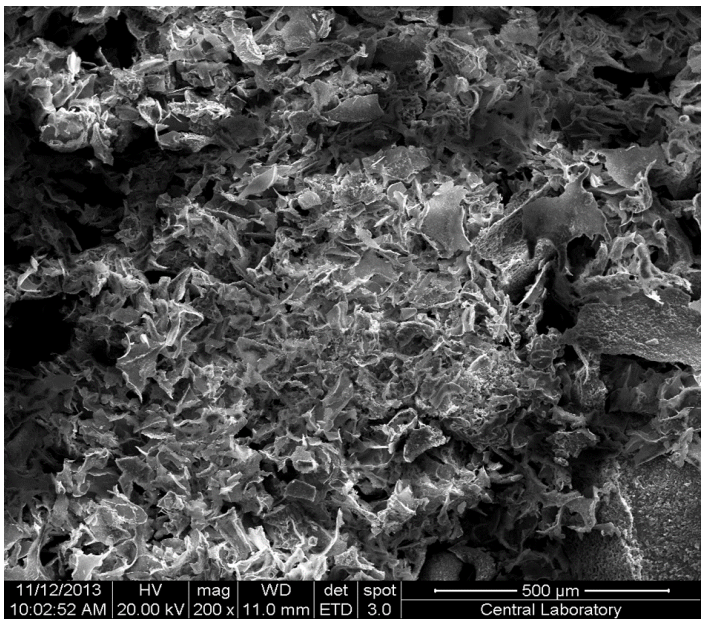

Figure 3.8 SEM image of zein slurry processed by microfluidizer at pH=6 (×200 magnification)

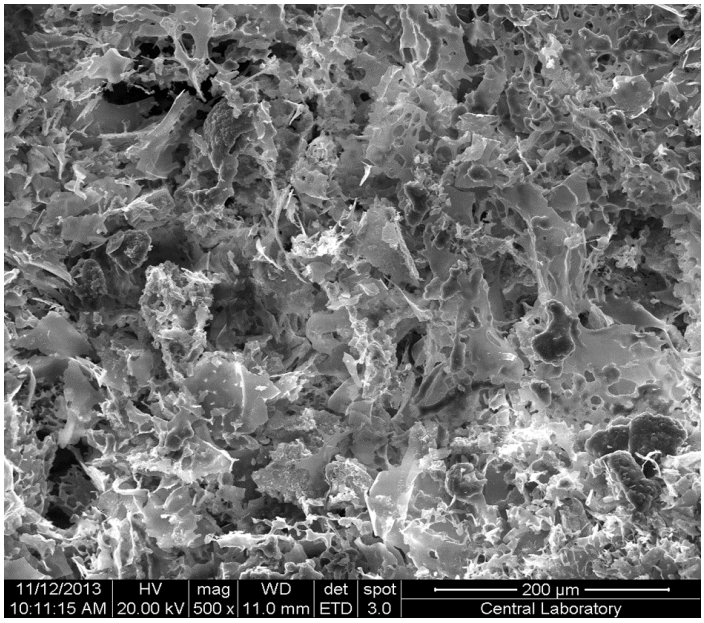

Figure 3.9 SEM image of zein slurry processed by microfluidizer at pH=6 (×500 magnification)

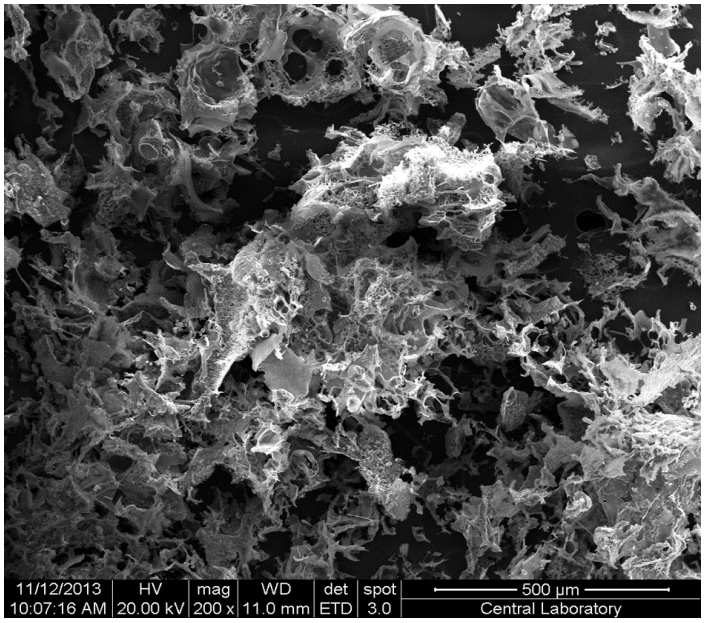

Figure 3.10 SEM image of zein slurry processed by microfluidizer at pH=8 (×200 magnification)

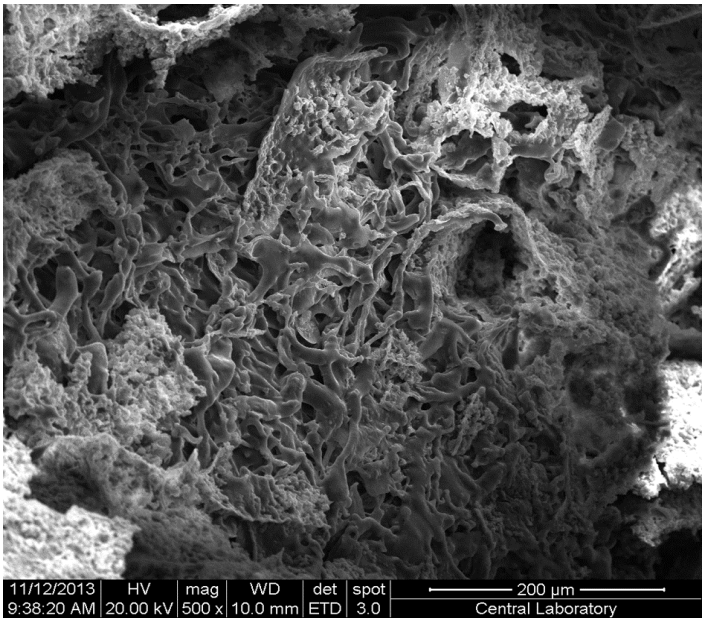

Figure 3.11 SEM image of zein slurry processed by microfluidizer at pH=8 (×500 magnification)

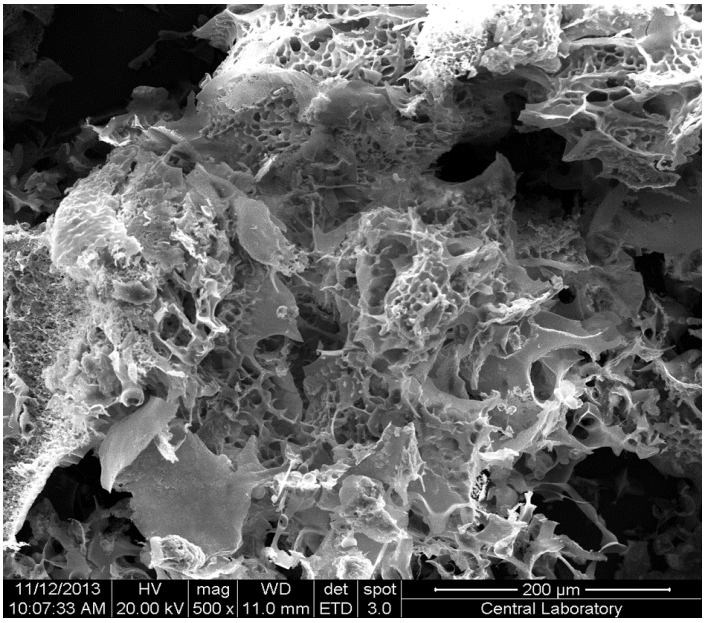

Figure 3.12 SEM image of zein slurry processed by microfluidizer at pH=10 (×200 magnification)

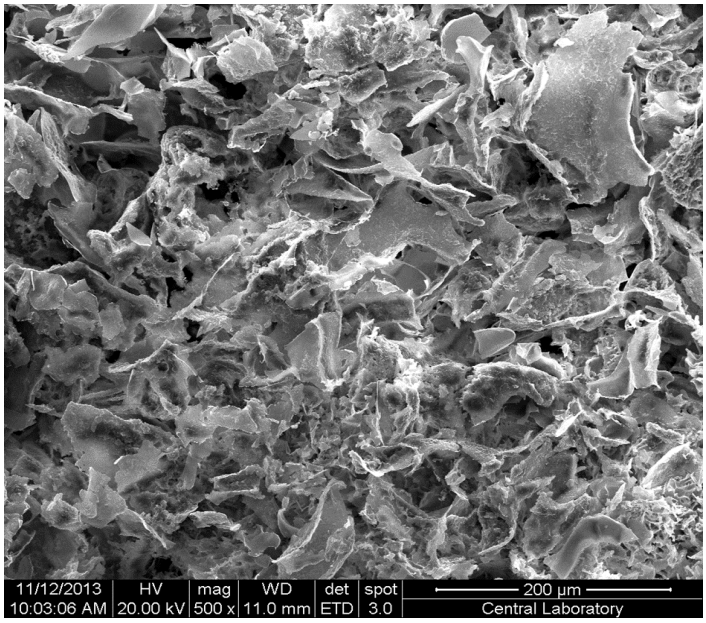

Figure 3.13 SEM image of zein slurry processed by microfluidizer at pH=10 (×500 magnification)

Lastly, scanning electron microscope (SEM) images are also beneficial to evaluate the results of other analyses. As stated before, microfluidization and alkaline treatment up to some point resulted in lower particle size and higher surface area, which were effective on textural and rheological properties, and also in staling mechanism of the products (Çıkrıkcı, 2013). The study of the Chau et al. (2006) pointed out that microfluidized corn bran had a potential as a functional ingredient to prevent and improve some characteristics and properties like viscosity, mouthfeel and texture.

3.2 Analyses on Emulsions

3.2.1 Emulsion Rheology

3.2.1.1 Flow Measurements

Shear stress (τ) vs shear rate (γ) data for all zein emulsions containing different oil and zein concentrations, and also prepared by different treatments were well fitted to the Herschel-Bulkley model at 25ºC (Eqn. 3.1):

$$\tau = \tau o + K(\dot{\gamma})^n \quad (3.1)$$

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 ($Pa.s^n$) and n is flow behavior index.

Table 3.1 presents the Herschel-Bulkley model parameters of zein emulsions, in which different oil and zein concentrations were used and also different treatments were applied. Flow behavior index (n) of emulsions was varying from 0.26 to 1.10. Because of that reason, it could be stated that a few of the emulsions showed shear thickening behavior (dilatant fluid) and most of them showed shear thinning behavior (pseudoplastic fluid). The viscosity of the shear thinning fluids decreases as applied shear is increased because applied shear resulted in breakage of the interactions between components. The vice versa is valid for shear thickening fluids (Çıkrıkcı, 2013; Demirkesen et al., 2010b). Table 3.1 indicated that the flow behavior index of all emulsions prepared by microfluidization were varying from 0.88 to 1.10, which were close to 1 (Newtonian-fluid). As stated before, microfluidization led to formation of more homogenous structure and increase in viscosity. On the other hand, alkaline treatment resulted in deamination and unfolding of proteins which changed the structure. Emulsions containing 15% oil & 10% zein, and 30% oil & 10% zein and prepared by pH arrangement to 8, showed the lowest n value as 0.26. That meant they differed from Newtonian fluids at most (n=1) and because of that reason they showed more complex structure than the others (Gómez et al., 2010).

Table 3.1 Herschel Bulkley parameters of emulsions at 25 ºC.

Formulations	Treatment	τ_0 (Pa)	K (Pa.s^n)	n	std. error
15% oil & 5% zein	mf	0.026	0.093	0.93	3.239
	pH6	1.085	2.803	0.53	2.792
	pH8	47.93	38.13	0.47	5.933
	pH10	0.619	7.557	0.55	2.456
30% oil & 5% zein	mf	0.109	0.396	0.93	3.239
	pH6	4.149	10.72	0.53	2.792
	pH8	71.77	57.09	0.47	5.933
	pH10	2.662	32.50	0.55	2.456
50% oil & 5% zein	mf	3.196	24.54	0.88	8.223
	pH6	193.1	499.0	0.53	2.792
	pH8	3192	2540	0.47	5.933
	pH10	413.3	1068	0.53	2.792
15% oil & 10% zein	mf	0.052	0.042	1.10	5.888
	pH6	1.109	5.229	0.62	3.628
	pH8	505.9	302.1	0.26	25.48
	pH10	19.37	32.96	0.42	2.093

Table 3.1 (cont'd) Herschel Bulkley parameters of emulsions at 25 ºC.

	mf	0.271	0.222	1.10	5.888
30% oil & 10% zein	pH6	5.442	25.65	0.62	3.628
	pH8	843.2	503.4	0.26	25.48
	pH10	114.5	194.9	0.42	2.093
	mf	32.08	245.4	0.88	8.223
50% oil & 10% zein	pH6	1334	3448	0.53	2.792
	pH8	43540	34640	0.47	5.933
	pH10	4918	12710	0.53	2.792
	mf	0.083	0.068	1.10	5.888
15% oil & 15% zein	pH6	1.220	5.752	0.62	3.628
	pH8	2327	113.4	0.81	24.48
	pH10	50.36	85.69	0.42	2.093
	mf	0.4341	0.3559	1.10	5.888
30% oil & 15% zein	pH6	11.43	53.87	0.62	3.628
	pH8	2220	398.6	0.80	30.43
	pH10	297.8	506.6	0.42	2.093
	mf	120.4	922.7	0.88	8.223
50% oil & 15% zein	pH6	3576	9240	0.53	2.792
	pH8	104900	83500	0.47	5.933
	pH10	11860	30640	0.53	2.792

Yield stress is one of the important rheological parameter to interpret the performance of a product (Kocak, 2010). It is defined as the stress level which has to be overcome to initiate the flow (Çıkrıkcı, 2013; Tabilo-Munizaga & Barbosa-Canovas, 2005). Generally, for the same emulsion preparations, the yield stress and consistency index values showed same pattern. pH=8 was the highest in terms of these values and it was pursued by pH=10, pH=6 and microfluidized zein emulsions, respectively. The explanation of resistance to flow could be the formation of smaller particles which led to homogenous and complex structure.

Also, increase in oil concentration caused to higher yield stress and consistency index. Similar results were found for increasing in zein concentrations. In the light of obtained results, it was concluded that increasing oil and zein concentraitons, and also decreasing particle size by microfluidization and additional alkalinity were the reasons behind higher consistency index and yield stress values.

The flow curves of emulsions containing different zein and oil concentrations, that prepared by different treatments are illustrated from Fig. 3.14 to Fig. 3.22.

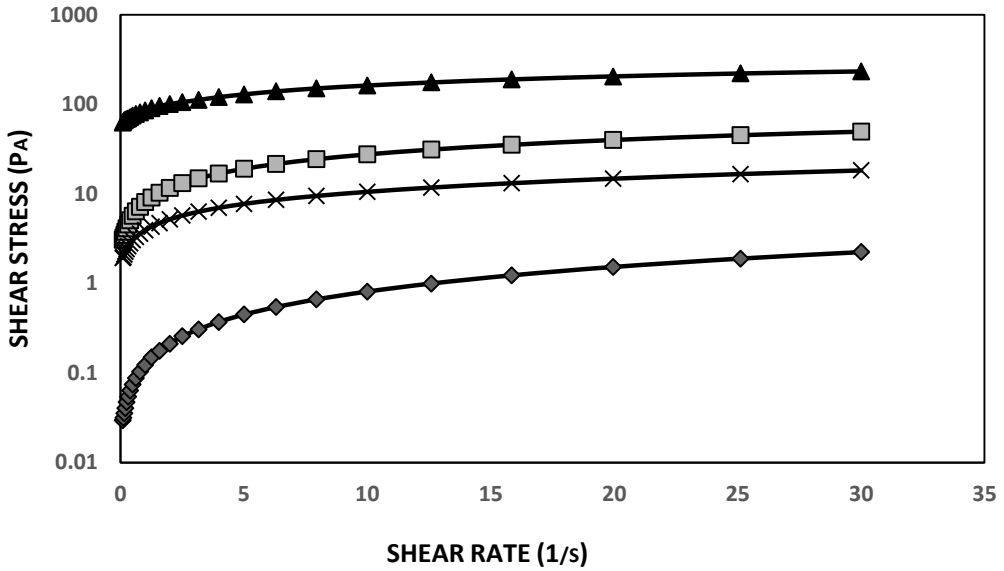

Figure 3.14 Flow curves obtained for emulsion containing 15% oil and 5% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

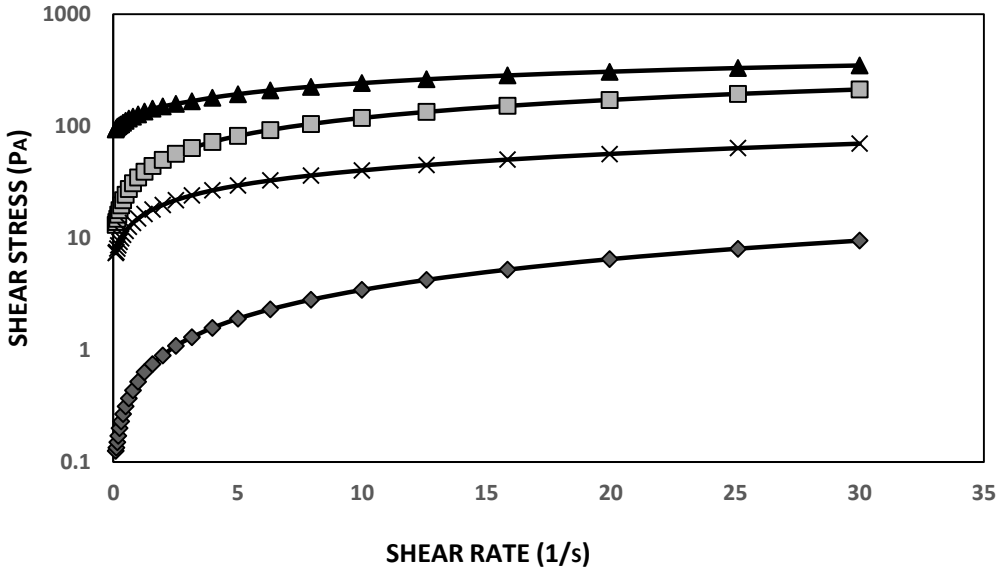

Figure 3.15 Flow curves obtained for emulsion containing 30% oil and 5% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

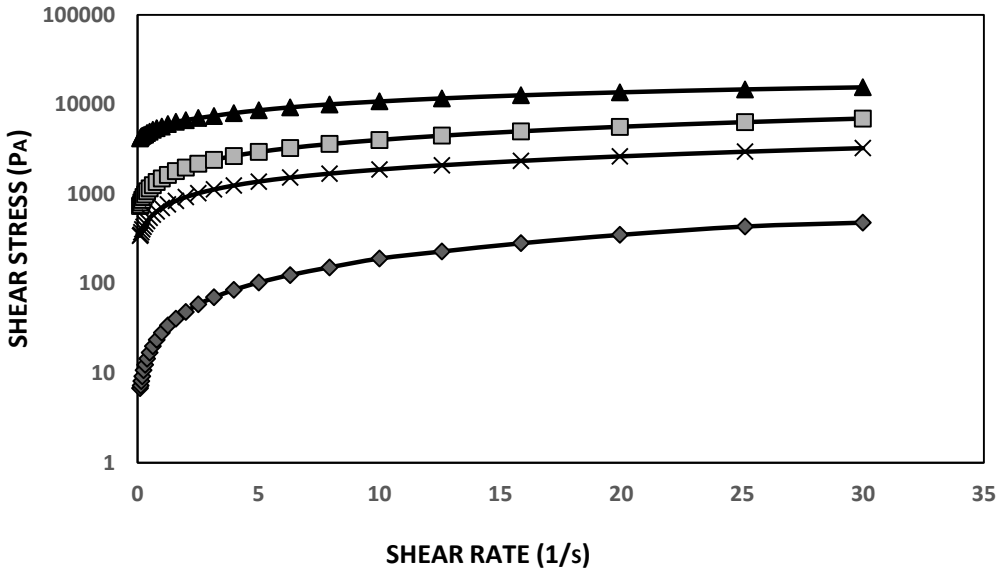

Figure 3.16 Flow curves obtained for emulsion containing 50% oil and 5% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

The flow curves of untreated zein emulsions could not be measured because of quick phase separation, which was seen in the emulsion due to hydrophobicity of zein without treatment. Due to this reason, emulsions prepared with untreated zein were ignored in this analysis.

As could be seen from previous three figures, emulsions containing 15, 30 and 50% oil concentrations with 5% microfluidized zein concentration were prepared at different pH values, and the flow curves of them were determined. In this point, it had to be expressed that the flow measurements of microfluidized zein emulsions could be obtained contrary to untreated zein. That meant microfluidization became successful and could be stated as an effective method for emulsion preparation as stated by McClements (2005), in spite of hydrophobic structure of zein. The study of Mert (2012) indicated that this technique provided more homogenous and smaller particles, which resulted in improvement of emulsion rheology. In the study of Masodi et al. (2002), microfluidization was indicated as a novel technology, which provided smaller particles and as a result of that higher viscous structure was obtained. Increasing viscosity was explained by improving water absorption capacity with finer particles. As stated in SEM analysis part, microfluidization led to formation of branched structure and as a result of that increased surface area, which were directly related with water holding capacity.

The shear stress vs shear rate figures showed that higher viscosity values were obtained from emulsions prepared by both microfluidization and alkalinity. If the alkalinity level was compared, it could be stated that alkalinity arrangement to pH=8 provided more viscous structure than the others. pH=8 was followed by pH=6, pH=10 and microfluidized zein emulsions, respectively. These results pointed out that increasing alkalinity resulted in formation of more viscous structure up to some point, and that point was determined as pH=8 in our study. As stated many times before, microfluidization led to finer particles. That particle distribution was improved by alkaline treatment. Therefore, emulsions prepared by alkaline treatment showed higher yield stress and consistency index values than emulsions prepared only by microfluidization. The reason behind these high values could be more interaction between water and zein by deamination and unfolding of proteins

due to alkalinity. By alkalinity, the structure of zein was reorganized and hydrophobicity was overcome in some degree. However, increasing alkalinity after a point resulted in decomposition in zein structure and return in viscosity. The flaky structure obtained by microfluidization was improved by alkaline treatment and viscosity of the structure increased that resulted in resistance to flow. Ciron et al. (2010) stated that microfluidization provided more consolidated network by decreasing particle size which led to more interactions through the structure.The study of San Martin-Gonzalez et al. (2009) on corn oil emulsions supported our findings related to flow measurements of emulsions. They changed the pressure in high pressure homogenizer to investigate the effect of pressure on emulsion rheology and stability. They pointed out that increasing pressure led to smaller particles and also higher viscosity up to some pressure level. However excessive pressure affected the structure in the direction of deformation, which provided lower viscosity. The same relationship was emphasized in the study of Mert (2012). This study also showed that increasing pressure resulted in high viscosity up to some point. These two studies on microfluidization pressure could be thought as our alkaline treatment. Increasing pressure could be thought as increasing alkalinity, which served to same purpose. As being in higher pressure, increasing alkalinity caused to increase in viscosity up to some point. This could be explained by overprocess of the samples. Smaller particles could be attained by these methods, but they had no ability to adsorb oil after a point.

The last point was that as could be observed from figures, increase in both oil and zein concentrations led to higher viscous structures. The viscosity of the emulsion, containing 15% of zein concentration and 50% of oil concentration, was highest and this value decreased by decreasing concentration amounts.

The results related to other concentration formulations showed same results with 5% of zein as could be seen from figures below.

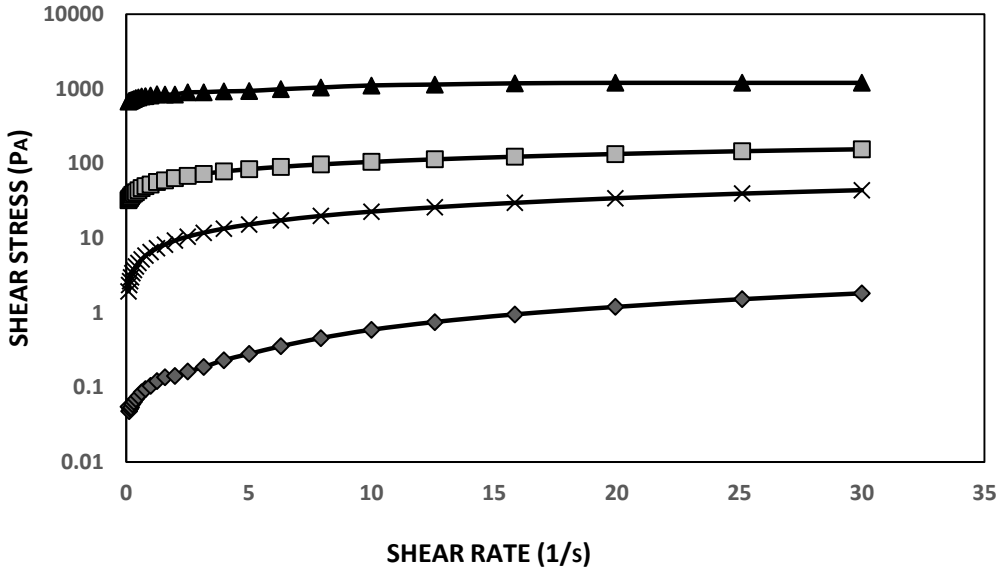

Figure 3.17 Flow curves obtained for emulsion containing 15% oil and 10% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

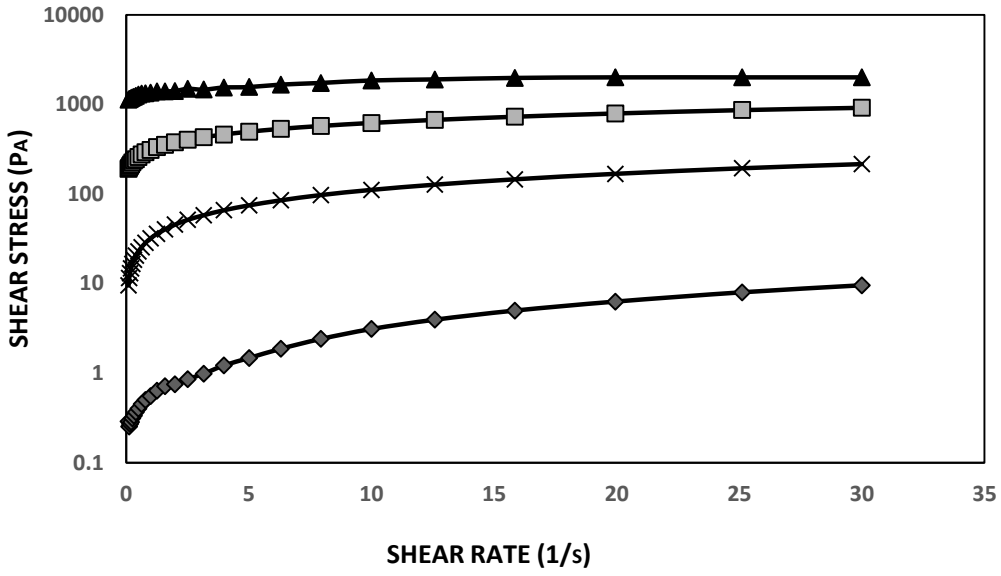

Figure 3.18 Flow curves obtained for emulsion containing 30% oil and 10% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

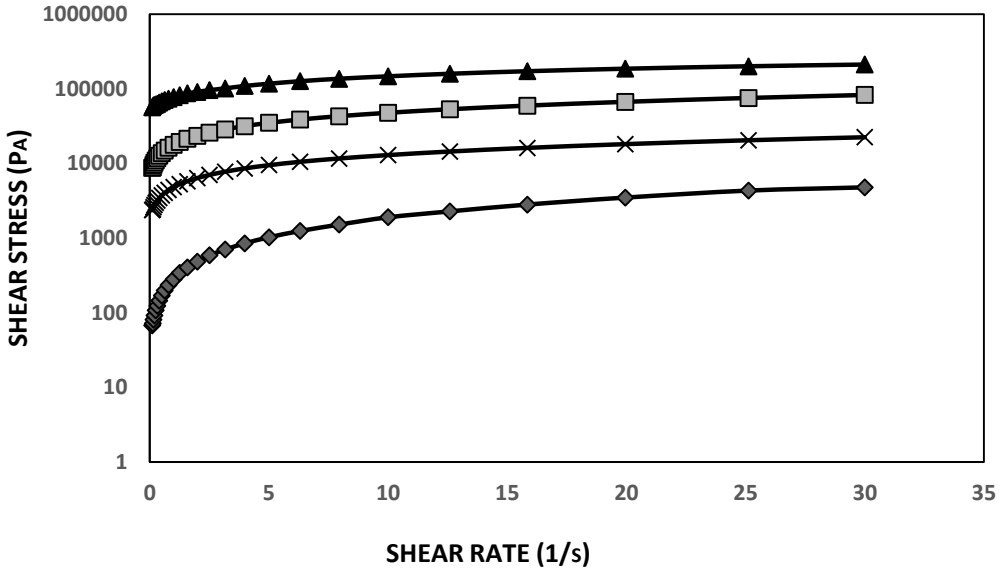

Figure 3.19 Flow curves obtained for emulsion containing 50% oil and 10% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

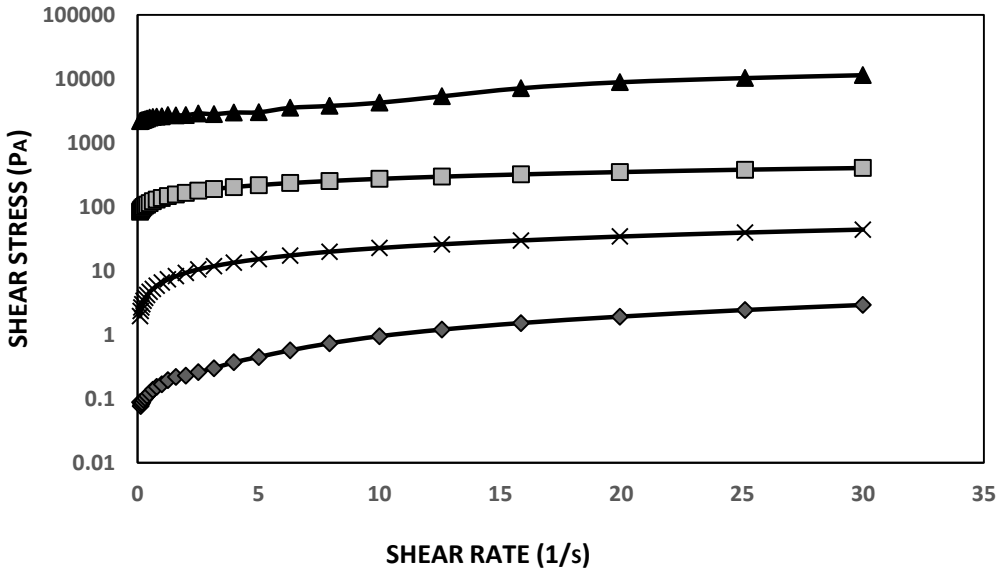

Figure 3.20 Flow curves obtained for emulsion containing 15% oil and 15% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

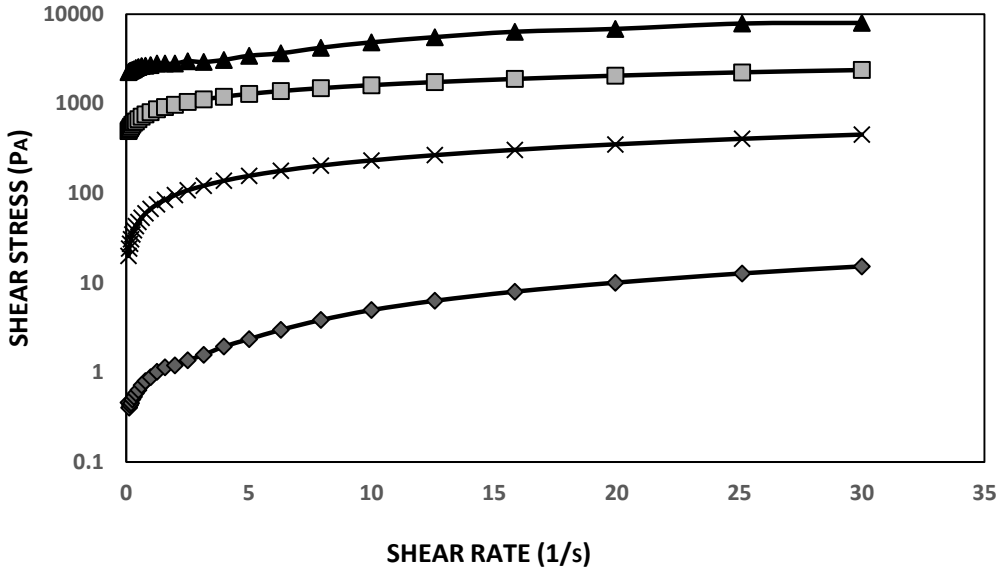

Figure 3.21 Flow curves obtained for emulsion containing 30% oil and 15% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

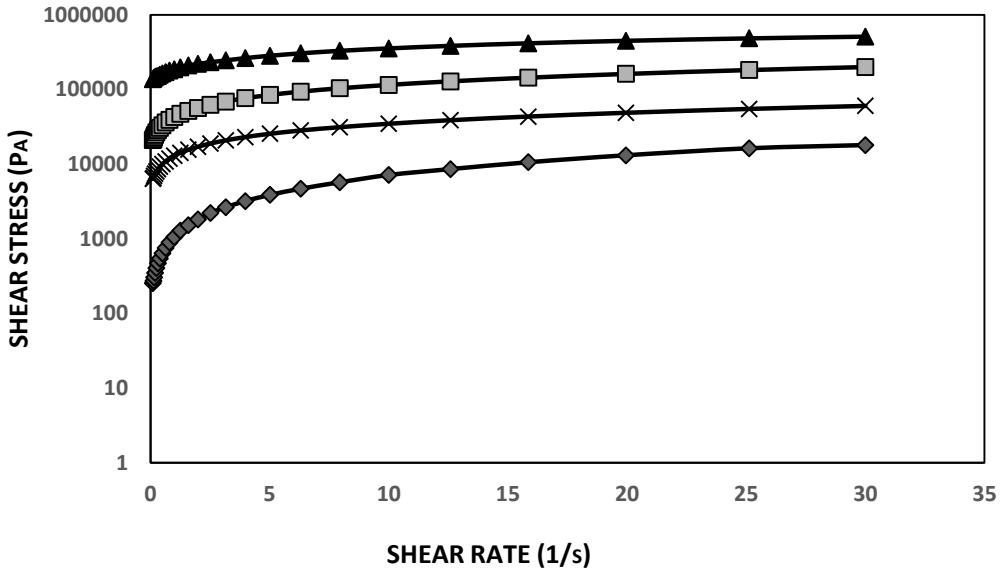

Figure 3.22 Flow curves obtained for emulsion containing 50% oil and 10% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

3.2.1.2 Viscoelastic Measurements

Elastic and viscous moduli values are represented from Fig. 3.23 to Fig. 3.40 for emulsions having different oil and zein concentrations, and also obtained by different treatments.

In all emulsions, increase in angular frequency caused increase in both elastic (G') and viscous (G") moduli values. Moreover, as could be seen easily from figures, elastic modulus value was higher than viscous modulus value for same concentrations, which was an indicator for elastic gel-like emulsion. As being in the flow measurements, increase in oil and zein concentrations also led to increase in G' and G". Therefore, the highest viscoelastic moduli values were obtained in emulsion prepared by mixing 50% oil and 15% zein concentrations.

When the effects of microfluidization and alkalinity on viscoelasticity of emulsion were analyzed, it could be stated that microfluidization provided a viscoelastic structure to our emulsions, which was not attained by untreated zein. The study of Mert (2012) indicated that microfluidizer creates higher shear rates for longer periods of time by its consistent pressure, and as a consequence of that it causes to higher moduli values in the samples. The study of Liu & Tang (2011) on whey protein emulsions indicated similar findings obtained by microfluidization technique.

Then, as could be observed from figures, the viscoelasticity attained by microfluidization improved by alkaline treatment. As being in the flow measurements, that effect lasted up to some point. The point at pH=8 had highest moduli values, and it was followed by pH=6, pH=10 and microfluidized zein, respectively. Similar to the findings in the previous part, increasing surface area and decreasing particle size by microfluidization and also alkalinity caused higher modili values up to some point. These treatments created more coalesced structure and increased interactions through the emulsions (Ciron et al., 2010). Also, the study of San Martin-Gonzalez et al. (2009) on corn oil emulsions supported our findings. Initial increase with alkalinity and then decrease after pH=8 in elastic and

viscous moduli values with excess treatment, would be the result of solubility property of zein. Solubility has an impact on functional properties of proteins such as emulsification, gelation and foam formation. Because of that reason, solubility plays an important role on functional properties of proteins (Shan et al., 2012). Similar findings were stated in the study of Shan et al. (2012) on ovomucin. They indicated that the solubility of the ovomucin decreased when pH value reached higher than 11, and undesirable changes occurred in the system such as protein hydrolysis, which resulted in deterioration in sensory and functional properties. Therefore, it should be stated that the highest solubility of zein could be around pH=8. Also, Roach et al. (2005) pointed out that hydrophobic interactions are important for protein conformation, because they directly related to solubility of the proteins. Our study showed that surface hydrophobicity of zein increased up to pH=8 and then showed a decreasing trend, which caused to decrease in solubility, and as a result of that decrease in moduli values was observed. The possible reason behind that situation could be the conformation between lipophilic and hydrophilic groups, which occurred mostly in the surface. Again, the study of Shan et al. (2012) on ovomucin showed similarity.

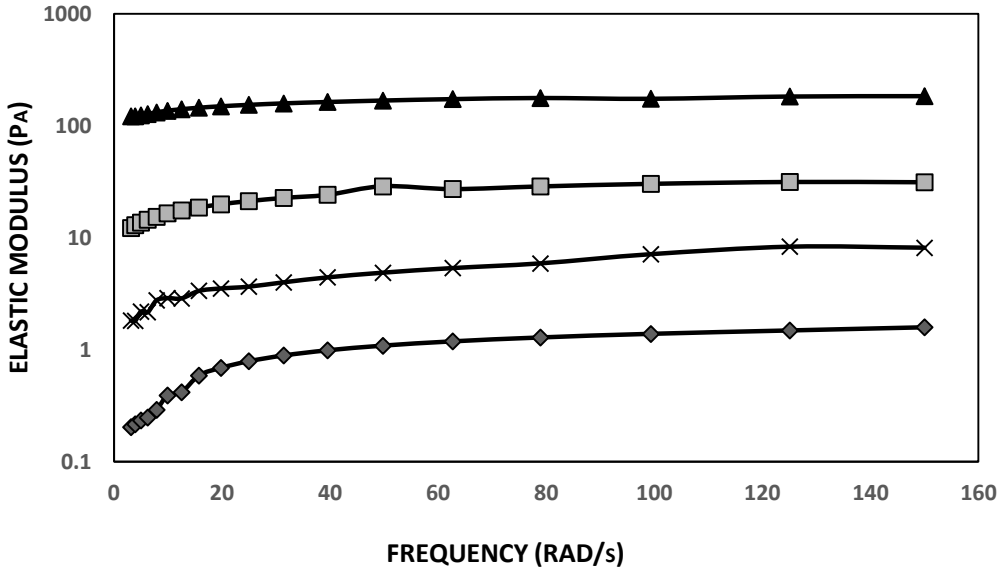

Figure 3.23 Elastic modulus obtained for emulsion containing 15% oil and 5% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

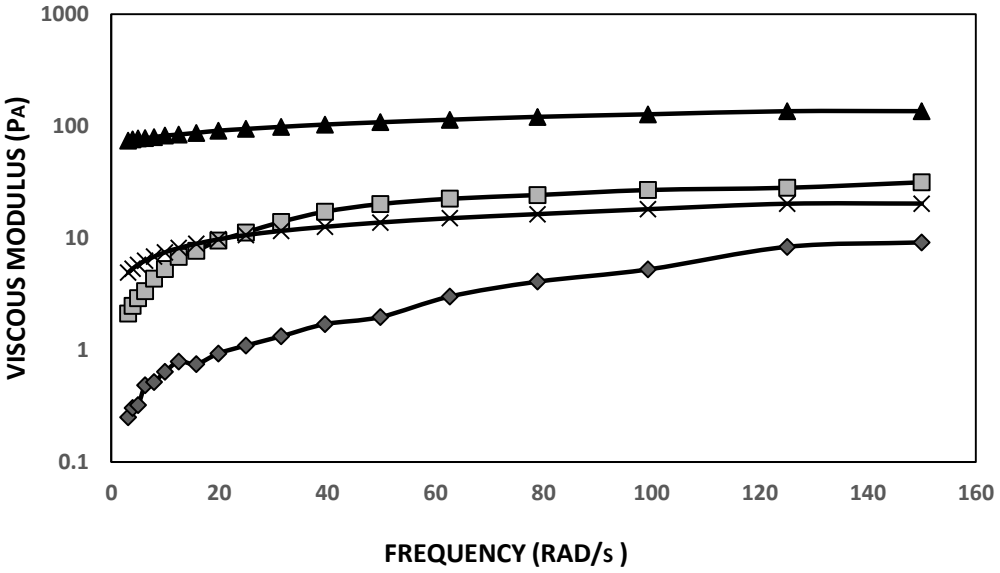

Figure 3.24 Viscous modulus obtained for emulsion containing 15% oil and 5% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

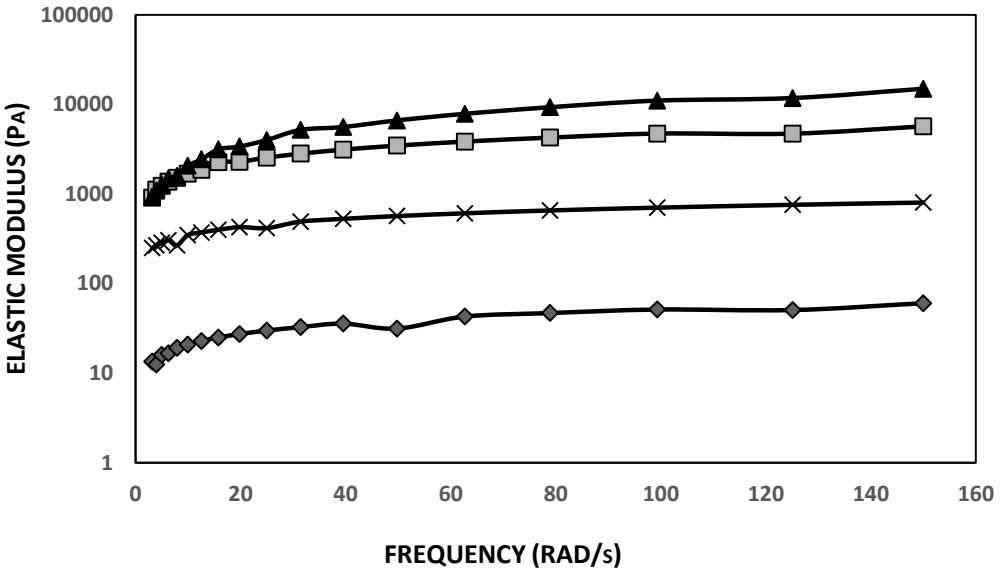

Figure 3.25 Elastic modulus obtained for emulsion containing 30% oil and 5% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

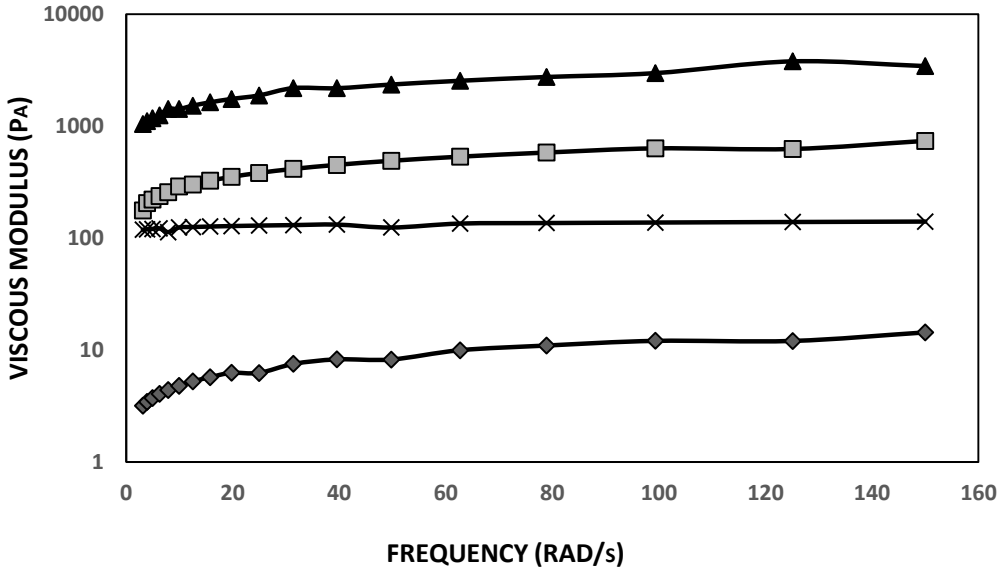

Figure 3.26 Viscous modulus obtained for emulsion containing 30% oil and 5% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

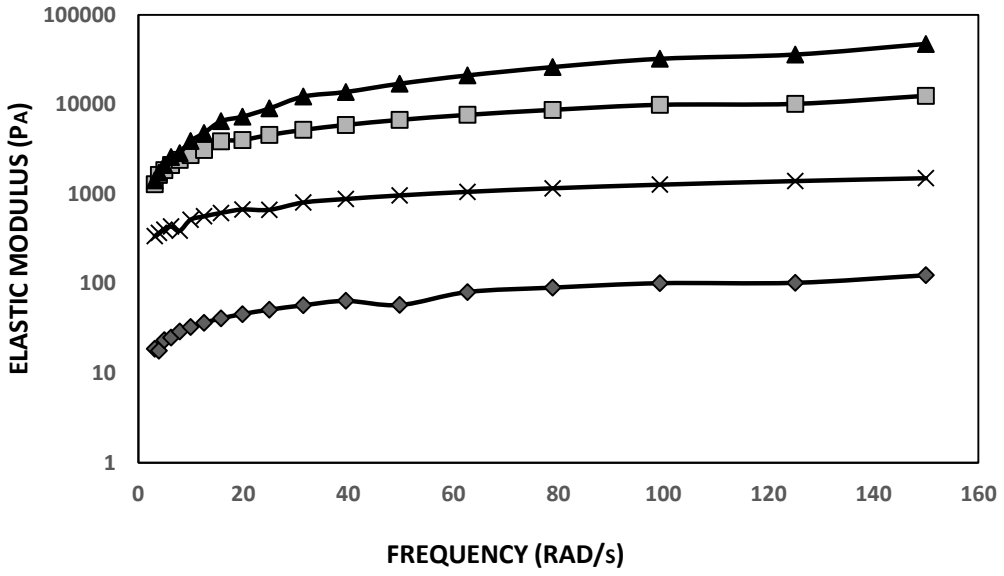

Figure 3.27 Elastic modulus obtained for emulsion containing 50% oil and 5% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

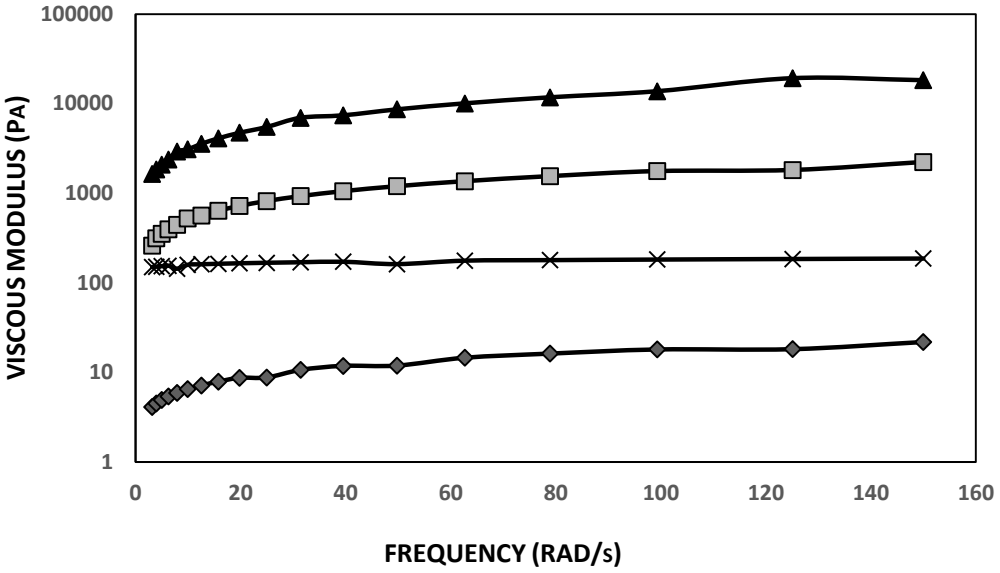

Figure 3.28 Viscous modulus obtained for emulsion containing 50% oil and 5% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

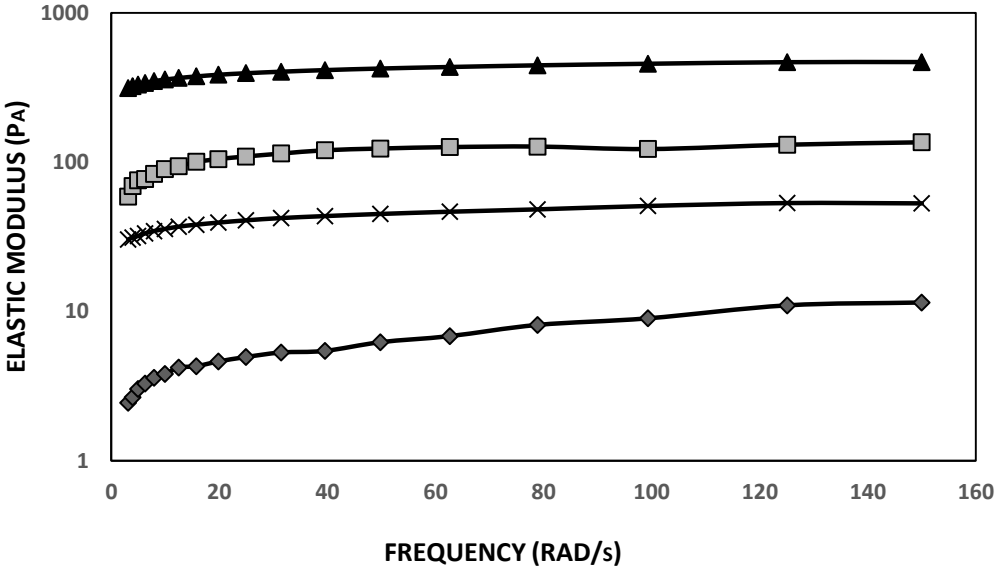

Figure 3.29 Elastic modulus obtained for emulsion containing 15% oil and 10% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

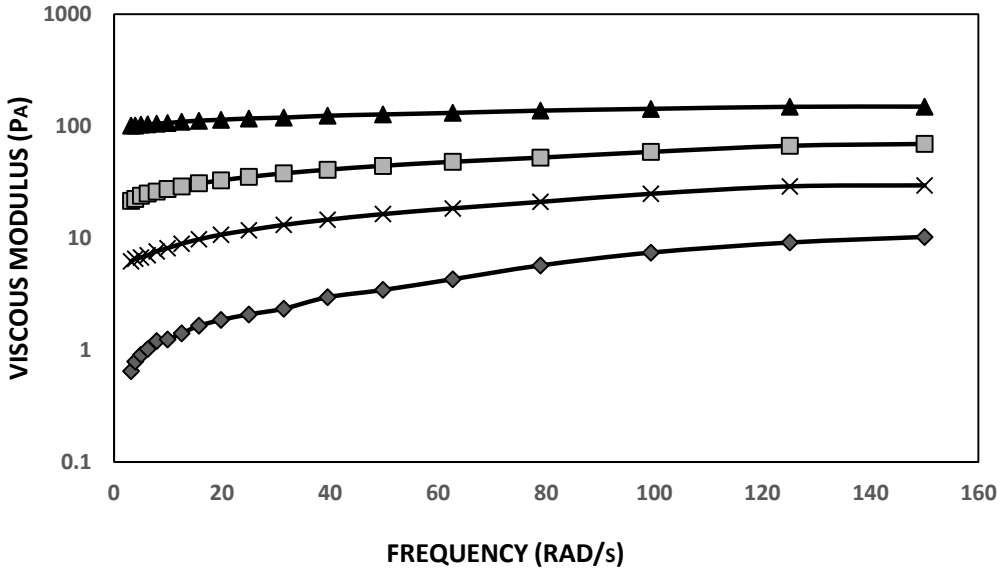

Figure 3.30 Viscous modulus obtained for emulsion containing 15% oil and 10% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

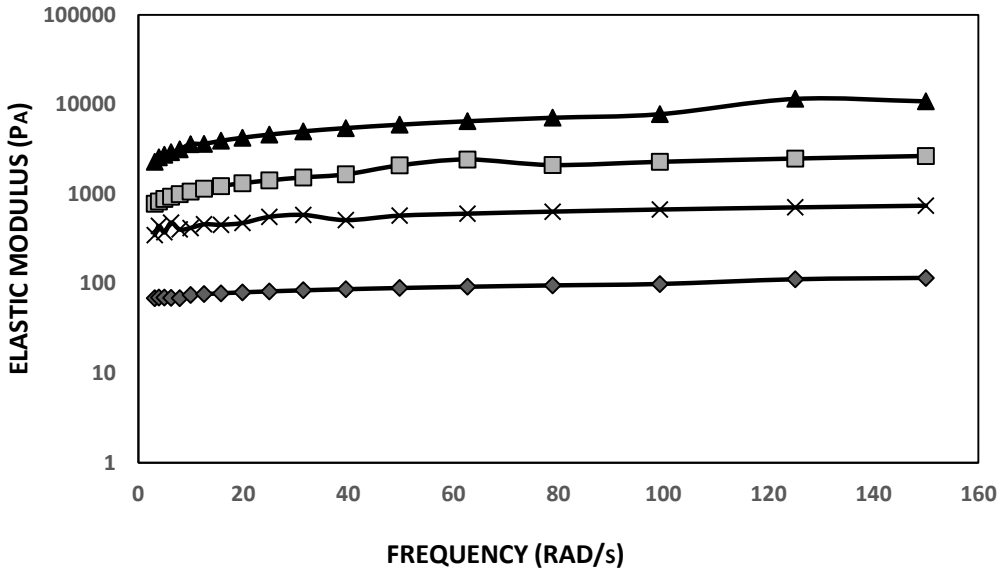

Figure 3.31 Elastic modulus obtained for emulsion containing 30% oil and 10% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

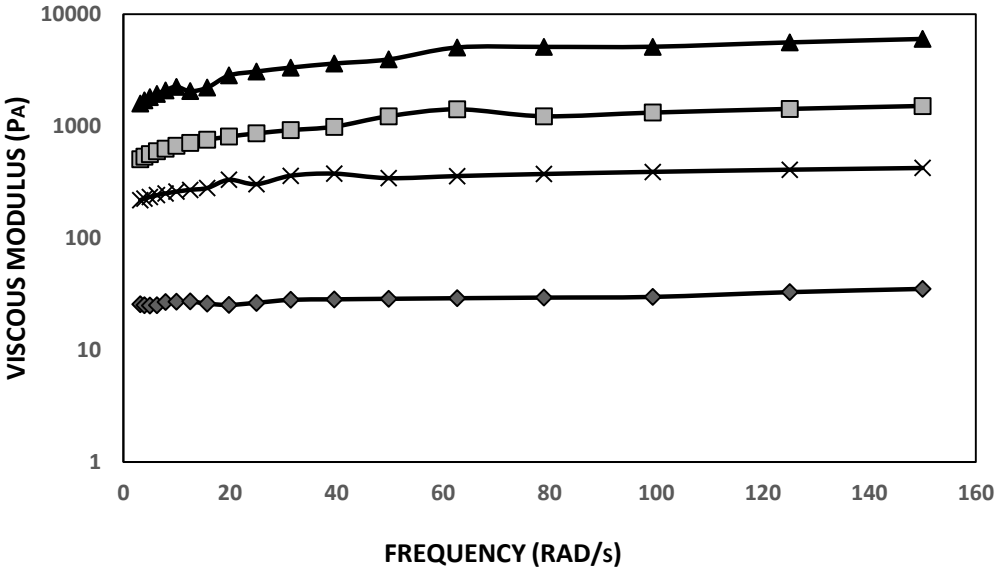

Figure 3.32 Viscous modulus obtained for emulsion containing 30% oil and 10% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

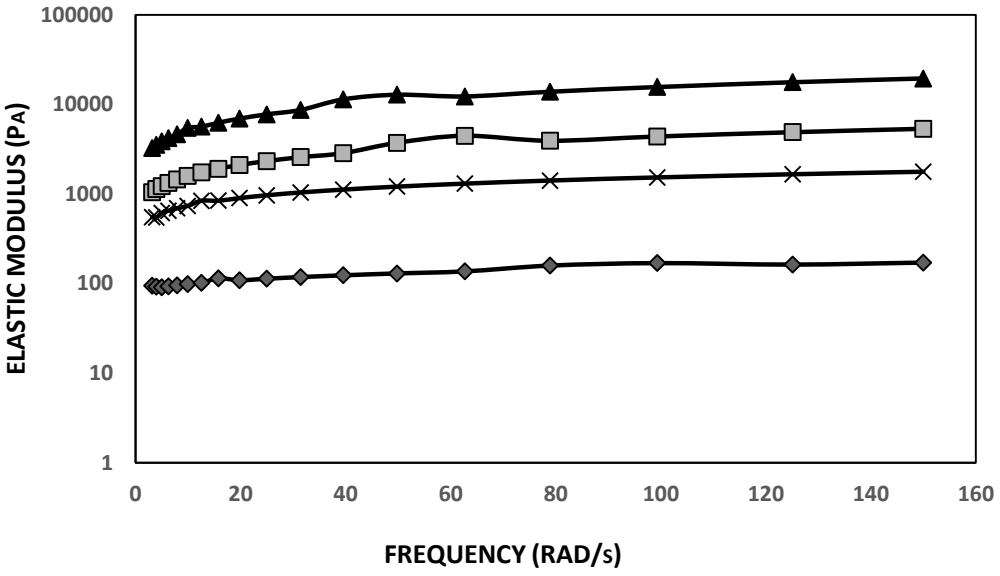

Figure 3.33 Elastic modulus obtained for emulsion containing 50% oil and 10% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

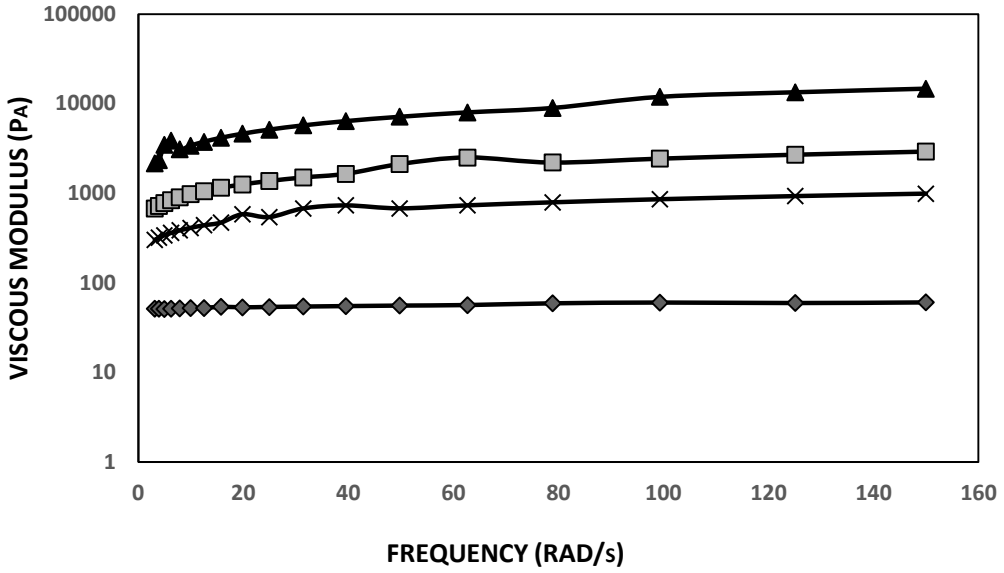

Figure 3.34 Viscous modulus obtained for emulsion containing 50% oil and 10% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

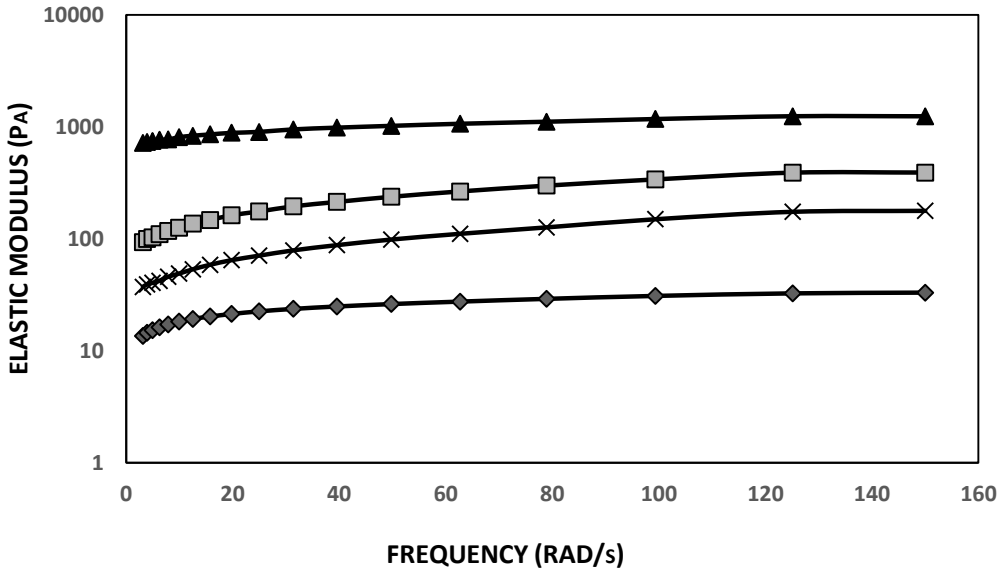

Figure 3.35 Elastic modulus obtained for emulsion containing 15% oil and 15% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

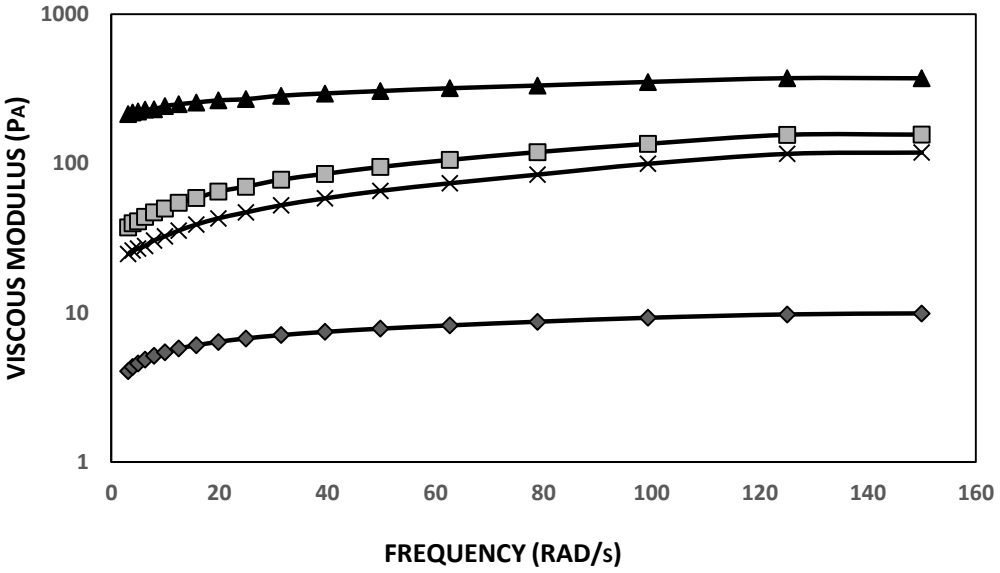

Figure 3.36 Viscous modulus obtained for emulsion containing 15% oil and 15% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

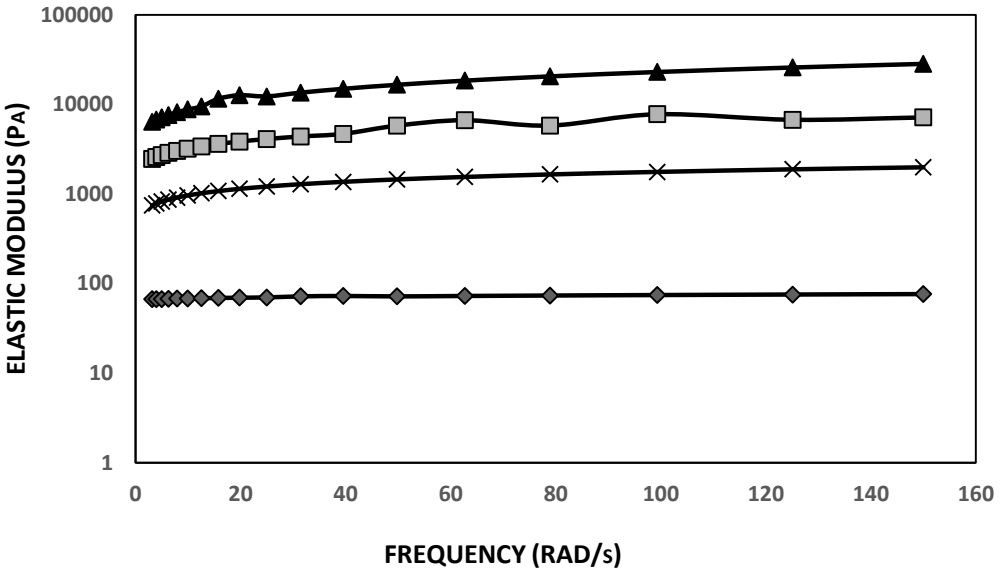

Figure 3.37 Elastic modulus obtained for emulsion containing 30% oil and 15% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

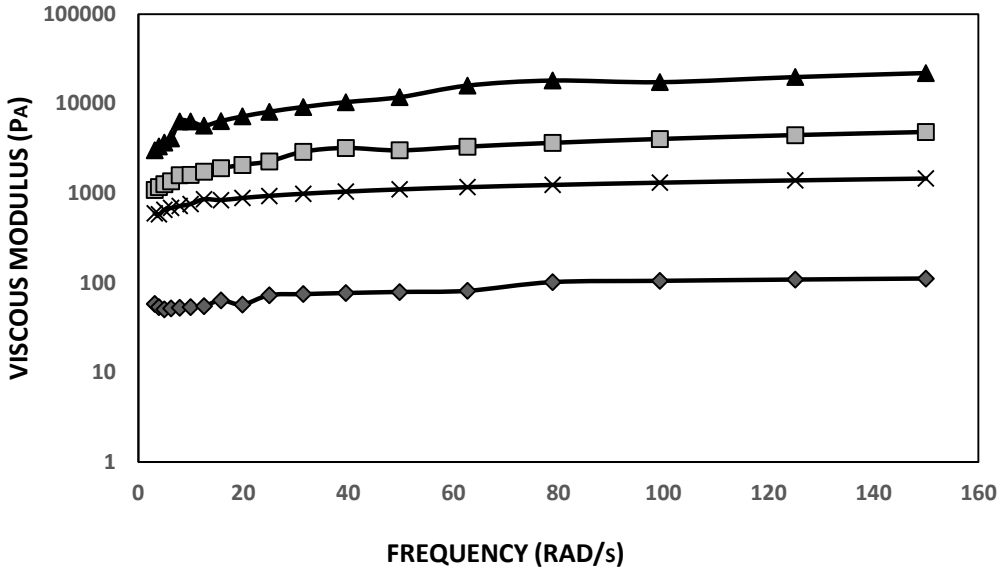

Figure 3.38 Viscous modulus obtained for emulsion containing 30% oil and 15% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

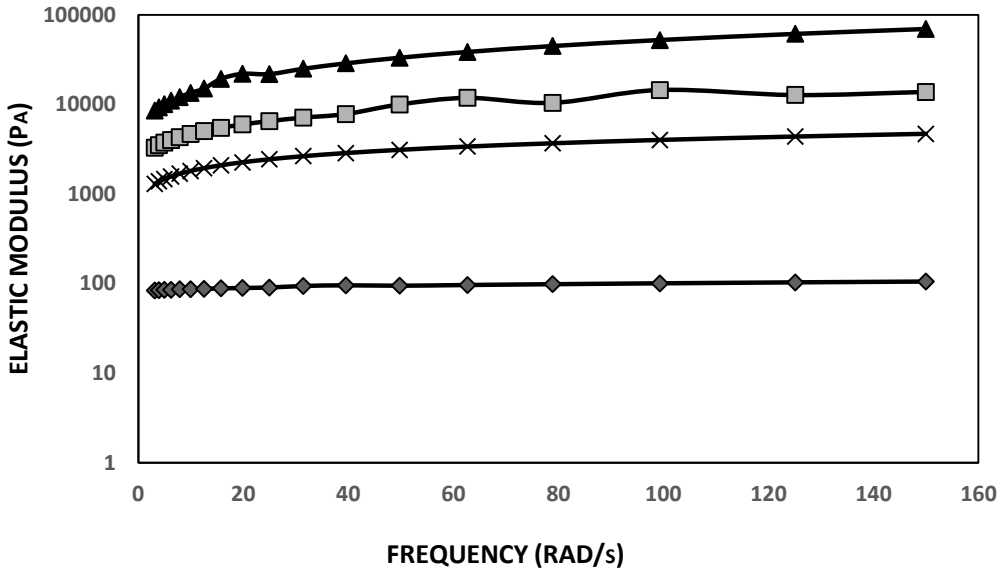

Figure 3.39 Elastic modulus obtained for emulsion containing 50% oil and 15% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

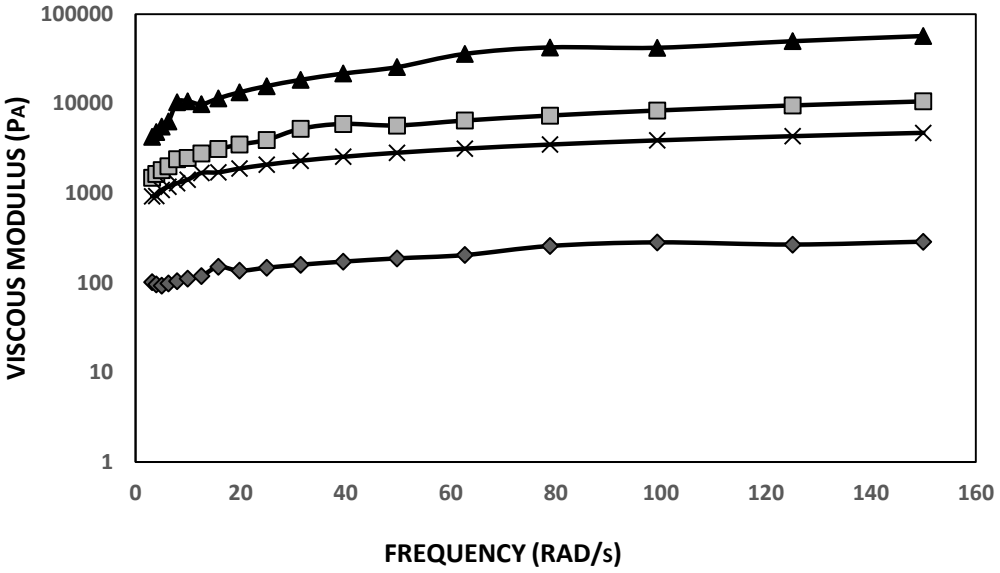

Figure 3.40 Viscous modulus obtained for emulsion containing 50% oil and 15% zein. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

3.2.2 Emulsion Stability

Because of the hydrophobicity of zein, separation between layers could be a commonly encountered stability problem for prepared emulsions (Mert, 2012). To overcome that problem, different treatments were applied to corn zein emulsions, and the stabilities of the emulsions were measured by dispersion analyzer.

The effects of different treatments on emulsion stability of corn zein are represented from Fig. 3.41 to Fig. 3.49 according to containing oil and zein concentrations. By changing oil and zein concentrations, optimum emulsion formulations was tried to find for zein emulsions in terms of emulsion stability. However, it should be stated that emulsion stability was influenced by several factors such as pH, droplet size, viscosity, interfacial tension and protein conformation (Hung & Zayas, 1991).

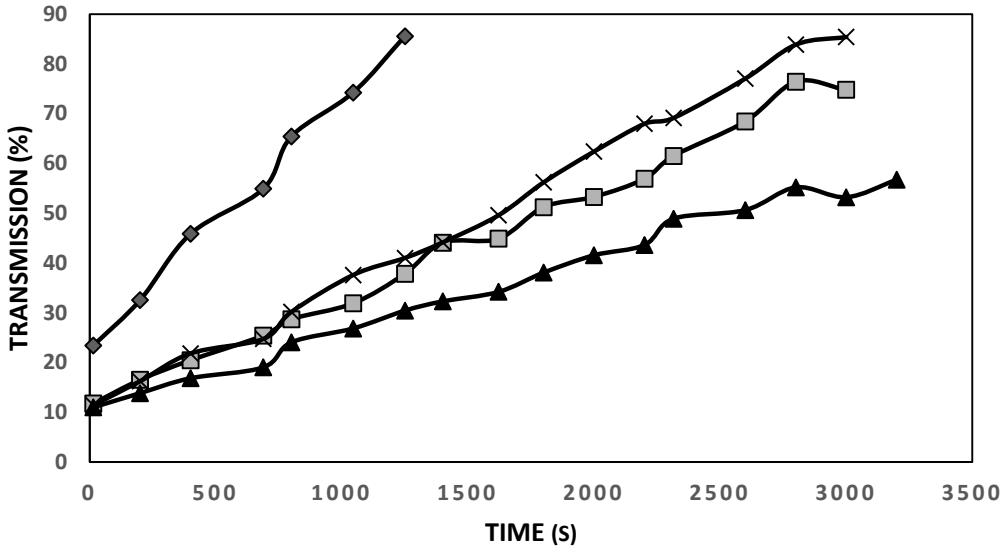

Figure 3.41 Change of total transmission of emulsion containing 15% oil and 5% zein with time. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

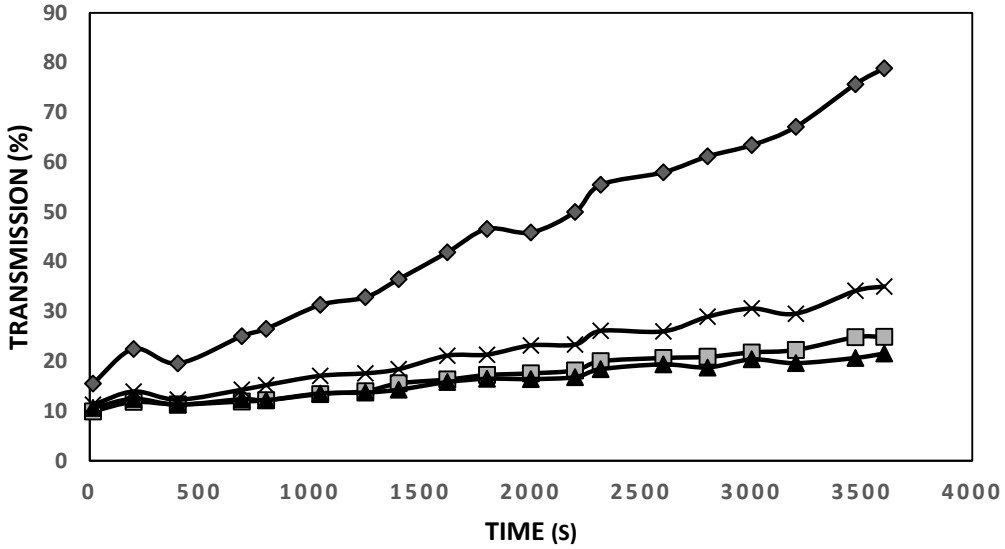

Figure 3.42 Change of total transmission of emulsion containing 30% oil and 5% zein with time. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

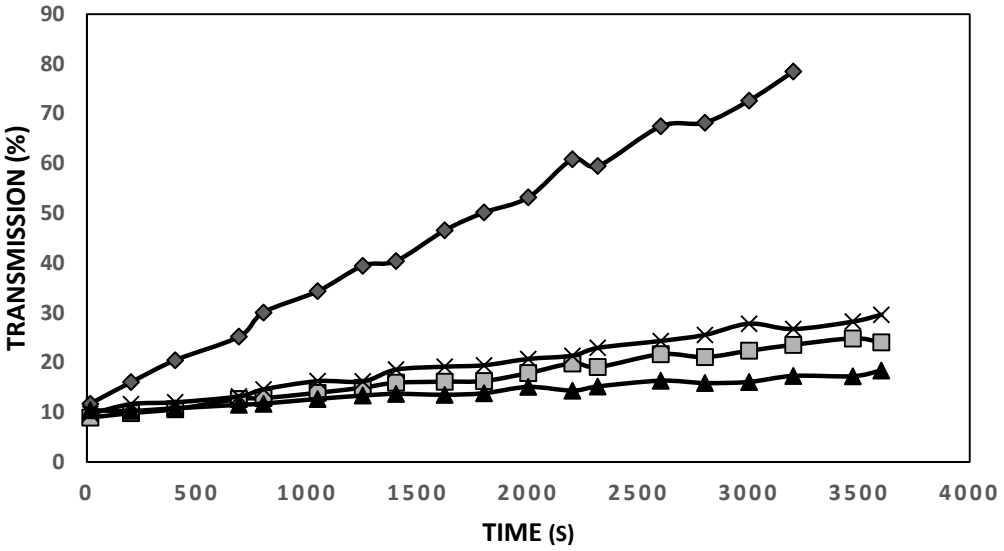

Figure 3.43 Change of total transmission of emulsion containing 50% oil and 5% zein with time. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

First of all, it should have been mentioned that since zein is a prolamine which means it cannot dissolved in water, but dissolve in alcohol solutions as stated several times before, emulsion preparations made with untreated zein produced nothing in terms of emulsion stability. These emulsions showed phase separation directly. Due to this reason, the emulsions made with untreated zein were ignored in this part of the study.

As a beginning point, it has been remarked that microfluidization is an effective treatment on emulsion preparation, which contributes to the formation of zein emulsions solitarily. pH arrangements were applied only to improve the emulsifying properties which gained by microfluidization. Microfluidization resulted in the formation of more homogenous structure through the emulsion by its combined forces, especially high pressure. As stated in the study of Mert (2012), microfluidization led to the formation of smaller and more homogenous particles by high pressure, and that resulted in improvement in emulsion stability. The

particle sedimentation pace was slowed down by obtaining smaller particles and higher phase viscosity, which improved emulsion stability. Floury et al. (2000)'s study on oil-in-water emulsions also stated that microfluidization provided fine droplets with smaller sizes, which led to higher emulsion stability.

As could be seen from above three figures, emulsions containing 15, 30 and 50% with 5% microfluidized zein concentration were prepared at different pH values, and the light transmission percentages were determined to analyze emulsion stabilities.

In all emulsions, emulsion prepared only by microfluidization was disintegrated and allowed to light passing rapidly. On the other hand, emulsion prepared by combining microfluidization and pH arrangements showed more stable emulsifying properties, and disintegration of these emulsions became slower than microfluidized zein emulsions. Increasing alkalinity of the zein resulted in improving emulsion stability up to some point. Anyone could notice that the slopes of the graphs are equal to the sedimentation pace of the emulsions. As could be seen from figures (Fig. 3.41, Fig. 3.42 and Fig. 3.43), arrangement of pH value to 8 gave the best result and it was followed by pH=6 and pH=10, respectively. That meant sedimentation pace of the pH=8 was slower and the pace increased at pH=6, at pH=10, and reached to its maximum at microfluidized zein. Results from figures revealed that emulsion stabilities of zein emulsions was highly dependent to pH, and also it could be stated that alkaline pH improved the emulsion stability when compared with acidic pH value. The findings in our study were fitted with the literature. The study of Shan et al. (2012) exhibited similar findings on ovomucin. The improvement with alkalinity showed a decrease after pH=8, but as could be observed from figures, it was still higher than its value at acidic pH.

Wu (2001) showed similar findings on emulsion stability of corn gluten meal. According to the finding of Wu (2001), corn zein showed poor emulsion stability between 3.2 and 6.2. After 6.9, increase in the emulsion stability became larger and reached its maximum at 7.8. Then, it decreased merely with increasing the alkalinity. As a comparison, the only difference between our study and Wu (2001)'s

study was that the decrease in the emulsion stability after 8 was not small in our study. The emulsion stability at pH=10 reduced to the back of the emulsion stability at pH=6. That difference could be the cause of microfluidization process, which was not applied in the study of Wu (2001). The structure of the zein would be deformed with the combination of microfluidization and excessive pH arrangements, and that could lead to higher backspacing. Also, Wu (2001) showed that particle size played an important role in the emulsion stability. Decreasing particle size with increasing alkalinity concluded more stable emulsions as being in our study and Mert (2012)'s study.

The increasing emulsion stability by alkalinity could be the result of deamination treatment and unfolding of the proteins. Proteins diminish their energy by folding and aggregating in the structure. Also, hydrophobic amino acids associate in the hidden part of the proteins not to encounter with aqueous surroundings. By increasing alkalinity, proteins are forced to break down and to open. The rearrangement in the structure of the protein and hydrophobic amino acids by unfolding provide the formation of more balanced emulsions (Cabra et al., 2007). Also, since hydrophobic amino acids are exposed to aqueous surroundings, surface hydrophobicity of the protein increases. By increasing the surface hydrophobicity of the protein, protein tends to adsorb to oil surface (Mine et al., 1991). These arrangements promote hydrophilic-lipophilic balances and that results in more interaction between protein and oil, and as a consequence of that more stable emulsions occur (Cabra et al., 2007). Also, the study of Sathe et al. (1982) emphasized the effects of hydrophilic-lipophilic balance, which is directly influenced by pH. Another factor that affect the emulsion stability could be the structure of biopolymer interfaces. The film forming ability of a protein has to be high to form a stable emulsion. As stated before, slight alkaline treatment resulted in higher protein solubility and dispersion of hydrophobic groups into water and oil interfaces. As a result of that, film forming ability of the protein was improved. On the other hand, if excess alkaline treatment was applied, protein hydrolysis was observed, which was a reason behind lower film forming ability (Shan et al., 2012).

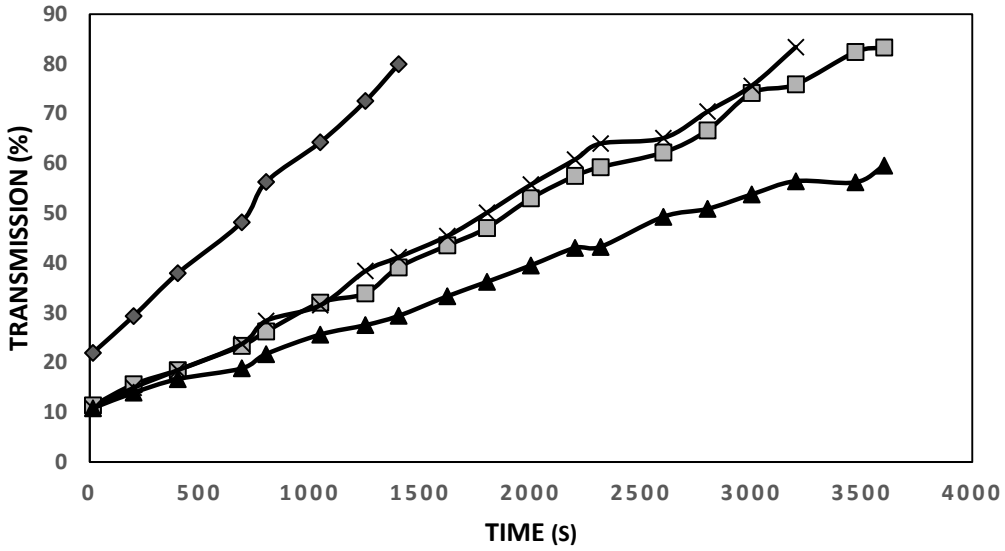

Figure 3.44 Change of total transmission of emulsion containing 15% oil and 10% zein with time. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

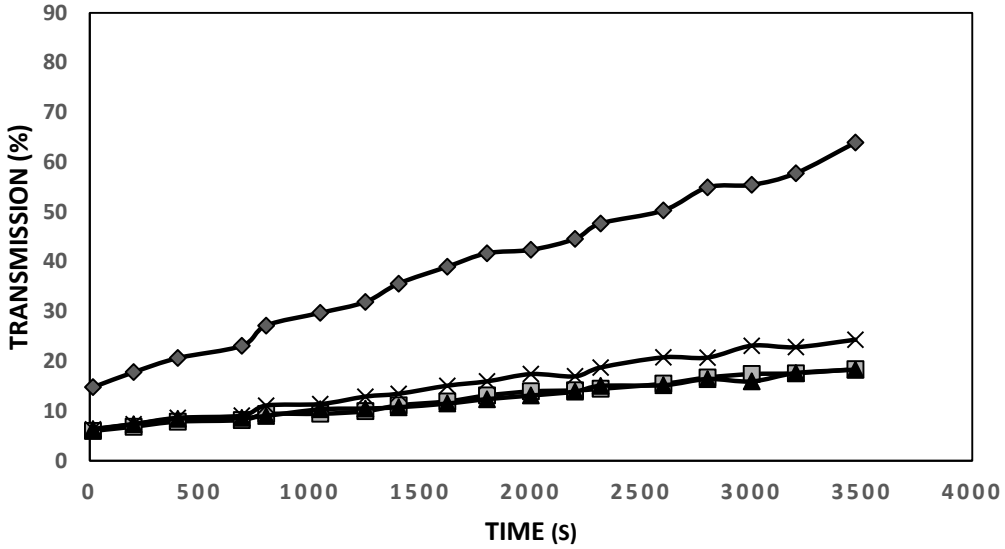

Figure 3.45 Change of total transmission of emulsion containing 30% oil and 10% zein with time. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

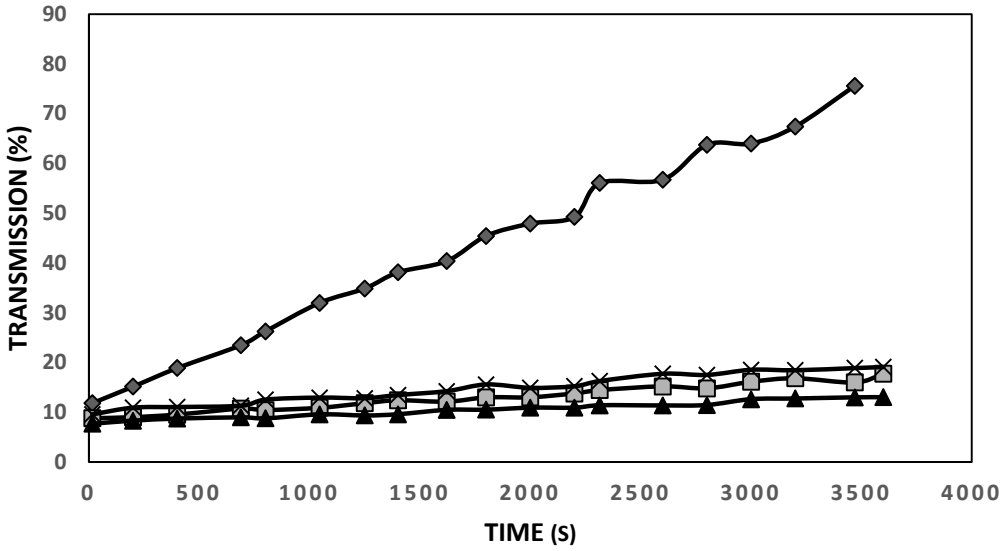

Figure 3.46 Change of total transmission of emulsion containing 50% oil and 10% zein with time. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

The same relationship was found in other concentration mixes. In all experiments, the emulsion stability at pH=8 was the best and at microfluidized zein was the worst regardless of concentration. The emulsion stability decreased in that order: pH=8 > pH=6 > pH=10 > mf.

Similar researches were done on whey protein isolate. Ma et al. (2011) indicated that shifting the acidity to neutral pH values or to alkalinity was an effective practice to improve the stability of the emulsions. The study of Mine et al. (1991) pointed out similar findings on emulsifying properties of ovalbumin. According to that study, emulsion stability of ovalbumin increased up to pH=5, but then decreased by increasing alkalinity.

The conspicuous point in the emulsion stability analysis was that emulsions having 15% oil concentrations showed more instability than 30% and 50% regardless of zein concentration as could be seen from Fig. 3.41, Fig. 3.44 and Fig. 3.47. Even,

microfluidized zein in 15% oil concentrations decomposed more rapidly than the others. The absence of oil for the adsorption of the proteins around the surrounding could be the reason behind that situation. On the other hand, emulsions having 30% and 50% oil concentrations almost showed same pattern when same zein concentrations were examined. When the analysis was done from the other perspective, emulsions having 5% and 15% zein concentrations were more instable than 10% regardless of oil concentration. The reason behind that could be two different concepts, which changed according to concentration levels. 5% zein concentrations could be unstable due to excess amount of oil found in emulsion, and that led to easy phase separation and coalescence. On the other hand, 15% zein concentrations could be unstable due to the absence of oil around the surroundings, and that caused to lack of adsorption of the proteins.

In conclusion, emulsion containing 10% zein concentration with 30% oil concentration (10:30:60) gave the best result in terms of emulsion stability according to our study.

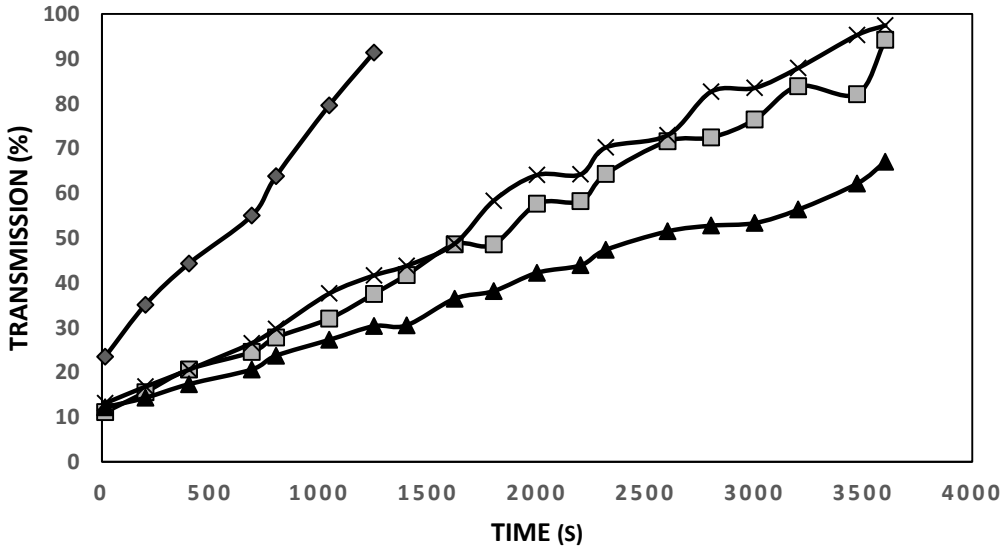

Figure 3.47 Change of total transmission of emulsion containing 15% oil and 15% zein with time. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

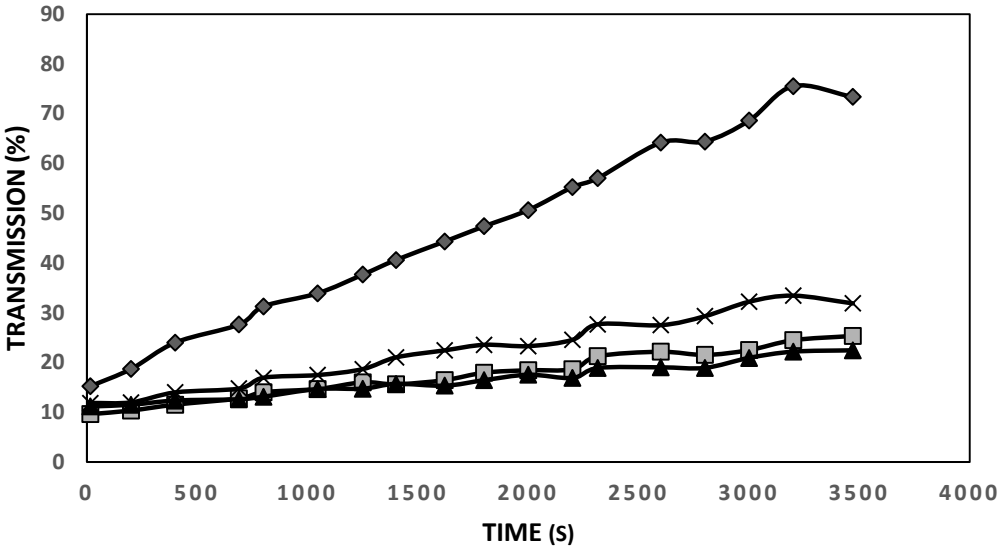

Figure 3.48 Change of total transmission of emulsion containing 30% oil and 15% zein with time. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

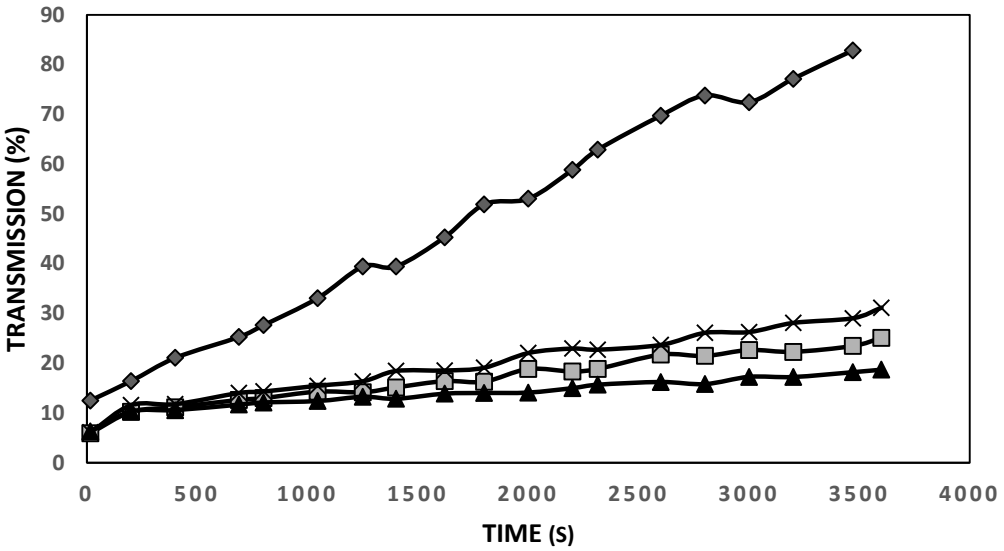

Figure 3.49 Change of total transmission of emulsion containing 50% oil and 15% zein with time. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

3.3 Analyses on Bread Dough and Bread

The rheological properties of corn bread dough, and some quality parameters (moisture content and crumb color) and textural properties of corn bread containing different hydrocolloids and starch amounts were determined in this section.

3.3.1 Bread Dough Rheology

The rheological properties of corn bread dough containing different starch amounts and hydrocolloids were determined in this section of the study by oscillatory measurements such as strain sweep and frequency sweep tests. Varying stress or strain were applied during oscillatory measurements to the samples to measure the effects of them on physical and chemical structure. Elastic modulus, viscous modulus and loss tangent values were determined by these measurements and comments were done according to their relationships (Létang et al., 1999). Corn bread dough rheology measurements were analyzed with respect to used gum, and strain and frequency sweep tests measurements for each of these gums are illustrated from Fig. 3.50 to Fig. 3.93.

3.3.1.1 Strain Sweep Tests

The estimation of linear viscoelastic region is the beginning and one of the most important point to determine the rheological properties of dough structure. As a general knowledge, when the measurements are done between the limits of linear viscoelastic region, the characteristics of dough sample do not change with varying stress and strain (Steffe, 1996). This is the reason behind the importance of determining linear viscoelastic region. Also, Mariotti et al. (2009) stated that the

stability of a sample is directly related to the length of the linear viscoelastic region, because elasticity is one of major indicator of sFig. And well dispersed system.

Corn bread dough rheology measurements related to strain sweep test were analyzed with respect to used gum between 0.01% and 10%, and represented from Fig. 3.50 to Fig. 3.71.

As could be seen from Fig. 3.50 to Fig. 3.55, the viscoelasticity of corn bread dough formulations without gum, in which different treatments were applied and also different starch amounts were used, were investigated by strain sweep test. The linear viscoelasticity of dough continued up to a certain point and showed a decreasing trend after that point. All figures showed that the viscoelasticity of the corn bread dough without gum was limited to approximately a strain of lower than 0.3%. Both elastic and viscous moduli values exhibited same trend. Generally, linear viscoelastic region gathered around the strain of 0.1%. The decrease in the linearity revealed after 0.5% and that decrease became larger above 1% strain. That meant the dough structure was brokedown beyond that deformation level. In other words, the applied forces within this limit were nondestructive to dough structure and applied processes could be reversed without any deformation (Lindahl & Eliasson, 1992).

The other parameter in the rheology experiments was treatment type. Any meaningful results were found for corn bread made by untreated zein and without gum, since the homogenous structure of bread dough during experiments was not attained. Therefore, due to the inability to form a well bread dough structure, untreated zein without gum was not tested for that experiment. As could be seen from figures, the dough prepared by microfluidization showed higher elastic and viscous moduli than the others, but the difference between them was too little. Doughs prepared by both microfluidization and pH arrangements had lower moduli values. That could be the result of the deamination treatment and unfolding of proteins by increasing alkalinity. By increasing alkalinity, more and more deformation was provided to dough structure and that led to lower moduli values.

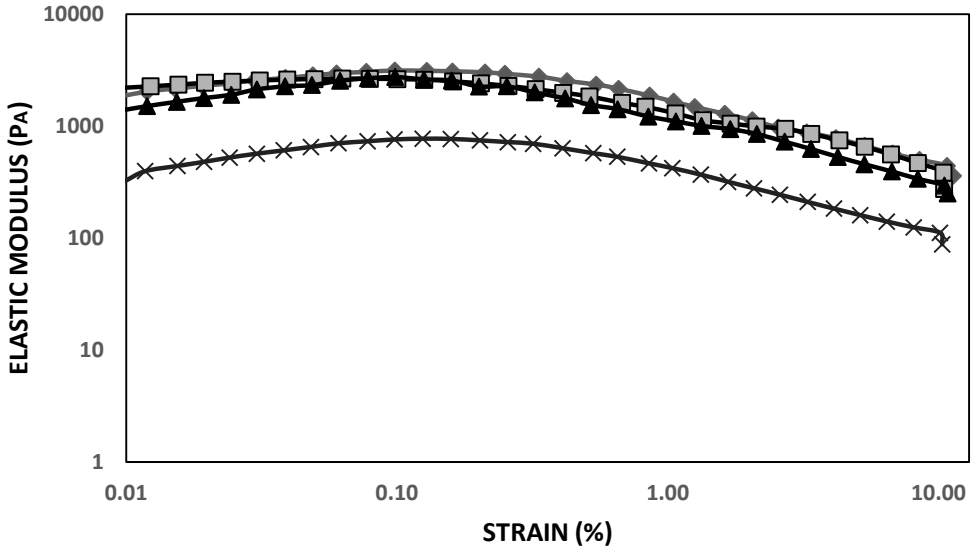

Figure 3.50 Elastic modulus obtained for bread dough samples containing 60 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

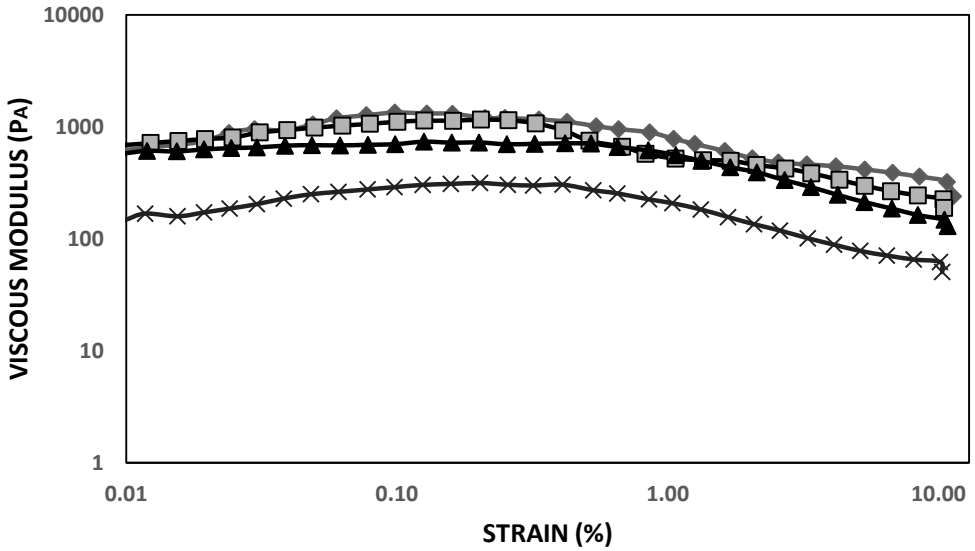

Figure 3.51 Viscous modulus obtained for bread dough samples containing 60 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

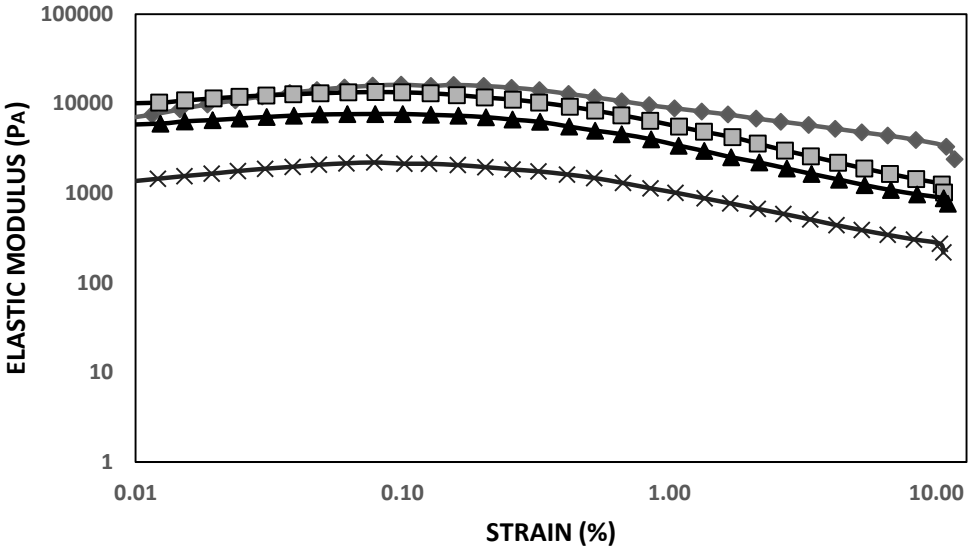

Figure 3.52 Elastic modulus obtained for bread dough samples containing 70 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

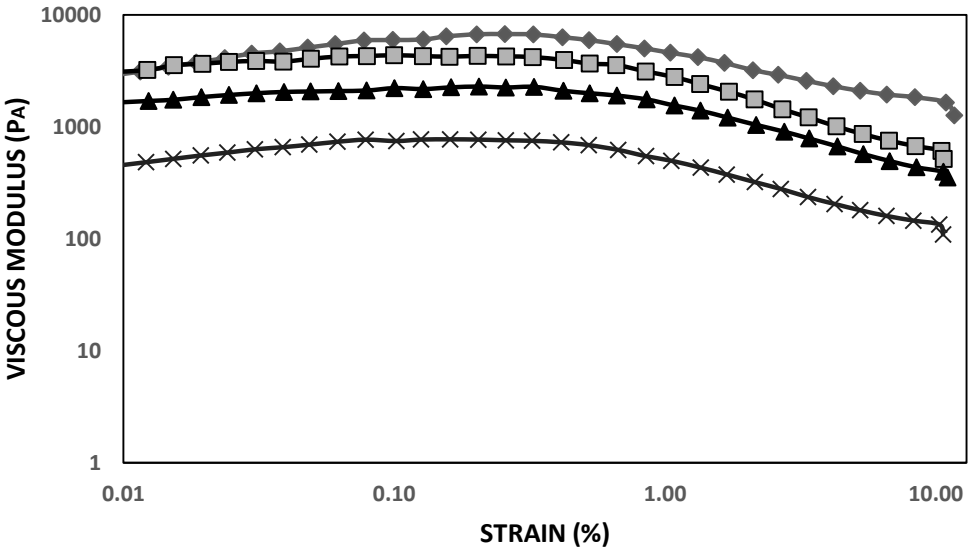

Figure 3.53 Viscous modulus obtained for bread dough samples containing 70 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

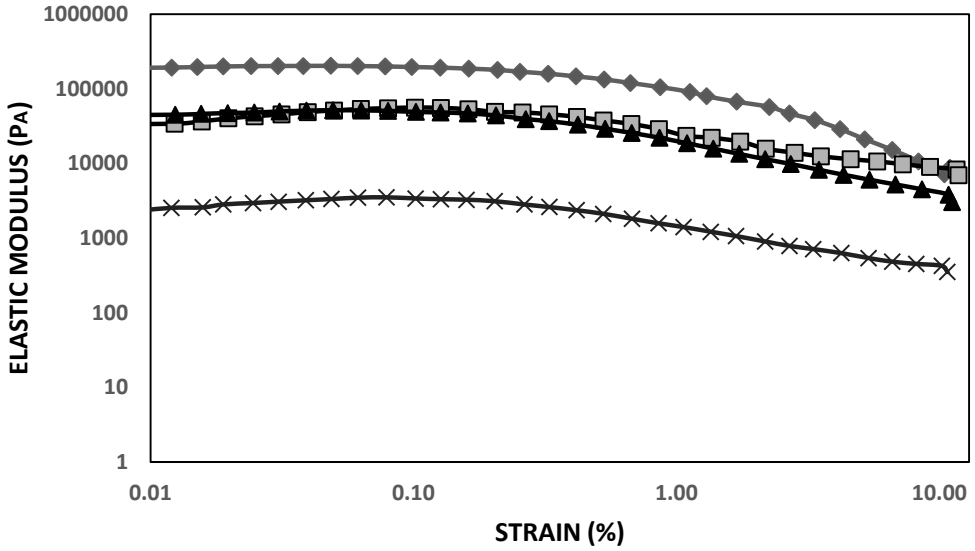

Figure 3.54 Elastic modulus obtained for bread dough samples containing 80 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

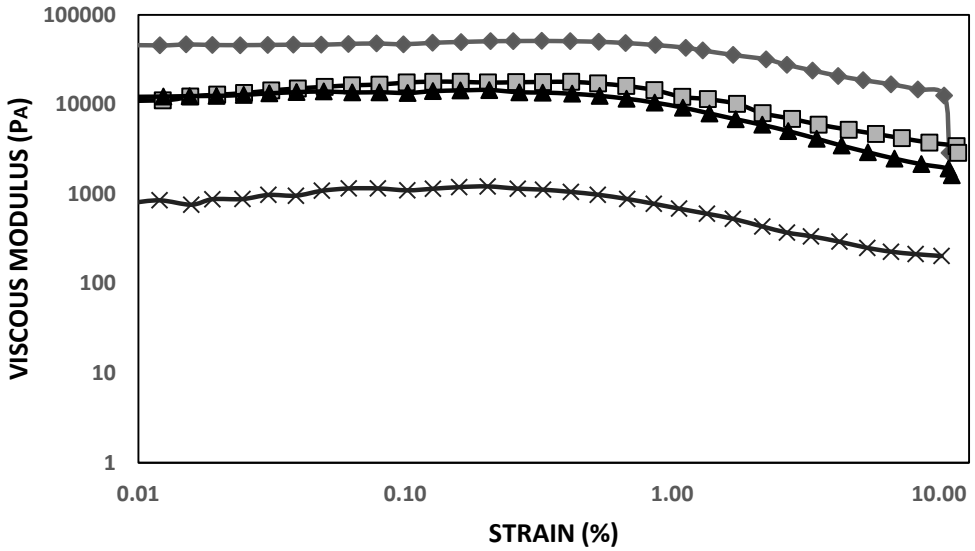

Figure 3.55 Viscous modulus obtained for bread dough samples containing 80 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

Moreover, as could be seen from figures, increasing starch amount resulted in higher elastic and viscous moduli values. However, that did not cause any deformation in the linearity of the viscoelastic region. On the other hand, elastic moduli values were greater than viscous moduli values, that meant samples showed a solid like behavior. It was more obvious and discussed clearly in the frequency sweep part.

The next six figures (Fig. 3.56 – Fig. 3.61) are the illustrations of the viscoelasticity of corn bread dough formulations with hpmc, in which different treatments were applied and also different starch amounts were used.

Dough with hpmc showed same pattern with dough prepared without gum. One of the small difference from the previous findings was that the linear viscoelastic region was seen below 0.4% strain for dough with hpmc, which was attained below 0.3% strain in without gum experiments, and that linearity corrupted more rapidly in without gum experiments. The addition of hpmc provided more linear stability to dough structure and the length of the linearity became longer. Also, the study of Crockett et al. (2011) indicated that the addition of hydrocolloids to dough structure led to increase in the elastic and viscous moduli values as being in our study. That was explained by obtaining more complex structure with hydrocolloids in terms of rheological properties.

The other and the more clear point as being different from experiments done without gum was the effect of microfluidization process. Because of inability of untreated zein to form a dough structure, control zein could not be obtained in the previous part. However, that problem was overcome with the addition of hpmc. As could be seen from figures related to hpmc, microfluidization led to increase in elastic and viscous moduli values. More homogenous and well formed dough structure by microfluidization could be the explanation of that.

The starch concentration affected the elastic and viscous moduli values in the same way, that meant they increased with more starch addition.

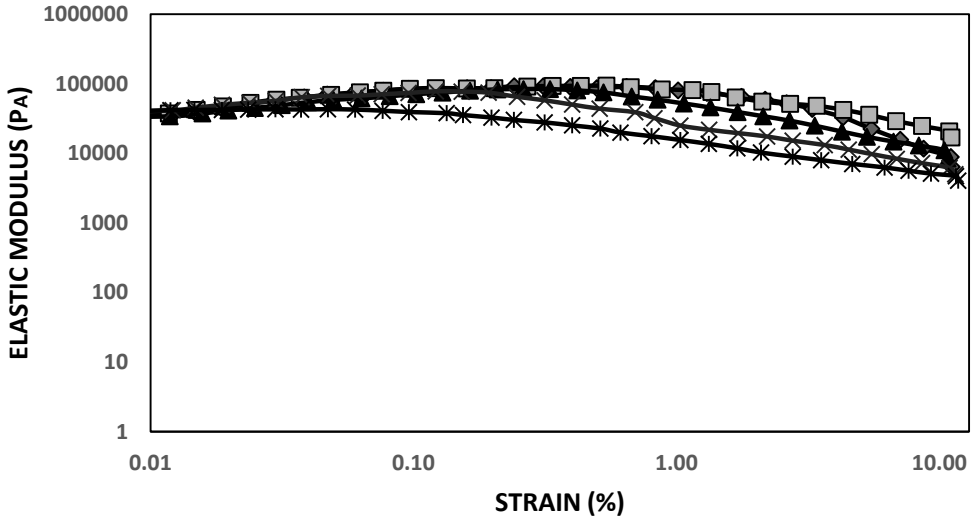

Figure 3.56 Elastic modulus obtained for bread dough samples containing 60 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

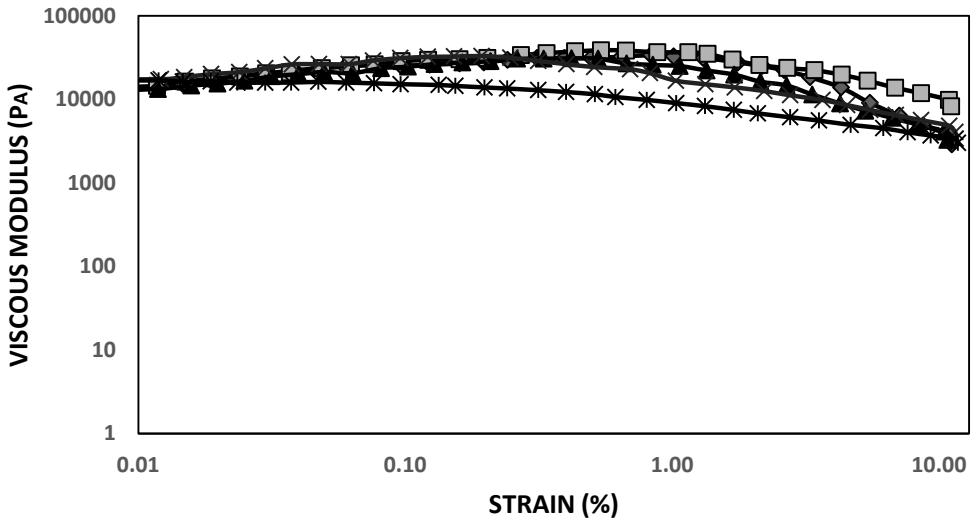

Figure 3.57 Viscous modulus obtained for bread dough samples containing 60 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

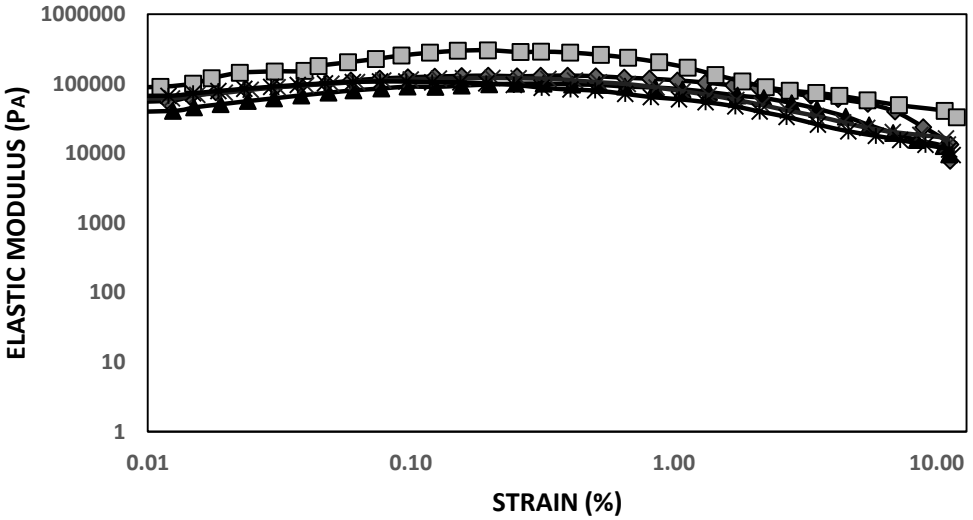

Figure 3.58 Elastic modulus obtained for bread dough samples containing 70 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

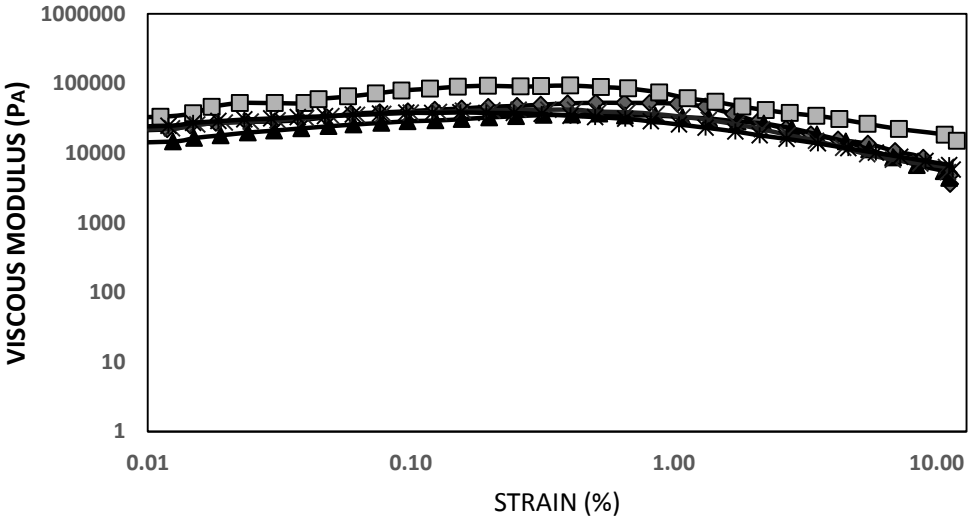

Figure 3.59 Viscous modulus obtained for bread dough samples containing 70 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

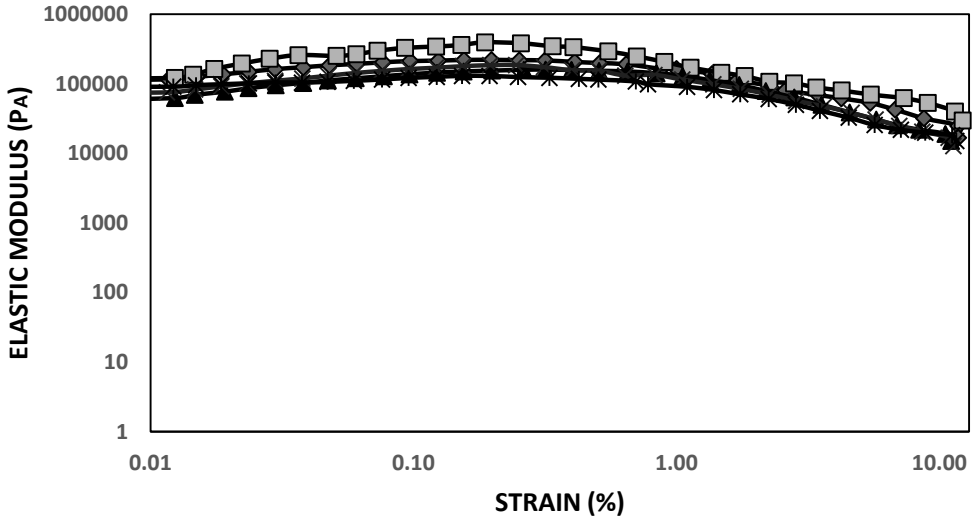

Figure 3.60 Elastic modulus obtained for bread dough samples containing 80 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

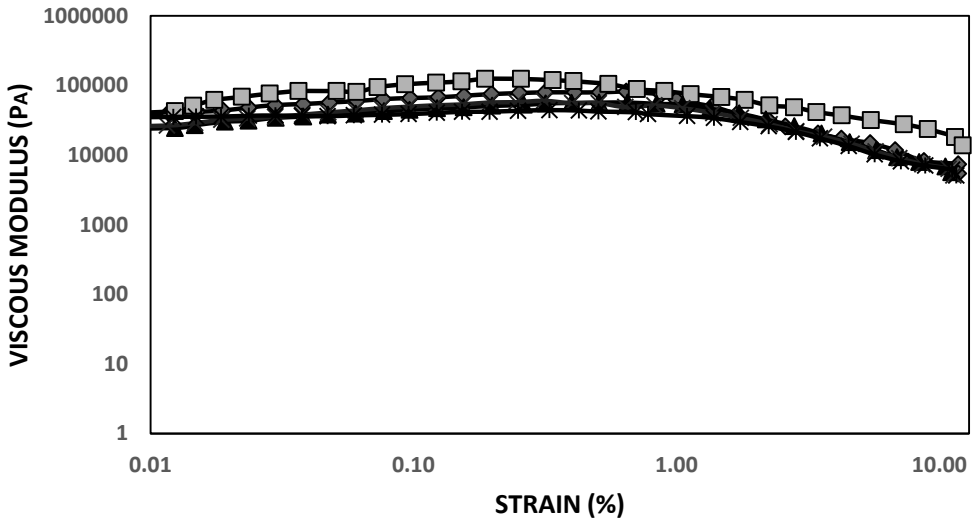

Figure 3.61 Viscous modulus obtained for bread dough samples containing 80 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

The results of the viscoelasticity of corn bread dough formulations with guar, in which different treatments were applied and also different starch amounts were used, could be seen from Fig. 3.62 to Fig. 3.67.

The linear viscoelasticity of the corn bread dough was improved by using of guar as could be seen from figures. The upper limit of the linearity was drove up to 0.8-0.9% strain and that meant length of the linearity was lengthened. Dough with guar showed same pattern with dough prepared without gum. As stated in the hpmc part, hydrocolloids like guar caused to increase in the values of elastic and viscous moduli.

Again, microfluidization led to higher moduli values, but alkaline treatment decreased these moduli values by changing structure. Moreover, increase in starch concentration caused to increase in elastic and viscous moduli values as being in other gums.

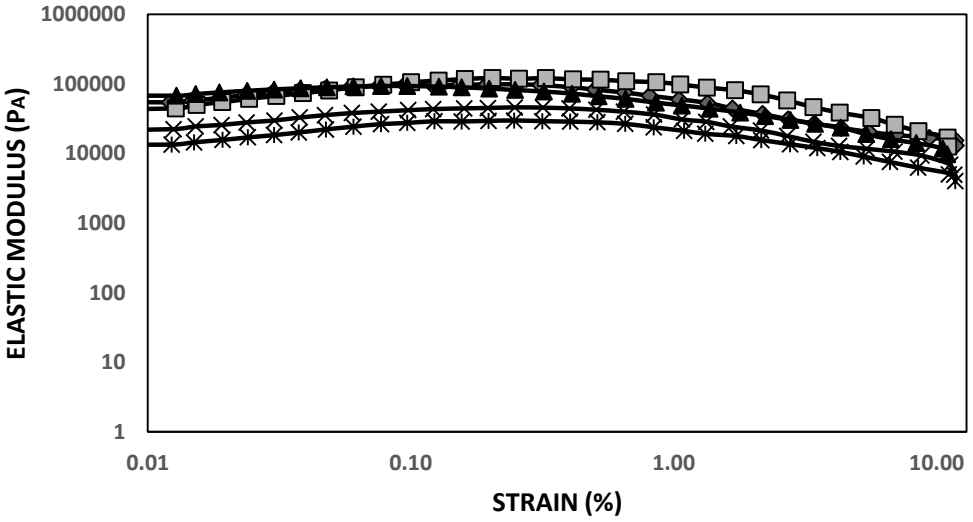

Figure 3.62 Elastic modulus obtained for bread dough samples containing 60 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

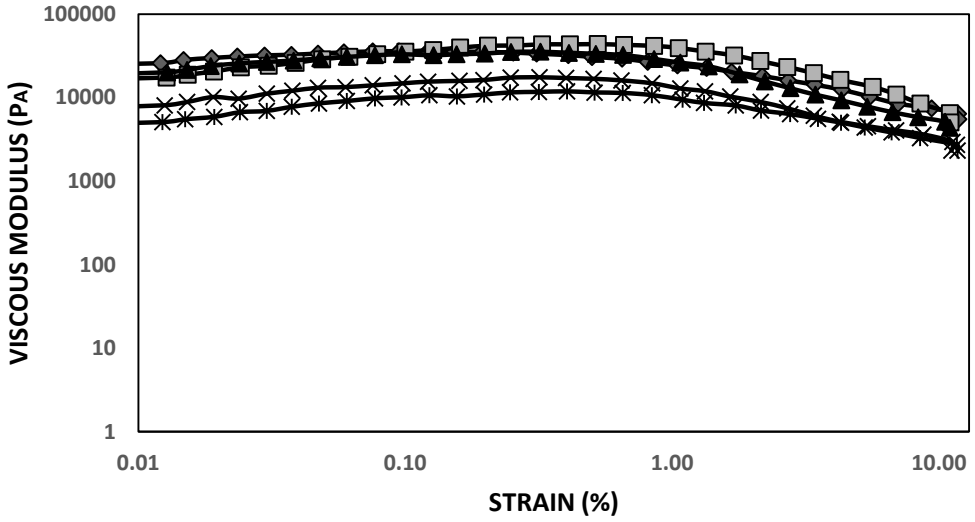

Figure 3.63 Viscous modulus obtained for bread dough samples containing 60 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (**ж**): pH=10

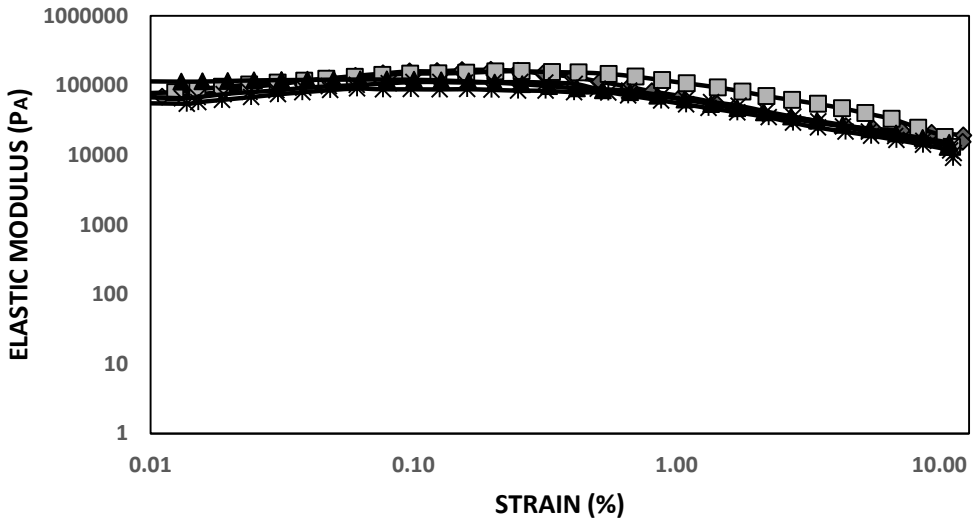

Figure 3.64 Elastic modulus obtained for bread dough samples containing 70 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (**ж**): pH=10

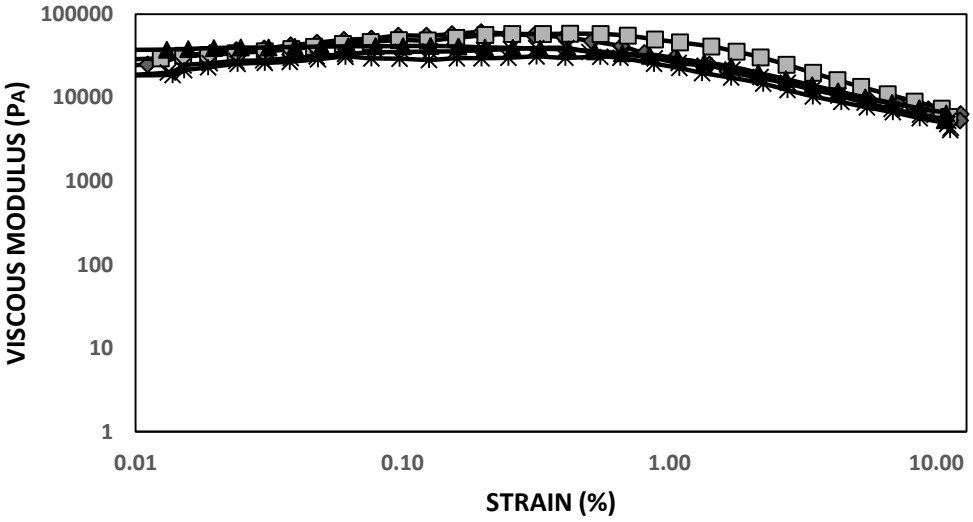

Figure 3.65 Viscous modulus obtained for bread dough samples containing 70 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

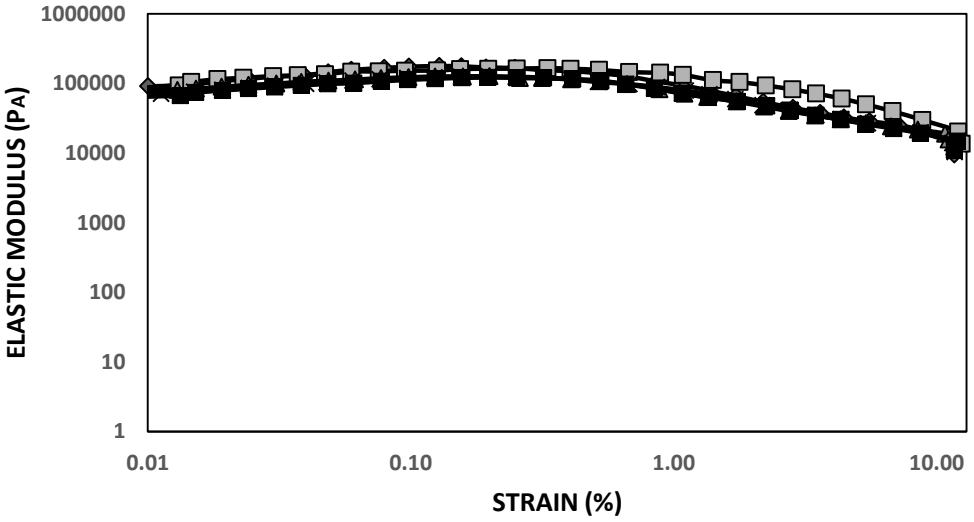

Figure 3.66 Elastic modulus obtained for bread dough samples containing 80 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

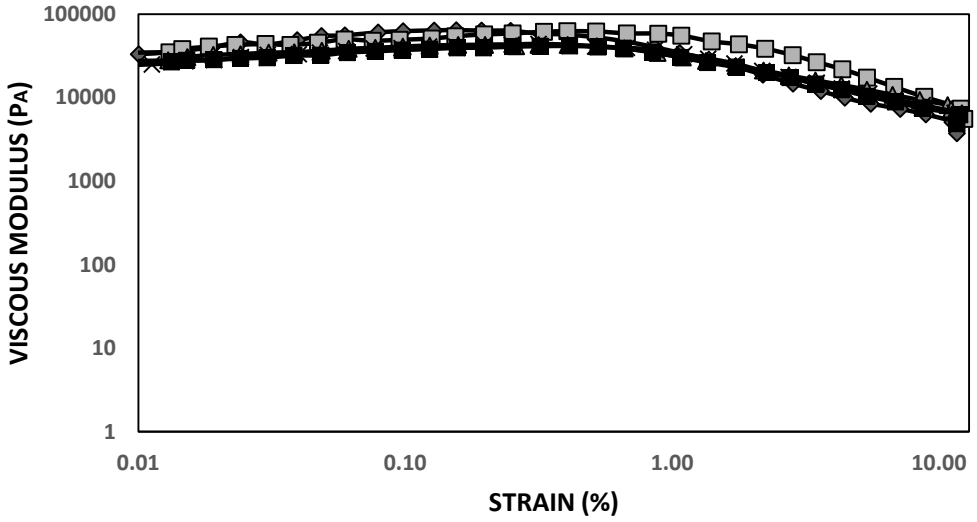

Figure 3.67 Viscous modulus obtained for bread dough samples containing 80 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

Figures related to the viscoelasticity of corn bread dough formulations with xanthan are represented in Fig. 3.68 and Fig. 3.69. Xanthan gum was only used in control and microfluidized zein experiments due to its inability to form a dough structure with additional alkaline treatment. That could be the result of deformation of the protein structure by that treatment too much. Similar to the case of hpmc, linear viscoelastic region reached to 0.4% strain and then linearity got lost.

The effects of microfluidization and starch amount could be observed from same figures. As being in other gums, when starch amount was increased, moduli values increased. Also, microfluidization led to increase in these values.

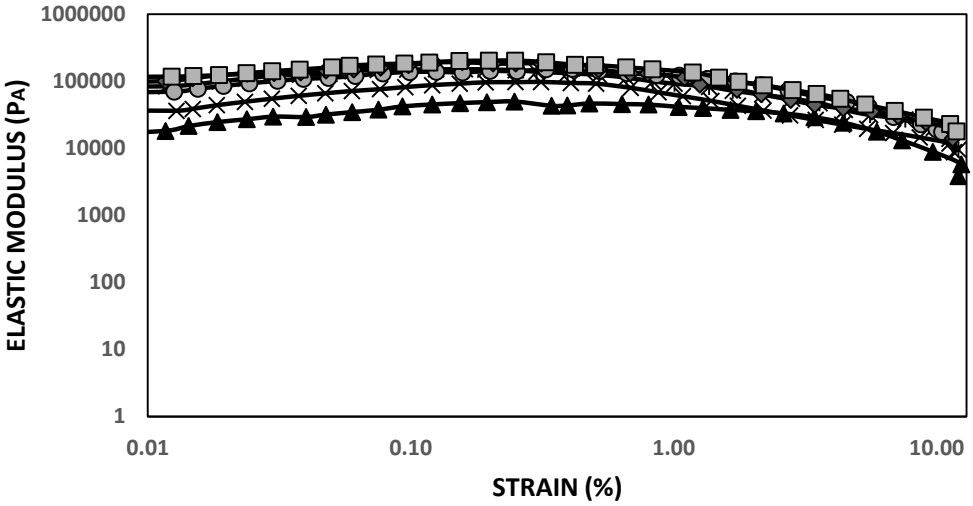

Figure 3.68 Elastic modulus obtained for bread dough samples with xanthan. (▲): 60 g control zein, (X): 60 g mf, (ж): 70 g control zein, (●): 70 g mf, (♦): 80 g control zein, (■): 80 g mf

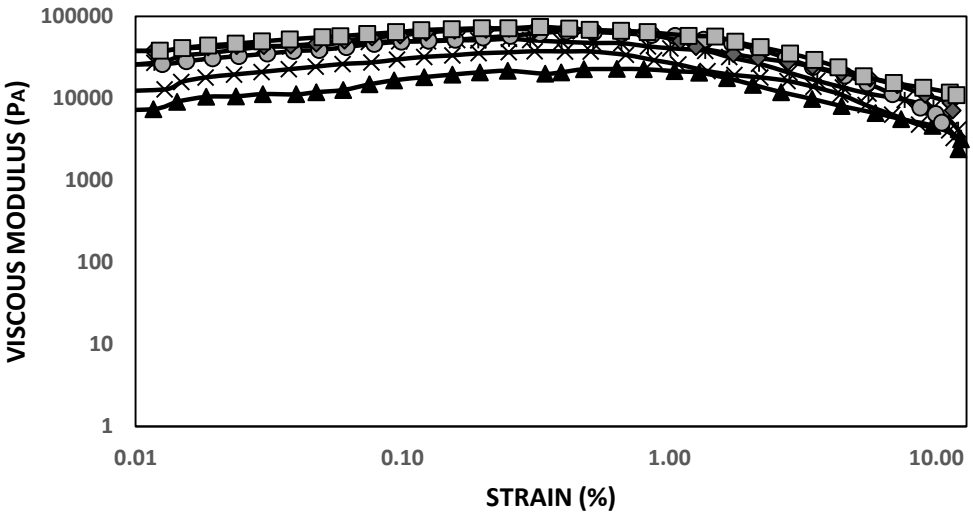

Figure 3.69 Viscous modulus obtained for bread dough samples with xanthan. (▲): 60 g control zein, (X): 60 g mf, (ж): 70 g control zein, (●): 70 g mf, (♦): 80 g control zein, (■): 80 g mf

The results of the linear viscoelasticity of corn bread dough formulations related to citrus fiber, in which different treatments were applied and also different starch amounts were used, could be seen in Fig. 3.70 to Fig. 3.71. Similar to xanthan gum, citrus fiber was also used only for control zein and microfluidization due to same reason with xanthan. As could be seen from the figures, linearity continued up to around the strain of 0.5%. Although it is not a hydrocolloid, citrus fiber provided same influence on the dough structure and increased elastic and viscous moduli values.

Similar to the xanthan, the effects of microfluidization and starch amount could be distinguished from figures. Increase in starch amount and application of microfluidization led to increase in elastic and viscous moduli values.

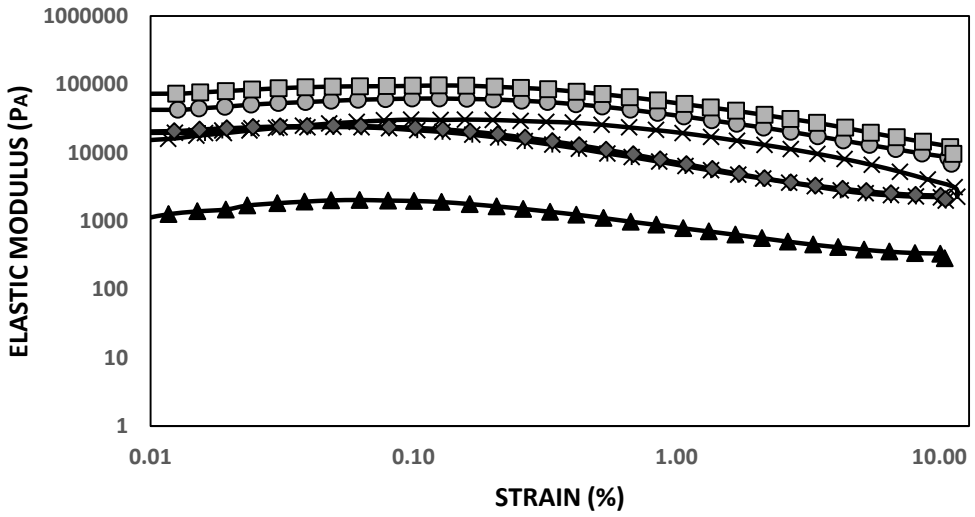

Figure 3.70 Elastic modulus obtained for bread dough samples with citrus fiber. (▲): 60 g control zein, (X): 60 g mf, (ж): 70 g control zein, (●): 70 g mf, (♦): 80 g control zein, (■): 80 g mf

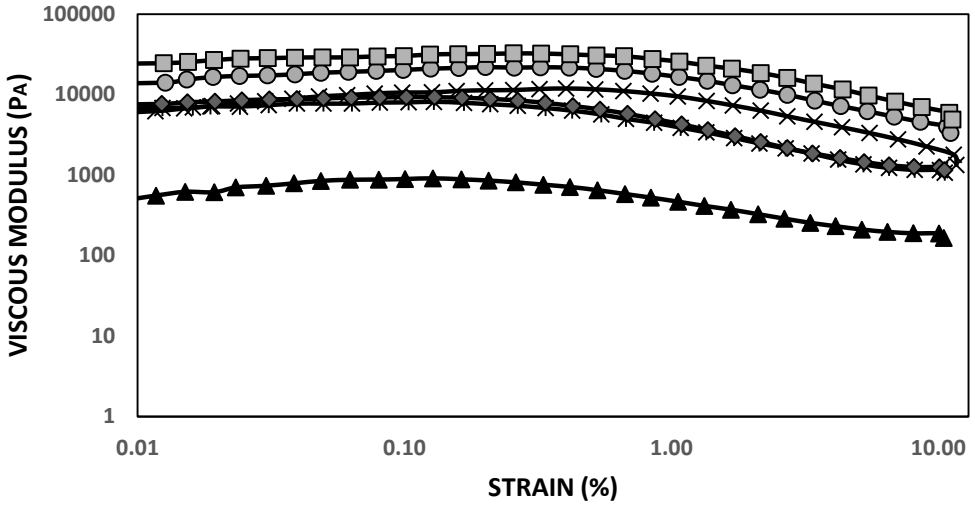

Figure 3.71 Viscous modulus obtained for bread dough samples with citrus fiber. (▲): 60 g control zein, (X): 60 g mf, (ж): 70 g control zein, (●): 70 g mf, (♦): 80 g control zein, (■): 80 g mf

In conclusion, microfluidization resulted in higher moduli values, but that increase was decreased by alkaline treatment by deformation of the structure. On the other hand, increase in the starch amount always led to increase in the elastic and viscous moduli. As stated in the beginning of this part, the length of the linear viscoelastic region is an important parameter to determine the stability (Mariotti et al., 2009). As could be seen from the figures, hydrocolloids increased that parameter. Especially, guar was so effective in terms of linear stability. Also, dough prepared by hpmc, xanthan and citrus fiber showed better results than dough without gum.

Similar findings were indicated by many researchers. Phan-Thien & Safari-Ardi (1998), Safari-Ardi & Phan-Thien (1998) and Weipert (1990) were the examples of the measurements of strain sweep test on the gluten-free dough formulations. Lazaridou et al. (2007) pointed out that gluten-free dough samples showed viscoelastic properties up to strain of 0.1%, and as being in our study, it showed a

larger decrease after 1% strain. Pruska-Kedzior et al. (2008)'s study on the formulations of gluten-free dough indicated the same point about viscoelastic region. Differently from on gluten-free studies, also some studies have been conducted to determine the linear viscoelastic region of the zein suspensions. The study of Zhong & Ikeda (2012) expressed that zein suspensions, prepared by the addition of ethanol solutions in different concentrations, showed a linear viscoelastic region below the strain of 0.003%. After that point, firstly a small decrease and then larger decrease was seen in elastic and viscous moduli values.

3.3.1.2 Frequency Sweep Test

0.1% strain was used as the controlled variable in frequency sweep tests and that strain value was within the linear viscoelastic region, which was found in the strain sweep tests. That meant frequency tests were not dependent to applied stress or strain.

Elastic (G') and viscous (G") moduli values of gluten-free corn bread samples containing different corn starch amounts and hydrocolloids, and also prepared by different treatments are illustrated from Fig. 3.72 to Fig. 3.93.

Frequency sweep test is an important and maybe the most common oscillatory test to determine the viscoelastic behavior of samples within viscoelastic range (Tunick, 2000). Because, it relates the elastic and viscous behaviors of a sample with varying frequency under strain or stress application (Gunasekaran & Ak, 2000). When the elastic behavior dominates the viscous behavior (G' > G"), gel or solid like behavior is observed. The vice versa is stated as liquid character (Steffe, 1996).

Elastic (G') and viscous (G") moduli values of gluten-free corn bread samples without gum are presented from Fig. 3.72 to Fig. 3.77. As could be seen from all figures, both elastic and viscous moduli values increased with increasing frequency, and the shape of the curves was similar to each other for all dough formulations. Only difference among them was the frequency dependence of them. Lazaridou et

al. (2007) stated that elastic moduli value and final quality of gluten-free breads show a strong correlation. Also, corn bread dough showed a solid like behavior since elastic moduli values was higher than viscous moduli values for same dough formulations. That meant loss tangent values of all corn bread dough formulations were lower than 1. Several studies have been performed on the rheology of bread dough. The study of Lazaridou et al. (2007) and Mariotti et al. (2009) on gluten-free bread formulations also showed similar findings. They stated that gluten-free dough produced solid elastic-like behavior under dynamic conditions. Also, rice flour dough exhibited same rheological properties as stated in the study of Gujral et al. (2003) and Sivaramakrishnan et al. (2004). Furthermore, the usage of different additives, such as resistant starch (Korus et al., 2009) and maltodextrin (Witczak et al., 2010) to improve the rheological and textural properties of gluten-free bread formulations gave same results. Moreover, wheat flour dough rheology had been investigated by many researchers and all of them reported that wheat dough also showed solid like behavior (Baltsavias et al., 1997; Edwards et al., 2003; Weipert, 1990). The study of Pruska-Kedzior et al. (2008) compared the rheological properties of wheat and gluten-free bread formulations and stated that elastic and viscous moduli values of gluten-free dough were really similar to wheat dough in terms of shape and frequency dependence. The only difference was observed on moduli values. Wheat dough exhibited higher moduli values than gluten-free doughs.

In addition to that, the increase in the starch concentration led to increase in elastic and viscous moduli values. Because of that reason, bread dough containing 80 g corn starch had higher moduli values than 70 g and 60 g. The similar study had been made by Witczak et al. (2012) to investigate the effects of modified starches on gluten-free dough formulations. According to this research, starch concentration was slightly effective on elastic and viscous moduli values.

The another parameter was treatment type, which was investigated to see the effect of microfluidization and alkalinity on the dough rheology. As stated before, untreated zein without gum was not able to form a homogenous mixture. Therefore, untreated zein was not tested in without gum experiments. Sivaramakrishnan et al.

(2004) and Demirkesen (2013) were encountered similar problems on rice dough formulations. As could be seen from figures, microfluidized zein resulted in higher moduli values than the others. It was followed by pH=6, pH=8 and pH=10. The study of Mert (2012) stated that microfluidization forms more homogenous and uniform particle size distribution by creating higher shear rates. Because of that reason, elastic and viscous moduli values of microfluidized dough samples had higher values. Also, this study showed that increasing pressure during microfluidization led to increasing moduli values up to some point and it resembled to our study. Increasing pressure could be thought as applying alkalinity to samples to open the structure. As being in that study, moduli values were reached to their highest by microfluidization, then it decreased by increasing alkalinity similar to pressure. This result could be expressed by overprocess of the samples. Overprocessing led to smaller particles, however those particles were not able to form strong matrices in the system (Mert, 2012).

When the rheologies of emulsions and doughs prepared with zein were compared, the results displayed differences. As a reminder, the moduli values showed the decreasing order of pH=8, pH=6, pH=10 and microfluidized zein in emulsion rheology. However, this result changed completely for dough rheology. This difference could be the consequence of usage of different ingredients in dough formulations different from emulsions. A sharp difference was easily observed on moduli values by microfluidization. The increase in alkalinity resulted in more solubilization for zein and because of that the moduli values in emulsion rheology increased by alkalinity up to some point. However, in dough rheology, although zein was easily solubilized by alkalinity, the structure of the other ingredients also had importance on rheological measurements. The same decreasing order was also found in strain sweep experiments in the previous part.

Another difference between doughs prepared by microfluidization and alkalinity was the frequency dependency of moduli values. As could be seen from figures, dough prepared by microfluidization had strong frequency dependence against doughs prepared by microfluidization and then treated by pH.

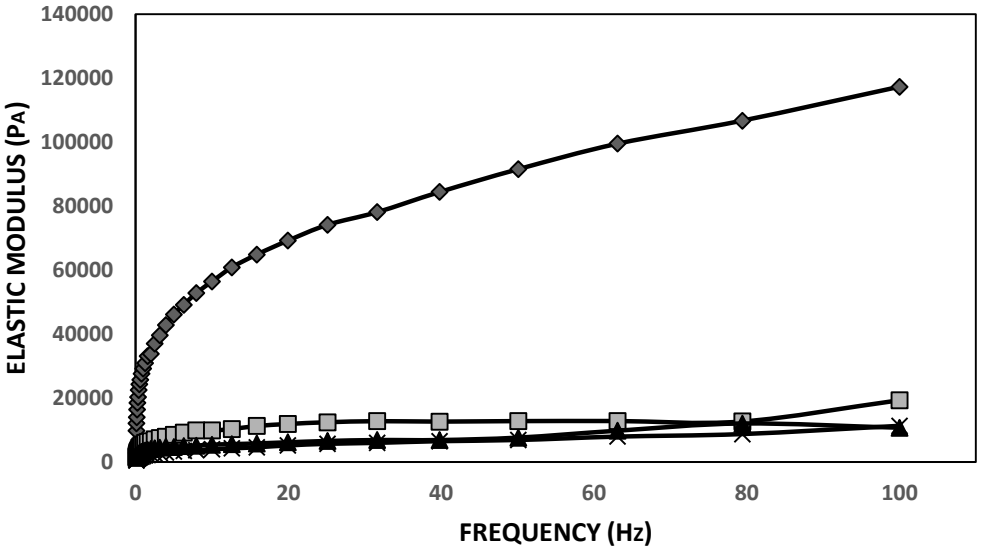

Figure 3.72 Elastic modulus obtained for bread dough samples containing 60 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

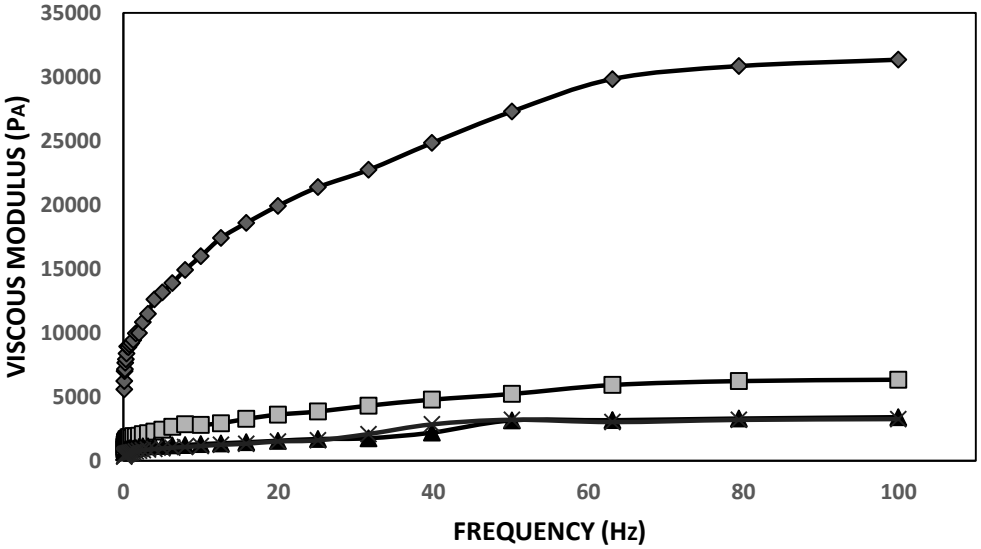

Figure 3.73 Viscous modulus obtained for bread dough samples containing 60 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

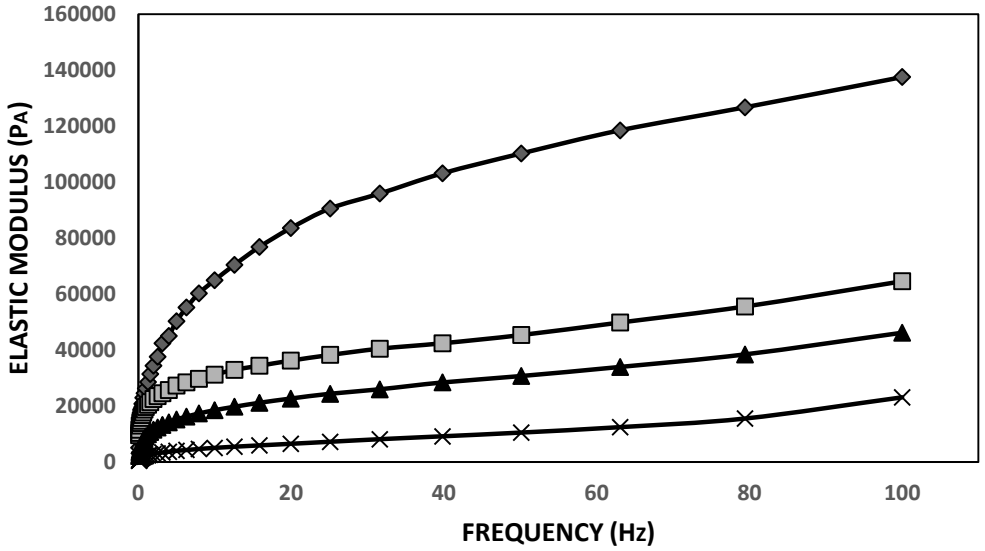

Figure 3.74 Elastic modulus obtained for bread dough samples containing 70 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

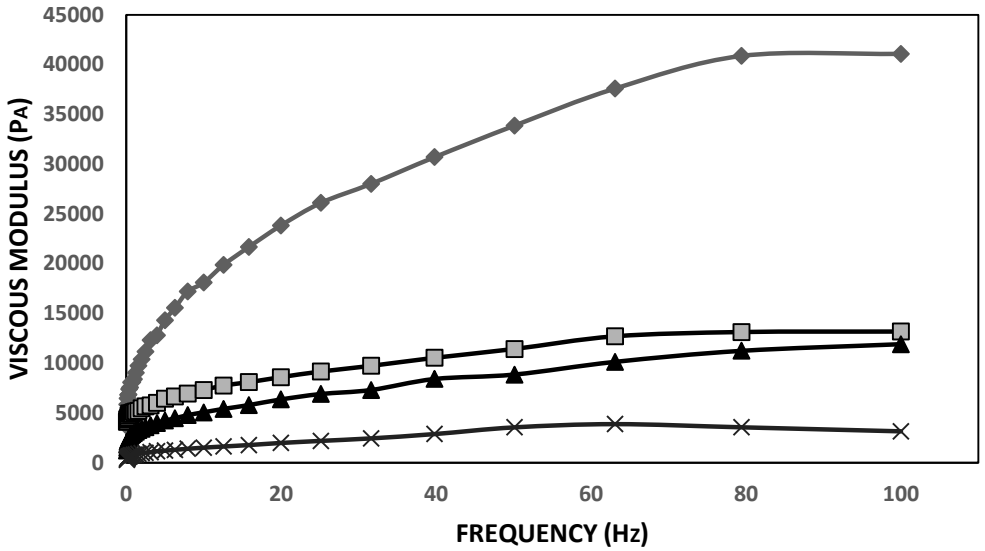

Figure 3.75 Viscous modulus obtained for bread dough samples containing 70 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

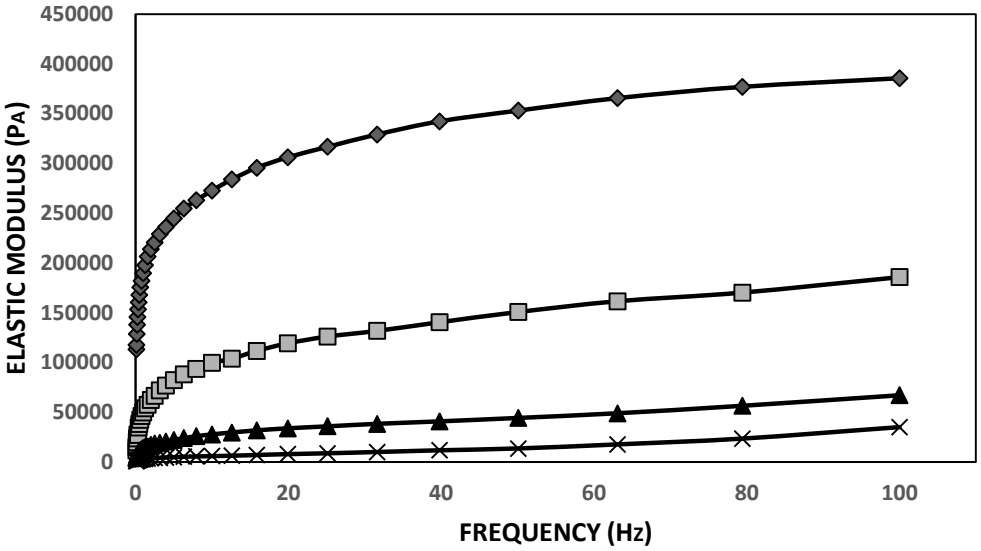

Figure 3.76 Elastic modulus obtained for bread dough samples containing 80 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

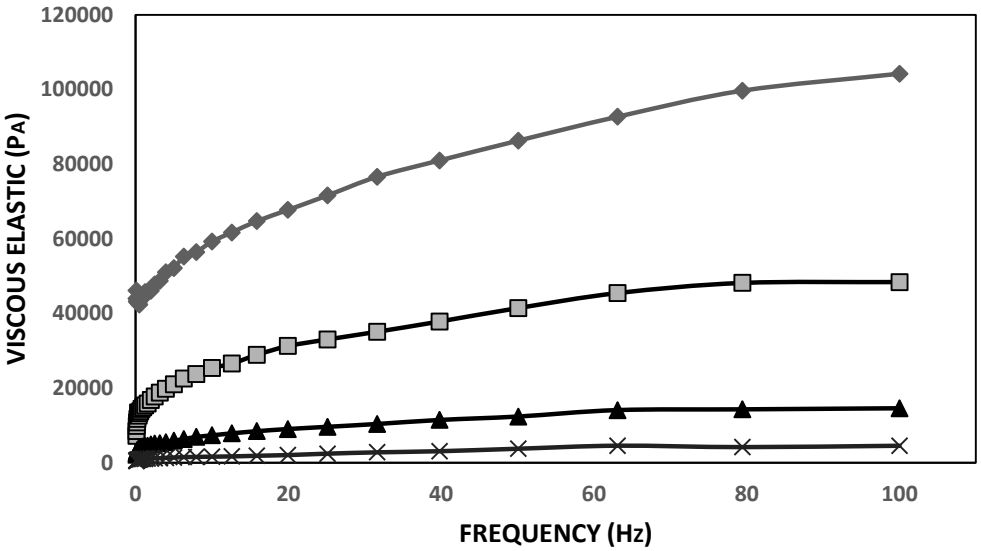

Figure 3.77 Viscous modulus obtained for bread dough samples containing 80 g corn starch without gum. (♦): mf, (■): pH=6, (▲): pH=8, (X): pH=10

Hydrocolloids are used as bread improvers to increase dough strength (Rosell et al., 2001). Because of that reason, we tried to investigate the effects of some gums like hpmc, guar and xanthan, and citrus fiber on rheological properties of gluten-free bread formulations.

From Fig. 3.78 to Fig.3.83, elastic (G') and viscous (G") moduli values of gluten-free corn bread samples with hpmc are represented. The results obtained from without gum experiments were again obtained for hpmc experiments. Both elastic and viscous moduli values increased with increasing frequency, and the shape of the curves was similar to each other for all dough formulations. Also, dough formulations presented solid like behavior. Besides, the increase in starch concentration led to increase in moduli values. Moreover, the application of microfluidization provided little increase in moduli values for 60 g starch concentration. However, 70 and 80 g starch concentrations showed more increase in these values.

The difference of hpmc experiment from without gum experiment was that formation of a well dough structure was succeeded by the aid of hpmc. Thus, untreated zein took part in this experiment. Again, microfluidized zein gave the higher moduli values and it was pursued by control zein, pH=6, pH=8 and pH=10, respectively. The formation of uniform particles provided higher moduli values, however, these values decreased by additional pH arrangements, which caused to deformation of complete system.

The studies of Andersson et al. (2011), Schober et al. (2008) and Schober et al. (2010) on zein-starch dough, Crockett et al. (2011) on rice flour dough, Ericksen et al. (2012) on zein dough and Schober et al. (2007) on sorghum dough to investigate the effect of hpmc on viscoelastic properties of gluten-free dough were in agreement with our findings.

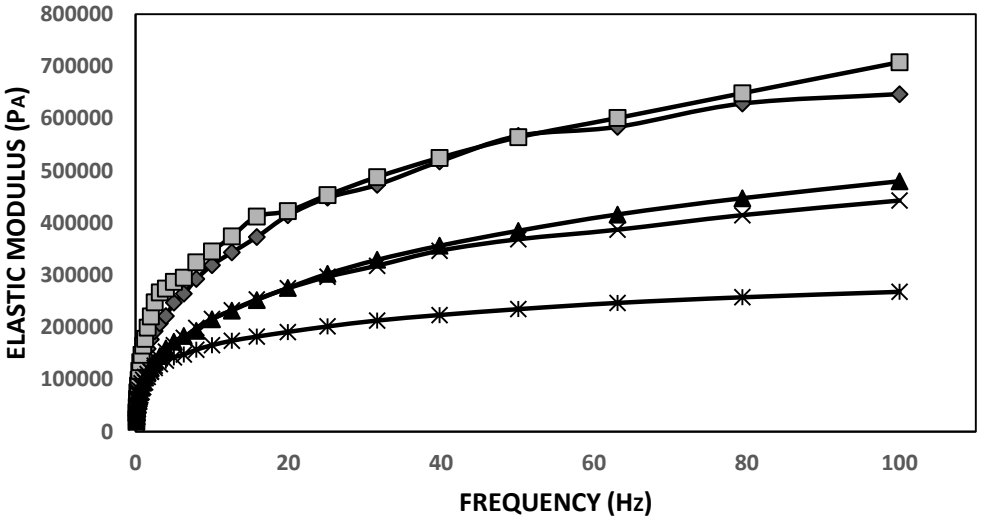

Figure 3.78 Elastic modulus obtained for bread dough samples containing 60 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

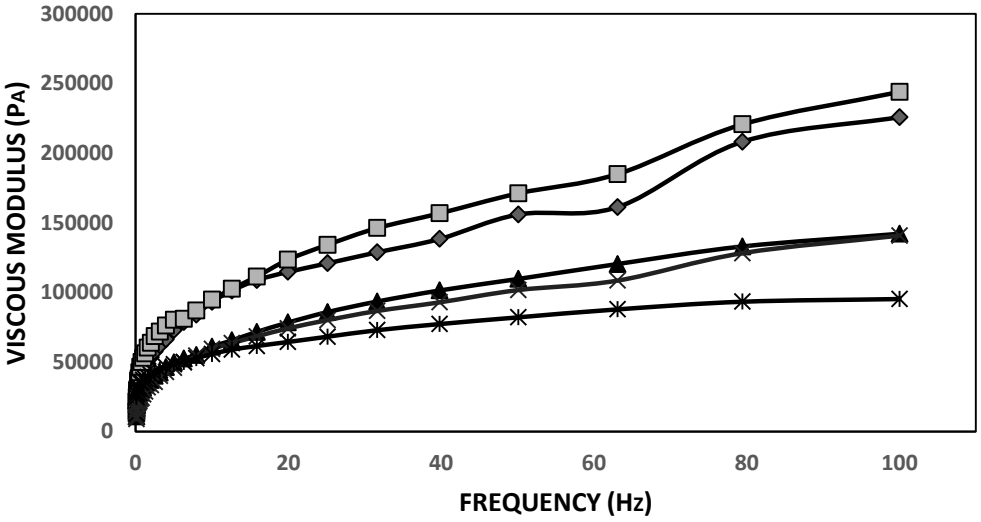

Figure 3.79 Viscous modulus obtained for bread dough samples containing 60 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

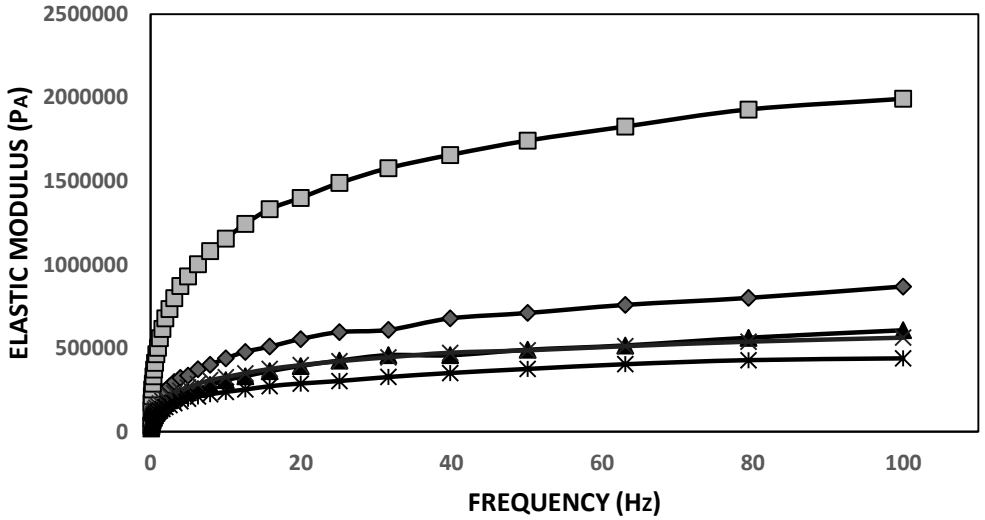

Figure 3.80 Elastic modulus obtained for bread dough samples containing 70 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

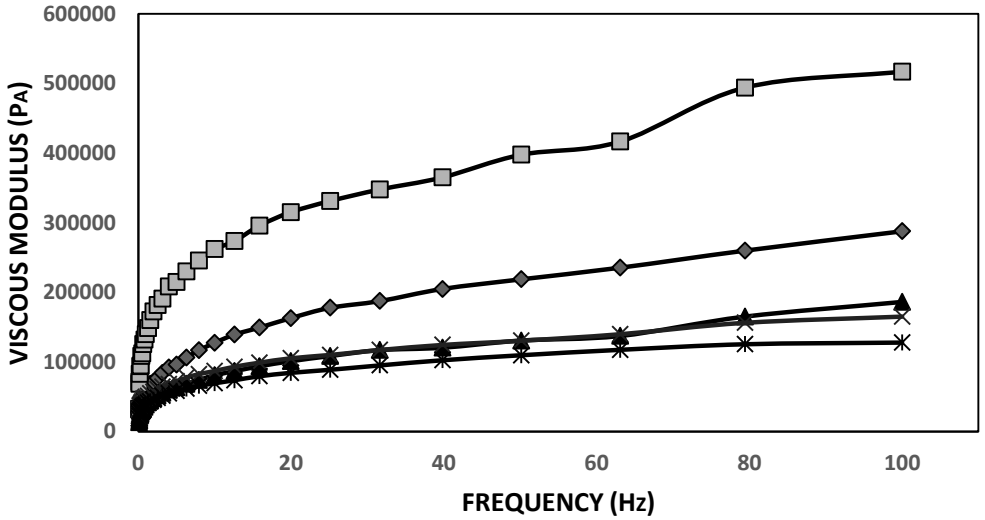

Figure 3.81 Viscous modulus obtained for bread dough samples containing 70 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

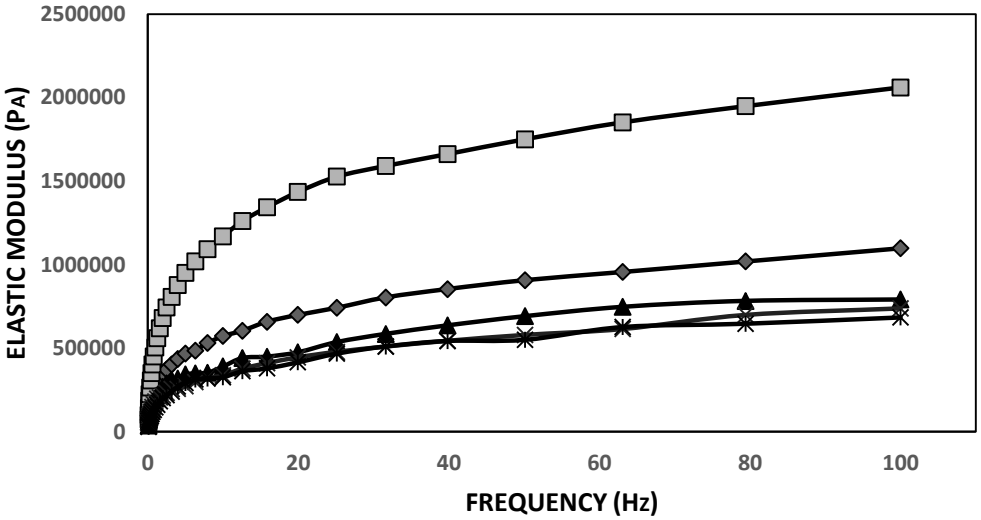

Figure 3.82 Elastic modulus obtained for bread dough samples containing 80 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

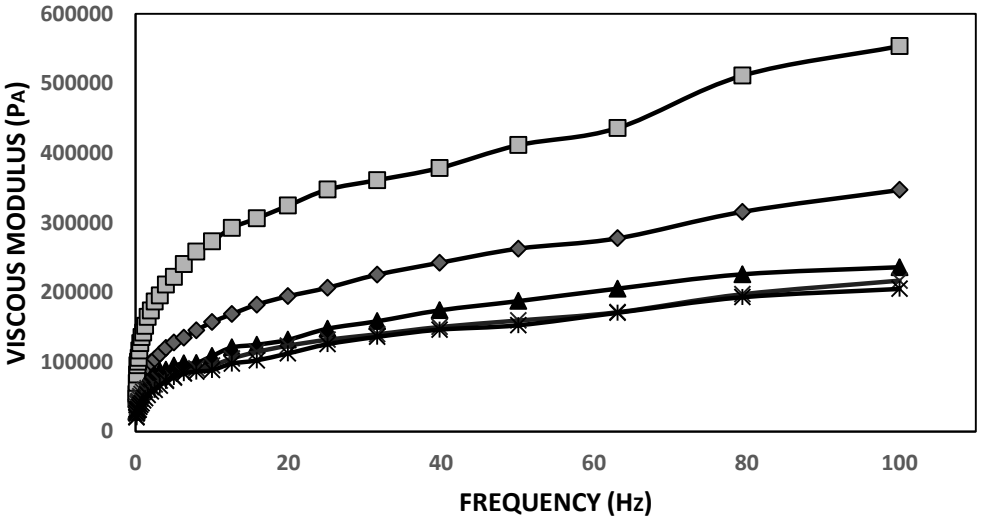

Figure 3.83 Viscous modulus obtained for bread dough samples containing 80 g corn starch with hpmc. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

The moduli values of gluten-free corn bread samples with guar are illustrated from Fig. 3.84 to Fig. 3.89. The results obtained with guar addition to gluten-free bread were completely same with hpmc experiments. As frequency increased, both G' and G" also increased. As could be seen from figures, similar curves were obtained for guar experiments. Elastic modulus was higher than viscous one, so that solid like behavior was observed. The increase in starch amount and usage of microfluidization as a treatment method resulted in higher moduli values as being in hpmc case. Again, alkaline treatment addition to microfluidization gave lower moduli values than microfluidized and also control zein.

The effects of guar on viscoelastic properties of gluten-free bread dough was also studied by Demirkesen et al. (2010a), and two studies were in agreement on the subject of guar.

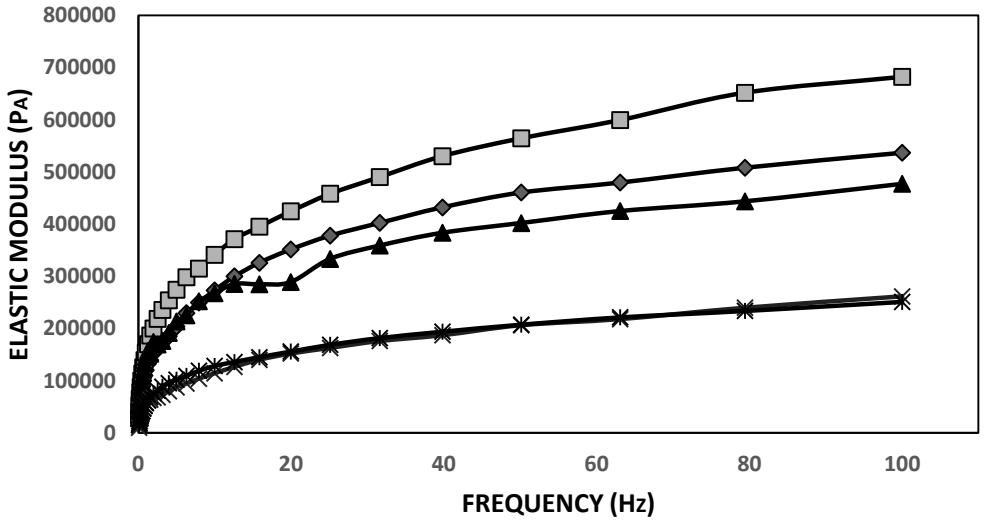

Figure 3.84 Elastic modulus obtained for bread dough samples containing 60 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

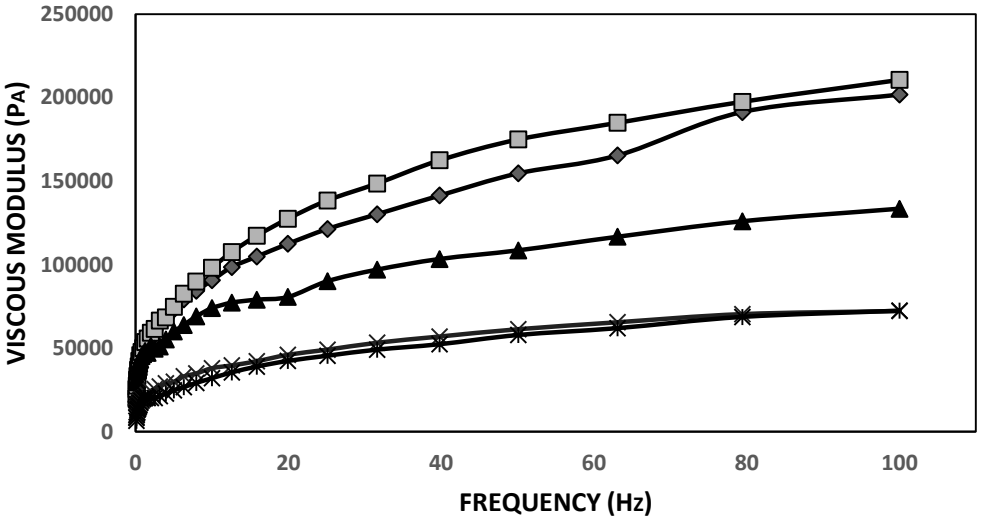

Figure 3.85 Viscous modulus obtained for bread dough samples containing 60 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

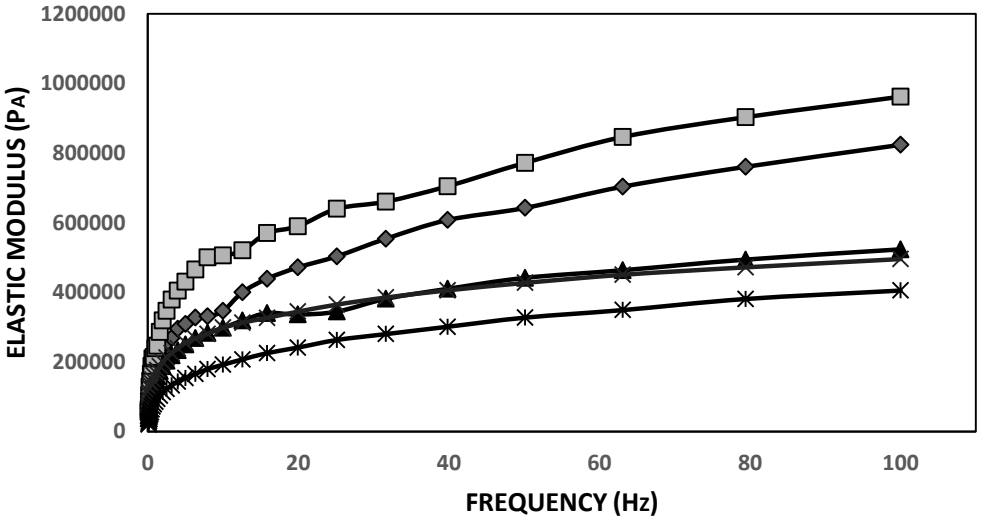

Figure 3.86 Elastic modulus obtained for bread dough samples containing 70 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

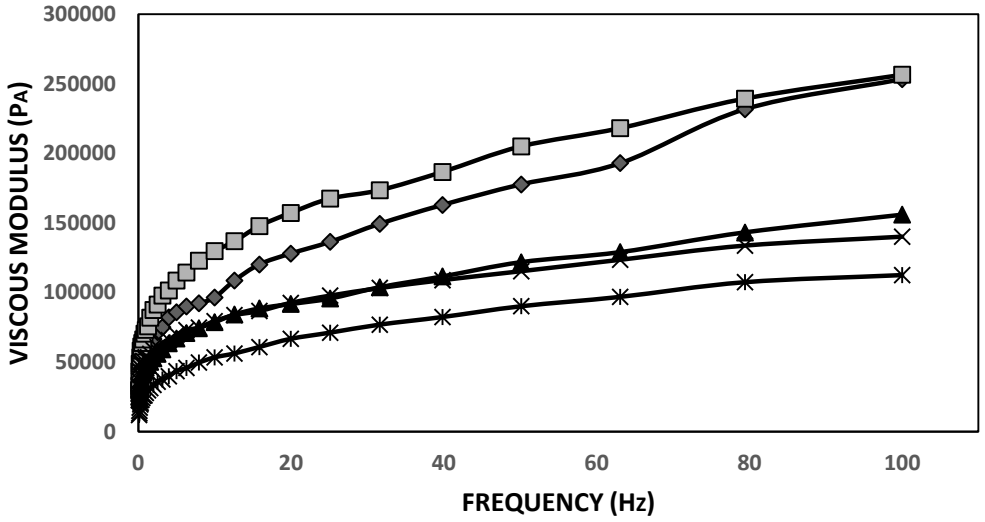

Figure 3.87 Viscous modulus obtained for bread dough samples containing 70 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

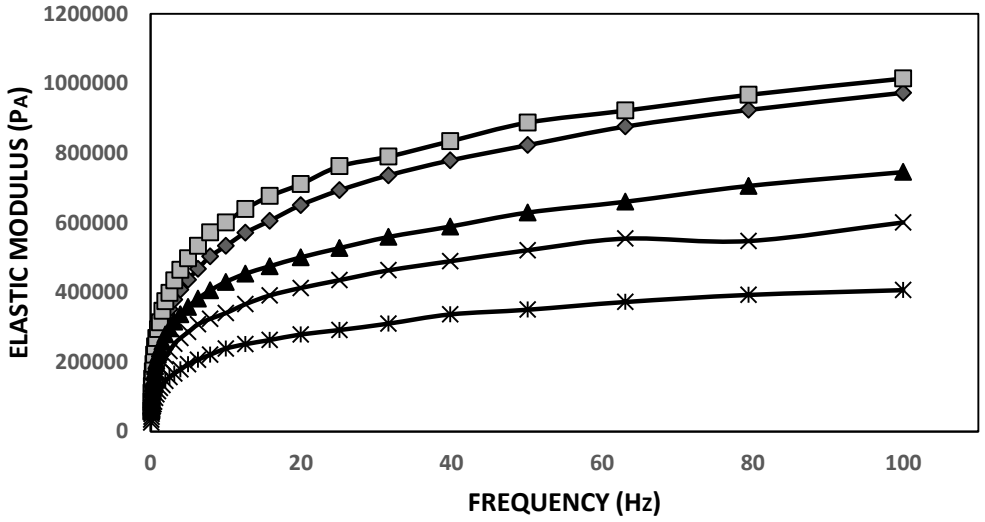

Figure 3.88 Elastic modulus obtained for bread dough samples containing 80 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

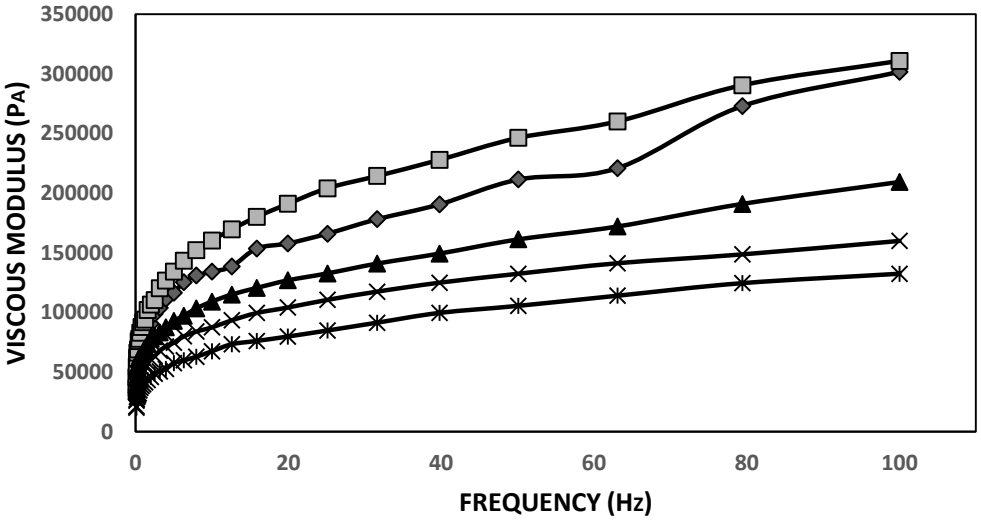

Figure 3.89 Viscous modulus obtained for bread dough samples containing 80 g corn starch with guar. (♦): control zein, (■): mf, (▲): pH=6, (X): pH=8, (ж): pH=10

Figures related to the elastic and viscous moduli values of corn bread dough formulations with xanthan are represented in Fig. 3.90 and Fig. 3.91. As stated in strain sweep experiments, xanthan gum was only used in control and microfluidized zein experiments due to its inability to form a dough structure with additional alkaline treatment. That could be the result of deformation of the structure by that treatment too much. The effects of microfluidization and starch amount could be observed from same figures since the parameter number was a few for that gum. As being in other gums, increase of the starch amount and application of microfluidization led to increase in moduli values.

The studies of Crockett et al. (2011), Sciarini et al. (2010), and Sciarini et al. (2012) displays paralellism to our study in terms of the effect of xanthan on viscoelastic properties of gluten-free bread dough.

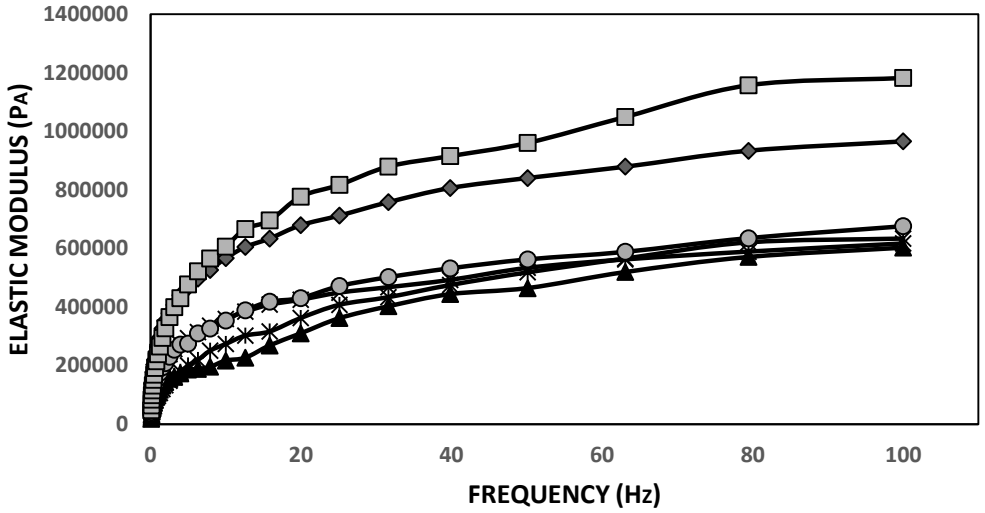

Figure 3.90 Elastic modulus obtained for bread dough samples with xanthan. (▲): 60 g control zein, (X): 60 g mf, (ж): 70 g control zein, (●): 70 g mf, (♦): 80 g control zein, (■): 80 g mf

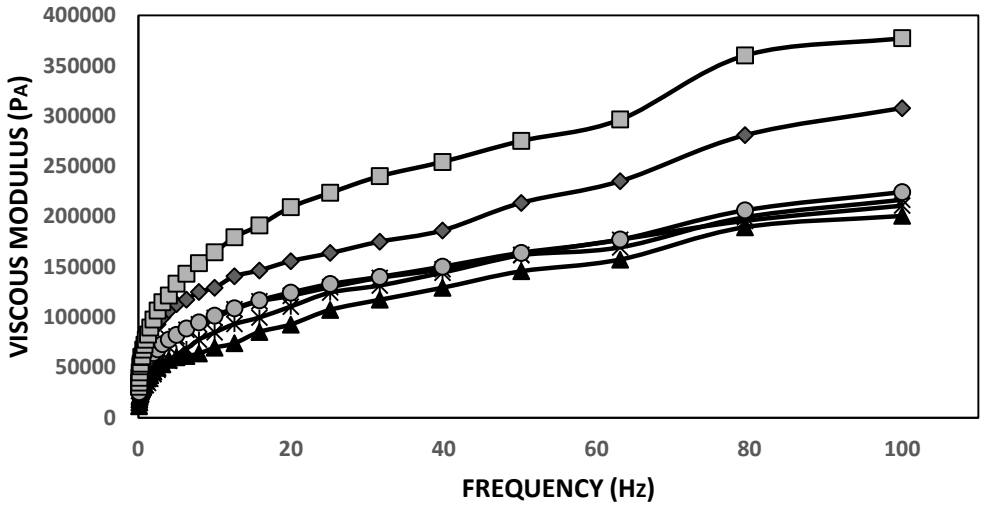

Figure 3.91 Viscous modulus obtained for bread dough samples with xanthan. (▲): 60 g control zein, (X): 60 g mf, (ж): 70 g control zein, (●): 70 g mf, (♦): 80 g control zein, (■): 80 g mf

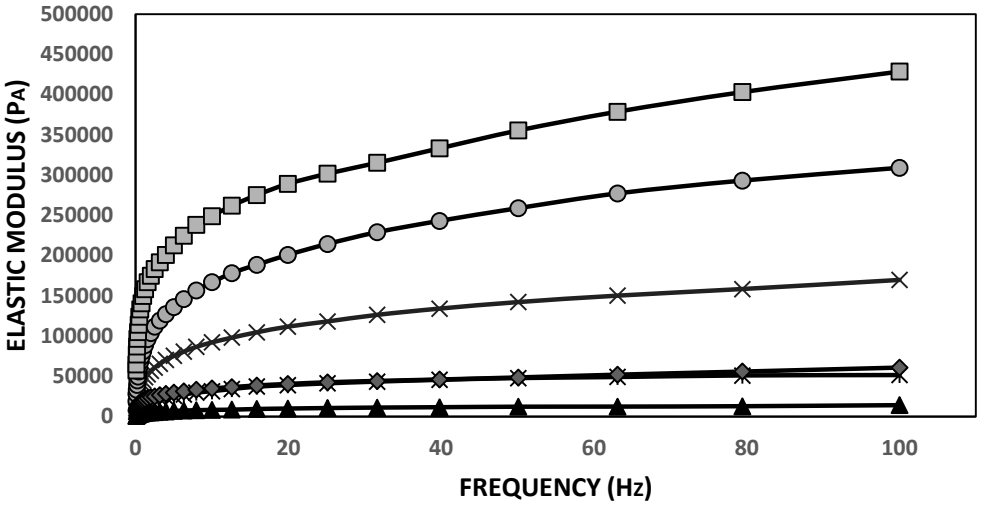

Figure 3.92 Elastic modulus obtained for bread dough samples with citrus fiber. (▲): 60 g control zein, (X): 60 g mf, (**ж**): 70 g control zein, (●): 70 g mf, (♦): 80 g control zein, (■): 80 g mf

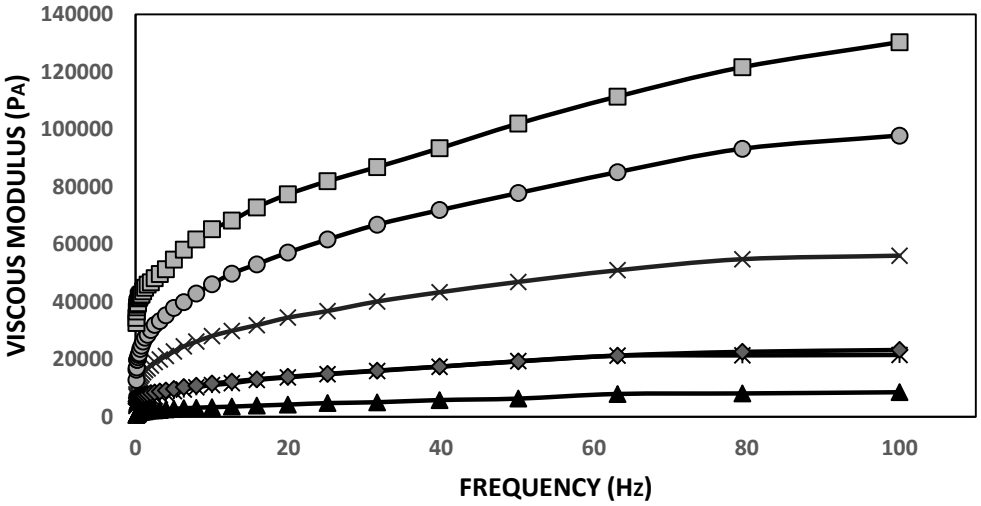

Figure 3.93 Viscous modulus obtained for bread dough samples with citrus fiber. (▲): 60 g control zein, (X): 60 g mf, (**ж**): 70 g control zein, (●): 70 g mf, (♦): 80 g control zein, (■): 80 g mf

The results of the elastic and viscous moduli values of corn bread dough formulations related to citrus fiber, in which different treatments were applied and also different starch amounts were used, could be seen in Fig. 3.92 and Fig. 3.93. Similar to xanthan gum, citrus fiber was also used only for control zein and microfluidization due to inability of dough formation. Although it is not a hydrocolloid, citrus fiber provided same influence on the dough structure and increased elastic and viscous moduli values.

Similar to the xanthan, the effects of microfluidization and starch amount could be observed from figures. Increase in starch amount and application of microfluidization led to increase in elastic and viscous moduli values.

In conclusion, it could be stated that microfluidization led to higher moduli values compared to untreated zein and zein treated by alkalinity. On the other side, increasing of starch amount always provided higher elastic and viscous moduli values. Moreover, as could be seen from figures, the addition of hpmc, guar, xanthan and citrus fiber to gluten-free dough formulations improved moduli values compared to dough without gum, which was also stated by Crockett et al. (2011). However, the frequency dependence was also increased with the addition of hydrocolloids.

Figures showed that the highest moduli values for corn bread dough formulations were observed with the addition of hpmc. Then, it was followed by xanthan, guar and citrus fiber. The values of dough with citrus fiber and dough without gum were close to each other, however citrus fiber increased moduli values a little bit. Demirkesen (2013) also indicated that hydrocolloids provided higher moduli values. However, the results of the study on rice flour dough pointed out that xanthan gave the highest values. Also, the study of the Lazaridou et al. (2007) supported the findings of Demirkesen (2013).

Also, temperature sweep tests had been studied on zein dough by Schober et al. (2008) to determine the glass transition temperature of zein and form a suitable gluten-free dough formulation by avoiding from this effect.

3.3.2 Moisture Content

The effects of different treatments and starch contents on moisture content of gluten-free corn bread were tabulated from Table 3.2 to Table 3.6 according to gum type. Some gum types were not tested for some treatments due to their inability to form a well bread dough and this was the reason why we could not compare all gum types with all treatments. Moisture contents of fresh bread samples without gum, with hpmc, guar, xanthan and citrus fiber were presented in tables below, respectively.

According to the results presented in Table 3.2, when starch amount increased, moisture content of the fresh bread samples decreased regardless of treatment type. That meant moisture content was the lowest in the 80 g starch formulation and the highest in the 60 g starch formulation in all treatments. There was a significant difference between starch amounts on moisture contents according to ANOVA results ($p \leq 0.05$). Since all formulations were same except starch amount, the moisture content was found lower in breads containing 80 g starch as expected.

On the other hand, when the treatment types were compared, it could be easily stated that treatment types did not affect the moisture content of the fresh bread samples made with same formulation. The means of the moisture contents were slightly different from each other, however statistical analysis showed that there was not a significant difference between treatment types on the moisture content of fresh bread samples ($p \leq 0.05$). This was an important point for our study because our purpose in that study did not affect the moisture content of the samples by varying treatments, on the contrary we tried to improve water holding capacity of the zein with microfluidization and alkalinity when same water amount was used.

Water holding capacity is a very important characteristics for foods. Because foods have lower holding capacity reaches its saturation point easily and the remaining water disperses through the surroundings (Wang et al., 2012). As would be stated in the texture part, higher water holding capacity resulted in higher hardness values in microfluidized and pH treated samples than untreated samples.

In the untreated samples, water interrelated mostly starch and other ingredients with each other, however it did not display any effect on zein because of its hydrophobicity. On the other hand, the microfluidized and alkaline treated samples, which had same amount of water in their structures, resulted in more interactions between all ingredients. Because of that reason, more consolidated network structure was observed in these samples, which was also expressed with SEM analysis in Fig. 3.2-Fig. 3.13.

Other moisture analyses related to gum types were stated below and every one of them showed the same results with bread samples made without gum in explained above. It had to be expressed that one more time for its clear understanding. As could be seen from Table 3.3 to Table 3.6, in all experiments, there was not a significant difference between treatment types. The only difference was coming from starch amounts ($p \leq 0.05$). Because, hpmc and guar experiments were made with all treatment types and starch amounts, the concept about moisture content could be understood more easily as stated above. Moisture contents showed similarities regarding of treatment types, but water holding capacity of the zein was enhanced by microfluidization and alkalinity due to their shear force and modification effects on the structure of zein. These effects resulted in overcome the hydrophobicity of zein by increasing water holding capacity and forming more qualified bread dough in spite of gluten-free structure, which are supported by rheological and textural analyses. The studies of Çıkrıkcı (2013) on hazelnut skin and Wang et al. (2012) on wheat bran also showed the effect of microfluidization process on water holding capacity.

Table 3.2 Moisture content of fresh bread samples without gum. Standard deviations were also indicated.

Treatment	Starch Amount	Moisture Content (%)
mf	60	48.97 ± 0.087^{abc}
	70	46.92 ± 0.260^{cde}
	80	44.64 ± 0.492^{f}
pH6	60	50.74 ± 0.043^{a}
	70	47.04 ± 0.035^{cd}
	80	44.54 ± 0.007^{f}
pH8	60	48.81 ± 0.630^{abc}
	70	47.21 ± 0.151^{cd}
	80	44.89 ± 0.125^{ef}
pH10	60	49.29 ± 0.774^{ab}
	70	47.68 ± 0.398^{bc}
	80	45.63 ± 0.275^{def}

*The means and standard deviations of two readings were calculated. Different letters represent significant difference ($p \leq 0.05$).

Table 3.3 Moisture content of fresh bread samples made with hpmc. Standard deviations were also indicated.

Treatment	Starch Amount	Moisture Content (%)
Control zein	60	49.44 ± 1.337^{a}
	70	47.29 ± 0.468^{abcde}
	80	44.03 ± 0.003^{fg}
mf	60	48.51 ± 0.565^{abc}
	70	45.91 ± 0.238^{cdefg}
	80	45.40 ± 0.085^{defg}
pH6	60	47.93 ± 0.157^{abcd}
	70	46.02 ± 0.190^{cdef}
	80	44.52 ± 0.005^{fg}
pH8	60	48.82 ± 0.265^{ab}
	70	45.12 ± 0.366^{efg}
	80	43.37 ± 0.275^{g}
pH10	60	48.25 ± 0.491^{abc}
	70	46.61 ± 0.228^{bcdef}
	80	45.11 ± 0.382^{efg}

*The means and standard deviations of two readings were calculated. Different letters represent significant difference ($p \leq 0.05$).

Table 3.4 Moisture content of fresh bread samples made with guar. Standard deviations were also indicated.

Treatment	Starch Amount	Moisture Content (%)
Control zein	60	50.66 ± 0.575^{a}
	70	48.37 ± 0.255^{c}
	80	46.65 ± 0.201^{de}
mf	60	50.07 ± 0.122^{ab}
	70	48.43 ± 0.175^{bc}
	80	46.23 ± 0.325^{e}
pH6	60	50.43 ± 0.047^{a}
	70	48.24 ± 0.039^{cd}
	80	46.02 ± 0.048^{e}
pH8	60	50.47 ± 0.429^{a}
	70	48.55 ± 0.446^{bc}
	80	47.37 ± 0.549^{cde}
pH10	60	51.00 ± 0.001^{a}
	70	48.43 ± 0.027^{bc}
	80	46.70 ± 0.209^{de}

*The means and standard deviations of two readings were calculated. Different letters represent significant difference ($p \leq 0.05$).

The final point was that although the comparison between gum types could not be done because of some inabilities of them as stated before, the water binding abilities of gums and fibers shows difference from each other, which can delay starch retrogradation, and that effect could be seen during storage (Sabanis, 2009). Also, since moisture content of a product is directly related with its shelf-life, this parameter plays an important role for products (Duman, 2013). As a consequence of that, gluten-free corn breads prepared with microfluidized and pH treated zein were expected to exhibit a slower staling mechanism.

Table 3.5 Moisture content of fresh bread samples made with xanthan. Standard deviations were also indicated.

Treatment	Starch Amount	Moisture Content (%)
Control zein	60	50.95 ± 0.207^{a}
	70	48.51 ± 0.237^{abc}
	80	46.62 ± 0.063^{bc}
mf	60	50.44 ± 0.178^{a}
	70	48.99 ± 0.828^{ab}
	80	46.15 ± 0.587^{c}

*The means and standard deviations of two readings were calculated. Different letters represent significant difference ($p \leq 0.05$).

Table 3.6 Moisture content of fresh bread samples made with citrus fiber. Standard deviations were also indicated.

Treatment	Starch Amount	Moisture Content (%)
Control zein	60	49.35 ± 0.004^{a}
	70	47.19 ± 0.402^{b}
	80	45.90 ± 0.003^{c}
mf	60	49.12 ± 0.173^{a}
	70	47.52 ± 0.004^{b}
	80	45.79 ± 0.054^{c}

*The means and standard deviations of two readings were calculated. Different letters represent significant difference ($p \leq 0.05$).

3.3.3 Crumb Color of Breads

The effects of different treatments and starch contents on crumb color of gluten-free corn bread are represented according to used gum in the formulation from Table 3.7 to Table 3.11. As stated in the moisture content part, some gum types were not tested for some treatments due to their inability to form a well bread dough and this was the reason why we could not compare all gum types with all treatments. Crumb color of fresh bread samples without gum, with hpmc, guar, xanthan and citrus fiber was presented in tables, respectively. Also, the visual observation could be done from Fig. A.1-Fig. A.5.

Table 3.7 Total color change of fresh bread samples without gum. Standard deviations were also indicated.

Treatment	Starch Amount	Total Color Change (ΔE)
mf	60	66.07 ± 1.172^{cd}
	70	54.97 ± 1.181^{fg}
	80	42.29 ± 1.484^{h}
pH6	60	83.07 ± 1.117^{a}
	70	75.24 ± 0.759^{b}
	80	62.41 ± 0.957^{de}
pH8	60	84.91 ± 0.834^{a}
	70	69.60 ± 0.916^{c}
	80	59.24 ± 1.284^{ef}
pH10	60	66.19 ± 1.000^{cd}
	70	52.64 ± 0.635^{g}
	80	42.25 ± 0.789^{h}

*The means and standard deviations of twelve readings were calculated. Different letters represent significant difference ($p \leq 0.05$).

Color has been always an important factor for the acceptance of a product by consumers (Ziobro et al., 2013). Because of that reason, the crumb color analysis was conducted on gluten-free corn samples. The ANOVA results of the total color change of fresh bread samples showed that treatment types and amount of starch used in the bread-making formulation were significantly effective on crumb color ($p \leq 0.05$).

As could be seen from Table 3.7 to Table 3.11, when the amount of starch increased in the bread-making formulation, the total color change of the bread samples was decreased. This was the result of the addition of starch, which gave the white color to bread and lightened its crumb color. According to that, total color change was highest in 60 g starch formulation and the lowest in 80 g formulation in all treatments. As a consequence, there was a significant difference between starch amounts on total color change of crumb color ($p \leq 0.05$).

The implementation of microfluidization and alkalinity processes to zein slurries for the bread-making process resulted in darker crumb color of bread. As could be seen from Table 3.8 and Table 3.9 in which hpmc and guar were applied to all treatments and starch amounts, microfluidization at pH=8 had higher total color change than the others. Then, it was followed by microfluidization at pH=6, microfluidization made at control pH value (4), microfluidization made at pH=10 and control zein (without treatment). In the formulation of bread made without gum also exhibited the same ranking, but it could not be applied to control zein as could be observed from Table 3.7. The same situation was present in the xanthan and citrus fiber formulations.

Lutein and zeaxanthin are the carotenoids, which are responsible from the yellow color of the zein, and also corn. The decrease in particle size by microfluidization and also alkaline treatment could result in revealing these carotenoids. Therefore, the yellowish color of the samples displayed itself more. It could be concluded that the color of zein became darker after the microfluidization process and this gave corn bread the darker yellow color. However, excessive alkaline treatment resulted in deterioration of these carotenoids and lightened the crumb color.

Several researches have been conducted to investigate the effect of particle size on color, and most of them showed that the decrease in particle size led to revealing the dominant component color. The study of Prasopsunwattana et al. (2009) on tannin showed that particle size is an important factor for the visibility of the compounds in the structure. Also, Hatcher et al. (2002) indicated that they were able to obtain brighter dough with decreasing particle size of the flour. On the other hand, some researchers resisted on this generalization with their studies. Majzoobi et al. (2013) stated that, increase in particle size resulted in increasing possibility of presence of dark pigments. By this way, the visibility of the dark pigments increased and color became darker.

The study of Mert (2012) on ketchup type products exhibited the similar findings, which showed microfluidization was effective on the total color change. This effect of microfluidization was also observed by Duman (2013) on the total color change of cacao fiber. Also, increased alkalinity could result in improved uniformity by breaking the structure with microfluidization up to some point. Beyond the limits, the structure became deformed. Because of that reason, total color change of the bread crumb was raised up to pH=8, but then decreased.

On the other hand, the brown color of the crust was formed by Maillard and caramelization reactions during baking. Since the temperature of the crumb of the bread did not exceed 100˚C in the baking process, Maillard and caramelization reactions in crumb structure did not occur, and because of that reason, our baking temperature was not an effective parameter on crumb color. The color property of the bread was directly related with applied treatment and used ingredients in the formulation (Gómez et al., 2008). Moreover, the effect of fermentation on color property was stated by Wollgast & Anklam (2000).

Table 3.8 Total color change of fresh bread samples made with hpmc. Standard deviations were also indicated.

Treatment	Starch Amount	Total Color Change (ΔE)
Control zein	60	49.37 ± 1.648^{gh}
	70	43.80 ± 3.027^{hi}
	80	35.49 ± 2.538^{j}
mf	60	76.51 ± 0.766^{ab}
	70	59.43 ± 0.824^{de}
	80	49.53 ± 1.020^{gh}
pH6	60	79.44 ± 0.803^{a}
	70	71.71 ± 0.818^{bc}
	80	59.96 ± 1.387^{de}
pH8	60	83.19 ± 0.900^{a}
	70	65.66 ± 0.989^{cd}
	80	56.90 ± 1.091^{ef}
pH10	60	64.46 ± 1.028^{d}
	70	51.36 ± 0.916^{fg}
	80	39.69 ± 1.412^{ij}

*The means and standard deviations of twelve readings were calculated. Different letters represent significant difference ($p \leq 0.05$).

Table 3.9 Total color change of fresh bread samples made with guar. Standard deviations were also indicated.

Treatment	Starch Amount	Total Color Change (ΔE)
Control zein	60	57.24 ± 1.462^{de}
	70	39.28 ± 1.241^{g}
	80	34.04 ± 1.166^{h}
mf	60	80.05 ± 0.698^{b}
	70	61.32 ± 0.861^{d}
	80	52.60 ± 1.041^{e}
pH6	60	87.64 ± 0.570^{a}
	70	79.84 ± 0.562^{b}
	80	66.45 ± 1.379^{c}
pH8	60	88.21 ± 0.876^{a}
	70	69.59 ± 1.099^{c}
	80	53.17 ± 1.193^{e}
pH10	60	69.63 ± 1.071^{c}
	70	53.66 ± 0.727^{e}
	80	46.06 ± 0.738^{f}

*The means and standard deviations of twelve readings were calculated. Different letters represent significant difference ($p \leq 0.05$).

Table 3.20 Total color change of fresh bread samples made with xanthan. Standard deviations were also indicated.

Treatment	Starch Amount	Total Color Change (ΔE)
Control zein	60	56.47 ± 1.614^{c}
	70	45.78 ± 1.800^{d}
	80	33.62 ± 1.243^{e}
mf	60	78.19 ± 1.141^{a}
	70	63.35 ± 0.514^{b}
	80	55.28 ± 0.595^{c}

*The means and standard deviations of twelve readings were calculated. Different letters represent significant difference ($p \leq 0.05$).

Moreover, some researchers found a relationship between the used gum and color of the crumb. Because some gums were not tested for some treatments in our study, the comparison between the effects of gums on crumb color could not be investigated. However, according to researches, addition of gums lightened the crumb color of the products (Lazaridou et al., 2007; Mandala et al., 2009). This situation was explained with the effects of hydrocolloids on water distribution by Mezaize et al. (2009).

In conclusion, regardless of used gum in the formulations, total color change of fresh bread samples decreased in that order: pH=8 > pH=6 > mf > pH=10 > control zein.

Table 3.11 Total color change of fresh bread samples made with citrus fiber. Standard deviations were also indicated.

Treatment	Starch Amount	Total Color Change (ΔE)
Control zein	60	54.07 ± 1.781^{c}
	70	45.59 ± 1.895^{d}
	80	34.27 ± 1.801^{e}
mf	60	80.14 ± 0.715^{a}
	70	72.53 ± 0.577^{b}
	80	52.10 ± 1.115^{c}

*The means and standard deviations of twelve readings were calculated. Different letters represent significant difference ($p \leq 0.05$).

3.3.4 Texture Profile of Breads

The textural properties of gluten-free corn bread containing different starch amounts and hydrocolloids were investigated in this part of the study by texture profile analysis (TPA). By this method, sensory properties such as hardness, fracturability, cohesiveness, springiness, chewiness and gumminess can be measured to determine the characteristics of the bread sample (Sahin & Sumnu, 2006). Textural measurements of gluten-free corn bread samples were analyzed with respect to used gum, and hardness, cohesiveness, springiness and chewiness

values for each gum at different storage durations were illustrated from Fig. 3.94 to Fig. 3.149, respectively.

First of all, as could be seen from Fig. 3.94 to Fig. 3.109, textural measurements of gluten-free corn bread samples without gum, in which different treatments were applied and also different starch amounts were used, were investigated for different storage durations.

Crumb hardness is an important propery for bakery products since it relates the required force to grind a food by a consumer. Because of that reason, hardness is generally related to freshness of a sample (Giannou & Tzia, 2007). Crumb hardness values obtained from TPA were monitored below for without gum experiments. Also, you could access to the images of bread samples from Fig. A.1.

First of all, the volume and structure of the bread samples without gum were analyzed from images. The breads obtained from microfluidized zein showed more consolidated network through the structure with pores. However, the images showed that the pores of the samples became smaller by addition of starch. Also, pH treatment resulted in decresing pore sizes and formation of more connected structure. Because of that reason, the hardness values of breads treated by pH was higher than microfluidized one.

As could be seen from figures, the increase in starch amount resulted in increase the hardness of the samples. Since increasing starch amount without changing the amounts of other ingredients led to more viscous dough, the bread made from that dough became harder. Statistical analysis showed that starch amount was significantly important for hardness of the bread samples ($p \leq 0.05$). Also, the study of Wronkowska et al. (2013) on buckwheat flour presented that the increasing flour led to higher hardness, which could be due to the increased volume. Same results were stated by Moore et al. (2006) on different types of gluten-free bread formulations. They indicated that gluten is the responsible constituent for the structural changes in bread crumb, and the interactions between starch and other macromolecular components are important for crumb structure. Whereas these changes are the result of polysaccharide constituents in gluten-free bread

formulations. Therefore, the researches were intensified on amylose and amylopectin. Ghiasi et al. (1984) indicated that amylopectin retrogradation and also amylose leaching were the reasons of staling process. In the cooling process, the effect of amylose retrogradation is seen too quickly. On the other hand, amylopectin retrogradation requires longer time. Also, the effect of amylopectin retrogradation is more effective on staling mechanism. Moore et al. (2006) pointed out that the excess presence of polysaccharides affects bread staling significantly, which was expressed by starch retrogradation and changes in water binding capacity. The another important factor was water in the staling. Because of its plasticizer property, water provides flexibility to other ingredients (Gray & BeMiller, 2003). As stated by Arendt et al. (2008), migration of water from crumb to crust and also amylopectin retrogradation lead to staling.

The degree of staling is one of the major problem related to gluten-free breads (Demirkesen et al., 2013). Because of that reason, there has been numerous researches on staling characteristics of gluten-free breads. Moreover, as the storage duration got longer, logically hardness of the samples increased. Results showed that there was a distinct difference in textural properties of bread samples during storage duration ($p \leq 0.05$). Similar findings had been published on buckwheat bread (Alvarez-Jubete et al., 2010; Wronkowska et al., 2013), chestnut and rice bread (Demirkesen et al., 2013), and corn bread (Moore et al., 2006).

The other parameter was treatment type. As indicated from the beginning, since untreated zein was not able to form a dough without gum, we could not compare the control zein with microfluidized and treated by alkaline. As could be observed from figures, breads made with microfluidized zein resulted in lower hardness than breads made with both microfluidized and treated by alkaline. Also, it could be stated that hardness values of bread samples showed a decreasing order of pH=6, pH=8, pH=10 and microfluidized zein. The water holding capacity of the zein was increased by microfluidization and improved with excess alkalinity up to some point. The effect of microfluidization on textural properties of cakes was also indicated by Çıkrıkcı (2013). According to ANOVA results, treatment type caused to significant difference in hardness values ($p \leq 0.05$).

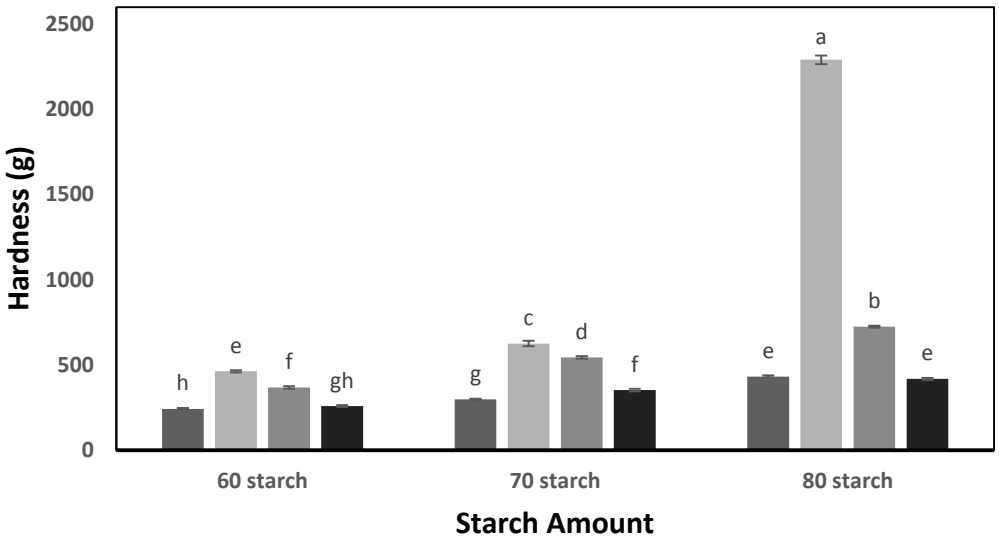

Figure 3.94 Hardness values of fresh bread samples without gum. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10

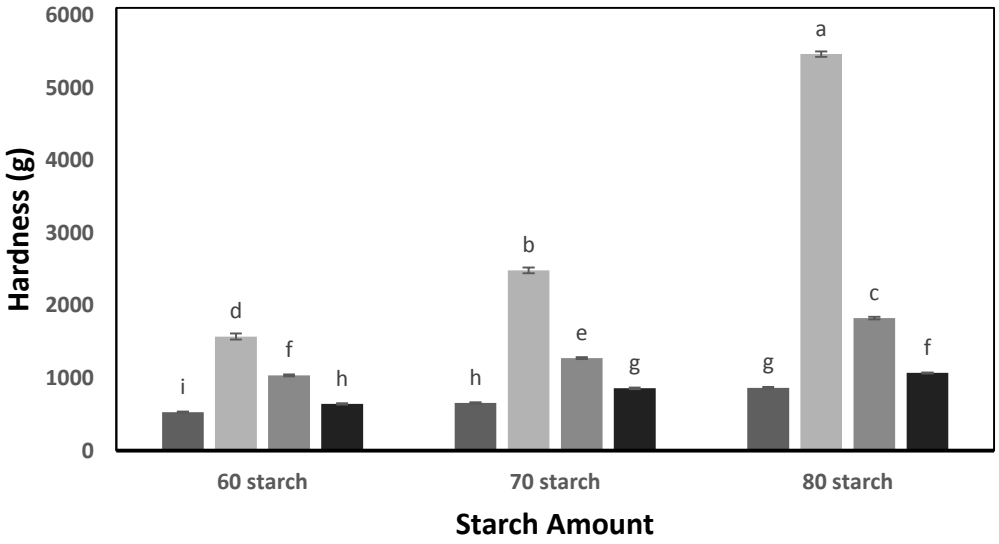

Figure 3.95 Hardness values of bread samples without gum stored for 1 day. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

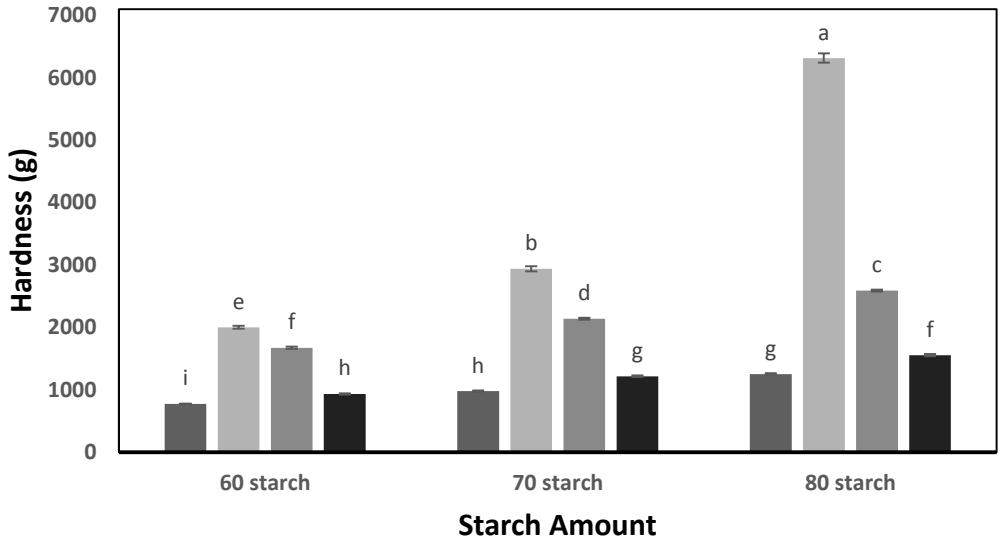

Figure 3.96 Hardness values of bread samples without gum stored for 2 days. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

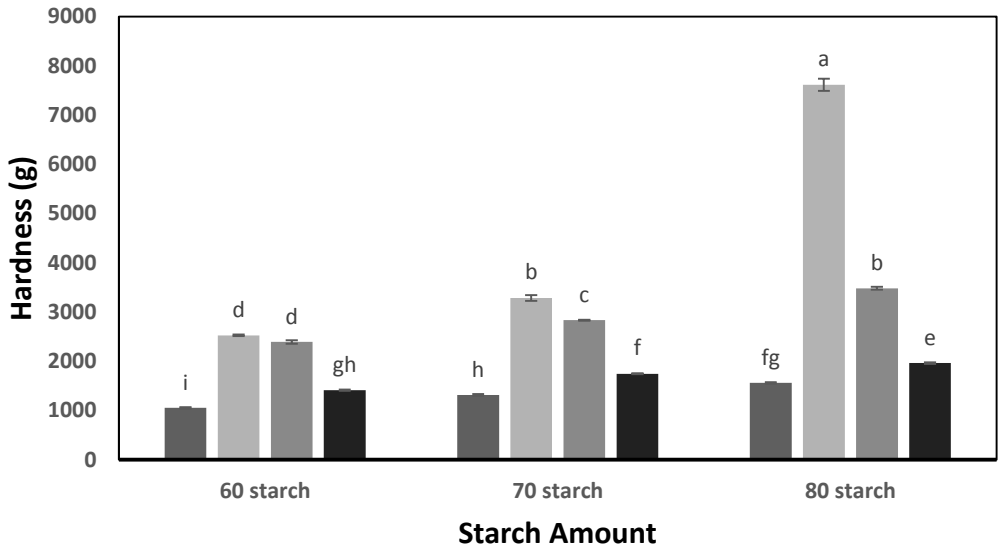

Figure 3.97 Hardness values of bread samples without gum stored for 3 days. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

The cohesiveness values of gluten-free corn bread samples without gum, in which different treatments were applied and also different starch amounts were used, are represented below according to storage durations (Fig.3.98-Fig.3.101).

Cohesiveness values showed decrease with increasing of starch amount on the day of baking. However, according to statistical analysis, there was no significant difference between the starch amounts of 60 g and 70 g for fresh samples. On the other hand, 80 g starch amount differed from others significantly on the day of baking ($p \leq 0.05$). When the other storage days were compared, it could be stated that the samples were indifferent from each other in terms of starch amounts ($p \leq 0.05$).

Also, the effect of storage duration in staling was investigated by comparing all parameters with each other, and it could be declared that increase in storage duration resulted in drop in cohesiveness value. There was a significant difference between storage durations except the third day ($p \leq 0.05$). Big decrease in cohesiveness value caused to high crumbling and it affects the consumer's acceptance (Ziobro et al., 2013). Similar findings were found on corn bread (Ziobro et al., 2013) and pseudocereal flours (Alvarez-Jubete et al., 2010).

Finally, the comparison between treatment types were done for cohesiveness values, it could be stated that pH=8 was the highest among all treatments. It was followed by microfluidized zein, pH=10 and pH=6, respectively. Cohesiveness values of all treatments showed a decreasing trend with storage duration, that meant their crumbling structure increased by time.

The studies of Majzoobi et al. (2013) and Mosharraf et al. (2009) mentioned that lower cohesiveness value could be the result of less adhesion through the bread structure, which was especially seen in gluten-free bread formulations.

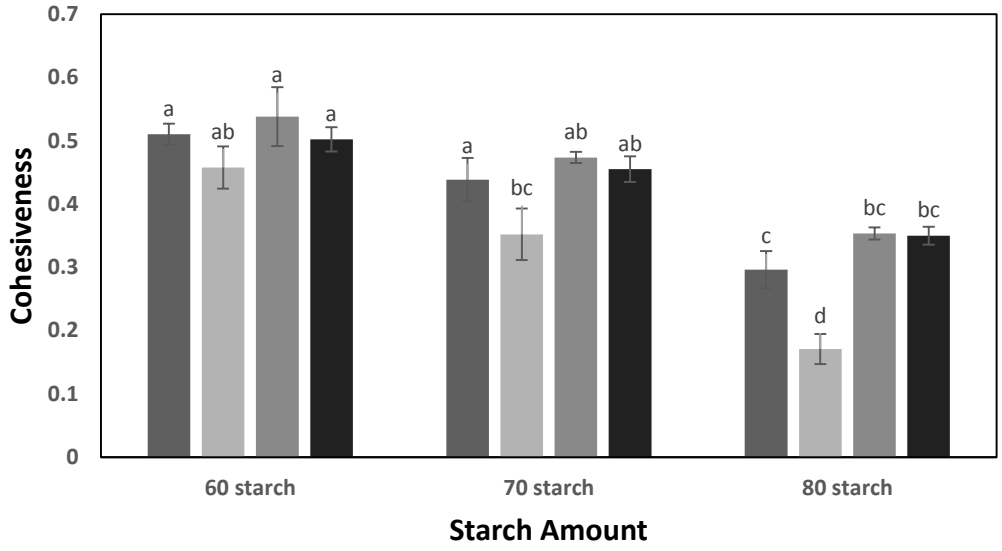

Figure 3.98 Cohesiveness values of fresh bread samples without gum. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

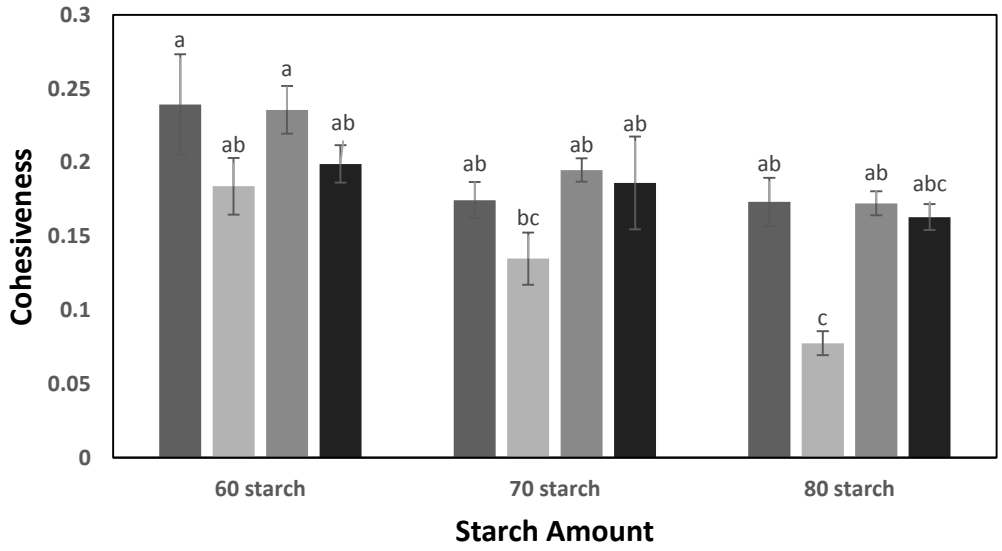

Figure 3.99 Cohesiveness values of bread samples without gum stored for 1 day. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

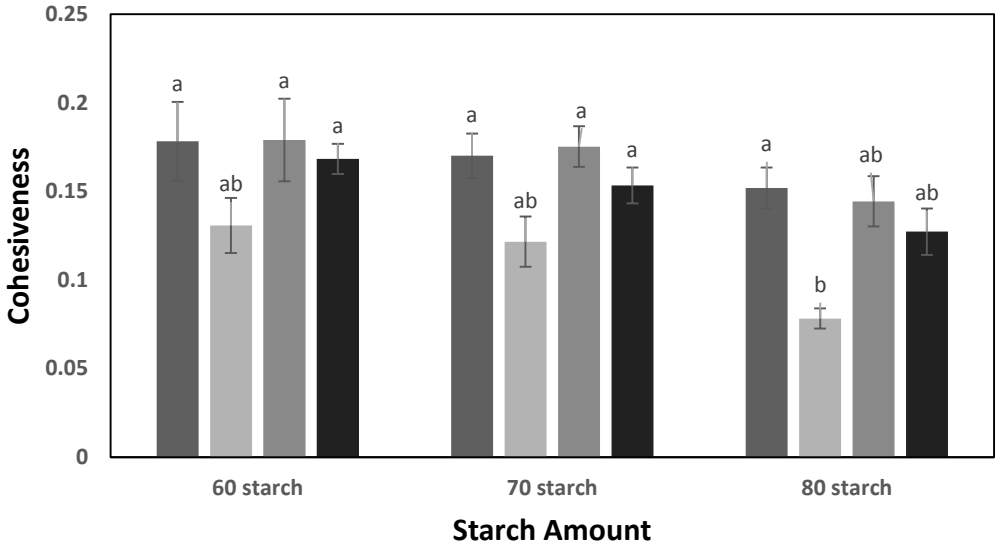

Figure 3.100 Cohesiveness values of bread samples without gum stored for 2 days.

■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

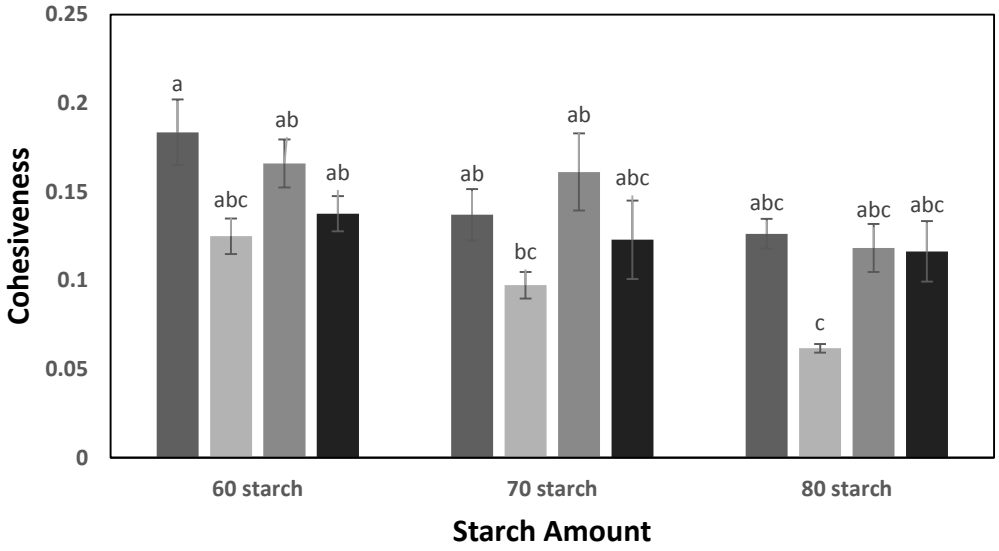

Figure 3.101 Cohesiveness values of bread samples without gum stored for 3 days.

■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

From Fig. 3.102 to Fig. 3.105, the springiness values of gluten-free corn bread samples without gum, in which different treatments were applied and also different starch amounts were used, are illustrated according to storage durations. Springiness values of gluten-free corn breads decreased slightly by increasing starch amount, but according to ANOVA, there was no significant difference between starch amounts in same storage period ($p \leq 0.05$). When the storage durations were compared, it could be concluded that the springiness values of gluten-free corn breads without gum showed a decreasing trend by time, that meant they lost their elasticity. The statistical analysis showed that the day of baking, the first and the third days were different from each other; however, the second day showed similarity to the first and third day ($p \leq 0.05$). Also, the springiness values of gluten-free corn bread without gum showed a decreasing order of pH=6, microfluidized zein, pH=10 and pH=8, respectively. However, the treatment type did not cause to any difference in terms of springiness. All samples were indifferent from each other ($p \leq 0.05$). The study of Ziobro et al. (2013) supported our findings about springiness, however the study of Alvarez-Jubete et al. (2010) showed difference.

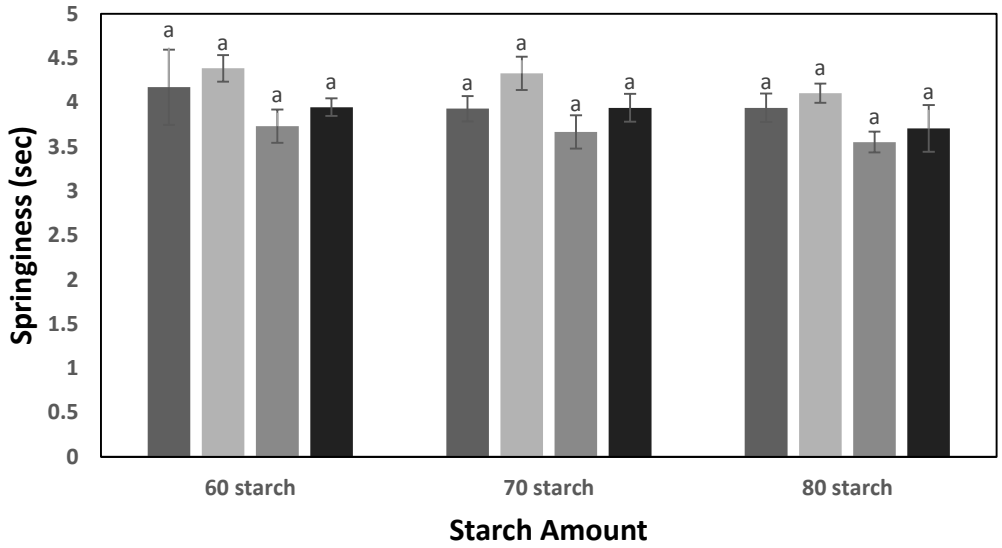

Figure 3.102 Springiness values of fresh bread samples without gum. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

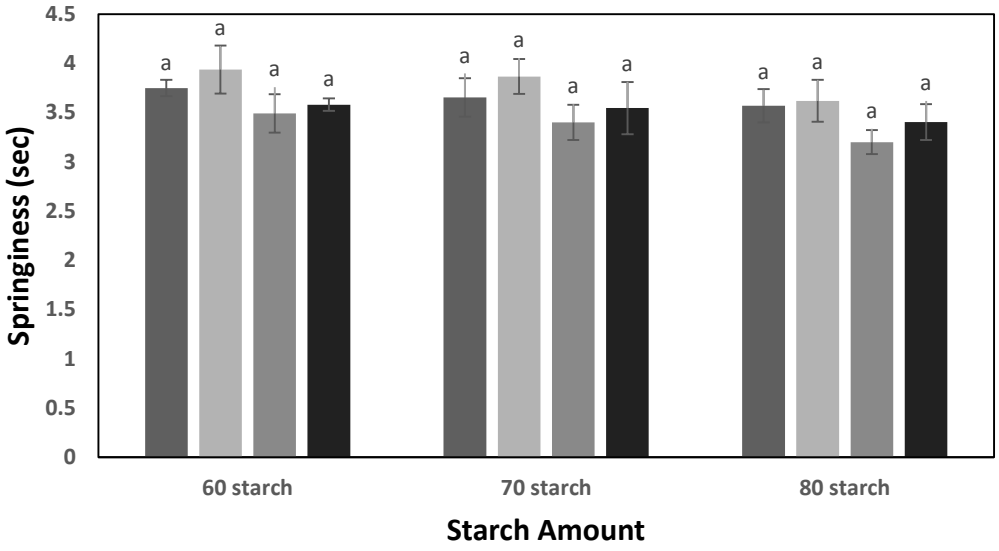

Figure 3.103 Springiness values of bread samples without gum stored for 1 day. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

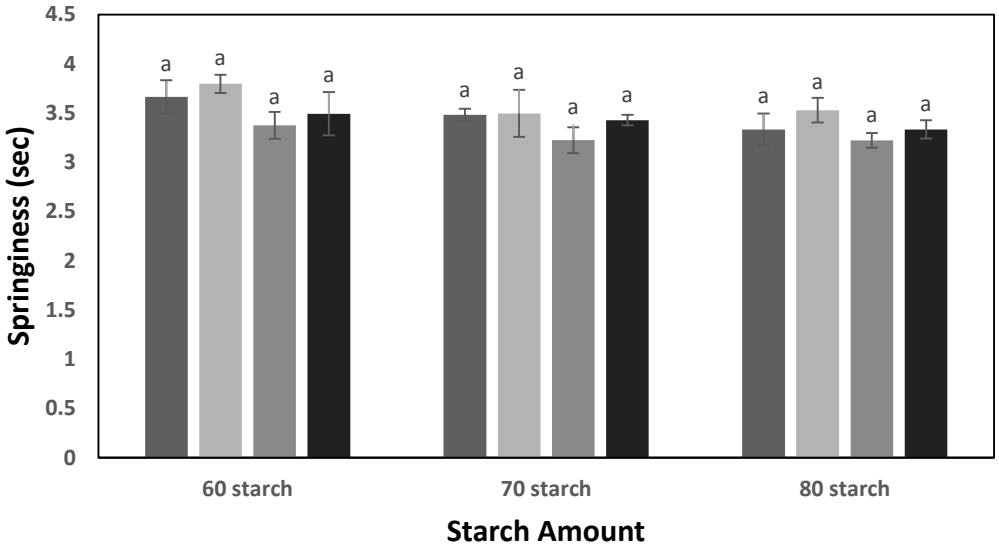

Figure 3.104 Springiness values of bread samples without gum stored for 2 days. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

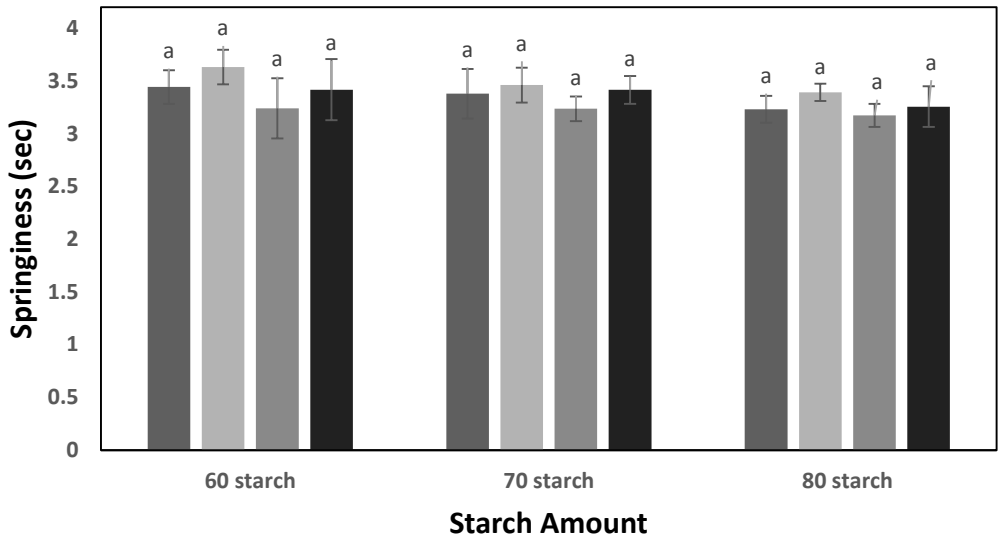

Figure 3.105 Springiness values of bread samples without gum stored for 3 days. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

The chewiness values of bread samples without gum, in which different treatments were applied and also different starch amounts were used, were illustrated below from Fig. 3.106 to Fig. 3.109 according to storage durations. Increasing of starch amount led to slight increase in chewiness, however statistical analysis indicated that there was no significant difference in terms of starch amount except bread sample, treated to pH=6 and containing 80 g corn starch ($p \leq 0.05$). Also, chewiness increased with storage duration. When the samples were compared in terms of treatment type, similar trend was observed with hardness values of same samples. As stated before, chewiness is the multiplication of hardness, cohesiveness and springiness (Sahin & Sumnu, 2006). In without gum experiments, as expressed above, although cohesiveness and springiness values showed difference with storage duration and starch amount, these effects were very low. Because of that reason, chewiness was dominated by hardness and similar trend with hardness was observed as being in the study of Ziobro et al. (2013).

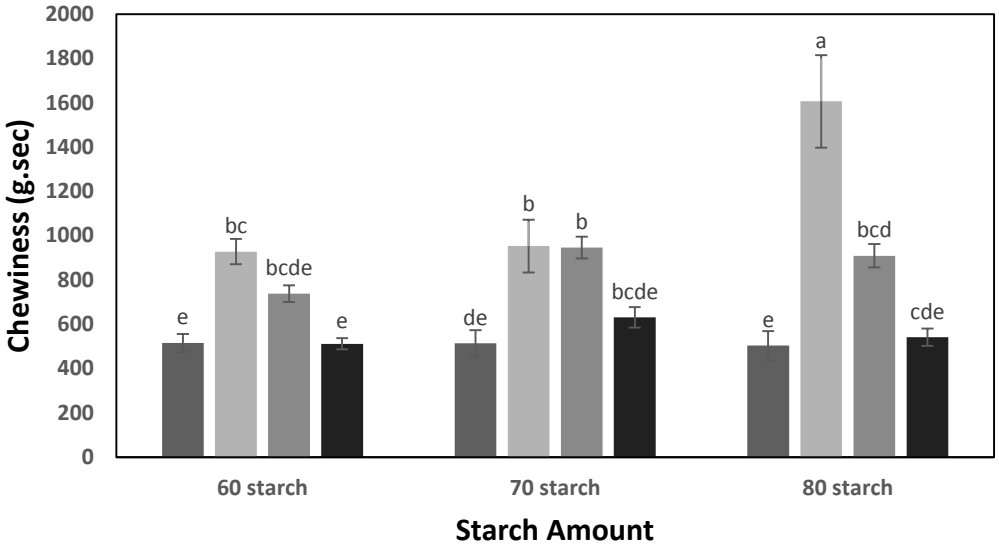

Figure 3.106 Chewiness values of fresh bread samples without gum. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

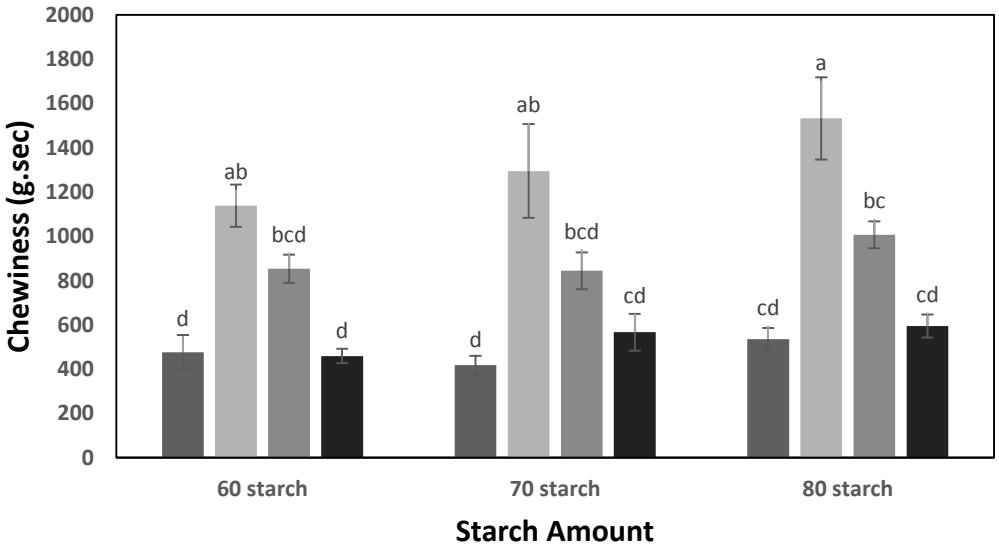

Figure 3.107 Chewiness values of bread samples without gum stored for 1 day. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

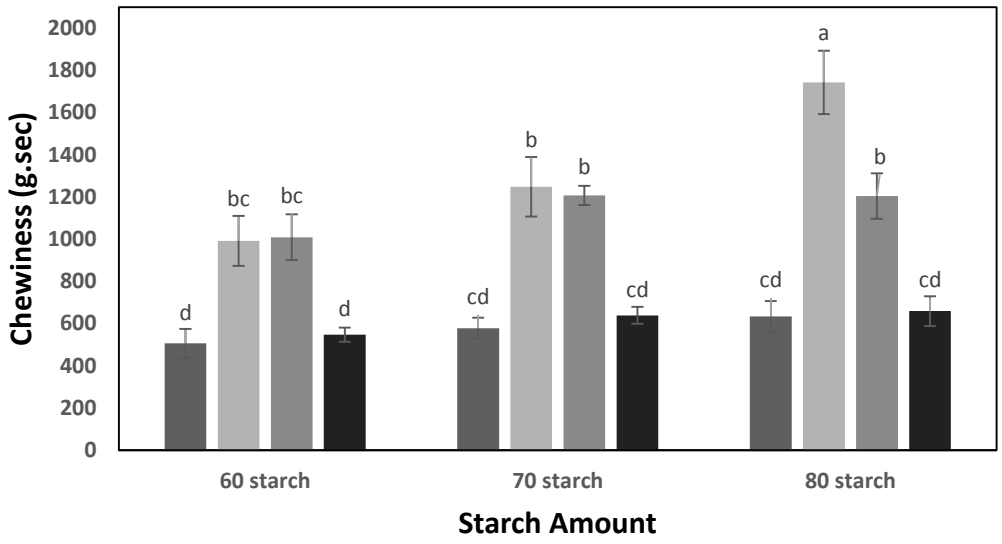

Figure 3.108 Chewiness values of bread samples without gum stored for 2 days. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

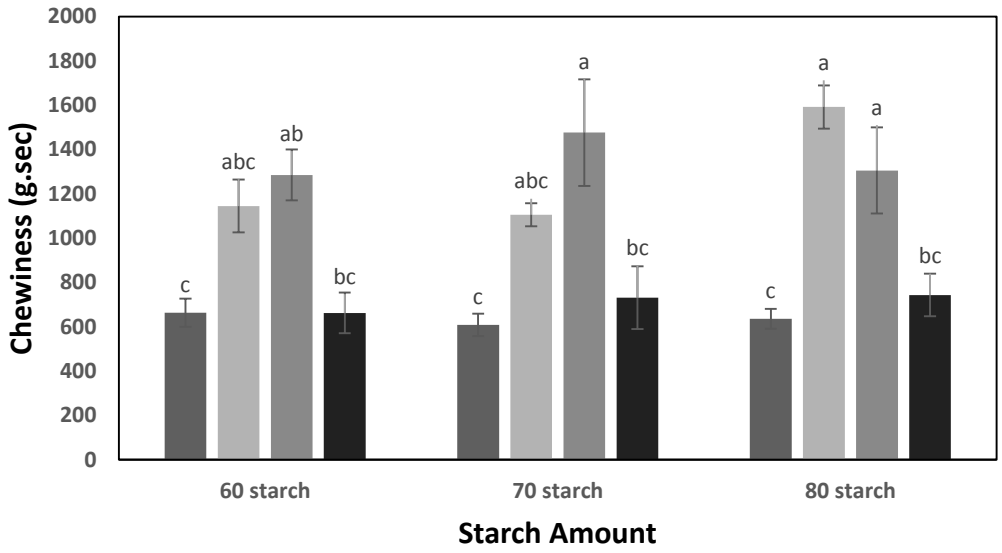

Figure 3.109 Chewiness values of bread samples without gum stored for 3 days. ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

Hydrocolloids are important additives for gluten-free bread systems since they provide many benefits. The lack of gluten network in gluten-free bread formulations results in lower bread volume. Hydrocolloids are perfectly suited to overcome this problem. Also, their water binding capacity is important to softer bread structure (Gallagher et al., 2003; Schober et al., 2007). Because of these and more reasons stated before, hydrocolloids have been used in gluten-free bread formulations to improve quality. Especially, the improvement on volume of gluten-free bread formulations by hydrocolloids was seen as an important factor. As could be seen from Fig. A.1 to Fig. A.5, the addition of hydrocolloids and also citrus fiber led to increase in bread volume by providing gas retention in the structure which was also stated by Gallagher et al. (2003) and Schober et al. (2007). Similar improvements on volume were observed by Kang et al. (1997) and Lazaridou et al. (2007) with the addition of xanthan, pectin, carboxymethylcellulose and agarose.

Crumb hardness values obtained from samples with hpmc, that prepared by different starch amounts and also different treatment types, are represented from Fig. 3.110 to Fig. 3.113.

The increase in starch amount resulted in higher hardness values and also the statistical analysis showed that there was a significant difference between starch amounts except samples obtained from control zein ($p \leq 0.05$). Also, the hardness increased as storage duration got longer and there had been a significant difference among storage durations ($p \leq 0.05$). The findings of Alvarez-Jubete et al. (2010), Wronkowska et al. (2013) and Demirkesen et al. (2013a) supported our results about storage duration.

Moreover, treatment type resulted in generally different hardness values. The experiments made with hpmc showed that pH=8 had highest hardness and it followed by pH=10, pH=6, microfluidized zein and control zein, respectively. The order of hardness of experiments made with hpmc was different than made without gum. It could be the result of interactions between gum and the other ingredients. It should have been stated that the working pH range of hydrocolloids are different from each other. In emulsion experiments, the rheology and stability of the

emulsions had been improved further, but the complexity expanded in bread-making experiment with addition of several ingredients. Because of that reason, alkaline treatment over microfluidization could not be understand exactly. It could be stated only that according to ANOVA, treatment types showed significant difference ($p \leq 0.05$). The study of Ciron et al. (2010) mentioned that the reason behind higher hardness values of microfluidized samples could be the increased homogeneity in micro structure.

When we compared the hardness values of breads obtained by the addition of hpmc with previous ones, a sharp decrease in hardness values was observed easily. For instance, the hardness values of fresh breads containing 60 g starch amount ranged between 50-200 for hpmc experiments, however it was between 200 and 500. The case was same for other starch amounts, treatment types and also storage durations. That meant bread samples containing hpmc exhibited lower hardness than without gum experiments when same conditions were applied. This could be explained by changes occurred with the addition of hpmc. Firstly, because of high water-binding capacity of hpmc, water in the crumb structure could not transfer to crust. Also, hpmc provided the formation of hydrogen bonds with starches, which resulted in delaying starch retrogradation (Sabanis & Tzia, 2010). The study of Demirkesen et al. (2010a) on chestnut flour showed that the handling ability and mixing properties of dough was improved by highly water-binding molecules such as gums, emulsifiers and fibers, which provided entrapment of air bubbles. Because of that reason, breads with lower hardness values were obtained by addition of hpmc. In addition to that, Demirkesen et al. (2010b), Demirkesen et al. (2013b), Guarda et al. (2004) Ozkoc et al. (2009), Santos et al. (2008) and Sumnu et al. (2010)'s studies supported the idea of that the hardness values decreased with hydrocolloids and fibers. Furthermore, gluten-free breads generally showed higher hardness values, and also it increased by storage duration. That property is not a desirable characteristic for a bakery product (Arendt et al., 2008). Because of that reason, addition of hydrocolloids which resulted in decrease in hardness was seen as an improvement on gluten-free breads.

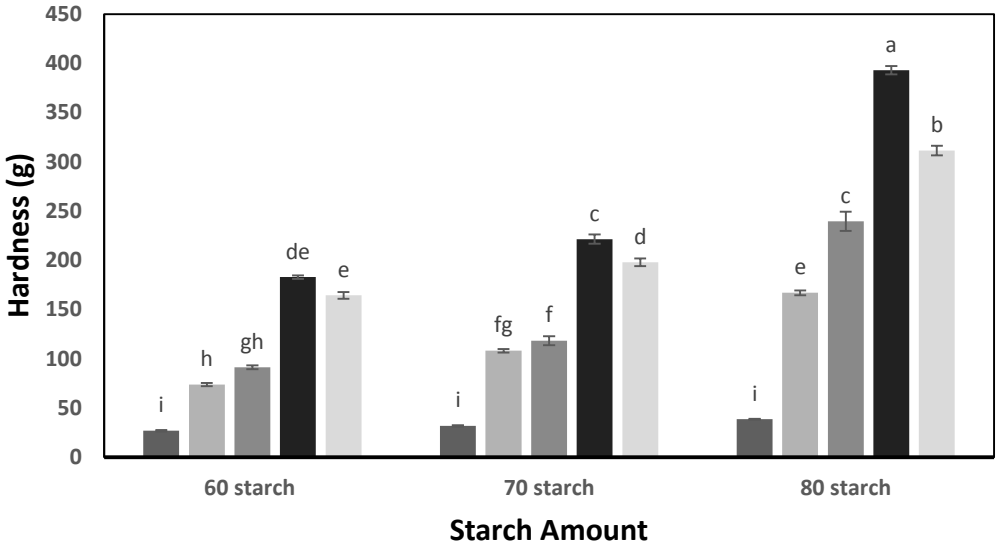

Figure 3.110 Hardness values of fresh bread samples with hpmc. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

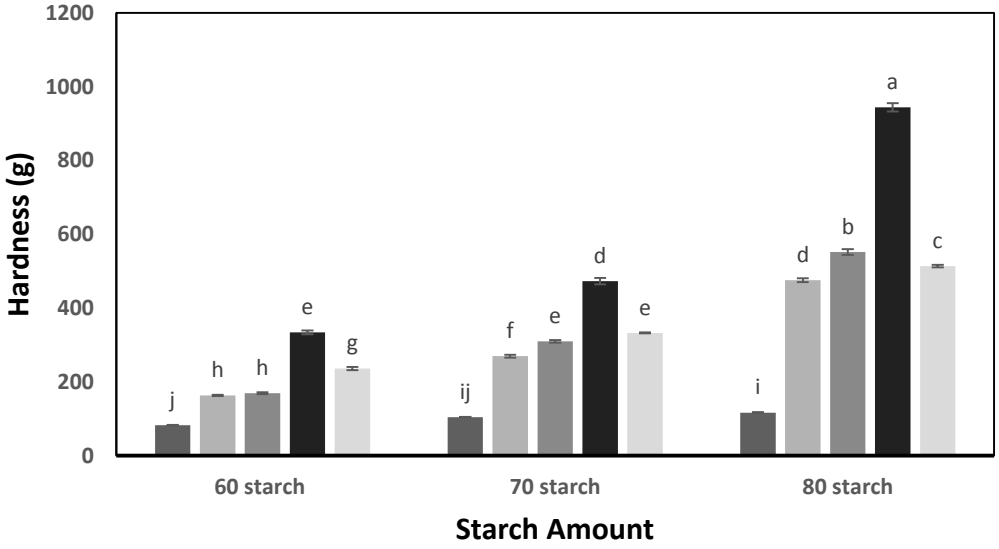

Figure 3.111 Hardness values of bread samples with hpmc stored for 1 day. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

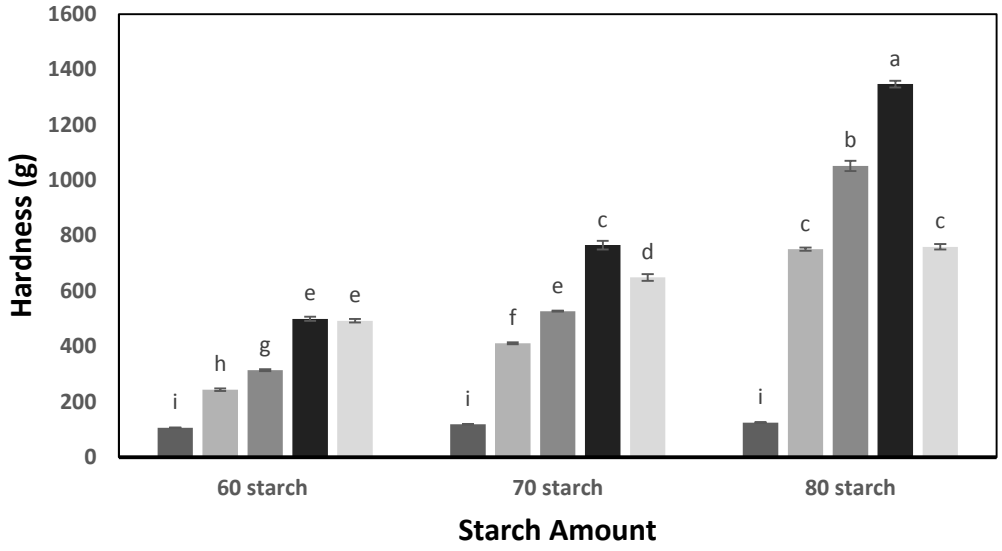

Figure 3.112 Hardness values of bread samples with hpmc stored for 2 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

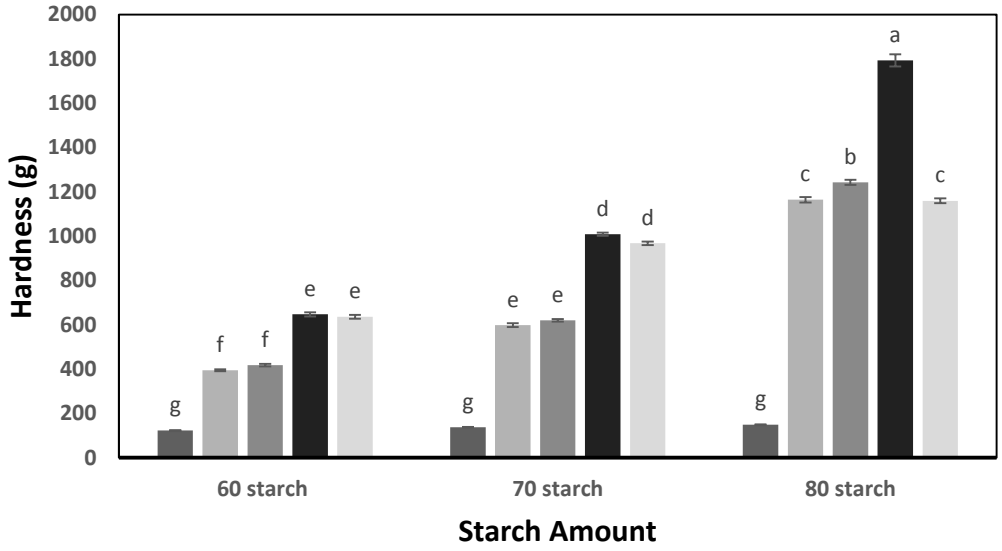

Figure 3.113 Hardness values of bread samples with hpmc stored for 3 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

Cohesiveness values of samples containing hpmc that prepared by different starch amounts and also different treatment types are shown below from Fig. 3.114 to Fig. 3.117. As being in experiments done without gum, cohesiveness value decreased with increased starch amount. Also, statistical analysis indicated that starch amount showed indifference related to storage duration. On the baking day, samples containing 60 g, 70 g and 80 g starch were significantly indifferent from each other. For the other days, significance of cohesiveness showed flunctuations, but generally starch amount was not important for cohesiveness as could be observed from figures and ANOVA tables ($p \leq 0.05$). On the other hand, ANOVA table, which showed the relationship of all parameters, indicated that storage durations exhibited completely difference results. When the storage duration got longer, the cohesiveness of the samples diminished. Thus, there was a significant difference among storage duration ($p \leq 0.05$). Treatment type resulted in difference for cohesiveness. The highest value was obtained from untreated samples and it was followed by microfluidized sample, pH=10, pH=8 and pH=6, respectively. As stated before, the interactions of the gums were not understood completely with increasing of alkalinity since the structure had several ingredients.

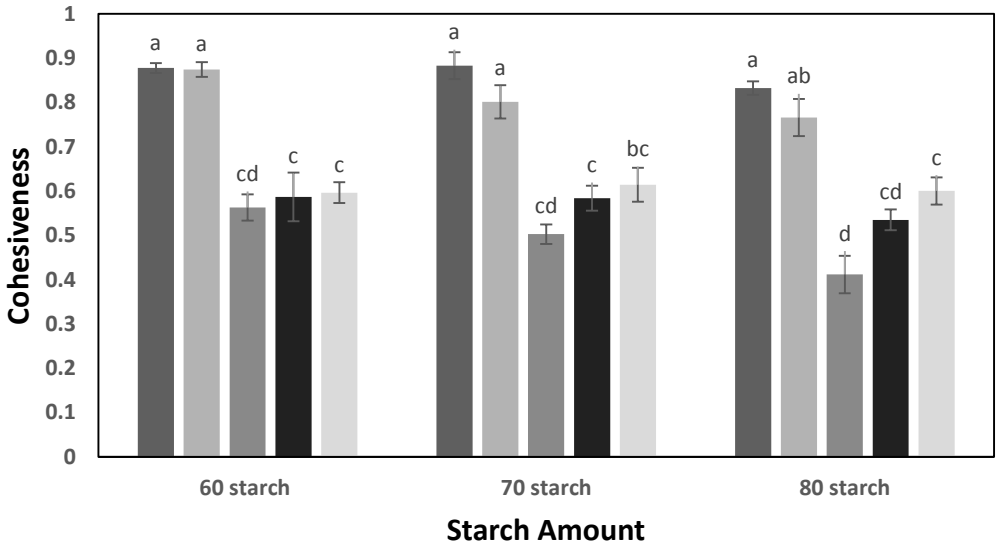

Figure 3.114 Cohesiveness values of fresh bread samples with hpmc. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

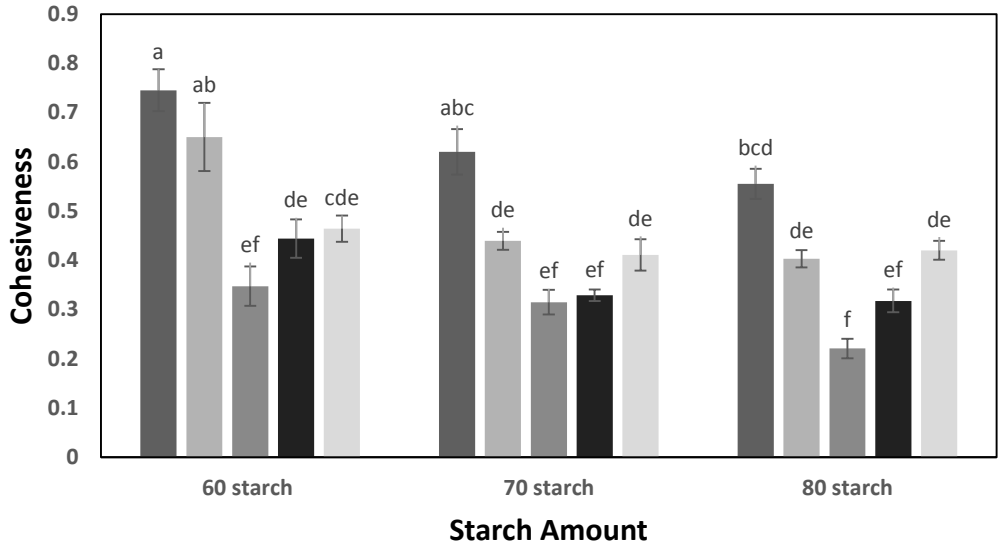

Figure 3.115 Cohesiveness values of bread samples with hpmc stored for 1 day. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

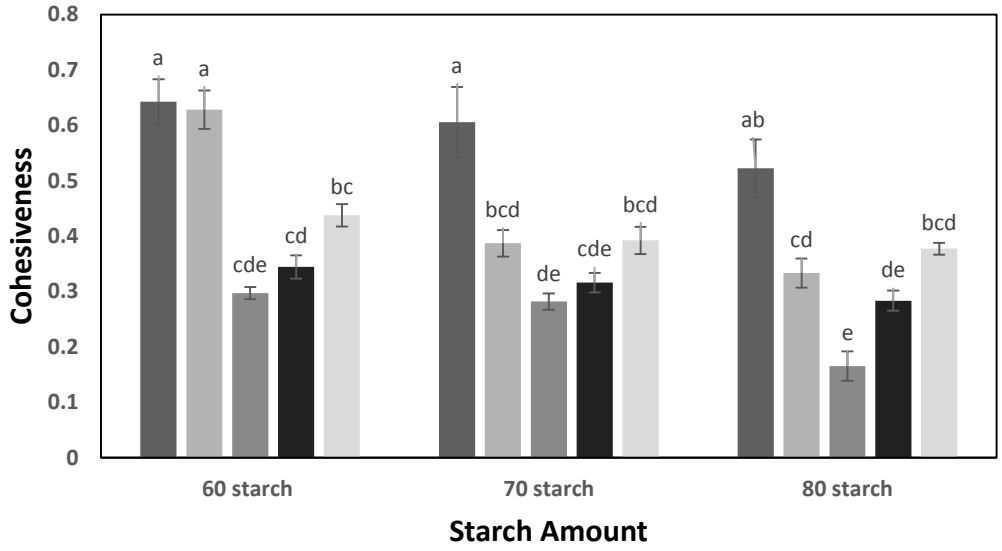

Figure 3.116 Cohesiveness values of bread samples with hpmc stored for 2 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

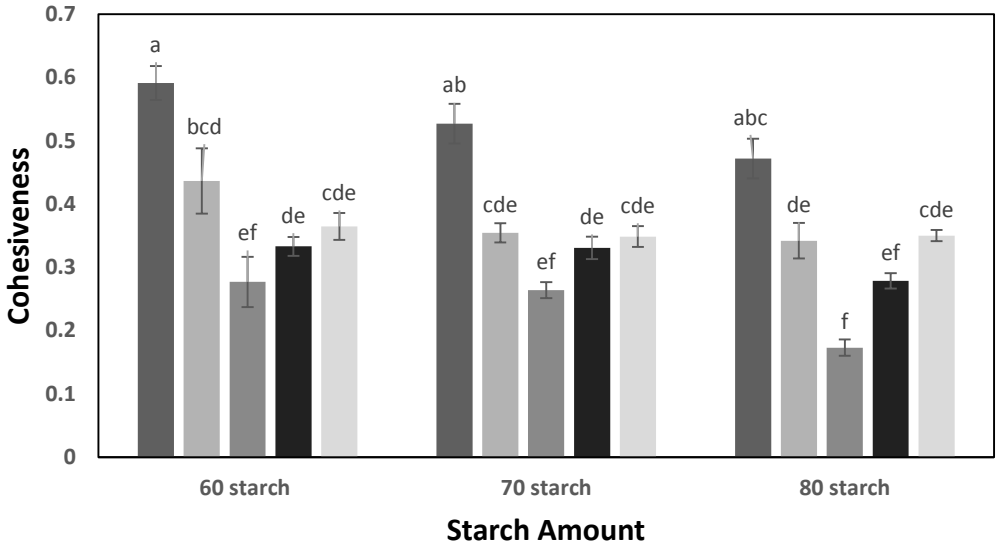

Figure 3.117 Cohesiveness values of bread samples with hpmc stored for 3 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

The springiness values of bread samples prepared with different treatments and starch amounts were represented below from Fig. 3.118 to Fig. 3.121. As could be seen from figures, springiness decreased slightly by addition of extra starch. However, the ANOVA results showed that there was no significant difference between starch amounts for all storage durations ($p \leq 0.05$). On the other side, higher storage duration led to lower springiness in a significant degree ($p \leq 0.05$). That meant the elasticity of the samples decreased by time. Lastly, springiness changed with treatment type. As could be seen from figures, the springiness values showed similarities within some groups. Untreated and microfluidized samples showed parallellism. Also, samples treated with alkaline were indifferent from each other. But, these two groups differed from each other.

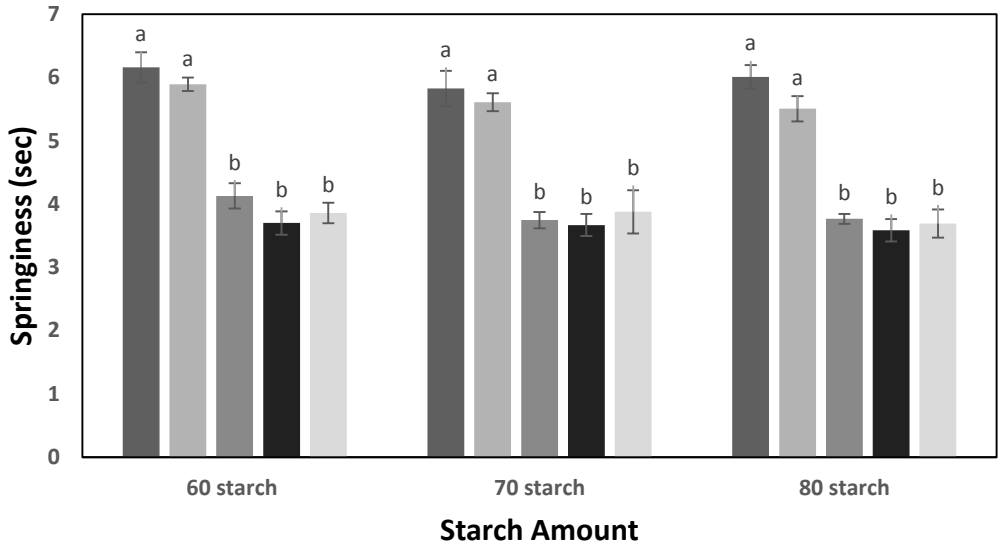

Figure 3.118 Springiness values of fresh bread samples with hpmc. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

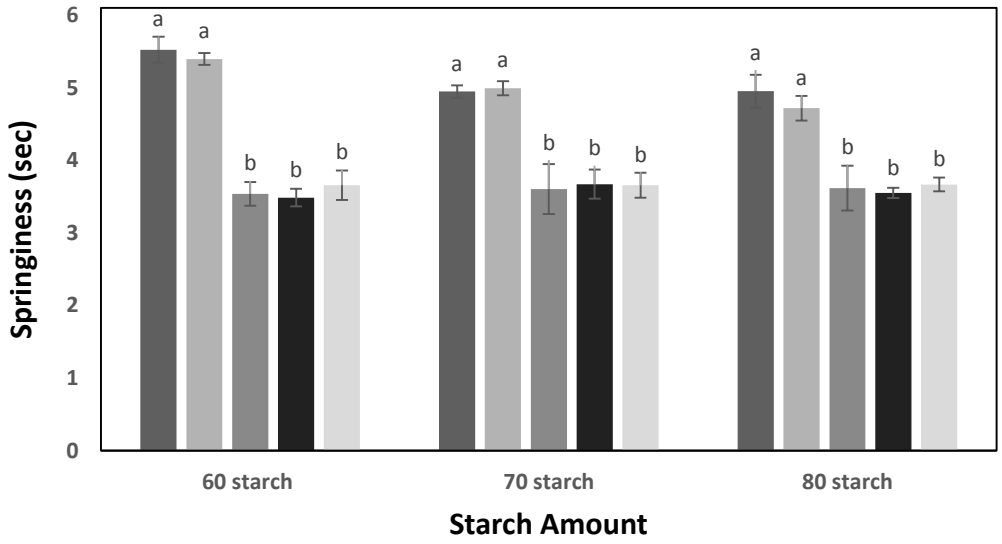

Figure 3.119 Springiness values of bread samples with hpmc stored for 1 day. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

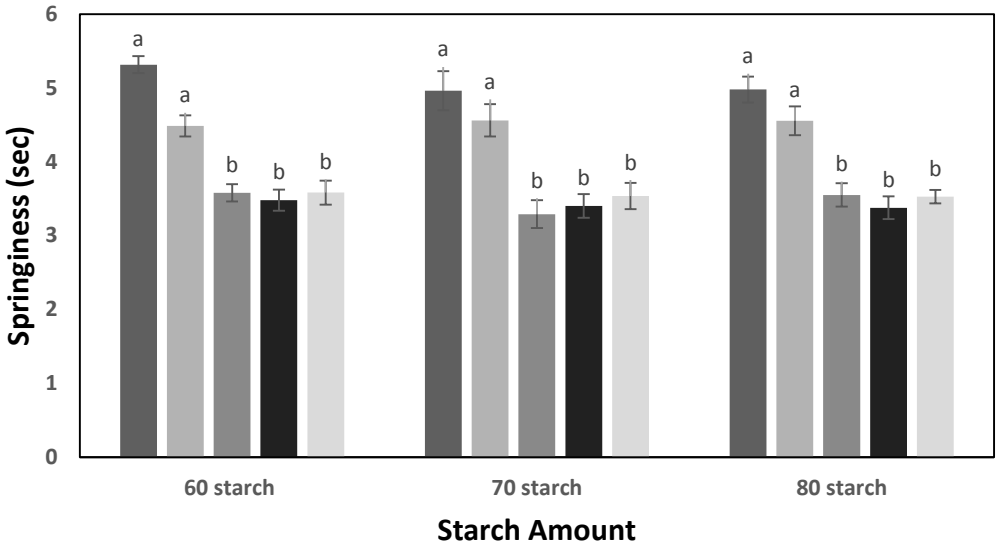

Figure 3.120 Springiness values of bread samples with hpmc stored for 2 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

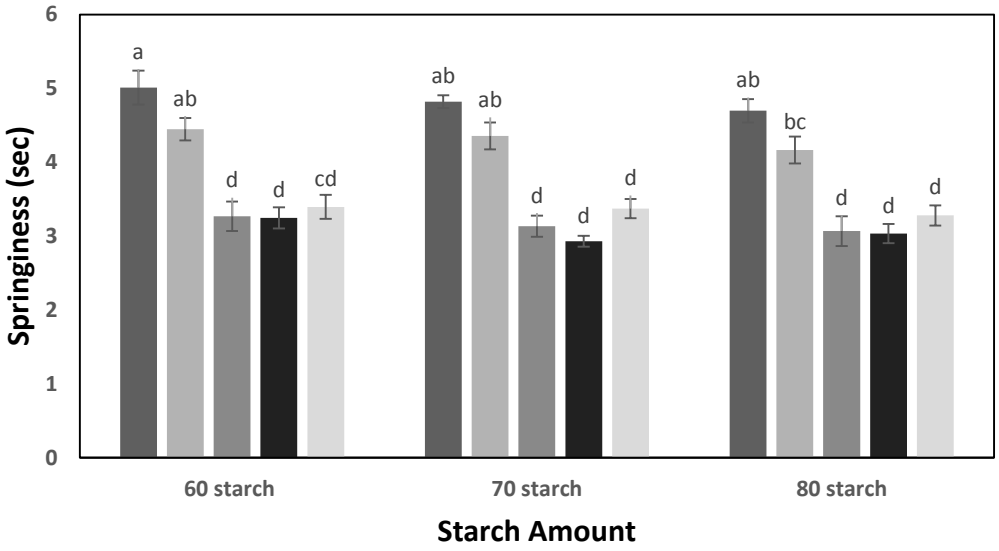

Figure 3.121 Springiness values of bread samples with hpmc stored for 3 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

The figures from Fig. 3.122 to Fig. 3.125 were related to the chewiness values of bread samples with hpmc, in which different treatments were applied and also different starch amounts were used, according to storage durations. Increasing starch amount caused to increase in chewiness values, but this increase was not a significant when the other parameters was kept constant ($p \leq 0.05$). Also, the statistical analysis showed that higher storage duration led to higher chewiness values, which was also an important factor for consumer's acceptability. When the analysis was made in terms of treatment type, it could be stated that microfluidized, treated by pH=8 and pH=10 had highest chewiness values. The untreated and treated by pH=6 followed them. Therefore, according to Table. B.50, microfluidized, treated by pH=8 and pH=10 were indifferent from each other. However, they differed from pH=6 and untreated samples. Also, untreated samples and treated to pH=6 were significantly different from each other ($p \leq 0.05$).

The studies of Andersson et al. (2011) and Schober et al. (2008) on zein-starch dough, Crockett et al. (2011) on rice flour dough, Ericksen et al. (2012) on zein dough and Schober et al. (2007) on sorghum dough to investigate the effect of hpmc on textural properties of gluten-free dough were in agreement with our findings.

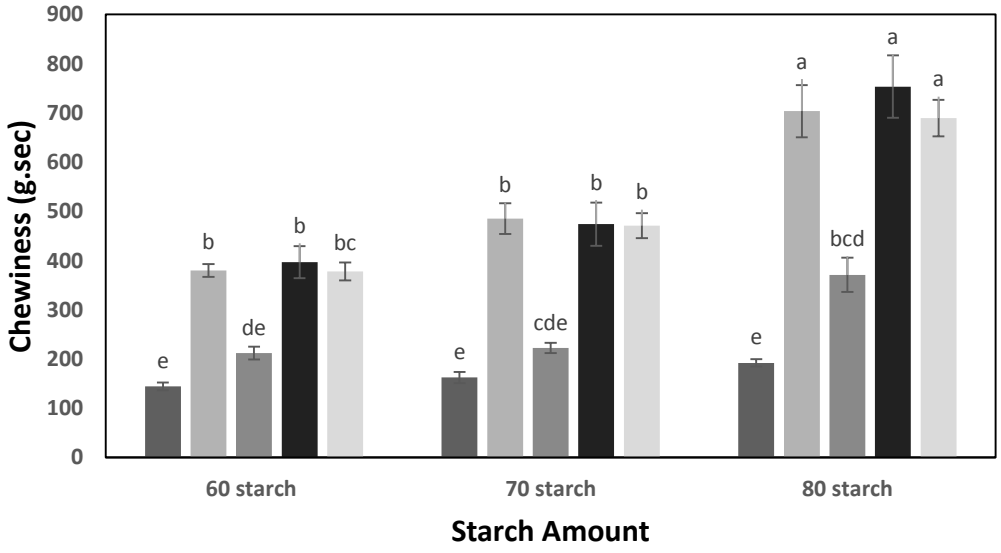

Figure 3.122 Chewiness values of fresh bread samples with hpmc. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

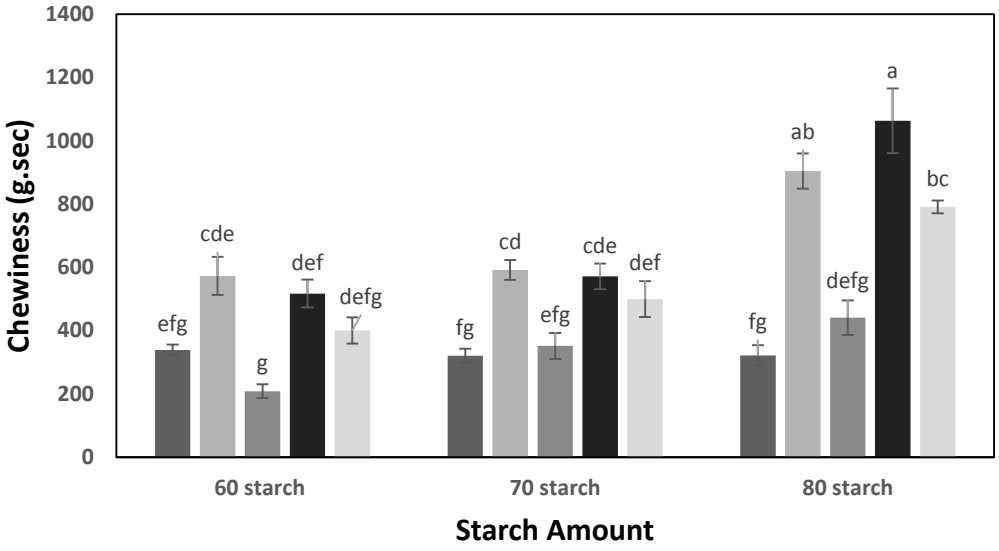

Figure 3.123 Chewiness values of bread samples with hpmc stored for 1 day. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

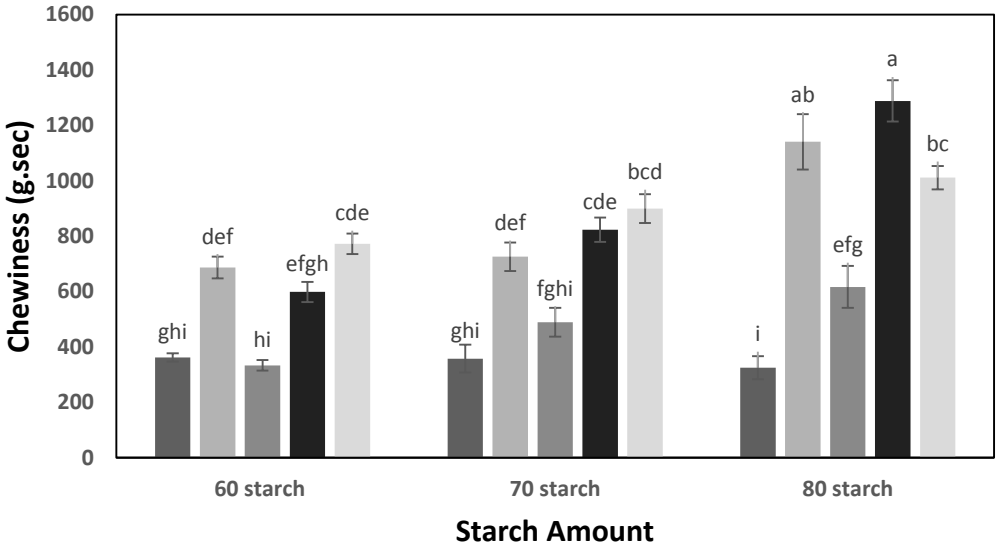

Figure 3.124 Chewiness values of bread samples with hpmc stored for 2 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

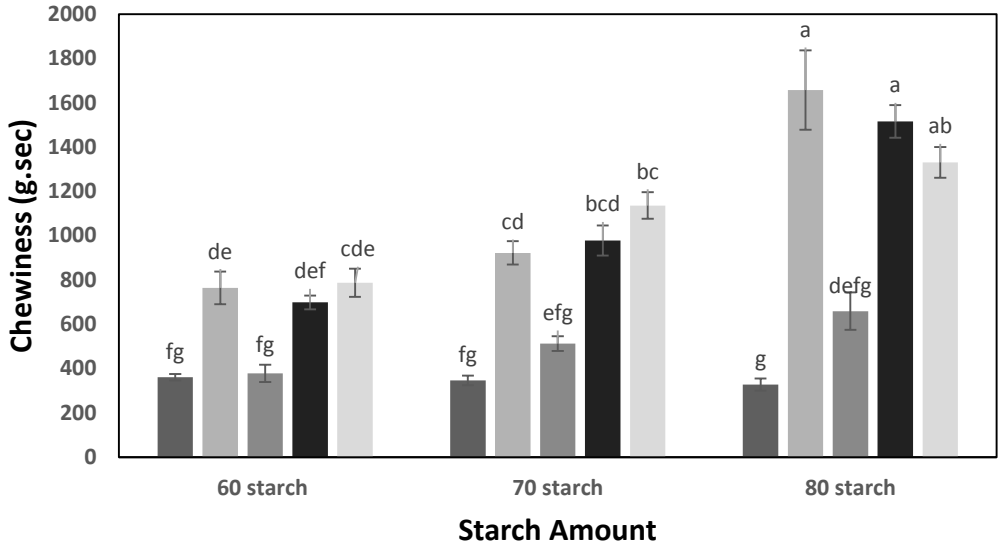

Figure 3.125 Chewiness values of bread samples with hpmc stored for 3 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

Crumb hardness values of gluten-free corn bread samples with guar that prepared by different starch amounts and also different treatment types are represented from Fig. 3.126 to Fig. 3.129. As could be observed from related figures, starch amount caused difference in hardness values of samples ($p \leq 0.05$). Samples containing 80 g corn starch had highest hardness and that value decreased by reduction in starch amount. Also, hardness was influenced excessively with storage duration. It became higher and higher as time elapsed. Therefore, we could easily state that storage duration had a significant role on hardness ($p \leq 0.05$). Finally, treatment type was analyzed, and it could be expressed that it differed by application of different treatments. Generally, microfluidized samples had the highest in 60 and 70 g starch contents and it was followed by pH=6, untreated, pH=8 and pH=10. The microfluidized sample replaced with pH=6 in 80 g starch amount. As a result, treatment type led to significant difference in hardness ($p \leq 0.05$).

Also, the figures related to cohesiveness, springiness and chewiness of gluten-free corn bread samples with guar are presented below. The similar results were found for these experiments done by replacement of guar instead of hpmc. Also, the significance of the starch amount, storage duration and also treatment type was analyzed for each measurements. As stated before many times, because of complex structure of bread since bread contains numerous ingredients within its structure, the effects of hpmc and guar showed difference in some extents. That could be explained by the required operation conditions of different hydrocolloids. Their acid-base interaction, operation temperature and interactions with macromolecules differ and these differences caused to changes in results between hpmc, guar, xanthan and citrus fiber.

The effects of guar on textural measurements of gluten-free bread dough was also studied by Demirkesen et al. (2010a), and two studies were in agreement on the subject of guar.

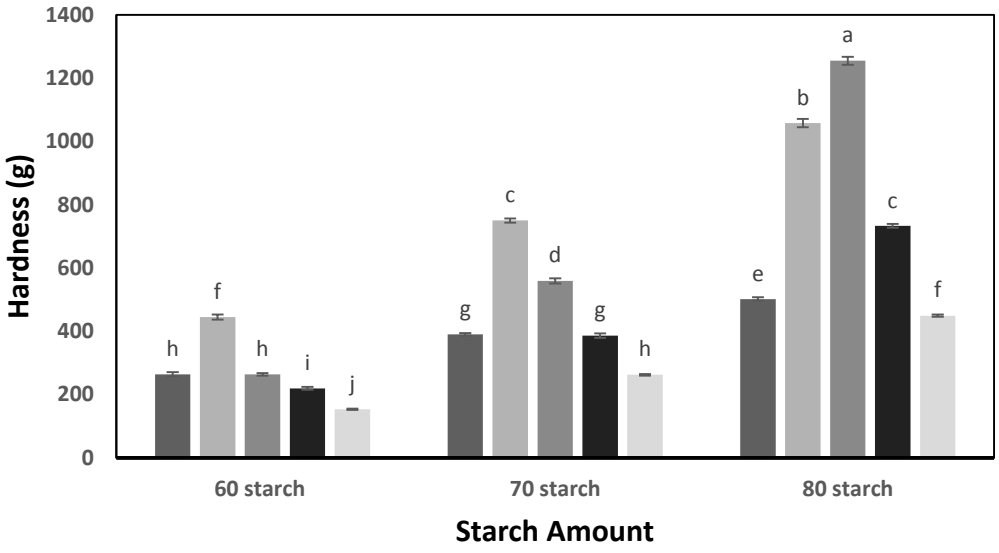

Figure 3.126 Hardness values of fresh bread samples with guar. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

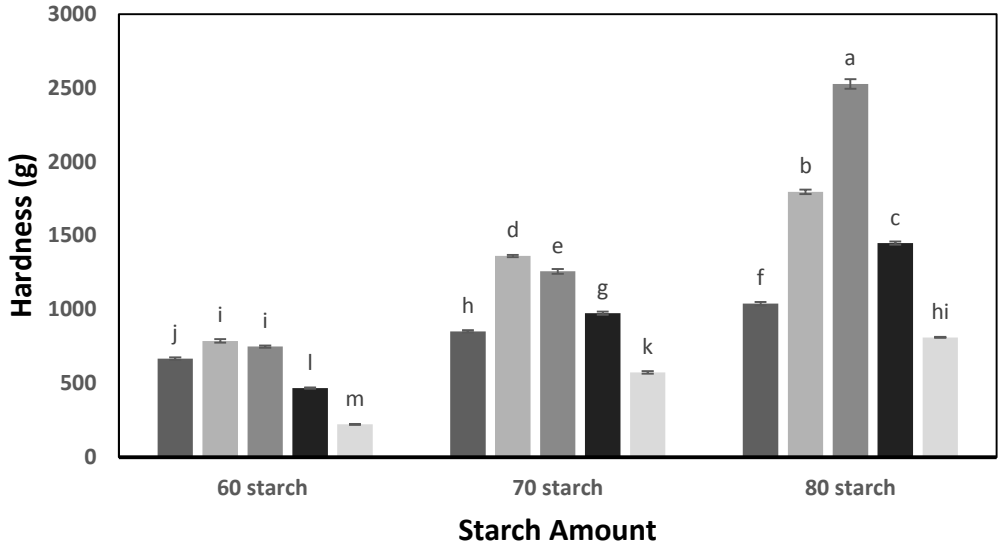

Figure 3.127 Hardness values of bread samples with guar stored for 1 day. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

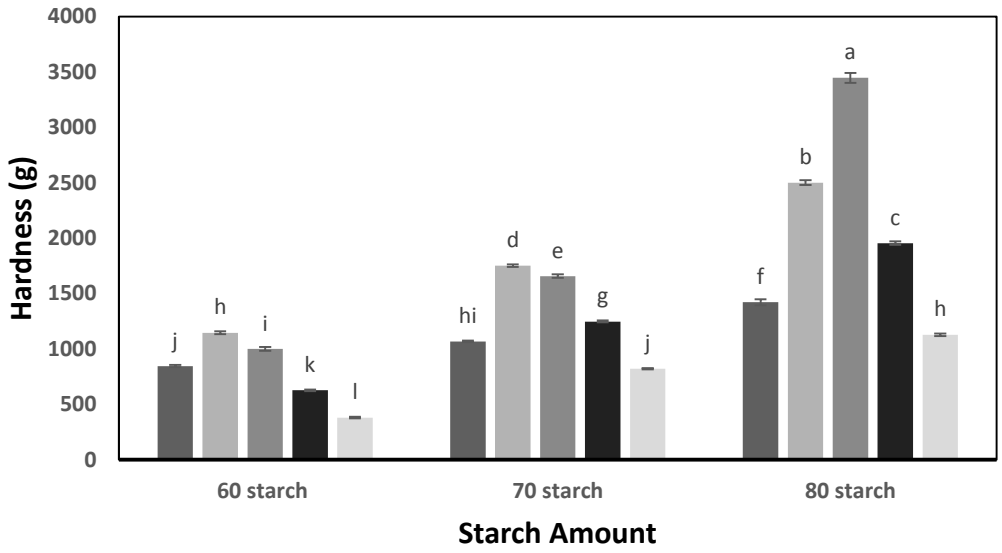

Figure 3.128 Hardness values of bread samples with guar stored for 2 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

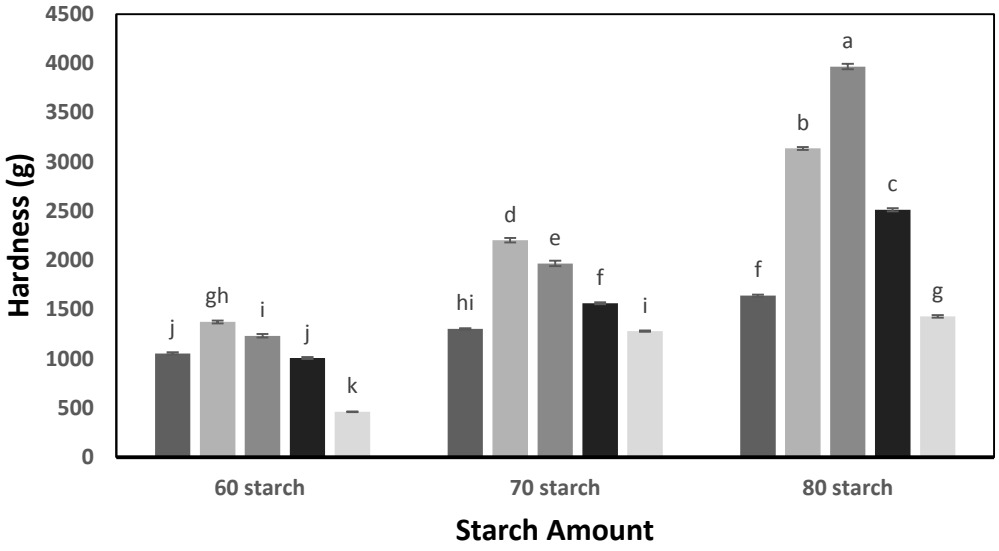

Figure 3.129 Hardness values of bread samples with guar stored for 3 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

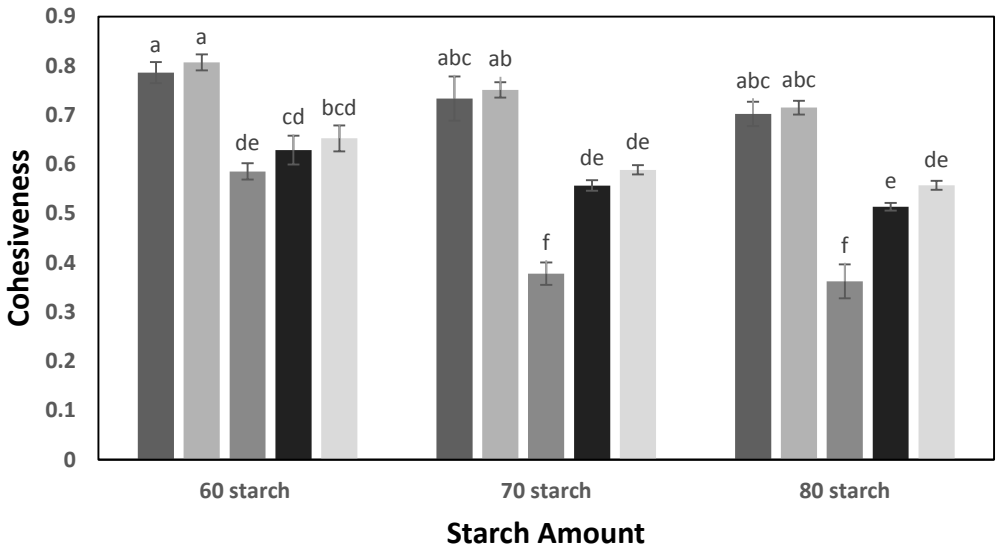

Figure 3.130 Cohesiveness values of fresh bread samples with guar. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

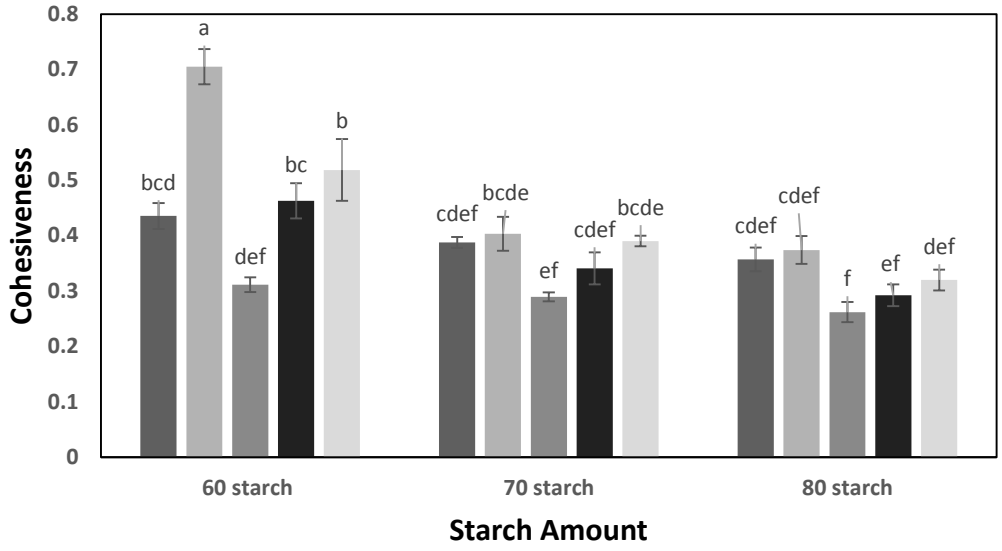

Figure 3.131 Cohesiveness values of bread samples with guar stored for 1 day. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

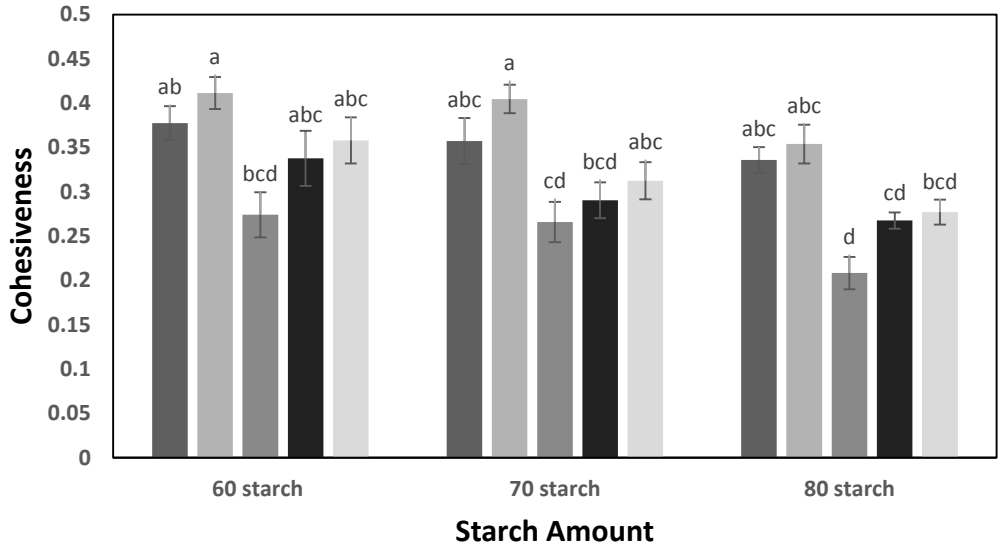

Figure 3.132 Cohesiveness values of bread samples with guar stored for 2 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

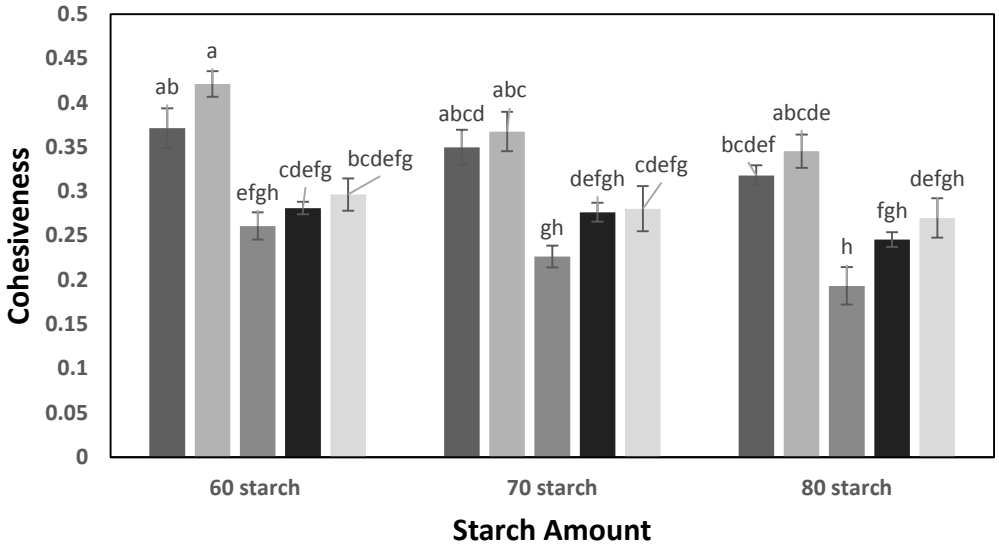

Figure 3.133 Cohesiveness values of bread samples with guar stored for 3 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

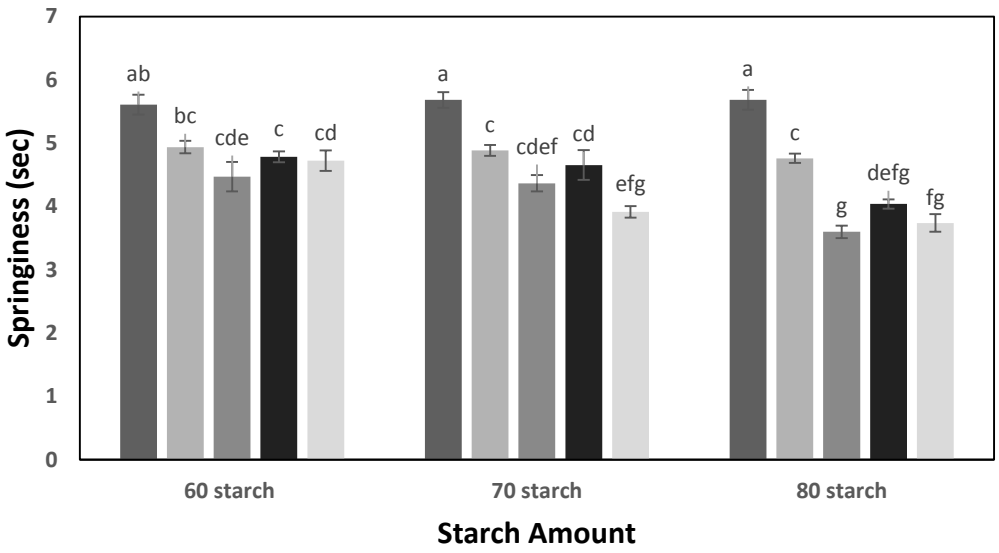

Figure 3.134 Springiness values of fresh bread samples with guar. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

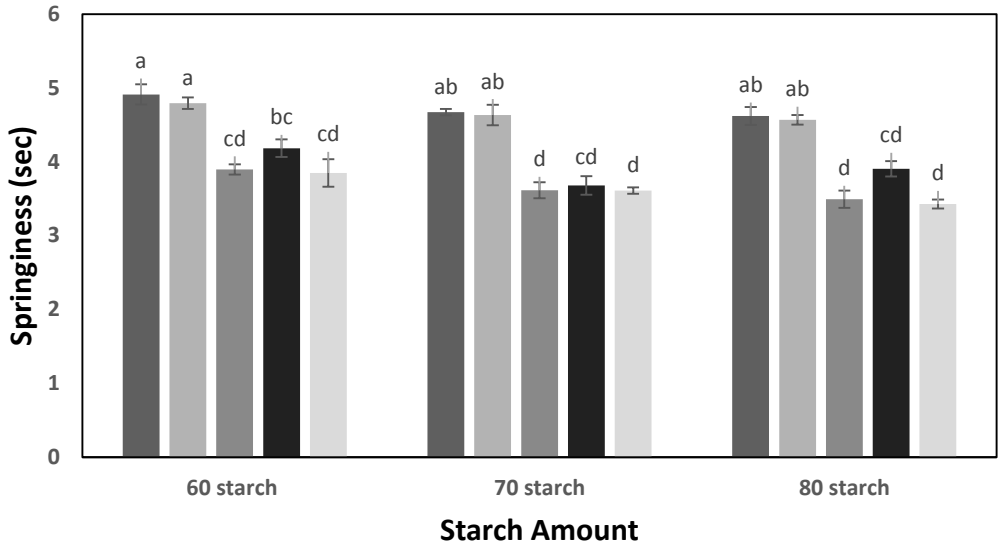

Figure 3.135 Springiness values of bread samples with guar stored 1 day. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

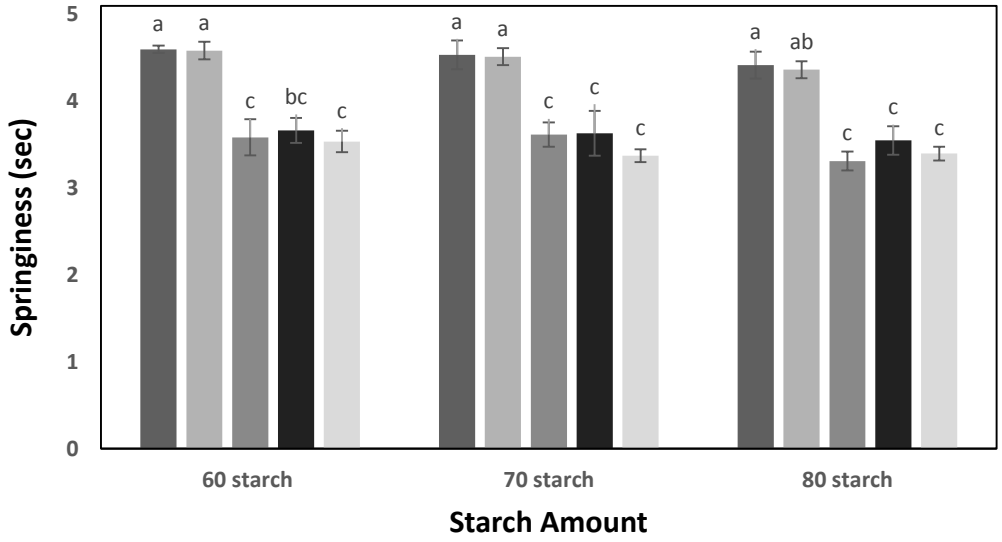

Figure 3.136 Springiness values of bread samples with guar stored 2 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

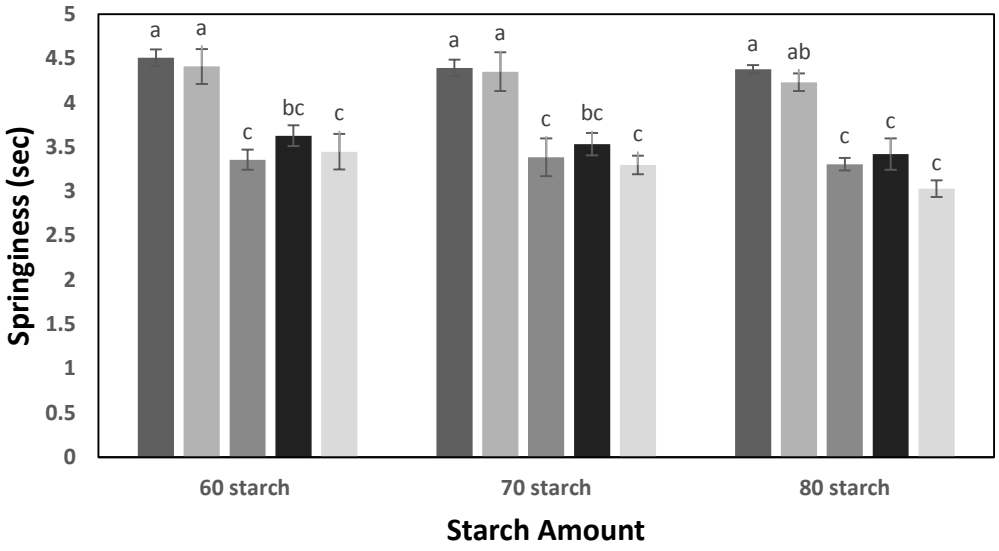

Figure 3.137 Springiness values of bread samples with guar stored 3 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

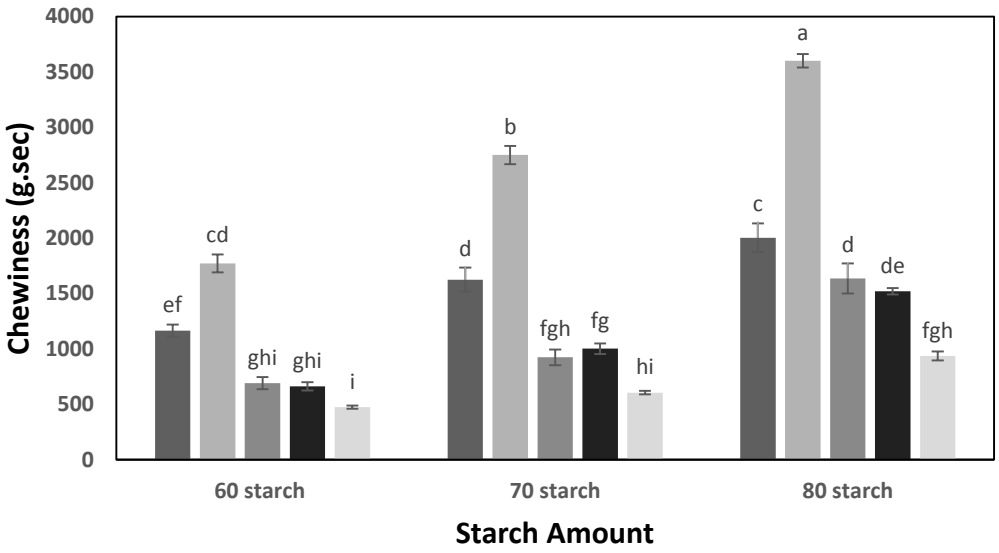

Figure 3.138 Chewiness values of fresh bread samples with guar. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

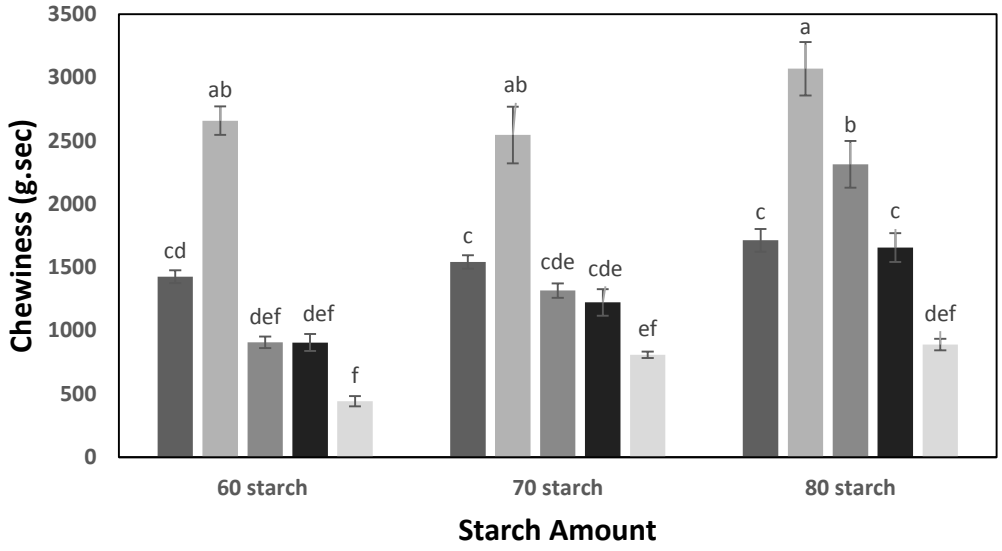

Figure 3.139 Chewiness values of bread samples with guar stored 1 day. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

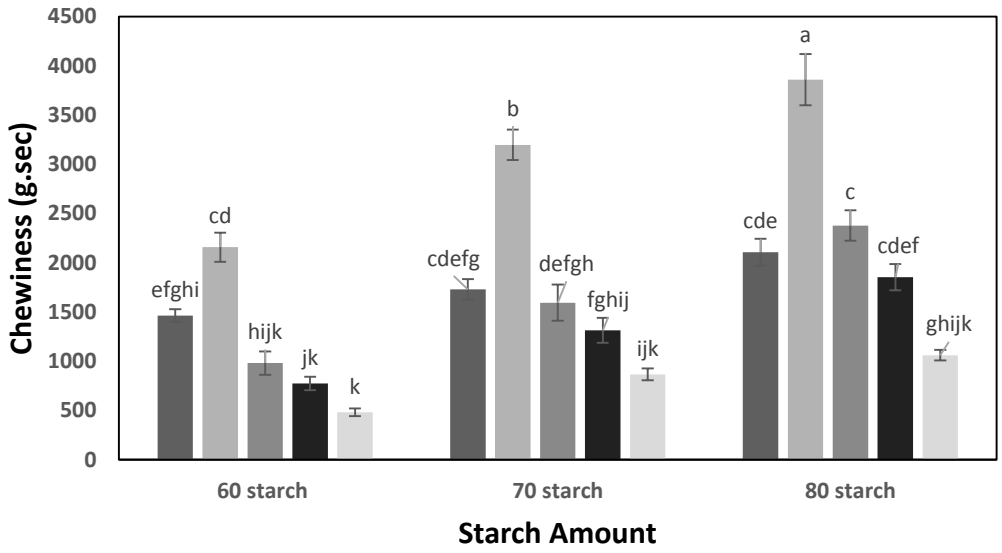

Figure 3.140 Chewiness values of bread samples with guar stored 2 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

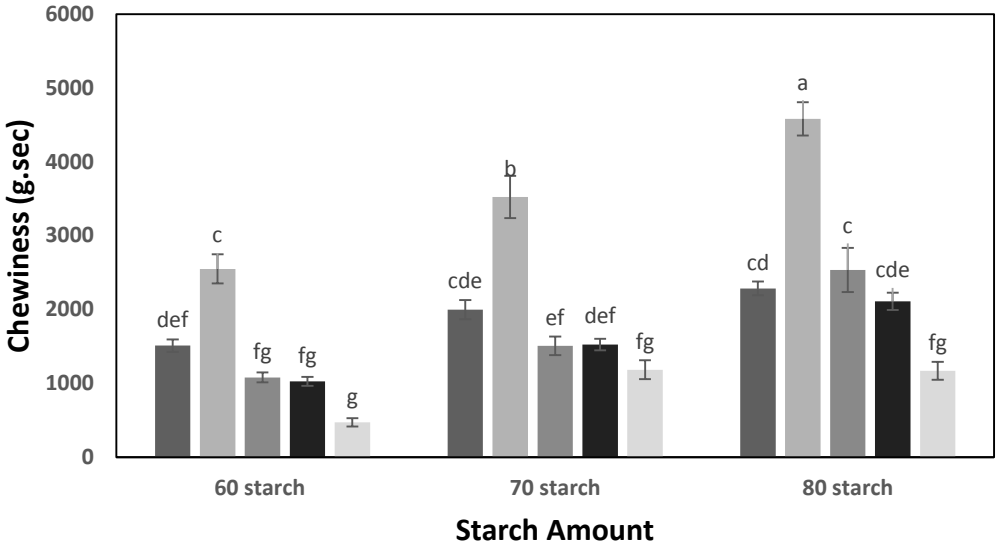

Figure 3.141 Chewiness values of bread samples with guar stored 3 days. ■: control zein, ■: mf, ■: pH=6, ■: pH=8, ■: pH=10.

From Fig. 3.142 to Fig. 3.145, the hardness, cohesiveness, springiness and chewiness values of gluten-free corn bread samples with xanthan, in which different treatments were applied and also different starch amounts were used, are presented. As could be understood from figures, xanthan and also citrus fiber experiments were done only on untreated and microfluidized samples. The reason behind that was the absence of the formation of dough structure by addition of alkaline with xanthan and citrus fiber.

As could be seen from Fig. 3.142, there was a significant difference between starch amounts, storage durations and treatment types ($p \leq 0.05$). All parameters led to differences in hardness value. As starch amount and storage duration increased, hardness value of the bread samples increased. On the other hand, microfluidization treatment caused to higher hardness values than untreated samples.

According to the Fig. 3.143, cohesiveness was influenced from all parameters significantly ($p \leq 0.05$). That meant starch amount, storage duration and treatment type were effective on cohesiveness. The exception was the third storage day. It showed similarity with the second storage day. As starch amount and storage duration increased, the cohesiveness decreased. On the other hand, microfluidized samples had higher cohesiveness values than untreated samples.

The springiness values of bread samples with xanthan are illustrated in Fig. 3.144. The figure and table showed that there was no significant difference between starch amounts, however, the treatment type was important significantly ($p \leq 0.05$). Both increasing starch amount and also treatment by microfluidization increased springiness values of bread samples. When comparison between storage durations were done, it could be stated that similarities and differences were observed. The day of baking was different from others. The first day resembled to second day, but differed from the third day. The second day was also indifferent from the third day. In general, springiness was lower by elapsed time.

Lastly, the chewiness values related to xanthan are represented in Fig. 3.145. The statistical analysis indicated that starch amount and treatment type caused to significant differences on chewiness. The effect of other parameter, storage duration, varied by time as being in springiness. All days except the third day were similar to each other and there was no significant difference between them, however, the third day showed differences ($p \leq 0.05$). Increase in starch amount caused to increase in chewiness value. As storage duration got longer, the chewiness increased. Also, the application of microfluidization led to higher chewiness values.

The studies of Crockett et al. (2011), Sciarini et al. (2010), and Sciarini et al. (2012) displays paralellism to our study in terms of the effect of xanthan on textural properties of gluten-free bread dough.

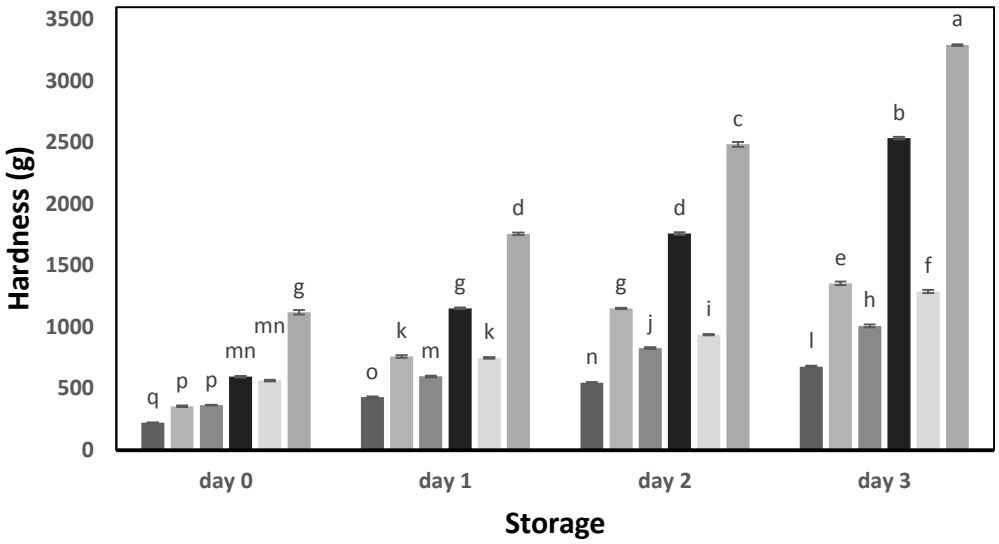

Figure 3.142 Hardness values of bread samples with xanthan. ■: 60 g control zein, ■: 60 g mf, ■: 70 g control zein, ■: 70 g mf, ■: 80 g control zein, ■: 80 g mf.

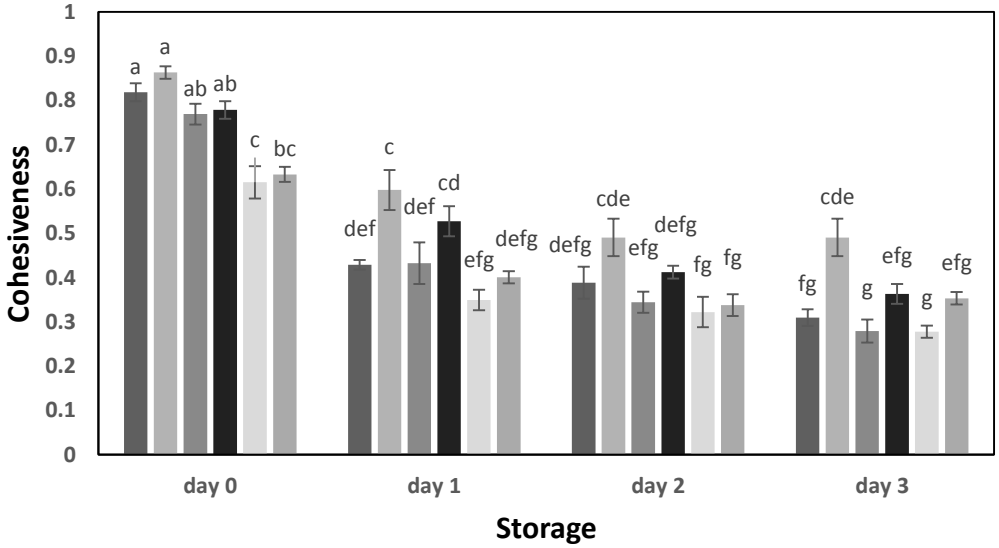

Figure 3.143 Cohesiveness values of bread samples with xanthan. ■: 60 g control zein, ■: 60 g mf, ■: 70 g control zein, ■: 70 g mf, ■: 80 g control zein, ■: 80 g mf.

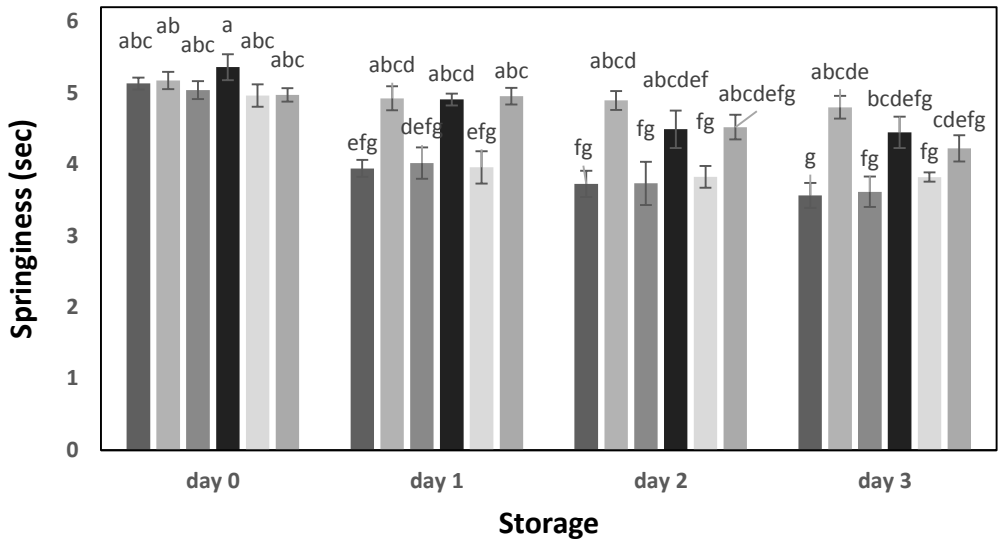

Figure 3.144 Springiness values of bread samples with xanthan. ■: 60 g control zein, ■: 60 g mf, ■: 70 g control zein, ■: 70 g mf, ■: 80 g control zein, ■: 80 g mf.

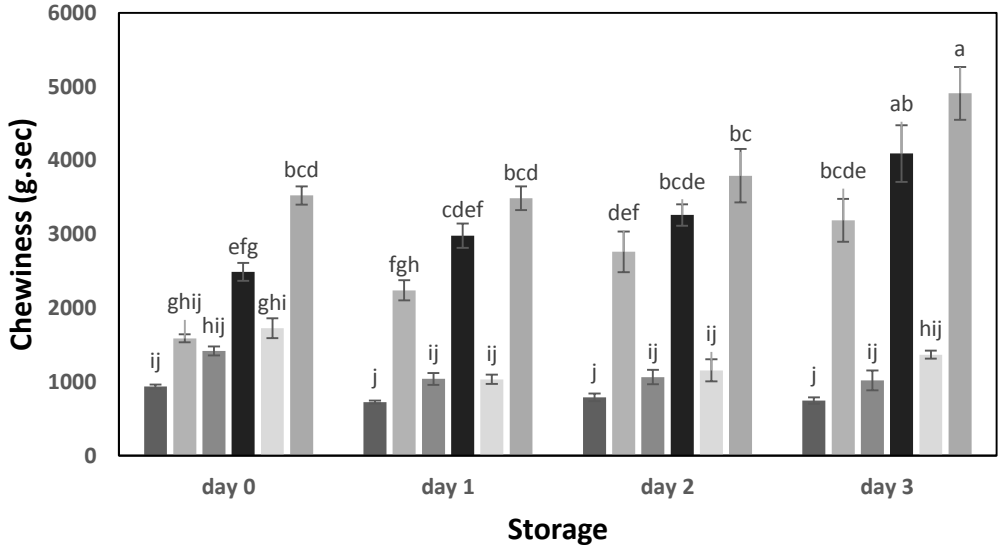

Figure 3.145 Chewiness values of bread samples with xanthan. ■: 60 g control zein, ■: 60 g mf, ■: 70 g control zein, ■: 70 g mf, ■: 80 g control zein, ■: 80 g mf.

The next four figures below are the illustrations of the hardness, cohesiveness, springiness and chewiness values of gluten-free corn bread samples with citrus fiber, in which different treatments were applied and also different starch amounts were used, were presented. As stated in previous part, citrus fiber was also used on microfluidized and untreated samples because of their inability to form a dough with alkaline. We mentioned the improvements of hydrocolloids on bread volume before. Like hydrocolloids, some fibers and emulsifiers also provide such improvements. The study of Sabanis et al. (2009) supported our findings on bread volume improvements as could be seen from Fig. A.5.

The significant difference between starch amounts, storage durations and treatment types on hardness were observed from Fig. 3.146 ($p \leq 0.05$). That meant all changes in parameters resulted in changes in hardness value. As starch amount and storage duration increased, the hardness increased as well. On the other hand, as could be seen from figure and related table, the addition of citrus fiber to the microfluidized samples caused to opposite result as obtained from xanthan. The hardness decreased by application of microfluidization in this time.

According to the ANOVA results, the cohesiveness value was influenced from starch amount, storage duration and treatment type. All parameters caused to significant differences on cohesiveness value except starch amount ($p \leq 0.05$). Exception was that samples containing 80 g starch was indifferent from 70 g. Higher starch amounts, longer storage durations and treatment by microfluidization led to lower cohesiveness values.

The springiness values of bread samples with citrus fiber are illustrated in Fig. 3.148. The figure and table showed that there was a significant difference between storage durations, however, the importance of the treatment type and starch amount changed. ($p \leq 0.05$). Bread samples containing 60 g starch had highest springiness values and different from other starch amounts, however, 70 and 80 g were indifferent from each other. When we compared storage duration, we could state that springiness decreased by time. But, only the day of baking showed difference from others.

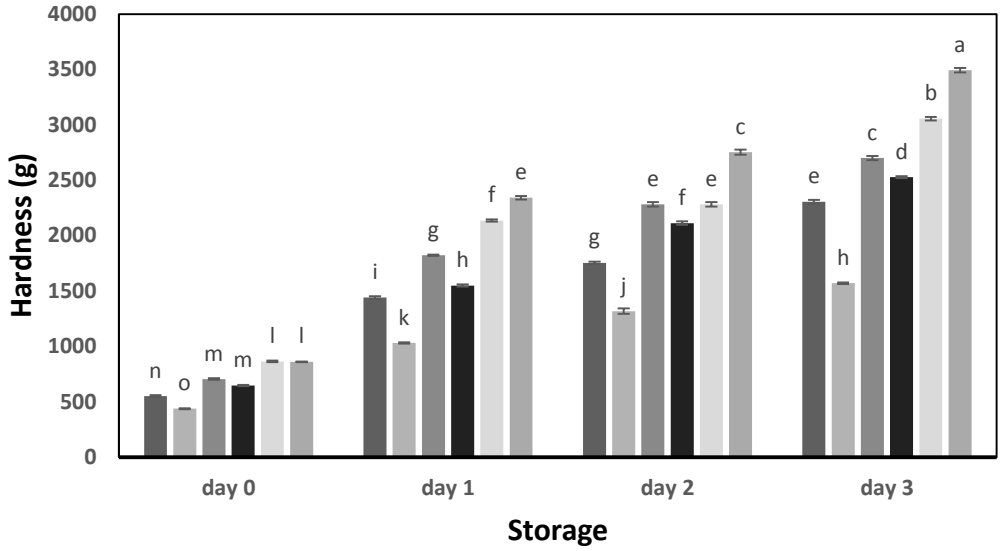

Figure 3.146 Hardness values of bread samples with citrus fiber. ■: 60 g control zein, ■: 60 g mf, ■: 70 g control zein, ■: 70 g mf, ■: 80 g control zein, ■: 80 g mf.

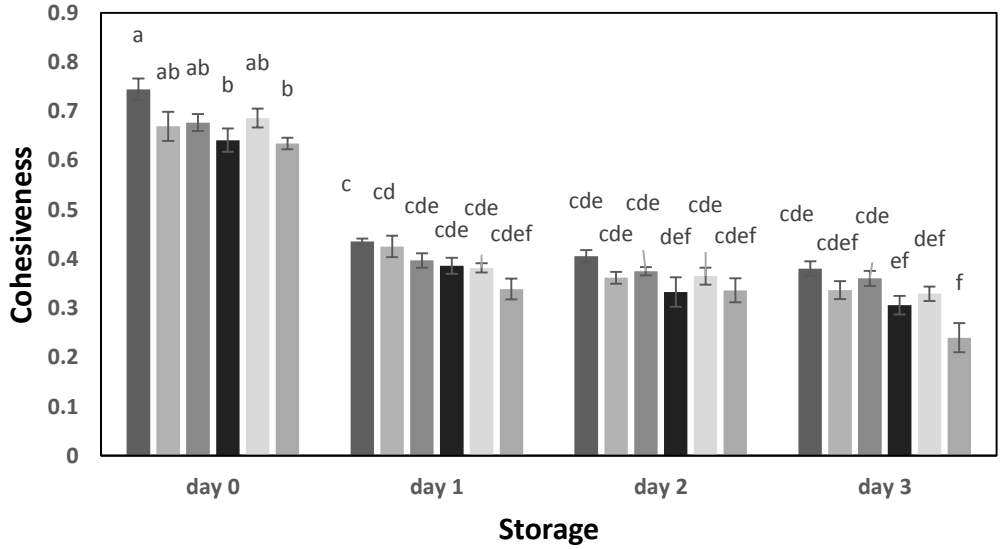

Figure 3.147 Cohesiveness values of bread samples with citrus fiber. ■: 60 g control zein, ■: 60 g mf, ■: 70 g control zein, ■: 70 g mf, ■: 80 g control zein, ■: 80 g mf.

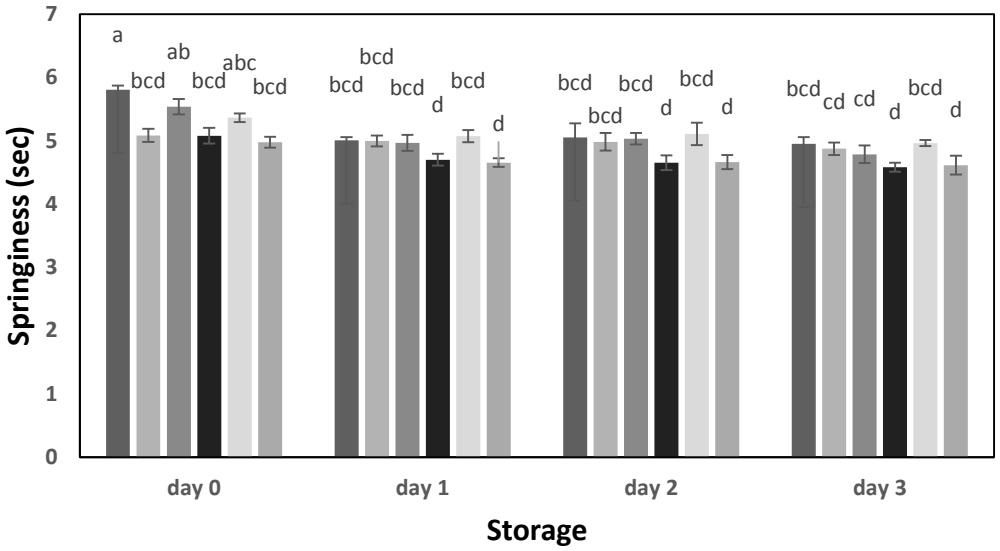

Figure 3.148 Springiness values of bread samples with citrus fiber. ■: 60 g control zein, ■: 60 g mf, ■: 70 g control zein, ■: 70 g mf, ■: 80 g control zein, ■: 80 g mf.

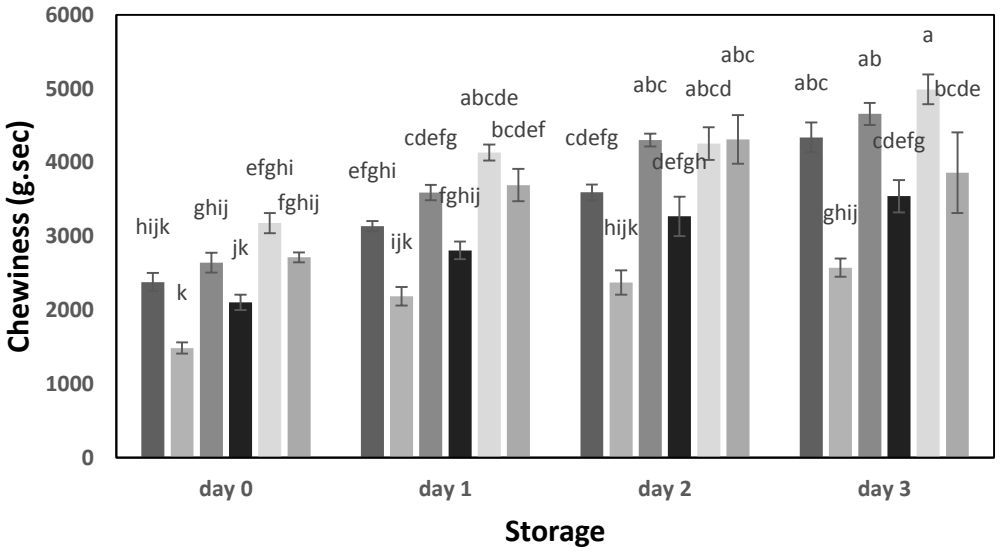

Figure 3.149 Chewiness values of bread samples with citrus fiber. ■: 60 g control zein, ■: 60 g mf, ■: 70 g control zein, ■: 70 g mf, ■: 80 g control zein, ■: 80 g mf.

The last figure (Fig. 3.149) was related to the chewiness values of bread samples containing citrus fiber. The figure and the related table showed that chewiness value was influenced by all parameters significantly ($p \leq 0.05$). As higher starch amount and longer storage durations caused to increase in chewiness value, application of microfluidization led to decrease in chewiness.

In conclusion, it could be stated that increase in starch amount always resulted in higher hardness, chewiness, and lower cohesiveness, springiness values. On the other hand, when the storage duration got longer, the same effect was observed on textural measurements. The difference came from the treatment type. Generally, microfluidization and alkaline treatment led to higher hardness, chewiness and lower cohesiveness and springiness than untreated samples. The most important point was that hydrocolloids provided higher volume to untreated samples, which could be found in Fig. A.1-Fig. A.5.

As the final point, to investigate and understand the effects of gums and mixing, different gum concentrations and mixing processes were applied. The results were tabulated from Fig. A.6 to Fig. A.8 with images. Hpmc and guar in terms of gum, and mixer and ultra-turrax in terms of mixing were selected for this experiment. As could be understood from Fig. A.6 and Fig. A.7, increase in hpmc and guar concentration led to higher volume to bread structure. Also, more homogenous network structure was observed with higher concentrations. On the other hand, ultra-turrax dominated mixer in terms of mixing as could be observed from Fig. A.8. The pores in bread structure was smaller with ultra-turrax mixing. Moreover, more homogenous structure was obtained with ultra-turrax mixing. However, since ultra-turrax could not be performed in all gums, mixer was used in texture analysis experiments.

In the light of these results, we could conclude that there are several parameter to improve the quality of gluten-free breads. However, all of them could not applied together.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

The effect of microfluidization and also alkaline treatment was clearly determined in the microstructural analyses of zein slurries by SEM. Also, the structural differences between untreated zein, zein passed only from colloidal mill, microfluidized zein, and zein treated by both microfluidization and alkalinity were clearly observed. The structure of the zein was opened by colloidal mill, microfluidization and alkaline treatment further and large zein blocks were broken down. Also, surface area of the zein was increased by these treatments. As a result of those, water holding capacity of the zein, which is hydrophobic in the beginning, was improved. However, the results showed that excessive alkaline treatment caused to deformation within the structure. Because of that reason, we concluded that pH=8 with microfluidization gave the best result on surface morphology.

In emulsion part of our study, we investigated emulsion rheology and stability. Rheological experiments showed that results were fitted to Herschel–Bulkley model. Also, most of them exhibited shear thinning behavior except some microfluidized zein emulsions. Moreover, elastic moduli (G') values were higher than viscous moduli (G") values, which denoted elastic-gel like behavior for emulsions. The increase in both oil and zein concentrations caused to higher viscoelastic moduli values. Besides, higher viscoelastic moduli values provided by microfluidization were improved by alkaline treatment up to some point. Moreover, the emulsion stability experiments indicated that microfluidization and alkaline treatment resulted in smaller particles, which provided more homogenous structure, and slower sedimentation pace up to some point.

The bread-making process could be divided into two part, which were related to dough and bread samples. In bread dough experiments, linear viscoelastic region was determined as a strain of lower than 0.3% for all formulations and viscoelastic measurements were done according to this result as selecting the controlled variable as a strain of 0.1%. All dough formulations showed that elastic moduli (G') values were higher than viscous moduli (G") values, which indicated solid like behavior. We could state that microfluidization led to higher moduli values compared to untreated zein and zein treated by alkalinity. On the other side, increasing starch amount always provided higher elastic and viscous moduli values. Also, the addition of hydrocolloids such as hpmc, guar, xanthan and citrus fiber to gluten-free dough formulations improved moduli values compared to dough without gum.

When the textural properties of gluten-free bread samples were analyzed, it was seen that as starch amount and storage duration increased, hardness and chewiness values of the samples certainly increased, but sometimes that increase was not significant according to statistical analysis. In case of springiness and cohesiveness, the picture was opposite. These values decreased with higher starch amount and longer storage duration. Microfluidization and alkaline treatment also caused to higher hardness and chewiness, and lower springiness and cohesiveness in general. The addition of hydrocolloids resulted in improvements in these textural properties. Furthermore, crumb color of bread samples prepared by microfluidization and alkaline treatment became darker than untreated samples.

In conclusion, using of microfluidization as a milling process for zein would be an effective operation. Also, emulsion properties, and rheological and textural properties of gluten-free bread formulations were improved by microfluidization and alkaline treatment. Moreover, the usage of hydrocolloids in gluten-free breads provided quality in terms of volume and texture, which could be seen from Fig. A.1- Fig. A.5.

As future study, to understand more clearly the staling charactesitics of gluten-free bread samples, X-ray and FT-IR analyses could be done.

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APPENDIX A

PICTURES OF SAMPLES

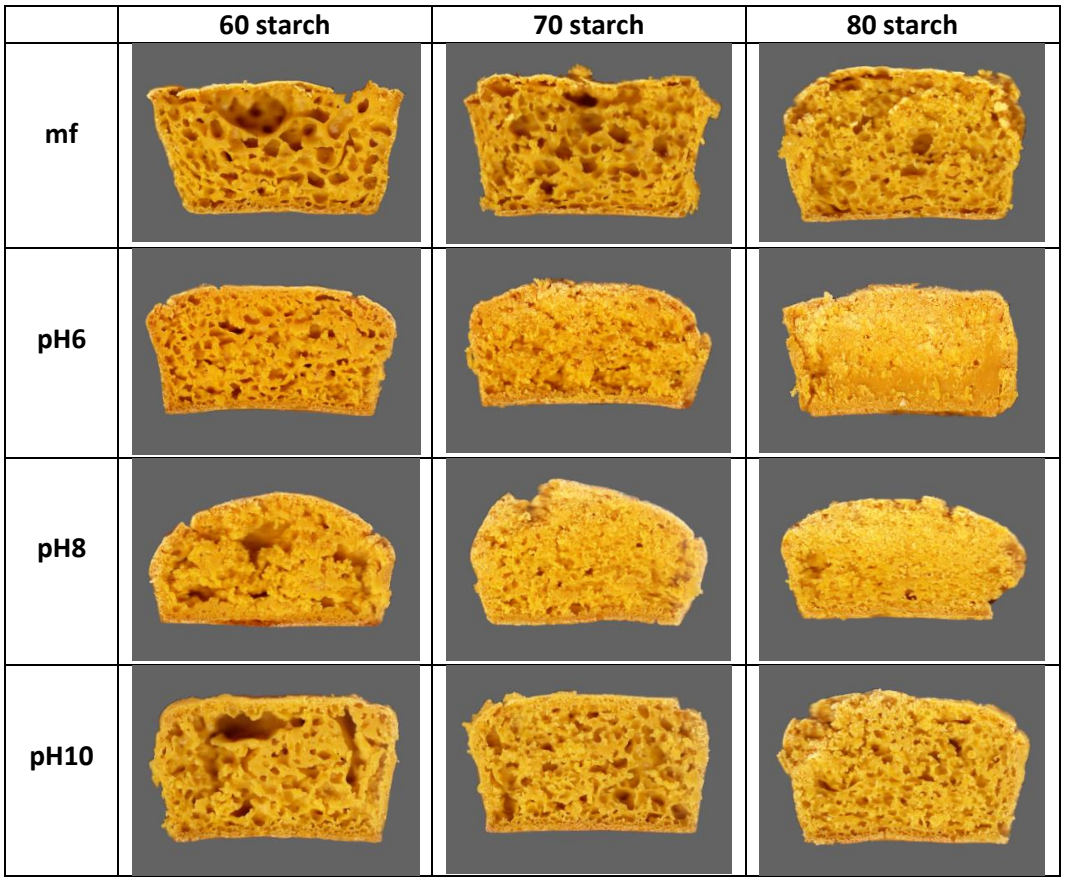


Figure A.1 Pictures of corn breads made without gum (control zein)

	60 starch	70 starch	80 starch
control zein			
mf			
pH6			
pH8			
pH10			

Figure A.2 Pictures of corn breads made with hpmc

	60 starch	70 starch	80 starch
control zein			
mf			
pH6			
pH8			
pH10			

Figure A.3 Pictures of corn breads made with guar

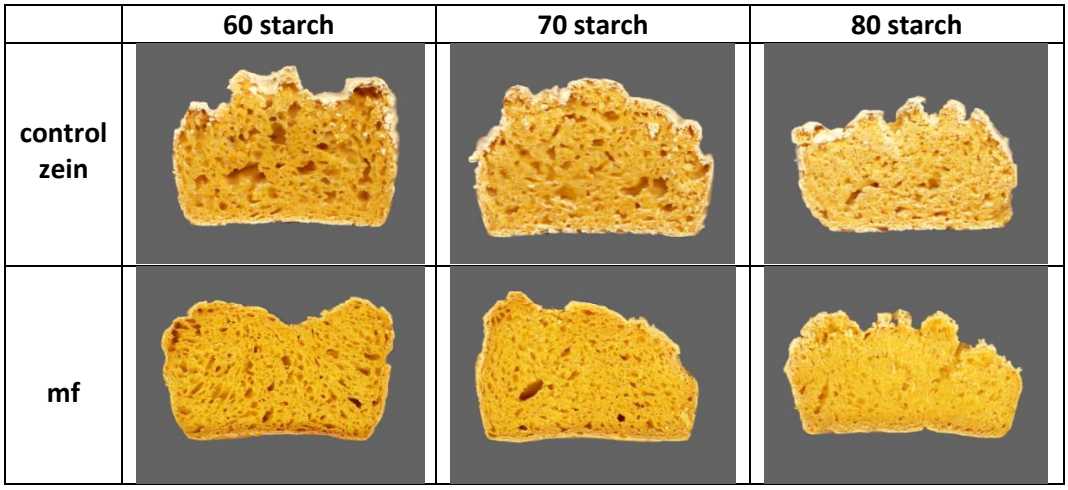


Figure A.4 Pictures of corn breads made with xanthan

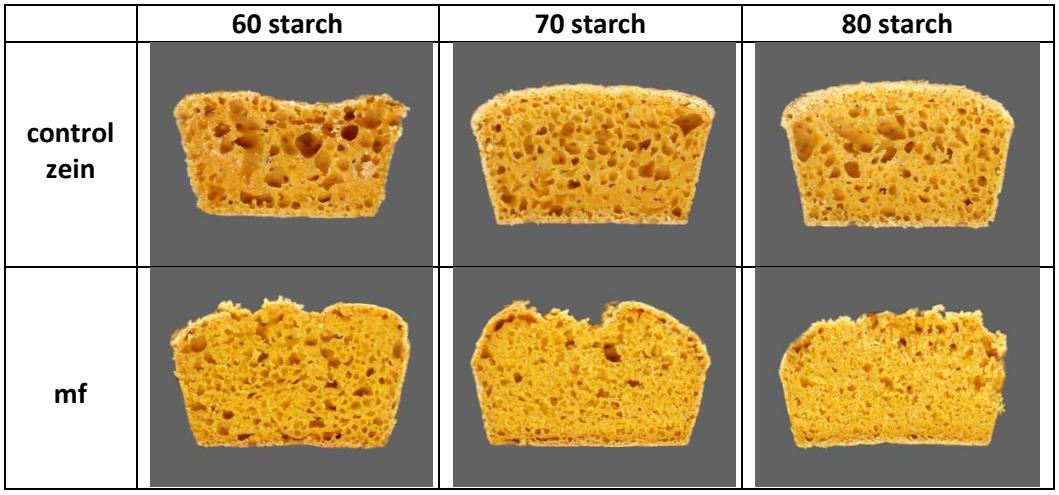


Figure A.5 Pictures of corn breads made with citrus fiber

	0 hpmc	0.7 hpmc	1.4 hpmc	2 hpmc
On 70 starch & mf				

Figure A.6 Pictures of corn breads to see the effect of hpmc

	0 guar	0.7 guar	1.4 guar	2 guar
On 70 starch & mf				

Figure A.7 Pictures of corn breads to see the effects of guar

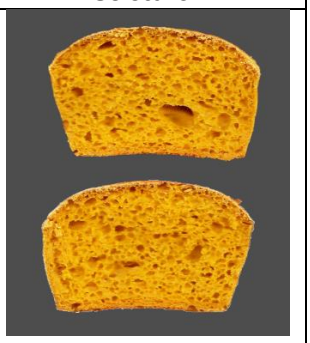
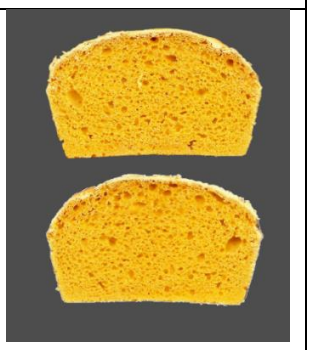
	60 starch	70 starch	80 starch
Mixer			
Ultra-turrax			

Figure A.8 Pictures of corn breads to see the effect of mixing

APPENDIX B

STATISTICAL ANALYSES

Appendix B contains only some examples of ANOVA results. All results could not take place in this part of the study. The related results were already stated in the tables and figures by spelling.

Table B.1 Results for Tukey's mean comparison test for moisture content of bread samples with hpmc

General Linear Model: moisture versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for moisture, using Adjusted SS for Tests

Source                   DF    Seq SS   Adj SS   Adj MS       F      P
treatment                 4    4.9815   4.9815   1.2454    2.95  0.056
starchamount              2   84.9475  84.9475  42.4737  100.46  0.000
treatment*starchamount    8    8.2974   8.2974   1.0372    2.45  0.064
Error                    15    6.3418   6.3418   0.4228
Total                    29  104.5682


S = 0.650219   R-Sq = 93.94%   R-Sq(adj) = 88.27%


Unusual Observations for moisture

Obs  moisture      Fit  SE Fit  Residual  St Resid
  1   50.7745  49.4373  0.4598    1.3372      2.91 R
  2   48.1001  49.4373  0.4598   -1.3372     -2.91 R

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

```
Grouping Information Using Tukey Method and 95.0% Confidence

treatment  N  Mean  Grouping
control    6  46.9  A
pH10       6  46.7  A
mf         6  46.6  A
pH6        6  46.2  A
pH8        6  45.8  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

starchamount   N  Mean  Grouping
60            10  48.6  A
70            10  46.2    B
80            10  44.5      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N  Mean  Grouping
control    60            2  49.4  A
pH8        60            2  48.8  A B
mf         60            2  48.5  A B C
pH10       60            2  48.2  A B C
pH6        60            2  47.9  A B C D
control    70            2  47.3  A B C D E
pH10       70            2  46.6    B C D E F
pH6        70            2  46.0      C D E F
mf         70            2  45.9      C D E F G
mf         80            2  45.4        D E F G
pH8        70            2  45.1          E F G
pH10       80            2  45.1          E F G
pH6        80            2  44.5            F G
control    80            2  44.0            F G
pH8        80            2  43.4              G

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

Table B.2 Results for Tukey's mean comparison test for total color change of bread samples with hpmc

General Linear Model: totalcolorchange versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80
```

Analysis of Variance for totalcolorchange, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
treatment	4	19420.6	19420.6	4855.2	197.53	0.000
starchamount	2	14984.8	14984.8	7492.4	304.83	0.000
treatment*starchamount	8	994.6	994.6	124.3	5.06	0.000
Error	165	4055.5	4055.5	24.6		
Total	179	39455.5				

S = 4.95773   R-Sq = 89.72%   R-Sq(adj) = 88.85%

Unusual Observations for totalcolorchange

Obs	totalcolorchange	Fit	SE Fit	Residual	St Resid
13	53.3956	43.8717	1.4312	9.5239	2.01 R
16	34.0701	43.8717	1.4312	-9.8015	-2.06 R
18	30.6651	43.8717	1.4312	-13.2066	-2.78 R
23	66.9314	43.8717	1.4312	23.0597	4.86 R
27	54.4295	35.5355	1.4312	18.8940	3.98 R
34	22.0599	35.5355	1.4312	-13.4756	-2.84 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	N	Mean	Grouping
pH6	36	70.4	A
pH8	36	68.7	A
mf	36	61.9	B
pH10	36	51.9	C
control	36	43.0	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	N	Mean	Grouping
60	60	70.7	A
70	60	58.4	B
80	60	48.3	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	N	Mean	Grouping
pH8	60	12	83.3	A
pH6	60	12	79.5	A
mf	60	12	76.5	A B
pH6	70	12	71.7	B C
pH8	70	12	65.8	C D
pH10	60	12	64.5	D
pH6	80	12	60.0	D E
mf	70	12	59.4	D E
pH8	80	12	56.9	E F
pH10	70	12	51.4	F G
mf	80	12	49.6	G H
control	60	12	49.5	G H

```
control    70            12  43.9                H I
pH10       80            12  39.7                  I J
control    80            12  35.5                    J

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

Table B.3 Results for Tukey's mean comparison test for hardness values of fresh bread samples with hpmc

General Linear Model: hardness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for hardness, using Adjusted SS for Tests

Source                  DF  Seq SS  Adj SS  Adj MS        F      P
treatment                4  606099  606099  151525  1674.75  0.000
starchamount             2  245727  245727  122863  1357.97  0.000
treatment*starchamount   8   77611   77611    9701   107.23  0.000
Error                   75    6786    6786      90
Total                   89  936223


S = 9.51188   R-Sq = 99.28%   R-Sq(adj) = 99.14%


Unusual Observations for hardness

Obs  hardness      Fit  SE Fit  Residual  St Resid
 49   258.778  239.536   3.883    19.242      2.22 R
 50   213.648  239.536   3.883   -25.888     -2.98 R
 52   215.616  239.536   3.883   -23.920     -2.75 R
 53   263.277  239.536   3.883    23.741      2.73 R
 54   260.650  239.536   3.883    21.114      2.43 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N   Mean  Grouping
pH8        18  265.8  A
pH10       18  224.5    B
pH6        18  149.7      C
mf         18  116.2        D
control    18   32.3          E

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence
```

```
starchamount   N   Mean  Grouping
80            30  229.8  A
70            30  135.4     B
60            30  107.8        C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N   Mean  Grouping
pH8        80            6  393.0  A
pH10       80            6  311.4     B
pH6        80            6  239.5        C
pH8        70            6  221.4        C
pH10       70            6  197.9           D
pH8        60            6  182.9           D E
mf         80            6  166.8              E
pH10       60            6  164.3              E
pH6        70            6  118.2                 F
mf         70            6  108.0                 F G
pH6        60            6   91.3                   G H
mf         60            6   73.8                      H
control    80            6   38.4                         I
control    70            6   31.6                         I
control    60            6   26.8                         I

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

Table B.4 Results for Tukey's mean comparison test for cohesiveness values of fresh bread samples with hpmc

General Linear Model: cohesiveness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for cohesiveness, using Adjusted SS for Tests

Source                  DF   Seq SS   Adj SS   Adj MS      F      P
treatment                4  1.88674  1.88674  0.47169  77.71  0.000
starchamount             2  0.07799  0.07799  0.03900   6.42  0.003
treatment*starchamount   8  0.04895  0.04895  0.00612   1.01  0.437
Error                   75  0.45524  0.45524  0.00607
Total                   89  2.46892


S = 0.0779092   R-Sq = 81.56%   R-Sq(adj) = 78.12%
```

Unusual Observations for cohesiveness

Obs	cohesiveness	Fit	SE Fit	Residual	St Resid	
28	0.622791	0.801584	0.031806	-0.178793	-2.51	R
32	0.583173	0.766134	0.031806	-0.182961	-2.57	R
54	0.245367	0.411411	0.031806	-0.166044	-2.33	R
55	0.782497	0.586740	0.031806	0.195757	2.75	R
58	0.392945	0.586740	0.031806	-0.193796	-2.72	R
81	0.759100	0.613977	0.031806	0.145123	2.04	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	N	Mean	Grouping
control	18	0.9	A
mf	18	0.8	A
pH10	18	0.6	B
pH8	18	0.6	B
pH6	18	0.5	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	N	Mean	Grouping
60	30	0.7	A
70	30	0.7	A B
80	30	0.6	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	N	Mean	Grouping
control	70	6	0.9	A
control	60	6	0.9	A
mf	60	6	0.9	A
control	80	6	0.8	A
mf	70	6	0.8	A
mf	80	6	0.8	A B
pH10	70	6	0.6	B C
pH10	80	6	0.6	C
pH10	60	6	0.6	C
pH8	60	6	0.6	C
pH8	70	6	0.6	C
pH6	60	6	0.6	C D
pH8	80	6	0.5	C D
pH6	70	6	0.5	C D
pH6	80	6	0.4	D

Means that do not share a letter are significantly different.

Table B.5 Results for Tukey's mean comparison test for springiness values of fresh bread samples with hpmc

General Linear Model: springiness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for springeness, using Adjusted SS for Tests

Source                  DF    Seq SS   Adj SS   Adj MS      F      P
treatment                4   92.5286  92.5286  23.1322  97.61  0.000
starchamount             2    0.9732   0.9732   0.4866   2.05  0.135
treatment*starchamount   8    0.5609   0.5609   0.0701   0.30  0.965
Error                   75   17.7732  17.7732   0.2370
Total                   89  111.8359


S = 0.486802   R-Sq = 84.11%   R-Sq(adj) = 81.14%


Unusual Observations for springeness

Obs  springeness      Fit   SE Fit  Residual  St Resid
  3      5.12000  6.15667  0.19874  -1.03667     -2.33 R
 11      4.84500  5.82417  0.19874  -0.97917     -2.20 R
 36      6.40000  5.50500  0.19874   0.89500      2.01 R
 81      2.75000  3.87583  0.19874  -1.12583     -2.53 R
 82      4.91000  3.87583  0.19874   1.03417      2.33 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N  Mean  Grouping
control    18   6.0  A
mf         18   5.7  A
pH6        18   3.9    B
pH10       18   3.8    B
pH8        18   3.6    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

starchamount   N  Mean  Grouping
60            30   4.7  A
70            30   4.5  A
80            30   4.5  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N  Mean  Grouping
```

```
control    60            6   6.2  A
control    80            6   6.0  A
mf         60            6   5.9  A
control    70            6   5.8  A
mf         70            6   5.6  A
mf         80            6   5.5  A
pH6        60            6   4.1    B
pH10       70            6   3.9    B
pH10       60            6   3.9    B
pH6        80            6   3.8    B
pH6        70            6   3.7    B
pH8        60            6   3.7    B
pH10       80            6   3.7    B
pH8        70            6   3.7    B
pH8        80            6   3.6    B

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

Table B.6 Results for Tukey's mean comparison test for chewiness values of fresh bread samples with hpmc

General Linear Model: chewiness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for chewiness, using Adjusted SS for Tests

Source                   DF   Seq SS   Adj SS  Adj MS      F      P
treatment                 4  2152008  2152008  538002  89.03  0.000
starchamount              2   937219   937219  468610  77.55  0.000
treatment*starchamount    8   233304   233304   29163   4.83  0.000
Error                    75   453199   453199    6043
Total                    89  3775730


S = 77.7345   R-Sq = 88.00%   R-Sq(adj) = 85.76%


Unusual Observations for chewiness

Obs  chewiness     Fit  SE Fit  Residual  St Resid
 28     343.70  487.08   31.73   -143.39     -2.02 R
 32     509.58  705.99   31.73   -196.41     -2.77 R
 36     864.63  705.99   31.73    158.64      2.24 R
 71    1051.62  757.29   31.73    294.33      4.15 R
 87     521.05  684.03   31.73   -162.98     -2.30 R

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

```
Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N   Mean  Grouping
pH8        18  542.5  A
mf         18  524.3  A
pH10       18  506.9  A
pH6        18  266.4    B
control    18  166.5      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

starchamount   N   Mean  Grouping
80            30  541.3  A
70            30  361.8    B
60            30  300.9      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N   Mean  Grouping
pH8        80            6  757.3  A
mf         80            6  706.0  A
pH10       80            6  684.0  A
mf         70            6  487.1    B
pH8        70            6  478.1    B
pH10       70            6  460.2    B
pH8        60            6  392.2    B
mf         60            6  379.8    B
pH10       60            6  376.5    B C
pH6        80            6  367.0    B C D
pH6        70            6  220.8      C D E
pH6        60            6  211.4        D E
control    80            6  192.2          E
control    70            6  162.6          E
control    60            6  144.7          E

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

Table B.7 Results for Tukey's mean comparison test for hardness values of bread samples with hpmc which stored for 1 day

General Linear Model: hardness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for hardness, using Adjusted SS for Tests
```

Source	DF	Seq SS	Adj SS	Adj MS	F	P
treatment	4	2127163	2127163	531791	3438.60	0.000
starchamount	2	1641094	1641094	820547	5305.72	0.000
treatment*starchamount	8	577273	577273	72159	466.59	0.000
Error	75	11599	11599	155		
Total	89	4357128				

S = 12.4360 R-Sq = 99.73% R-Sq(adj) = 99.68%

Unusual Observations for hardness

Obs	hardness	Fit	SE Fit	Residual	St Resid	
51	576.199	551.900	5.077	24.299	2.14	R
53	522.241	551.900	5.077	-29.659	-2.61	R
64	501.177	473.065	5.077	28.112	2.48	R
65	499.139	473.065	5.077	26.074	2.30	R
68	907.685	943.787	5.077	-36.102	-3.18	R
71	983.868	943.787	5.077	40.081	3.53	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	N	Mean	Grouping
pH8	18	583.6	A
pH10	18	360.6	B
pH6	18	343.7	C
mf	18	302.8	D
control	18	101.2	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	N	Mean	Grouping
80	30	520.2	A
70	30	297.9	B
60	30	197.0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	N	Mean	Grouping
pH8	80	6	943.8	A
pH6	80	6	551.9	B
pH10	80	6	513.3	C
mf	80	6	475.4	D
pH8	70	6	473.1	D
pH8	60	6	334.0	E
pH10	70	6	332.6	E
pH6	70	6	309.6	E
mf	70	6	269.6	F
pH10	60	6	235.9	G
pH6	60	6	169.5	H
mf	60	6	163.2	H
control	80	6	116.8	I
control	70	6	104.5	I J

```
control    60           6   82.3                    J

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

Table B.8 Results for Tukey's mean comparison test for cohesiveness values of bread samples with hpmc which stored for 1 day

General Linear Model: cohesiveness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for cohesiveness, using Adjusted SS for Tests

Source                  DF   Seq SS   Adj SS   Adj MS      F      P
treatment                4  1.26778  1.26778  0.31695  45.99  0.000
starchamount             2  0.34676  0.34676  0.17338  25.16  0.000
treatment*starchamount   8  0.09949  0.09949  0.01244   1.80  0.089
Error                   75  0.51691  0.51691  0.00689
Total                   89  2.23094


S = 0.0830187   R-Sq = 76.83%   R-Sq(adj) = 72.50%


Unusual Observations for cohesiveness

Obs  cohesiveness       Fit    SE Fit   Residual  St Resid
  2      0.583173  0.745247  0.033892  -0.162074     -2.14 R
 10      0.775822  0.620127  0.033892   0.155695      2.05 R
 22      0.824327  0.650345  0.033892   0.173982      2.30 R
 24      0.413144  0.650345  0.033892  -0.237201     -3.13 R
 40      0.530669  0.347294  0.033892   0.183375      2.42 R
 60      0.286011  0.443758  0.033892  -0.157747     -2.08 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N  Mean  Grouping
control    18   0.6  A
mf         18   0.5    B
pH10       18   0.4    B C
pH8        18   0.4      C D
pH6        18   0.3        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence
```

```
starchamount   N  Mean  Grouping
60            30   0.5  A
70            30   0.4    B
80            30   0.4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N  Mean  Grouping
control    60            6   0.7  A
mf         60            6   0.7  A B
control    70            6   0.6  A B C
control    80            6   0.6    B C D
pH10       60            6   0.5      C D E
pH8        60            6   0.4        D E
mf         70            6   0.4        D E
pH10       80            6   0.4        D E
pH10       70            6   0.4        D E
mf         80            6   0.4        D E
pH6        60            6   0.3          E F
pH8        70            6   0.3          E F
pH8        80            6   0.3          E F
pH6        70            6   0.3          E F
pH6        80            6   0.2            F

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

Table B.9 Results for Tukey's mean comparison test for springiness values of bread samples with hpmc which stored for 1 day

General Linear Model: springiness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for springeness, using Adjusted SS for Tests

Source                  DF   Seq SS   Adj SS   Adj MS      F      P
treatment                4  47.6366  47.6366  11.9091  57.96  0.000
starchamount             2   0.7417   0.7417   0.3708   1.80  0.172
treatment*starchamount   8   2.0928   2.0928   0.2616   1.27  0.270
Error                   75  15.4108  15.4108   0.2055
Total                   89  65.8820


S = 0.453297   R-Sq = 76.61%   R-Sq(adj) = 72.24%


Unusual Observations for springeness
```

Obs	springeness	Fit	SE Fit	Residual	St Resid
15	4.10000	4.95000	0.18506	-0.85000	-2.05 R
45	2.70000	3.60333	0.18506	-0.90333	-2.18 R
47	5.11000	3.60333	0.18506	1.50667	3.64 R
50	5.03000	3.61667	0.18506	1.41333	3.42 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	N	Mean	Grouping
control	18	5.1	A
mf	18	5.0	A
pH10	18	3.7	B
pH6	18	3.6	B
pH8	18	3.6	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	N	Mean	Grouping
60	30	4.3	A
70	30	4.2	A
80	30	4.1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	N	Mean	Grouping
control	60	6	5.5	A
mf	60	6	5.4	A
mf	70	6	5.0	A
control	80	6	4.9	A
control	70	6	4.9	A
mf	80	6	4.7	A
pH8	70	6	3.7	B
pH10	80	6	3.7	B
pH10	70	6	3.7	B
pH10	60	6	3.7	B
pH6	80	6	3.6	B
pH6	70	6	3.6	B
pH8	80	6	3.6	B
pH6	60	6	3.5	B
pH8	60	6	3.5	B

Means that do not share a letter are significantly different.

Table B.10 Results for Tukey's mean comparison test for chewiness values of bread samples with hpmc which stored for 1 day

General Linear Model: chewiness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for chewiness, using Adjusted SS for Tests

Source                  DF   Seq SS   Adj SS  Adj MS      F      P
treatment                4  2565751  2565751  641438  47.11  0.000
starchamount             2  1497566  1497566  748783  54.99  0.000
treatment*starchamount   8   687005   687005   85876   6.31  0.000
Error                   75  1021214  1021214   13616
Total                   89  5771537


S = 116.688   R-Sq = 82.31%   R-Sq(adj) = 79.00%


Unusual Observations for chewiness

Obs  chewiness      Fit  SE Fit  Residual  St Resid
 68     834.23  1070.89   47.64   -236.66     -2.22 R
 72    1504.62  1070.89   47.64    433.73      4.07 R
 84     741.47   505.05   47.64    236.42      2.22 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N   Mean  Grouping
pH8        18  718.9  A
mf         18  689.3  A
pH10       18  565.2    B
pH6        18  332.1      C
control    18  327.2      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

starchamount   N   Mean  Grouping
80            30  705.5  A
70            30  467.8    B
60            30  406.4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N    Mean  Grouping
pH8        80            6  1070.9  A
mf         80            6   905.3  A B
pH10       80            6   786.2    B C
```

```
mf          70            6   591.8      C D
pH8         70            6   572.1      C D E
mf          60            6   570.9      C D E
pH8         60            6   513.8        D E F
pH10        70            6   505.0        D E F
pH6         80            6   440.2        D E F G
pH10        60            6   404.2        D E F G
pH6         70            6   350.2          E F G
control     60            6   336.9          E F G
control     80            6   325.0            F G
control     70            6   319.7            F G
pH6         60            6   206.0              G

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

Table B.11 Results for Tukey's mean comparison test for hardness values of bread samples with hpmc which stored for 2 days

General Linear Model: hardness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for hardness, using Adjusted SS for Tests

Source                  DF    Seq SS   Adj SS   Adj MS        F      P
treatment                4   5592936  5592936  1398234  3024.28  0.000
starchamount             2   3509298  3509298  1754649  3795.18  0.000
treatment*starchamount   8   1497541  1497541   187193   404.88  0.000
Error                   75     34675    34675      462
Total                   89  10634450


S = 21.5020   R-Sq = 99.67%   R-Sq(adj) = 99.61%


Unusual Observations for hardness

Obs  hardness      Fit  SE Fit  Residual  St Resid
 50   1107.36  1051.52    8.78     55.84      2.84 R
 51   1009.69  1051.52    8.78    -41.82     -2.13 R
 53   1092.26  1051.52    8.78     40.75      2.08 R
 54   1005.55  1051.52    8.78    -45.97     -2.34 R
 62    693.28   765.57    8.78    -72.29     -3.68 R
 79    689.41   648.74    8.78     40.68      2.07 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N   Mean  Grouping
```

```
pH8         18  870.7  A
pH10        18  633.5    B
pH6         18  630.6    B
mf          18  468.6      C
control     18  116.5        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

starchamount   N   Mean  Grouping
80            30  806.8  A
70            30  494.2    B
60            30  330.9      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N    Mean  Grouping
pH8        80            6  1347.1  A
pH6        80            6  1051.5    B
pH8        70            6   765.6      C
pH10       80            6   759.6      C
mf         80            6   751.2      C
pH10       70            6   648.7        D
pH6        70            6   526.9          E
pH8        60            6   499.4          E
pH10       60            6   492.3          E
mf         70            6   411.0            F
pH6        60            6   313.5              G
mf         60            6   243.5                H
control    80            6   124.8                  I
control    70            6   118.7                  I
control    60            6   105.9                  I

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

Table B.12 Results for Tukey's mean comparison test for cohesiveness values of bread samples with hpmc which stored for 2 days

General Linear Model: cohesiveness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for cohesiveness, using Adjusted SS for Tests

Source                 DF   Seq SS   Adj SS   Adj MS      F      P
treatment               4  1.24366  1.24366  0.31091  54.93  0.000
```

starchamount	2	0.26900	0.26900	0.13450	23.76	0.000
treatment*starchamount	8	0.15834	0.15834	0.01979	3.50	0.002
Error	75	0.42452	0.42452	0.00566		
Total	89	2.09552				

S = 0.0752346 R-Sq = 79.74% R-Sq(adj) = 75.96%

Unusual Observations for cohesiveness

Obs	cohesiveness	Fit	SE Fit	Residual	St Resid
4	0.798413	0.642561	0.030714	0.155852	2.27 R
7	0.444299	0.605634	0.030714	-0.161335	-2.35 R
9	0.865985	0.605634	0.030714	0.260351	3.79 R
18	0.766433	0.522223	0.030714	0.244210	3.56 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	N	Mean	Grouping
control	18	0.6	A
mf	18	0.4	B
pH10	18	0.4	B
pH8	18	0.3	C
pH6	18	0.2	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	N	Mean	Grouping
60	30	0.5	A
70	30	0.4	B
80	30	0.3	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	N	Mean	Grouping
control	60	6	0.6	A
mf	60	6	0.6	A
control	70	6	0.6	A
control	80	6	0.5	A B
pH10	60	6	0.4	B C
pH10	70	6	0.4	B C D
mf	70	6	0.4	B C D
pH10	80	6	0.4	B C D
pH8	60	6	0.3	C D
mf	80	6	0.3	C D
pH8	70	6	0.3	C D E
pH6	60	6	0.3	C D E
pH8	80	6	0.3	D E
pH6	70	6	0.3	D E
pH6	80	6	0.2	E

Means that do not share a letter are significantly different.

Table B.13 Results for Tukey's mean comparison test for springiness values of bread samples with hpmc which stored for 2 days

General Linear Model: springiness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for springeness, using Adjusted SS for Tests

Source                  DF   Seq SS   Adj SS   Adj MS      F      P
treatment                4  41.0666  41.0666  10.2667  59.55  0.000
starchamount             2   0.2949   0.2949   0.1475   0.86  0.429
treatment*starchamount   8   0.5511   0.5511   0.0689   0.40  0.917
Error                   75  12.9298  12.9298   0.1724
Total                   89  54.8425


S = 0.415207   R-Sq = 76.42%   R-Sq(adj) = 72.02%


Unusual Observations for springeness

Obs  springeness      Fit   SE Fit  Residual  St Resid
  8      4.10000  4.96333  0.16951  -0.86333     -2.28 R
 10      6.04500  4.96333  0.16951   1.08167      2.85 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N  Mean  Grouping
control    18   5.1  A
mf         18   4.5    B
pH10       18   3.5      C
pH6        18   3.5      C
pH8        18   3.4      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

starchamount   N  Mean  Grouping
60            30   4.1  A
80            30   4.0  A
70            30   4.0  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N  Mean  Grouping
control    60            6   5.3  A
control    80            6   5.0  A
control    70            6   5.0  A
mf         70            6   4.6  A
```

```
mf          80            6   4.6  A
mf          60            6   4.5  A
pH10        60            6   3.6     B
pH6         60            6   3.6     B
pH6         80            6   3.6     B
pH10        70            6   3.5     B
pH10        80            6   3.5     B
pH8         60            6   3.5     B
pH8         70            6   3.4     B
pH8         80            6   3.4     B
pH6         70            6   3.3     B

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

Table B.14 Results for Tukey's mean comparison test for chewiness values of bread samples with hpmc which stored for 2 days

General Linear Model: chewiness versus treatment; starchamount

```
Factor         Type   Levels  Values
treatment      fixed       5  control; mf; pH10; pH6; pH8
starchamount   fixed       3  60; 70; 80


Analysis of Variance for chewiness, using Adjusted SS for Tests

Source                   DF   Seq SS   Adj SS   Adj MS      F      P
treatment                 4  4858602  4858602  1214650  71.90  0.000
starchamount              2  1615163  1615163   807582  47.80  0.000
treatment*starchamount    8   998232   998232   124779   7.39  0.000
Error                    75  1267015  1267015    16894
Total                    89  8739012


S = 129.975   R-Sq = 85.50%   R-Sq(adj) = 82.80%


Unusual Observations for chewiness

Obs  chewiness      Fit  SE Fit  Residual  St Resid
 31     889.01  1138.56   53.06   -249.55     -2.10 R
 36    1560.44  1138.56   53.06    421.88      3.56 R
 49     847.18   598.19   53.06    248.99      2.10 R
 68    1516.17  1278.23   53.06    237.94      2.01 R
 72     985.12  1278.23   53.06   -293.11     -2.47 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N   Mean  Grouping
pH8        18  896.9  A
pH10       18  889.8  A
```

```
mf          18  848.5  A
pH6         18  475.1    B
control     18  349.6      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

starchamount   N   Mean  Grouping
80            30  870.6  A
70            30  657.3    B
60            30  548.0      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N    Mean  Grouping
pH8        80            6  1278.2  A
mf         80            6  1138.6  A B
pH10       80            6  1010.9    B C
pH10       70            6   890.5    B C D
pH8        70            6   817.7      C D E
pH10       60            6   768.0      C D E
mf         70            6   722.3        D E F
mf         60            6   684.8        D E F
pH6        80            6   598.2          E F G
pH8        60            6   594.9          E F G H
pH6        70            6   493.6            F G H I
control    70            6   362.4              G H I
control    60            6   359.1              G H I
pH6        60            6   333.6                H I
control    80            6   327.3                  I

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

Table B.15 Results for Tukey's mean comparison test for hardness values of bread samples with hpmc which stored for 3 days

General Linear Model: hardness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for hardness, using Adjusted SS for Tests

Source                  DF    Seq SS    Adj SS   Adj MS        F      P
treatment                4  10202773  10202773  2550693  4017.01  0.000
starchamount             2   6723362   6723362  3361681  5294.21  0.000
treatment*starchamount   8   2371735   2371735   296467   466.90  0.000
```

```
Error                   75     47623     47623      635
Total                   89  19345493
```

```
S = 25.1987   R-Sq = 99.75%   R-Sq(adj) = 99.71%
```

Unusual Observations for hardness

Obs	hardness	Fit	SE Fit	Residual	St Resid	
35	1114.03	1163.86	10.29	-49.83	-2.17	R
54	1291.51	1242.68	10.29	48.82	2.12	R
67	1708.78	1793.29	10.29	-84.51	-3.67	R
68	1736.37	1793.29	10.29	-56.92	-2.47	R
69	1850.94	1793.29	10.29	57.65	2.51	R
71	1871.32	1793.29	10.29	78.03	3.39	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	N	Mean	Grouping
pH8	18	1149.6	A
pH10	18	920.5	B
pH6	18	760.1	C
mf	18	718.3	D
control	18	135.3	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	N	Mean	Grouping
80	30	1101.3	A
70	30	665.9	B
60	30	443.1	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	N	Mean	Grouping				
pH8	80	6	1793.3	A				
pH6	80	6	1242.7		B			
mf	80	6	1163.9			C		
pH10	80	6	1158.9			C		
pH8	70	6	1008.8				D	
pH10	70	6	967.1				D	
pH8	60	6	646.8					E
pH10	60	6	635.4					E
pH6	70	6	619.9					E
mf	70	6	597.2					E
pH6	60	6	417.6				F	
mf	60	6	393.9				F	
control	80	6	147.6					G
control	70	6	136.5					G
control	60	6	121.9					G

Means that do not share a letter are significantly different.

Table B.16 Results for Tukey's mean comparison test for cohesiveness values of bread samples with hpmc which stored for 3 days

General Linear Model: cohesiveness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for cohesiveness, using Adjusted SS for Tests

Source                  DF    Seq SS    Adj SS    Adj MS      F      P
treatment                4  0.831045  0.831045  0.207761  53.13  0.000
starchamount             2  0.089917  0.089917  0.044959  11.50  0.000
treatment*starchamount   8  0.035238  0.035238  0.004405   1.13  0.356
Error                   75  0.293296  0.293296  0.003911
Total                   89  1.249496


S = 0.0625349   R-Sq = 76.53%   R-Sq(adj) = 72.15%


Unusual Observations for cohesiveness

Obs  cohesiveness       Fit    SE Fit  Residual  St Resid
  7      0.653466  0.526793  0.025530  0.126673      2.22 R
 21      0.686208  0.436478  0.025530  0.249730      4.37 R
 41      0.431109  0.276829  0.025530  0.154279      2.70 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N  Mean  Grouping
control    18   0.5  A
mf         18   0.4    B
pH10       18   0.4    B C
pH8        18   0.3      C
pH6        18   0.2        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

starchamount   N  Mean  Grouping
60            30   0.4  A
70            30   0.4  A
80            30   0.3    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N  Mean  Grouping
control    60            6   0.6  A
control    70            6   0.5  A B
control    80            6   0.5  A B C
```

```
mf          60            6   0.4    B C D
pH10        60            6   0.4      C D E
mf          70            6   0.4      C D E
pH10        80            6   0.4      C D E
pH10        70            6   0.3      C D E
mf          80            6   0.3        D E
pH8         60            6   0.3        D E
pH8         70            6   0.3        D E
pH8         80            6   0.3          E F
pH6         60            6   0.3          E F
pH6         70            6   0.3          E F
pH6         80            6   0.2            F

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

Table B.17 Results for Tukey's mean comparison test for springiness values of bread samples with hpmc which stored for 3 days

General Linear Model: springiness versus treatment; starchamount

```
Factor         Type   Levels  Values
treatment      fixed       5  control; mf; pH10; pH6; pH8
starchamount   fixed       3  60; 70; 80


Analysis of Variance for springeness, using Adjusted SS for Tests

Source                   DF   Seq SS   Adj SS   Adj MS      F      P
treatment                 4  44.9647  44.9647  11.2412  73.78  0.000
starchamount              2   0.7878   0.7878   0.3939   2.59  0.082
treatment*starchamount    8   0.2386   0.2386   0.0298   0.20  0.991
Error                    75  11.4272  11.4272   0.1524
Total                    89  57.4182


S = 0.390337   R-Sq = 80.10%   R-Sq(adj) = 76.38%


Unusual Observations for springeness

Obs  springeness      Fit   SE Fit  Residual  St Resid
  6      4.16500  5.01083  0.15935  -0.84583     -2.37 R
 39      4.07500  3.26667  0.15935   0.80833      2.27 R
 53      3.82000  3.06583  0.15935   0.75417      2.12 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N  Mean  Grouping
control    18   4.8  A
mf         18   4.3    B
pH10       18   3.3      C
```

```
pH6         18   3.2      C
pH8         18   3.1      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

starchamount   N  Mean  Grouping
60            30   3.9  A
70            30   3.7  A
80            30   3.6  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N  Mean  Grouping
control    60            6   5.0  A
control    70            6   4.8  A B
control    80            6   4.7  A B
mf         60            6   4.4  A B
mf         70            6   4.4  A B
mf         80            6   4.2    B C
pH10       60            6   3.4      C D
pH10       70            6   3.4        D
pH10       80            6   3.3        D
pH6        60            6   3.3        D
pH8        60            6   3.2        D
pH6        70            6   3.1        D
pH6        80            6   3.1        D
pH8        80            6   3.0        D
pH8        70            6   2.9        D

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

Table B.18 Results for Tukey's mean comparison test for chewiness values of bread samples with hpmc which stored for 3 days

General Linear Model: chewiness versus treatment; starchamount

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80


Analysis of Variance for chewiness, using Adjusted SS for Tests

Source                  DF    Seq SS   Adj SS   Adj MS      F      P
treatment                4   9631773  9631773  2407943  80.27  0.000
starchamount             2   3960165  3960165  1980082  66.01  0.000
treatment*starchamount   8   2110895  2110895   263862   8.80  0.000
Error                   75   2249906  2249906    29999
```

```
Total                   89  17952738


S = 173.201   R-Sq = 87.47%   R-Sq(adj) = 85.13%


Unusual Observations for chewiness

Obs  chewiness      Fit  SE Fit  Residual  St Resid
 21    1100.94   755.75   70.71    345.19      2.18 R
 31    1098.97  1670.46   70.71   -571.49     -3.61 R
 32    2221.45  1670.46   70.71    550.99      3.48 R
 34    2054.31  1670.46   70.71    383.85      2.43 R
 35    1323.89  1670.46   70.71   -346.57     -2.19 R
 53    1051.86   665.17   70.71    386.70      2.45 R
 71    1831.27  1508.69   70.71    322.57      2.04 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N    Mean  Grouping
mf         18  1115.8  A
pH10       18  1084.2  A
pH8        18  1061.7  A
pH6        18   514.7    B
control    18   344.3      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

starchamount   N    Mean  Grouping
80            30  1100.6  A
70            30   779.0    B
60            30   592.8      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

treatment  starchamount  N    Mean  Grouping
mf         80            6  1670.5  A
pH8        80            6  1508.7  A
pH10       80            6  1330.6  A B
pH10       70            6  1133.8    B C
pH8        70            6   981.3    B C D
mf         70            6   921.0      C D
pH10       60            6   788.1      C D E
mf         60            6   755.7        D E
pH8        60            6   695.0        D E F
pH6        80            6   665.2        D E F G
pH6        70            6   512.1          E F G
pH6        60            6   366.7            F G
control    60            6   358.3            F G
control    70            6   346.5            F G
control    80            6   328.1              G

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

Table B.19 Results for Tukey's mean comparison test for hardness values of bread samples with hpmc for all parameters

General Linear Model: hardness versus treatment; starchamount; storage

```
Factor        Type   Levels  Values
treatment     fixed       5  control; mf; pH10; pH6; pH8
starchamount  fixed       3  60; 70; 80
storage       fixed       4  0; 1; 2; 3


Analysis of Variance for hardness, using Adjusted SS for Tests

Source                            DF    Seq SS    Adj SS   Adj MS         F
treatment                          4  14862867  14862867  3715717  11071.55
starchamount                       2   9732525   9732525  4866263  14499.77
storage                            3  16995132  16995132  5665044  16879.86
treatment*starchamount             8   3418895   3418895   427362   1273.39
treatment*storage                 12   3666104   3666104   305509    910.31
starchamount*storage               6   2386955   2386955   397826   1185.38
treatment*starchamount*storage    24   1105265   1105265    46053    137.22
Error                            300    100683    100683      336
Total                            359  52268426

Source                               P
treatment                        0.000
starchamount                     0.000
storage                          0.000
treatment*starchamount           0.000
treatment*storage                0.000
starchamount*storage             0.000
treatment*starchamount*storage   0.000
Error
Total


S = 18.3197   R-Sq = 99.81%   R-Sq(adj) = 99.77%


Unusual Observations for hardness

Obs  hardness      Fit  SE Fit  Residual  St Resid
158    907.68   943.79    7.48    -36.10     -2.16 R
161    983.87   943.79    7.48     40.08      2.40 R
230   1107.36  1051.52    7.48     55.84      3.34 R
231   1009.69  1051.52    7.48    -41.82     -2.50 R
232   1017.35  1051.52    7.48    -34.17     -2.04 R
233   1092.26  1051.52    7.48     40.75      2.44 R
234   1005.55  1051.52    7.48    -45.97     -2.75 R
242    693.28   765.57    7.48    -72.29     -4.32 R
247   1309.25  1347.09    7.48    -37.84     -2.26 R
250   1381.22  1347.09    7.48     34.13      2.04 R
251   1311.73  1347.09    7.48    -35.36     -2.11 R
259    689.41   648.74    7.48     40.68      2.43 R
262    611.18   648.74    7.48    -37.55     -2.25 R
302   1198.02  1163.86    7.48     34.16      2.04 R
305   1114.03  1163.86    7.48    -49.83     -2.98 R
324   1291.51  1242.68    7.48     48.82      2.92 R
325    685.83   646.84    7.48     39.00      2.33 R
337   1708.78  1793.29    7.48    -84.51     -5.05 R
```

338	1736.37	1793.29	7.48	-56.92	-3.40 R
339	1850.94	1793.29	7.48	57.65	3.45 R
340	1830.35	1793.29	7.48	37.06	2.22 R
341	1871.32	1793.29	7.48	78.03	4.67 R
355	1113.91	1158.87	7.48	-44.97	-2.69 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	N	Mean	Grouping
pH8	72	717.4	A
pH10	72	534.8	B
pH6	72	471.0	C
mf	72	401.5	D
control	72	96.3	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	N	Mean	Grouping
80	120	664.5	A
70	120	398.3	B
60	120	269.7	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

storage	N	Mean	Grouping
3	90	736.8	A
2	90	544.0	B
1	90	338.4	C
0	90	157.7	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	N	Mean	Grouping
pH8	80	24	1119.3	A
pH6	80	24	771.4	B
pH10	80	24	685.8	C
mf	80	24	639.3	D
pH8	70	24	617.2	E
pH10	70	24	536.6	F
pH8	60	24	415.8	G
pH6	70	24	393.6	H
pH10	60	24	382.0	H
mf	70	24	346.5	I
pH6	60	24	248.0	J
mf	60	24	218.6	K
control	80	24	106.9	L
control	70	24	97.8	L M
control	60	24	84.2	M

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	storage	N	Mean	Grouping
pH8	3	18	1149.6	A
pH10	3	18	920.5	B
pH8	2	18	870.7	C
pH6	3	18	760.1	D
mf	3	18	718.3	E
pH10	2	18	633.5	F
pH6	2	18	630.6	F
pH8	1	18	583.6	G
mf	2	18	468.6	H
pH10	1	18	360.6	I
pH6	1	18	343.7	I
mf	1	18	302.8	J
pH8	0	18	265.8	K
pH10	0	18	224.5	L
pH6	0	18	149.7	M
control	3	18	135.3	M N
control	2	18	116.5	N O
mf	0	18	116.2	N O
control	1	18	101.2	O
control	0	18	32.3	P

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	storage	N	Mean	Grouping
80	3	30	1101.3	A
80	2	30	806.8	B
70	3	30	665.9	C
80	1	30	520.2	D
70	2	30	494.2	E
60	3	30	443.1	F
60	2	30	330.9	G
70	1	30	297.9	H
80	0	30	229.8	I
60	1	30	197.0	J
70	0	30	135.4	K
60	0	30	107.8	L

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	storage	N	Mean
pH8	80	3	6	1793.3
pH8	80	2	6	1347.1
pH6	80	3	6	1242.7
mf	80	3	6	1163.9
pH10	80	3	6	1158.9
pH6	80	2	6	1051.5
pH8	70	3	6	1008.8
pH10	70	3	6	967.1
pH8	80	1	6	943.8
pH8	70	2	6	765.6
pH10	80	2	6	759.6
mf	80	2	6	751.2
pH10	70	2	6	648.7
pH8	60	3	6	646.8
pH10	60	3	6	635.4

pH6	70	3	6	619.9
mf	70	3	6	597.2
pH6	80	1	6	551.9
pH6	70	2	6	526.9
pH10	80	1	6	513.3
pH8	60	2	6	499.4
pH10	60	2	6	492.3
mf	80	1	6	475.4
pH8	70	1	6	473.1
pH6	60	3	6	417.6
mf	70	2	6	411.0
mf	60	3	6	393.9
pH8	80	0	6	393.0
pH8	60	1	6	334.0
pH10	70	1	6	332.6
pH6	60	2	6	313.5
pH10	80	0	6	311.4
pH6	70	1	6	309.6
mf	70	1	6	269.6
mf	60	2	6	243.5
pH6	80	0	6	239.5
pH10	60	1	6	235.9
pH8	70	0	6	221.4
pH10	70	0	6	197.9
pH8	60	0	6	182.9
pH6	60	1	6	169.5
mf	80	0	6	166.8
pH10	60	0	6	164.3
mf	60	1	6	163.2
control	80	3	6	147.6
control	70	3	6	136.5
control	80	2	6	124.8
control	60	3	6	121.9
control	70	2	6	118.7
pH6	70	0	6	118.2
control	80	1	6	116.8
mf	70	0	6	108.0
control	60	2	6	105.9
control	70	1	6	104.5
pH6	60	0	6	91.3
control	60	1	6	82.3
mf	60	0	6	73.8
control	80	0	6	38.4
control	70	0	6	31.6
control	60	0	6	26.8

treatment	starchamount	storage	Grouping
pH8	80	3	A
pH8	80	2	B
pH6	80	3	C
mf	80	3	D
pH10	80	3	D
pH6	80	2	E
pH8	70	3	E F
pH10	70	3	F G
pH8	80	1	G
pH8	70	2	H
pH10	80	2	H
mf	80	2	H
pH10	70	2	I
pH8	60	3	I
pH10	60	3	I J
pH6	70	3	I J
mf	70	3	J

```
pH6         80            1                          K
pH6         70            2                          K L
pH10        80            1                          K L M
pH8         60            2                            L M
pH10        60            2                            L M
mf          80            1                              M
pH8         70            1                              M
pH6         60            3                                N
mf          70            2                                N
mf          60            3                                N
pH8         80            0                                N
pH8         60            1                                  O
pH10        70            1                                  O
pH6         60            2                                  O
pH10        80            0                                  O P
pH6         70            1                                  O P
mf          70            1                                    P Q
mf          60            2                                      Q R
pH6         80            0                                      Q R S
pH10        60            1                                      Q R S
pH8         70            0                                        R S T
pH10        70            0                                         S T U
pH8         60            0                                         T U V
pH6         60            1                                         U V W
mf          80            0                                       U V W X
pH10        60            0                                     U V W X Y
mf          60            1                                     U V W X Y
control     80            3                                     V W X Y Z
control     70            3                                       W X Y Z
control     80            2                                    X Y Z AA
control     60            3                                       Y Z AA
control     70            2                                          Z AA
pH6         70            0                                          Z AA
control     80            1                                     Z AA AB
mf          70            0                                     Z AA AB
control     60            2                                     Z AA AB
control     70            1                                     Z AA AB
pH6         60            0                                       AA AB
control     60            1                                       AA AB
mf          60            0                                       AB AC
control     80            0                                       AC AD
control     70            0                                       AC AD
control    60          0                                           AD

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

Table B.20 Results for Tukey's mean comparison test for cohesiveness values of bread samples with hpmc for all parameters

General Linear Model: cohesiveness versus treatment; starchamount; ...

```
Factor        Type   Levels  Values
```

treatment	fixed	5	control; mf; pH10; pH6; pH8
starchamount	fixed	3	60; 70; 80
storage	fixed	4	0; 1; 2; 3

Analysis of Variance for cohesiveness, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
treatment	4	4.95254	4.95254	1.23814	219.79	0.000
starchamount	2	0.69178	0.69178	0.34589	61.40	0.000
storage	3	5.06732	5.06732	1.68911	299.85	0.000
treatment*starchamount	8	0.24166	0.24166	0.03021	5.36	0.000
treatment*storage	12	0.27669	0.27669	0.02306	4.09	0.000
starchamount*storage	6	0.09189	0.09189	0.01531	2.72	0.014
treatment*starchamount*storage	24	0.10035	0.10035	0.00418	0.74	0.806
Error	300	1.68996	1.68996	0.00563		
Total	359	13.11219				

S = 0.0750547 R-Sq = 87.11% R-Sq(adj) = 84.58%

Unusual Observations for cohesiveness

Obs	cohesiveness	Fit	SE Fit	Residual	St Resid	
28	0.622791	0.801584	0.030641	-0.178793	-2.61	R
32	0.583173	0.766134	0.030641	-0.182961	-2.67	R
38	0.702307	0.563120	0.030641	0.139187	2.03	R
54	0.245367	0.411411	0.030641	-0.166044	-2.42	R
55	0.782497	0.586740	0.030641	0.195757	2.86	R
58	0.392945	0.586740	0.030641	-0.193796	-2.83	R
81	0.759100	0.613977	0.030641	0.145123	2.12	R
92	0.583173	0.745247	0.030641	-0.162074	-2.37	R
100	0.775822	0.620127	0.030641	0.155695	2.27	R
102	0.480826	0.620127	0.030641	-0.139300	-2.03	R
110	0.800936	0.650345	0.030641	0.150591	2.20	R
111	0.505496	0.650345	0.030641	-0.144849	-2.11	R
112	0.824327	0.650345	0.030641	0.173982	2.54	R
114	0.413144	0.650345	0.030641	-0.237201	-3.46	R
130	0.530669	0.347294	0.030641	0.183375	2.68	R
150	0.286011	0.443758	0.030641	-0.157747	-2.30	R
184	0.798413	0.642561	0.030641	0.155852	2.27	R
187	0.444299	0.605634	0.030641	-0.161335	-2.35	R
189	0.865985	0.605634	0.030641	0.260351	3.80	R
198	0.766433	0.522223	0.030641	0.244210	3.56	R
291	0.686208	0.436478	0.030641	0.249730	3.64	R
311	0.431109	0.276829	0.030641	0.154279	2.25	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	N	Mean	Grouping
control	72	0.7	A
mf	72	0.5	B

pH10	72	0.4	C
pH8	72	0.4	D
pH6	72	0.3	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	N	Mean	Grouping
60	120	0.5	A
70	120	0.5	B
80	120	0.4	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

storage	N	Mean	Grouping
0	90	0.7	A
1	90	0.4	B
2	90	0.4	C
3	90	0.4	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	N	Mean	Grouping
control	60	24	0.7	A
control	70	24	0.7	A B
mf	60	24	0.6	A B
control	80	24	0.6	B
mf	70	24	0.5	C
pH10	60	24	0.5	C
mf	80	24	0.5	C D
pH10	70	24	0.4	C D E
pH10	80	24	0.4	C D E
pH8	60	24	0.4	C D E F
pH8	70	24	0.4	D E F G
pH6	60	24	0.4	E F G
pH8	80	24	0.4	F G
pH6	70	24	0.3	G
pH6	80	24	0.2	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	storage	N	Mean	Grouping
control	0	18	0.9	A
mf	0	18	0.8	A
control	1	18	0.6	B
pH10	0	18	0.6	B C
control	2	18	0.6	B C
pH8	0	18	0.6	B C D
control	3	18	0.5	C D E
mf	1	18	0.5	D E F
pH6	0	18	0.5	D E F
mf	2	18	0.4	E F G
pH10	1	18	0.4	F G H

pH10	2	18	0.4	G H I
mf	3	18	0.4	G H I J
pH8	1	18	0.4	G H I J
pH10	3	18	0.4	H I J
pH8	2	18	0.3	I J K
pH8	3	18	0.3	I J K
pH6	1	18	0.3	J K
pH6	2	18	0.2	K
pH6	3	18	0.2	K

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	storage	N	Mean	Grouping
60	0	30	0.7	A
70	0	30	0.7	A B
80	0	30	0.6	B
60	1	30	0.5	C
60	2	30	0.5	C D
70	1	30	0.4	D E
60	3	30	0.4	E
70	2	30	0.4	E F
80	1	30	0.4	E F G
70	3	30	0.4	E F G
80	2	30	0.3	F G
80	3	30	0.3	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	storage	N	Mean
control	70	0	6	0.9
control	60	0	6	0.9
mf	60	0	6	0.9
control	80	0	6	0.8
mf	70	0	6	0.8
mf	80	0	6	0.8
control	60	1	6	0.7
mf	60	1	6	0.7
control	60	2	6	0.6
mf	60	2	6	0.6
control	70	1	6	0.6
pH10	70	0	6	0.6
control	70	2	6	0.6
pH10	80	0	6	0.6
pH10	60	0	6	0.6
control	60	3	6	0.6
pH8	60	0	6	0.6
pH8	70	0	6	0.6
pH6	60	0	6	0.6
control	80	1	6	0.6
pH8	80	0	6	0.5
control	70	3	6	0.5
control	80	2	6	0.5
pH6	70	0	6	0.5
control	80	3	6	0.5
pH10	60	1	6	0.5
pH8	60	1	6	0.4
mf	70	1	6	0.4
pH10	60	2	6	0.4

mf	60	3	6	0.4
pH10	80	1	6	0.4
pH6	80	0	6	0.4
pH10	70	1	6	0.4
mf	80	1	6	0.4
pH10	70	2	6	0.4
mf	70	2	6	0.4
pH10	80	2	6	0.4
pH10	60	3	6	0.4
mf	70	3	6	0.4
pH10	80	3	6	0.4
pH10	70	3	6	0.3
pH6	60	1	6	0.3
pH8	60	2	6	0.3
mf	80	3	6	0.3
mf	80	2	6	0.3
pH8	60	3	6	0.3
pH8	70	3	6	0.3
pH8	70	1	6	0.3
pH8	80	1	6	0.3
pH8	70	2	6	0.3
pH6	70	1	6	0.3
pH6	60	2	6	0.3
pH8	80	2	6	0.3
pH6	70	2	6	0.3
pH8	80	3	6	0.3
pH6	60	3	6	0.3
pH6	70	3	6	0.3
pH6	80	1	6	0.2
pH6	80	3	6	0.2
pH6	80	2	6	0.2

treatment	starchamount	storage	Grouping
control	70	0	A
control	60	0	A
mf	60	0	A
control	80	0	A
mf	70	0	A B
mf	80	0	A B C
control	60	1	A B C D
mf	60	1	B C D E
control	60	2	B C D E F
mf	60	2	B C D E F G
control	70	1	C D E F G H
pH10	70	0	C D E F G H I
control	70	2	C D E F G H I J
pH10	80	0	C D E F G H I J
pH10	60	0	C D E F G H I J K
control	60	3	C D E F G H I J K
pH8	60	0	D E F G H I J K L
pH8	70	0	D E F G H I J K L
pH6	60	0	E F G H I J K L M
control	80	1	E F G H I J K L M
pH8	80	0	E F G H I J K L M N
control	70	3	E F G H I J K L M N O
control	80	2	E F G H I J K L M N O P
pH6	70	0	E F G H I J K L M N O P Q
control	80	3	F G H I J K L M N O P Q R
pH10	60	1	G H I J K L M N O P Q R
pH8	60	1	H I J K L M N O P Q R S
mf	70	1	I J K L M N O P Q R S T
pH10	60	2	I J K L M N O P Q R S T
mf	60	3	J K L M N O P Q R S T
pH10	80	1	K L M N O P Q R S T

pH6	80	0	L M N O P Q R S T
pH10	70	1	L M N O P Q R S T
mf	80	1	M N O P Q R S T
pH10	70	2	M N O P Q R S T U
mf	70	2	M N O P Q R S T U
pH10	80	2	N O P Q R S T U
pH10	60	3	N O P Q R S T U
mf	70	3	O P Q R S T U
pH10	80	3	O P Q R S T U
pH10	70	3	P Q R S T U V
pH6	60	1	P Q R S T U V
pH8	60	2	Q R S T U V
mf	80	3	Q R S T U V
mf	80	2	Q R S T U V W
pH8	60	3	Q R S T U V W
pH8	70	3	Q R S T U V W
pH8	70	1	Q R S T U V W
pH8	80	1	R S T U V W
pH8	70	2	R S T U V W
pH6	70	1	R S T U V W
pH6	60	2	R S T U V W
pH8	80	2	S T U V W
pH6	70	2	S T U V W
pH8	80	3	S T U V W
pH6	60	3	S T U V W
pH6	70	3	T U V W
pH6	80	1	U V W
pH6	80	3	V W
pH6	80	2	W

Means that do not share a letter are significantly different.

Table B.21 Results for Tukey's mean comparison test for springiness values of fresh bread samples with hpmc for all parameters

General Linear Model: springiness versus treatment; starchamount; ...

Factor	Type	Levels	Values
treatment	fixed	5	control; mf; pH10; pH6; pH8
starchamount	fixed	3	60; 70; 80
storage	fixed	4	0; 1; 2; 3

Analysis of Variance for springeness, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
treatment	4	217.4162	217.4162	54.3540	283.38	0.000
starchamount	2	2.5343	2.5343	1.2671	6.61	0.002
storage	3	34.7167	34.7167	11.5722	60.33	0.000

```
treatment*starchamount             8    1.3070    1.3070   0.1634    0.85
0.558
treatment*storage                 12    8.7804    8.7804   0.7317    3.81
0.000
starchamount*storage               6    0.2633    0.2633   0.0439    0.23
0.967
treatment*starchamount*storage    24    2.1364    2.1364   0.0890    0.46
0.986
Error                            300   57.5410   57.5410   0.1918
Total                            359  324.6953
```

```
S = 0.437954   R-Sq = 82.28%   R-Sq(adj) = 78.79%
```

```
Unusual Observations for springeness

Obs  springeness      Fit   SE Fit  Residual  St Resid
  3      5.12000  6.15667  0.17879  -1.03667     -2.59 R
 11      4.84500  5.82417  0.17879  -0.97917     -2.45 R
 36      6.40000  5.50500  0.17879   0.89500      2.24 R
 79      4.70000  3.87583  0.17879   0.82417      2.06 R
 81      2.75000  3.87583  0.17879  -1.12583     -2.82 R
 82      4.91000  3.87583  0.17879   1.03417      2.59 R
 88      4.54000  3.69000  0.17879   0.85000      2.13 R
105      4.10000  4.95000  0.17879  -0.85000     -2.13 R
135      2.70000  3.60333  0.17879  -0.90333     -2.26 R
137      5.11000  3.60333  0.17879   1.50667      3.77 R
140      5.03000  3.61667  0.17879   1.41333      3.54 R
141      2.81500  3.61667  0.17879  -0.80167     -2.01 R
188      4.10000  4.96333  0.17879  -0.86333     -2.16 R
190      6.04500  4.96333  0.17879   1.08167      2.71 R
276      4.16500  5.01083  0.17879  -0.84583     -2.12 R
309      4.07500  3.26667  0.17879   0.80833      2.02 R

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

```
Grouping Information Using Tukey Method and 95.0% Confidence

treatment   N  Mean  Grouping
control    72   5.3  A
mf         72   4.9    B
pH10       72   3.6      C
pH6        72   3.5      C
pH8        72   3.4      C

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

```
Grouping Information Using Tukey Method and 95.0% Confidence

starchamount    N  Mean  Grouping
60            120   4.3  A
70            120   4.1    B
80            120   4.1    B

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

```
Grouping Information Using Tukey Method and 95.0% Confidence

storage   N  Mean  Grouping
0        90   4.6  A
```

1	90	4.2	B
2	90	4.0	C
3	90	3.7	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	N	Mean	Grouping
control	60	24	5.5	A
control	80	24	5.2	A B
control	70	24	5.1	A B
mf	60	24	5.1	B
mf	70	24	4.9	B
mf	80	24	4.7	B
pH6	60	24	3.6	C
pH10	60	24	3.6	C
pH10	70	24	3.6	C
pH10	80	24	3.5	C
pH6	80	24	3.5	C
pH8	60	24	3.5	C
pH6	70	24	3.4	C
pH8	70	24	3.4	C
pH8	80	24	3.4	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	storage	N	Mean	Grouping
control	0	18	6.0	A
mf	0	18	5.7	A
control	1	18	5.1	B
control	2	18	5.1	B
mf	1	18	5.0	B C
control	3	18	4.8	B C
mf	2	18	4.5	C D
mf	3	18	4.3	D E
pH6	0	18	3.9	E F
pH10	0	18	3.8	E F G
pH10	1	18	3.7	F G H
pH8	0	18	3.6	F G H
pH6	1	18	3.6	F G H I
pH8	1	18	3.6	F G H I
pH10	2	18	3.5	F G H I
pH6	2	18	3.5	F G H I
pH8	2	18	3.4	F G H I
pH10	3	18	3.3	G H I
pH6	3	18	3.2	H I
pH8	3	18	3.1	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	storage	N	Mean	Grouping
60	0	30	4.7	A
70	0	30	4.5	A B
80	0	30	4.5	A B C
60	1	30	4.3	B C D
70	1	30	4.2	C D E

80	1	30	4.1	D E
60	2	30	4.1	D E F
80	2	30	4.0	D E F G
70	2	30	4.0	D E F G
60	3	30	3.9	E F G
70	3	30	3.7	F G
80	3	30	3.6	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	storage	N	Mean
control	60	0	6	6.2
control	80	0	6	6.0
mf	60	0	6	5.9
control	70	0	6	5.8
mf	70	0	6	5.6
control	60	1	6	5.5
mf	80	0	6	5.5
mf	60	1	6	5.4
control	60	2	6	5.3
control	60	3	6	5.0
mf	70	1	6	5.0
control	80	2	6	5.0
control	70	2	6	5.0
control	80	1	6	5.0
control	70	1	6	4.9
control	70	3	6	4.8
mf	80	1	6	4.7
control	80	3	6	4.7
mf	70	2	6	4.6
mf	80	2	6	4.6
mf	60	2	6	4.5
mf	60	3	6	4.4
mf	70	3	6	4.4
mf	80	3	6	4.2
pH6	60	0	6	4.1
pH10	70	0	6	3.9
pH10	60	0	6	3.9
pH6	80	0	6	3.8
pH6	70	0	6	3.7
pH8	60	0	6	3.7
pH10	80	0	6	3.7
pH8	70	1	6	3.7
pH8	70	0	6	3.7
pH10	80	1	6	3.7
pH10	60	1	6	3.7
pH10	70	1	6	3.7
pH6	80	1	6	3.6
pH6	70	1	6	3.6
pH8	80	0	6	3.6
pH10	60	2	6	3.6
pH6	60	2	6	3.6
pH6	80	2	6	3.6
pH8	80	1	6	3.6
pH6	60	1	6	3.5
pH10	70	2	6	3.5
pH10	80	2	6	3.5
pH8	60	1	6	3.5
pH8	60	2	6	3.5
pH8	70	2	6	3.4
pH10	60	3	6	3.4

pH8	80	2	6	3.4
pH10	70	3	6	3.4
pH6	70	2	6	3.3
pH10	80	3	6	3.3
pH6	60	3	6	3.3
pH8	60	3	6	3.2
pH6	70	3	6	3.1
pH6	80	3	6	3.1
pH8	80	3	6	3.0
pH8	70	3	6	2.9

treatment	starchamount	storage	Grouping
control	60	0	A
control	80	0	A B
mf	60	0	A B C
control	70	0	A B C D
mf	70	0	A B C D E
control	60	1	A B C D E F
mf	80	0	A B C D E F G
mf	60	1	A B C D E F G H
control	60	2	A B C D E F G H I
control	60	3	B C D E F G H I J
mf	70	1	B C D E F G H I J
control	80	2	B C D E F G H I J
control	70	2	C D E F G H I J
control	80	1	C D E F G H I J
control	70	1	C D E F G H I J
control	70	3	D E F G H I J K
mf	80	1	E F G H I J K L
control	80	3	E F G H I J K L M
mf	70	2	F G H I J K L M N
mf	80	2	F G H I J K L M N O
mf	60	2	G H I J K L M N O P
mf	60	3	H I J K L M N O P
mf	70	3	I J K L M N O P Q
mf	80	3	J K L M N O P Q R
pH6	60	0	J K L M N O P Q R
pH10	70	0	K L M N O P Q R S
pH10	60	0	K L M N O P Q R S
pH6	80	0	L M N O P Q R S
pH6	70	0	L M N O P Q R S
pH8	60	0	L M N O P Q R S
pH10	80	0	L M N O P Q R S
pH8	70	1	M N O P Q R S
pH8	70	0	M N O P Q R S
pH10	80	1	M N O P Q R S
pH10	60	1	N O P Q R S
pH10	70	1	N O P Q R S
pH6	80	1	N O P Q R S
pH6	70	1	N O P Q R S
pH8	80	0	N O P Q R S
pH10	60	2	N O P Q R S
pH6	60	2	N O P Q R S
pH6	80	2	N O P Q R S
pH8	80	1	N O P Q R S
pH6	60	1	N O P Q R S
pH10	70	2	N O P Q R S
pH10	80	2	O P Q R S
pH8	60	1	P Q R S
pH8	60	2	P Q R S
pH8	70	2	Q R S
pH10	60	3	Q R S
pH8	80	2	Q R S
pH10	70	3	Q R S

pH6	70	2	R	S
pH10	80	3	R	S
pH6	60	3	R	S
pH8	60	3	R	S
pH6	70	3	R	S
pH6	80	3		S
pH8	80	3		S
pH8	70	3		S

Means that do not share a letter are significantly different.

Table B.22 Results for Tukey's mean comparison test for chewiness values of bread samples with hpmc for all parameters

General Linear Model: chewiness versus treatment; starchamount; storage

Factor	Type	Levels	Values
treatment	fixed	5	control; mf; pH10; pH6; pH8
starchamount	fixed	3	60; 70; 80
storage	fixed	4	0; 1; 2; 3

Analysis of Variance for chewiness, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
treatment	4	17163951	17163951	4290988	257.91	0.000
starchamount	2	7394890	7394890	3697445	222.23	0.000
storage	3	9276210	9276210	3092070	185.85	0.000
treatment*starchamount	8	3179698	3179698	397462	23.89	0.000
treatment*storage	12	2044182	2044182	170349	10.24	0.000
starchamount*storage	6	615224	615224	102537	6.16	0.000
treatment*starchamount*storage	24	849738	849738	35406	2.13	0.002
Error	300	4991333	4991333	16638		
Total	359	45515227				

S = 128.988 R-Sq = 89.03% R-Sq(adj) = 86.88%

Unusual Observations for chewiness

Obs	chewiness	Fit	SE Fit	Residual	St Resid	
71	1051.62	757.29	52.66	294.33	2.50	R
158	834.23	1070.89	52.66	-236.66	-2.01	R

162	1504.62	1070.89	52.66	433.73	3.68	R
174	741.47	505.05	52.66	236.42	2.01	R
211	889.01	1138.56	52.66	-249.55	-2.12	R
216	1560.44	1138.56	52.66	421.88	3.58	R
229	847.18	598.19	52.66	248.99	2.11	R
248	1516.17	1278.23	52.66	237.94	2.02	R
252	985.12	1278.23	52.66	-293.11	-2.49	R
291	1100.94	755.75	52.66	345.19	2.93	R
301	1098.97	1670.46	52.66	-571.49	-4.85	R
302	2221.45	1670.46	52.66	550.99	4.68	R
304	2054.31	1670.46	52.66	383.85	3.26	R
305	1323.89	1670.46	52.66	-346.57	-2.94	R
323	1051.86	665.17	52.66	386.70	3.28	R
332	714.82	981.32	52.66	-266.51	-2.26	R
341	1831.27	1508.69	52.66	322.57	2.74	R
351	869.41	1133.84	52.66	-264.43	-2.25	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	N	Mean	Grouping
pH8	72	805.0	A
mf	72	794.5	A
pH10	72	761.5	A
pH6	72	397.1	B
control	72	296.9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	N	Mean	Grouping
80	120	804.5	A
70	120	566.5	B
60	120	462.0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

storage	N	Mean	Grouping
3	90	824.1	A
2	90	692.0	B
1	90	526.6	C
0	90	401.3	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	N	Mean	Grouping
pH8	80	24	1153.8	A
mf	80	24	1105.1	A
pH10	80	24	953.0	B
pH10	70	24	747.4	C
pH8	70	24	712.3	C D
mf	70	24	680.6	C D E
mf	60	24	597.8	D E F
pH10	60	24	584.2	E F

pH8	60	24	549.0	F
pH6	80	24	517.6	F G
pH6	70	24	394.2	G H
control	60	24	299.7	H
control	70	24	297.8	H
control	80	24	293.1	H
pH6	60	24	279.4	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

treatment	storage	N	Mean	Grouping
mf	3	18	1115.8	A
pH10	3	18	1084.2	A
pH8	3	18	1061.7	A
pH8	2	18	896.9	B
pH10	2	18	889.8	B
mf	2	18	848.5	B C
pH8	1	18	718.9	C D
mf	1	18	689.3	D E
pH10	1	18	565.2	E F
pH8	0	18	542.5	E F
mf	0	18	524.3	F
pH6	3	18	514.7	F
pH10	0	18	506.9	F
pH6	2	18	475.1	F G
control	2	18	349.6	G H
control	3	18	344.3	G H
pH6	1	18	332.1	G H
control	1	18	327.2	G H
pH6	0	18	266.4	H I
control	0	18	166.5	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

starchamount	storage	N	Mean	Grouping
80	3	30	1100.6	A
80	2	30	870.6	B
70	3	30	779.0	B C
80	1	30	705.5	C D
70	2	30	657.3	D E
60	3	30	592.8	E F
60	2	30	548.0	F G
80	0	30	541.3	F G
70	1	30	467.8	G H
60	1	30	406.4	H I
70	0	30	361.8	H I
60	0	30	300.9	I

Means that do not share a letter are significantly different.
Grouping Information Using Tukey Method and 95.0% Confidence

treatment	starchamount	storage	N	Mean
mf	80	3	6	1670.5
pH8	80	3	6	1508.7
pH10	80	3	6	1330.6
pH8	80	2	6	1278.2
mf	80	2	6	1138.6
pH10	70	3	6	1133.8

pH8	80	1	6	1070.9
pH10	80	2	6	1010.9
pH8	70	3	6	981.3
mf	70	3	6	921.0
mf	80	1	6	905.3
pH10	70	2	6	890.5
pH8	70	2	6	817.7
pH10	60	3	6	788.1
pH10	80	1	6	786.2
pH10	60	2	6	768.0
pH8	80	0	6	757.3
mf	60	3	6	755.7
mf	70	2	6	722.3
mf	80	0	6	706.0
pH8	60	3	6	695.0
mf	60	2	6	684.8
pH10	80	0	6	684.0
pH6	80	3	6	665.2
pH6	80	2	6	598.2
pH8	60	2	6	594.9
mf	70	1	6	591.8
pH8	70	1	6	572.1
mf	60	1	6	570.9
pH8	60	1	6	513.8
pH6	70	3	6	512.1
pH10	70	1	6	505.0
pH6	70	2	6	493.6
mf	70	0	6	487.1
pH8	70	0	6	478.1
pH10	70	0	6	460.2
pH6	80	1	6	440.2
pH10	60	1	6	404.2
pH8	60	0	6	392.2
mf	60	0	6	379.8
pH10	60	0	6	376.5
pH6	80	0	6	367.0
pH6	60	3	6	366.7
control	70	2	6	362.4
control	60	2	6	359.1
control	60	3	6	358.3
pH6	70	1	6	350.2
control	70	3	6	346.5
control	60	1	6	336.9
pH6	60	2	6	333.6
control	80	3	6	328.1
control	80	2	6	327.3
control	80	1	6	325.0
control	70	1	6	319.7
pH6	70	0	6	220.8
pH6	60	0	6	211.4
pH6	60	1	6	206.0
control	80	0	6	192.2
control	70	0	6	162.6
control	60	0	6	144.7

treatment	starchamount	storage	Grouping
mf	80	3	A
pH8	80	3	A B
pH10	80	3	B C
pH8	80	2	B C D
mf	80	2	C D E
pH10	70	3	C D E
pH8	80	1	C D E F
pH10	80	2	D E F G

pH8	70	3	D E F G H
mf	70	3	E F G H I
mf	80	1	E F G H I
pH10	70	2	E F G H I J
pH8	70	2	F G H I J K
pH10	60	3	F G H I J K L
pH10	80	1	F G H I J K L
pH10	60	2	F G H I J K L M
pH8	80	0	G H I J K L M N
mf	60	3	G H I J K L M N
mf	70	2	G H I J K L M N O
mf	80	0	H I J K L M N O P
pH8	60	3	H I J K L M N O P Q
mf	60	2	H I J K L M N O P Q
pH10	80	0	H I J K L M N O P Q
pH6	80	3	I J K L M N O P Q R
pH6	80	2	J K L M N O P Q R S
pH8	60	2	J K L M N O P Q R S
mf	70	1	J K L M N O P Q R S
pH8	70	1	K L M N O P Q R S
mf	60	1	K L M N O P Q R S
pH8	60	1	L M N O P Q R S T
pH6	70	3	L M N O P Q R S T
pH10	70	1	L M N O P Q R S T U
pH6	70	2	L M N O P Q R S T U V
mf	70	0	L M N O P Q R S T U V
pH8	70	0	M N O P Q R S T U V
pH10	70	0	N O P Q R S T U V W
pH6	80	1	O P Q R S T U V W X
pH10	60	1	P Q R S T U V W X
pH8	60	0	Q R S T U V W X
mf	60	0	R S T U V W X
pH10	60	0	R S T U V W X
pH6	80	0	R S T U V W X
pH6	60	3	R S T U V W X
control	70	2	R S T U V W X
control	60	2	S T U V W X
control	60	3	S T U V W X
pH6	70	1	S T U V W X
control	70	3	S T U V W X
control	60	1	S T U V W X
pH6	60	2	S T U V W X
control	80	3	S T U V W X
control	80	2	S T U V W X
control	80	1	S T U V W X
control	70	1	S T U V W X
pH6	70	0	T U V W X
pH6	60	0	T U V W X
pH6	60	1	U V W X
control	80	0	V W X
control	70	0	W X
control	60	0	X

Means that do not share a letter are significantly different.