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M.Sc. in Food Engineering

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REPUBLIC OF TURKEY

GAZİANTEP UNIVERSITY

GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES

INVESTIGATION OF THE USE OF RESISTANT STARCH IN THE PRODUCTION OF GLUTEN-FREE BISCUITS

M.Sc. THESIS

IN

FOOD ENGINEERING

BY

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This is to certify that we have read this thesis and that in our consensus/majority opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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

Kadir Can ASLANPAY

ABSTRACT

INVESTIGATION OF THE USE OF RESISTANT STARCH IN THE PRODUCTION OF GLUTEN-FREE BISCUITS

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In this study, resistant starch (RS) were used as a functional component and a source of dietary fiber in the production of gluten-free biscuits. The amount of gluten-free flour in the biscuit formulation was reduced by 10, 20 and 30% and RS was used instead of gluten-free flour; and the baking time of the biscuits was kept constant, and they were baked at 175, 200 and 225ºC. Biscuits were compared by measuring the quality parameters in terms of moisture, water activity, pH, color, diameter, height, texture and dietary fiber. When samples are baked at different baking temperatures observed that as temperature increased, moisture, water activity, pH, biscuit diameter, L*, hardness and fracturability decrease; biscuit height and a* and b* values increased. Generally, as the proportion of RS increases at each baking temperature, moisture, water activity and L* value increased; pH, a*, b*, biscuit diameter and height decreased. As the proportion of RS addition increases, hardness value increased, fracturability value decreased compared to the control value. But with the exception of control biscuits, as the amount of RS increases, the hardness value decreases. Despite this decrease, the results are above the control biscuit values. Dietary fiber amounts of biscuits baked at 200ºC were measured. Control, 10, 20 and 30% RS added biscuits dietary fiber amounts were measured as 1.51, 2.79, 5.35 and 5.84%, respectively. The amount of dietary fiber increased with increasing the amount of RS. As a result, according to the study, it was observed that the use of RS instead of gluten-free flour had positive effects and enabled the production of healthy biscuits.

Key Words: Functional Food, Dietary Fiber, Resistant Starch, Gluten-Free Biscuit

ÖZET

GLUTENSİZ BİSKÜVİ ÜRETİMİNDE DİRENÇLİ NİŞASTA KULLANIMININ ARAŞTIRILMASI

ASLANPAY, Kadir Can
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Tez Yöneticisi: Doç. Dr. Ali Coşkun DALGIÇ
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104 sayfa

Bu çalışmada glutensiz bisküvi üretiminde fonksiyonel bir bileşen ve diyet lif kaynağı olarak dirençli nişasta (DN) kullanılmıştır. Yapılan çalışmada, bisküvi reçetesindeki glutensiz un miktarı %10, 20 ve 30 oranında azaltılarak yerine DN kullanılmış olup, pişirme süresi sabit tutularak, 175, 200, 225ºC olmak üzere 3 farklı sıcaklıkta pişirilmiştir. Kontrol grubu bisküviler tamamen glutensiz un ile yapılmıştır. Bisküvilerin nem, su aktivitesi, pH, renk, çap, yükseklik, tekstür ve diyet lif gibi kalite parametreleri ölçülerek karşılaştırılmıştır. Yapılan numuneler farklı sıcaklıklarda pişirildiğinde, sıcaklık arttıkça nem, su aktivitesi, pH, bisküvi çapı, L*, sertlik ve kırılganlık değerinin azaldığı; bisküvi yüksekliği, a* ve b* değerlerinin ise arttığı görülmüştür. Genel olarak her sıcaklıkta DN ilave oranı arttıkça nem, su aktivitesi ve L* değeri artmış olup; pH, a*, b*, bisküvi çapı ve yüksekliği azalmıştır. DN ekleme oranı arttıkça kontrol değerine göre sertlik değeri artmış olup, kırılganlık değeri ise azalmıştır. Fakat kontrol bisküviler haricinde kendi aralarında DN miktarı arttıkça sertlik değeri azalmaktadır, bu azalmaya rağmen kontrol bisküvi değerlerinin üzerinde bulunmaktadır. 200ºC de pişirilen bisküvilerin diyet lif miktarları ölçülmüştür. Kontrol, %10, 20 ve 30 DN eklenen bisküvilerin diyet lif miktarları sırası ile %1,51, 2,79, 5,35 ve 5,84 olarak ölçülmüştür. DN miktarının artması ile diyet lif miktarı artmıştır. Sonuç olarak, yapılan çalışmaya göre glutensiz un yerine DN kullanımının olumlu etkilerinin olduğu görülmüş olup, daha sağlıklı bisküvi üretilmesine olanak sağlamıştır.

Anahtar Kelimeler: Fonksiyonel Gıda, Diyet Lif, Dirençli Nişasta, Glutensiz Bisküvi

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LIST OF ABBREVIATIONS

AACC	American Association for Clinical Chemistry
AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
a*	Redness
b*	Yellowness
CD	Celiac Disease
FAO	Food and Agriculture Organization
FFC	Functional Food Center
FOSHU	Food for Specified Health Uses
GEQ	Good Extrusion Qualities
GG	Guar Gum
GI	Glycemic Index
HDL	High Density Lipoproteins
HGT	High Gelatinization Temperature
HWBH	Higher Water Binding Capacity
IDL	Intermediate Density Lipoproteins
IFIC	International Food Information Council Foundation
ILSI	International Life Sciences Institute
LDL	Low Density Lipoproteins
LGB	Locust Bean Gum

LWHC	Lower Water Holding Capacity
L*	Lightness
mm	Millimeter
rpm	Revolution Per Minute
RS	Resistant Starch
SCFA	Short-Chain Fatty Acids
SD	Standard Deviation
TDF	Total Dietary Fiber
VLDL	Very Low Density Lipoproteins
WHO	World Health Organization
XG	Xanthan Gum

CHAPTER 1

LITERATURE REVIEW

1.1 Biscuit

The word biscuit comes from the Latin ‘panis biscoctus’, which means twice-baked. It is thought that biscuits have been baked for thousands of years and were originally baked in a hot oven and then cooled in a cool oven (Manley, 2000). Nowadays, this process is not available in modern biscuit production factories because with the development of technology, biscuits are baking in oven zones with different baking temperatures according to the desired quality parameters and cooled in cooling belts and packaged with seasoning or creaming process.

Biscuit is one of the most popular bakery items consumed in a wide range of society. The interest in biscuits in the snack product category is increasing day by day. The main reason for this may the increase in the participation of women in working life, the decrease in the time allocated for cooking and the increase in income levels. Furthermore, it has become more preferred due to biscuit that’s, ready to eat, good nutritional quality, and availability in different varieties (with cream, chocolate, marshmallow etc.) and affordable cost. The most popular snack which is biscuits that produce from factory of many varieties (Sudha et al., 2007). Biscuit is a term used to describe a cereal-based baked product, and the cereal content varies according to the type of biscuit, but should be at least 60% in cereals such as wheat, oats or barley. The final product moisture content can vary between 1-5% and cream, marshmallow, fillings can be used as filling or depositing between biscuits. The low moisture content ensures that biscuits are generally free from microbiological spoilage and confers a long shelf life on the product, provided of course that they are protected from uptake of moisture from a damp atmosphere or damp surroundings. Their low moisture content also gives biscuits a relatively high energy density compared other baked foods. Biscuit has higher fat content than other baked products. In addition to the features mentioned above, biscuit has high calorie density. This feature has been the reason for choice for many travelers and explorers who that grateful for the biscuits (Wade, 1988).

In the Turkish Standards Institute biscuit is defined as into to the wheat flour edible ingredients such as leavening, vegetable oil, sugar, salt if you need rich biscuits whey powder, egg, starch etc. It is defined as the product obtained by shaping and baking after being kneaded with water (TS 2383, 2017). Biscuits consumption is increasing day by day. Because of long storage without staling, offer to the consumer pleasant and varied flavors has an important place in ready to eat food sector. In Turkey, it is included in the category of food consumed daily. And the average per capita biscuit consumption is around 5-6 kg per year (Doğan and Uğur, 2005).

The major ingredients for biscuit making are flour, sugar and fat. In addition to these, it is also possible to adding other minor ingredients such as milk or milk powder, some type starch, flavoring or coloring agents, salt, texture improving agents, leavening agents and nutrient-enhancing additives to the biscuit formula.

1.1.1 Wheat Flour

Throughout the history, food supply and nutrition have been the most important problem. In response to the rapid increase in the world population; limited natural resources and reached the top of arable agricultural areas. It has become extremely important to increase the yield and quality in agricultural products by using breeding techniques (Sertakan, 2006). Wheat is a culture plants that is commonly used in human nutrition. In our country and world, wheat is the first in terms of cultivated area and production. According to data from the 2018, wheat production forecast was approximately 20 million tons in Turkey (Chamber of Agricultural Engineers, 2018).

Wheat flour is the main component with the most amount in biscuit recipes. A normal wheat grain (undamaged) consists of three distinct and different parts. These are bran (12%) which is the outer husk, endosperm (85.5%) which is the white center and the germ (2.5%). The wheat flour is obtained by grinding endosperm while by removing the brown surface coating (bran) and germ is separated during process and reducing the particle size to a fine powder. Extraction rate of wheat flour is expressed in kilograms of flour obtained from 100 kg wheat. This rate refers to separation of the bran layer so, that is indicative of the pure flour. The extraction rate increases, rate of endosperm also increases in flour. Wheat flour with 70-75% extraction rate can be used to make nearly most of type biscuits. In practice, whole meal flour is, almost 100% extraction because of all wheat grain is used during milling. Wheat varies in its quality as a result of variety, farming practice and climatic conditions. Flour mills may, to some extent, select wheat to make a wheat flour of the desired quality, but the conditions in the mill also affect the flour produced. It is possible to make all type biscuit from most types of flour but differences in protein content and moisture, in particular, affect the consistencies of dough. Because the changes in flour quality are of great importance for biscuit manufacturers. Therefore, biscuit producers always wants from flour suppliers to provide flour of the same standards. For some special types of biscuits, special purpose flours can be produced by suppliers by mixing different wheat types. In this way, the special type flour demands of the biscuit manufacturer are supplied (Manley, 2000). In the milling process, wheat can be defined as hard and soft depending on the physical properties of wheat grains. This difference is related to the season and weather conditions in which wheat seeds are sown. The names of the wheat types called winter and spring wheat come from these seasons. Winter wheat varieties, also called soft wheat, have lower protein content and softer grains than spring wheat varieties. The protein content of spring wheat, that is hard wheat, has a protein content of approximately 10-14%. The protein content of soft wheat which is at low protein levels is in the range of 8-11%. When hard wheat is processed and flour is made (known as milling), the grain of wheat is crushes and most of starch particles are damaged and cause high water absorption properties due to the water-holding capacity of starch. In contrast, when soft wheat grains are milled, softer type of flour made and that is less damaged starch granules and this type has lower water retention capacity (Manley, 2000).

For biscuit producers the most interesting feature of wheat flour is protein quality. Because when the producer wants to develop a biscuit, who will first want to test or know the protein value and quality of wheat flour. In the presence of water, these proteins combine to form gluten network. Total protein in wheat consists of approximately 80-85% gluten proteins. The flour from wheat meets water in the dough process and during the mixing process forms a unique viscous and elastic mass knownas the gluten network. The strength and elasticity of dough it is the gluten formation in dough are determined by the flour specification, especially the amount of water in the recipe and mixing and shaping type processes. Protein in wheat flour produced from different wheat varieties or protein in wheat flour of the same variety grown under different conditions shows significant differences when combined with water in the recipe. Gluten permits the retention of air and CO_2 bubbles during baking of a biscuit dough to give open textured and good eating profile products. The most important flour component gluten which is affecting such as its workability, elasticity and extensibility of biscuit dough (from its viscoelastic properties) and quality characteristics of the final product its internal structure appearance, volume and texture. Gluten consists of two separate proteins: gliadin and glutenin. Gliadin contributes to the viscous structure, while glutenin is known to provide the elastic structure of the gluten protein (Türksoy and Özkaya, 2006). The physical and chemical properties of wheat flour, which is one of the main components of the biscuit, affect the various quality characteristics of the final product. The choice of suitable flour for biscuits is important. As a flour profile used in biscuit making, flour containing a low quality protein, so flour is weak and extensible gluten network, low ash content, moisture balance and fine grain flour can be used. For some special biscuits special purpose flours may be use, in other words flours with specific properties, may be used, but generally, a typical specification for the biscuit flour to be used for the biscuit should include some or all of the properties and values shown in Table 1.1.

Table 1.1 Typical specification of flour used for biscuits (Manley, 2000)

Parameters	**Value ranges**
Wheat type	maximum amount of soft wheat varieties
Moisture content	14% ± 0.5
Protein (N*5.7)	7.5-12%
Ash content	0.46-0.55
Particle size range	
particles greater than 250µm	less than 1%
particles greater than 50µm	40% ± 0.5

1.1.2 Fats and Oils

Fats and oils are main nutrients source such as proteins and carbohydrates for humans. The nutritive importance of fats and oils is based on their role as fuel molecules. Fats and oils which are the highest energy source in human nutrition, these have 9 kcal/g energy value. Fats and oils taken with foods is the source of essential fatty acids that are necessary for nutrition. In addition, fats and oils are responsible for the transport of fat-soluble vitamins which is A, D, E and K (Belitz, 2009).

In bakery, generally shortening term is used instead of fats and oil. The term "shortening" refers to the fat or oil ability to shorten the strands of gluten in wheat flour. The shortening that are used in bakery products range in their consistency from liquid oils to high melting point solid fats. Although the chemical composition is the same, the fats are semi-solid at room temperature and the edible oils are completely liquid. The reason they are named differently in this way is due to the solid fat content at room temperature (Jacob and Leelavathi, 2007).

Generally when making a biscuit recipe, generally the third largest component is fat, after wheat flour and sugar. Fat, a food component, plays an important role in the preparation and consumption of food (Manley, 2000).

Fats improve a wide range of characteristics properties to the added foods. These features can be said as dough rheology, desirable appearance, flavor, aroma, texture and mouth feel. The first sensory characteristics perceived by the consumer are usually visual cues, followed closely by aroma sensations. These initial perceptions will set up expectations about the product (Lucca and Beverly, 1994).

The rheological properties of the biscuit dough affect the processing of the dough and thus affect the quality of the baked goods. According to the study, the type and amount of fat used in the biscuit formulation has a strong effect on the viscoelastic properties of the biscuit dough (Baltsavias et al., 1997). During the making of the biscuit dough, generally the fats are added before the water. In other words, wheat flour becomes a mixture with oil before water. This raw material adding sequence, so it does prevent the development of gluten network and provides the poor elastic dough suitable for biscuit dough. However, this sequence is not suitable for the production of some elastic biscuit dough because it shrinks after lamination or stick to the mould (Faubion and Hoseney 1990).

In terms of sensory quality in the final product, fat is one of the main components having an effect on the overall biscuit texture. In earlier studies, the amount of fat or oil is changed or adding fat replacer in biscuit recipe, it has been observed that biscuits have a great effect on textural properties. In these studies, it has been determined that biscuit tenderness increases with increasing oil level however, with the decrease in the amount of fat in the biscuit recipe with higher breaking strength. In biscuit making important parameter is melting profile of fats and oils. The melting profile of fats and oils in biscuit recipe impacts how air molecules homogeneously penetrates to the fat, in addition to dough rheology, mouth feeling and especially in terms of the peroxide value which is quality parameter gives information about how long shelf life. Within melting temperature range, decreasing in the temperature it gives information about how much oil passes from solid form to liquid form. The other important parameter is solid fat content of fats in biscuit making. At specified temperatures, it is the ratio of solid fat to whole fat. And as the amount of liquid form of fat increases, it affects the spreadability of fat. If the biscuits are produced with a high solid fat content of fat, breaking force of biscuits will be higher. Fats in solid and liquid form at certain temperatures have different effects on the mixing of biscuit dough. Liquid form supports mixing process with lubrication effect. The solid form traps air into the oil molecules during the mixing process. With effective mixing, the air molecules are trapped in the oil and help to obtain excellent volume of the biscuit. The solid fat content has a significant relationship with texture, shelf life and other quality parameters of the baked product. The presence of the solid and liquid form of same fat in the biscuit recipe offers positive functions in baked products (Khatkar and Devi, 2016).

1.2 Functional Foods

In recent years, consumer demand for food production has changed significantly. Today's consumers are demanding foods with two main characteristics. The first is concerned with the traditional nutrition aspects of food. The second and more important is; consumers want to bring additional health benefits or preserve their health from food taken regularly (Aparicio-Saguila’n et al., 2007). Nowadays, food is designed not only to satisfy hunger and to take the nutrients necessary for humans. Humans while consume foods wants to prevent nutritional diseases and improves their physical and mental well-being. The importance of functional food products has increased. The fact that people give more importance own health and they have turned to natural products and functional foods rather than medicinal products such as drugs. The concept of functional food has emerged, consumers health awareness and against more nutritious consumption. And the food industry has accelerated research and development in the area of healthier and more innovative functional foods (Meral, 2011).

The concept of functional food was first promoted in 1984 by Japanese scientists who studied the relationships between nutrition, sensory satisfaction, fortification and modulation of physiological systems. Functional food concept which is the product of sustainable and good nutrition studies to overcome the problems caused by Japan's inadequate natural resources. In 1991, the Ministry of Health of Japan was the first in the world for health-related foods introduced rules for approval of a specific health-related food category called FOSHU (Foods for Specific Health Use). FOSHU included the creation of special health demands for such food. In Japan, traditional functional food is seen as a different class of products and means that a "FOSHU" symbol may be shown on the food label after it has been approved as functional food. Functional foods as discussed in the early 1990's in the Unites States and in the middle 1990's in Europe (Siro et al., 2008).

Basically, functional foods is defined as nutrients that can benefit health beyond basic nutrition. Functional foods is also described similar in appearance to conventional food that is intended to be consumed as part of a normal diet in daily life, but has been modified to improve physiological roles beyond the provision of basic nutrient requirements. Functional foods enable the final consumer to lead a healthier life with the foods they eat without changing the eating habits (Bech-Larsen and Grunert, 2003).

With the development and importance of functional products in the world market, the conceptualization efforts for these products have gained speed. In order to determine the boundaries of the product group, the definition development process has gained importance. Each scientific sector uses a different functional food definition.

International Food Information Council Foundation - IFIC; food and beverages that benefit beyond the basic nutritional requirements.

International Life Sciences Institute - ILSI; a food product can be defined as functional food if it acts by making one or more functions of the body healthier and better or by reducing the risk of disease. Even in European Union legislation, the definition of functional food still does not have a clear and agreed framework.

Two thousand years ago, Hippocrates was on the right path when he said “Let food be thy medicine and medicine be thy food”. However, Functional Food Center - FFC change that to "Let functional food be thy medicine." (Martirosyan and Singh, 2015).

However, if we look at the common points of definitions, functional foods are definitely (Özdemir et al., 2009);

- Food should not be consumed infrequently, it should be a nutrient that is frequently used in daily nutrition.
- It should contribute to the nutrition of the individual, help to preserve and improve health.
- If the food is processed by functional properties, there should be no loss of nutritive properties.
- Physicochemical, quantitative and qualitative properties of food components should be determined.
- Food consumption should be proven to be reliable.
- The appropriate daily intake should be determined for nutritional properties.
- Its impact must be approved by the world of science.

There are two main approaches to functional food production. The first of these is the functional products obtained by the presence of more or less functional components in food. The second is the products obtained by the addition of a variety of functional components from the outside into the food (Meral, 2011).The products in the first approach are natural products. The second approach is the products produced as a result of a specific design. Functional food components are potentially beneficial components. It found naturally in foods or can be added to foods as functional ingredients which carotenoids, dietary fiber, fatty acids, flavonoids, isothiocyanates, phenolic acids, plant stanols and sterols, polyols, prebiotics and probiotics, phytoestrogens, soy protein, vitamins and minerals (Guine et al., 2010).

By using the components which have a functional feature in the production of bakery products, during the consumption of these foods, it is ensured to be taken into the body in the components that are beneficial on human health. The most commonly used, functional component are nutritional fibers. Low calorie foods, dietary fiber and antioxidant content enhanced foods, gluten-free foods, diabetic foods are among the foods considered to be functional foods (Doğan, 2016).

1.3 Dietary Fiber

In the current social trends, more attention has been given to the effects of food nutrition on human health. This can be attributed to the development of the economy, living conditions and improving consumer demand. Some components such as phenolic substances, dietary fiber, vitamins and minerals found in foods give functional properties to food due to their positive effects on health (Wang et al., 2014).

Dietary fiber has functional properties, it plays a key role in the development of valuable foods for human health. Dietary fiber containing foods are known as functional foods. Dietary fiber is the food fraction that is from part of plant material. Dietary fibers are a group of food components that are not digested in the small intestine, but which can be fermented in the large intestine. Fibers are present naturally in various amounts in all plant foods. Some plants containing significant amounts of dietary fiber these are legumes, cereals, some fruits (especially apples and bananas) and fruits, some vegetables (such as broccoli, carrots and root vegetables, potatoes and onions), seed husk and bran (Fadaei and Salehifar, 2012).

Dietary fibers have three origins: vegetable, animal and synthetic. RS, hemicellulose, lignin, β-glukan, pectin, gums, mucilages and oligosaccharides are considered as a nutritional fiber of plant origin. Vegetable origin dietary fiber sources cellulose, fruits, vegetables, cereal grains, legumes, woody ingredient foods (celery), cereal outer layers, sugar beet and potato, rice, unripe bananas, baked and chilled pasta, whole grains, barley and oat cell wall, fruit vegetable intracellular tissue and cell walls, plant extrudates, pulses, onions and garlic. Dietary fiber sources of animal origin is sea crustacean which is the most common natural polymer after cellulose in nature.

Resistant maltodextrins are synthetic dietary fiber sources. Dietary fiber does not belong to a defined chemical group. But, dietary fiber is a combination of chemically heterogeneous substances such as celluloses, hemicelluloses, pectins, lignins, gums and polysaccharides from seaweeds or bacteria. Celluloses, hemicelluloses and pectins are also referred to as structural polysaccharides because they are components of plant cell walls. Other polysaccharides that are not constituents of cell walls are but also classified as dietary fiber; secreted gums (e.g. gum arabic), reserve gums (bean gums, guar gums), and seaweeds (carrageenans, agars, alginates). Some scientists have also added resistant starch to this group (Thebaudin et al., 1997).

According to research, dietary fiber is taken in sufficient amounts in nutrition, there are many human health benefits. The most important of these; It reduces constipation and irritable bowel syndrome, reduces cholesterol, reduces coronary diseases and prevents cardiovascular heart disease, obesity and diabetes adn colon cancer and reduces the risk of developing breast cancer (Guine et al., 2010).

Dietary fiber is examined in two groups as water-soluble and water-insoluble. Dietary fibers insoluble in water: resistant starch, celluloses, lignins, some hemicellulose; soluble in water: pectins, gums and mucilage. Basically, water-insoluble dietary fibers are involved in the protection of the intestine. Water-soluble dietary fiber is effective in lowering cholesterol in the blood and reducing the absorption of glucose in the intestine.

Insoluble dietary fibers may hold up to water 20 times their weight. Because of low energy value and water retention feature of the dietary fiber, it increases the viscosity of stomach contents and delay the emptying of stomach. Since the stomach is not empty, the eating desire of the individual decreases. Even it is known that, when supported with plenty of water, a feeling of satiety will be created for longer. Water-insoluble dietary fibers cause an increase in the stool mass as a direct fecal material and creates a feeling of satiety in the human body. It prevents the formation of diseases such as constipation and hemorrhoids due to accelerating and facilitating feces. In diets with high dietary fiber, bile acids are absorbed by the fibers, so they do not return and are excreted by feces. As a result, cholesterol in the blood is converted into bile acids in the liver and bile acid is produced again. Thus, it has been shown that it reduces blood cholesterol levels by more than 20% (Candal, 2016; Dülger and Şahan, 2011; Burdurlu and Karadeniz, 2003).

The popularity of fiber-rich foods has started to increase in social life. The reason for this is the increased awareness among consumers of the relationship between food, lifestyle and health. Therefore, RS has gained importance as a new source of dietary fiber. RS have received great interest both in terms of their potential health benefits (similar to soluble fibers) and their functional properties. RS as dietary fibers helps control the functioning of the digestive system, microbial flora, blood cholesterol level, glycemic index and diabetes. In addition to the potential health benefits of resistant starches, another positive advantage is its low effect on the sensory and eating properties of food compared to conventional fiber sources such as whole grains, fruits or bran. Some desirable physicochemical properties in the preparation of certain specialty foods include swelling capacity, viscosity, gel formation and water binding capacity (Fuentes-Zaragoza et al., 2010).

As a result; the presence of RS, which acts as a dietary fiber, in foods with higher rates helps improve human health and quality of life. By the production and consumption of such products, it is considered that the incidence of many diseases, which become a major problem in society, can be significantly reduced (Candal, 2016).

1.4 Resistant Starch

Dietary fiber, which has an important place for a healthy life with consumption of foods, it can be enriched with this aspect by adding it to the food. In order for functional foods to be classified as functional foods or components, they must contain a component that has positive health effects or eliminate a component that has a negative health effect. Nowadays, RS which is widely used in foods containing high dietary fiber, can be used in functional foods recipes as functional component. Functional properties of RS are summarized in figure 1.1. Starch is the major source of carbohydrate in the human diet. Starch is stored as a reserve material in a variety of seed, tubers, fruit and rhizomes. It is the main constituent of cereal and potato products. Starch is being energy source for people all over the world (Sofi, 2017).

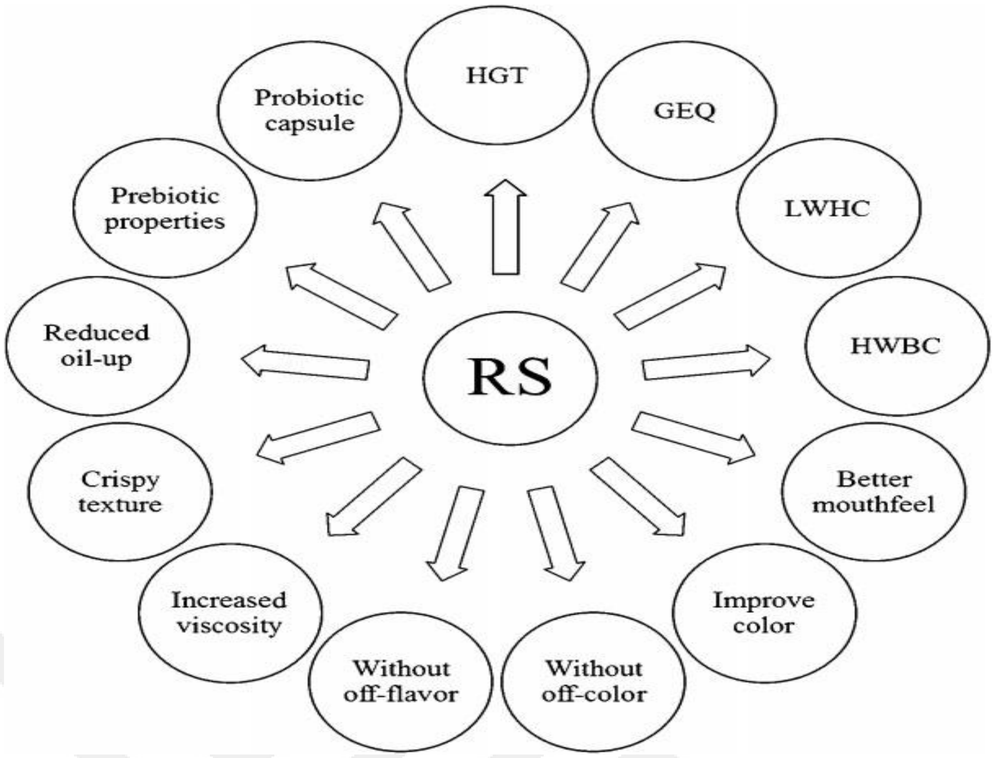

Figure 1.1 Functional properties of resistant starch

HGT: High gelatinization temperature, GEQ: Good extrusion qualities, LWHC: Lower water holding capacity, HWBC: Higher water binding capacity

Starch occurs in many plant tissues as a granule, usually between 1 and 100 µm in diameter, depending upon the plant source. Starch is a polysaccharide consisting of d-glucose units, referred to as homoglucan or glucopyranose, and has two major bio macromolecules that is amylose and amylopectin. Amylose is the straight chain polyglucan monomers (which is polysaccharides composed of D-glucose), it consists of about 1000 glucose units connected to each other by α-(1, 4) glycosidic bonds. Amylopectin, branched polyglucan, comprised approximately 4000 glucose units linked to each other by glucose units, in the straight chain α-(1,4) and in branching points α-(1,6) glycosidic bonds (Zieba, 2009).

Native starch granules do not dissolve in cold water. When starch granules are heated in water, they absorb 30% of its dry weight and swell (Carlstedt et al., 2015). With the dissolution process, the amylose passes to water. The amylose is dissolved in the solution as a random coil polymer. And amylose interacts with each other and traps water between them. In which viscosity increases, this phenomenon is called gelatinization. Gelatinization is an irreversible process (Xie et al., 2013). The structure resulting from gelatinization is unstable and becomes gel structure with cooling during storage. Depending on the cooling and storage time of the gelatinized starch; interaction between starch chains is increasing. Upon cooling, the polymer chains are strengthened by hydrogen bonds and re-associate as a double-stranded structure. This reassociation process commonly termed retrogradation (Haralampu, 2000). As a result of retrograde, the crystal structure becomes more resistant to enzymes (Candal, 2016).

Starch granules are more easily digested when gelatinized. The gelatinized starches are in an unstable form, which upon cooling re-form crystalline they become resistant to hydrolysis by amylase, so they are more resistant to digestion. This process is known as retrogradation. Generally, amylose-rich starches are naturally more resistant to digestion and at the same time more susceptible to retrogradation (Nugent, 2005).

Corn starch gels between 66-77^{o}C, high amylose corn starch gel forms between 67-105^{o}C on the retrograde of the gel structure, both polymers of the starch have effect about gelatinization (Wianecki and Kołakowski, 2007; Kibar et al, 2010). Two starch polymers, amylose and amylopectin, retrograde kinetics are quite different from the each other. Amylose/amylopectin ratio is important for retrogradation. Amylopectin is not prone to retrogradation and may have a few days to be retrograde. Amylose is highly susceptible to retrograde and can be retrograde within hours. The resistance of starch to digestion depends on the ratio between the starch polymers. Starches that are rich in amylose content are naturally more resistant to digestion and more inclined to retrogradation. Starch retrogradation is directly related to the structural formation of RS (Kotancılar et al., 2009).

1.4.1 Resistant Starch Definition and Types

The concept of RS has created a new interest in the bioavailability of starch and its use as a source of dietary fiber. In the researches, it was seen that the physiological functions of RS were similar with dietary fiber (Kotancılar et al., 2009). Generally, digestible starches are broken down by the enzymes α-amylase, glucoamylase and sucrose-isomaltase to provide free glucose for digestion in the small intestine (Nugent, 2005). But, all starches taken into the human body with food are not digested and absorbed in the small intestine (Ratnayake and Jackson, 2008). Resistant starch is an example of said starch. It is an enzymatically indigestible type of starch which is resist digestion in the stomach and small intestine in healthy individuals (Nugent, 2005; Sofi, 2017). RS can reach the large intestine because it cannot be hydrolyzed by enzymes in the human digestive system. And can be used as a fermentation substrate for large intestinal micro flora (Henningsson et al., 2002; Hu et al., 2015). In the study of the use of RS in different foods and consumption habits in different countries; RS was used to increase fiber content and provide beneficial effect to some health problems (Murphy et al., 2008). RS is divided into five subgroups according to their chemical and physical properties: resistant starch type 1, 2, 3, 4 and 5 (Ashwar, 2016; Sofi, 2017; Zieba, 2009). There are five types of RS that have been introduced by scientific studies. Table 1.2 summarizes each RS types, sources, and descriptions.

Resistant Starch Type 1 (RS1): RS1 is term given to physically inaccessible starch which is entrapped within whole or partly milled grains or seeds (Sofi, 2017). Figure 1.2 shows microscopic view of the physically inaccessible RS1 in cell or tissue (Sajilata et al., 2006).

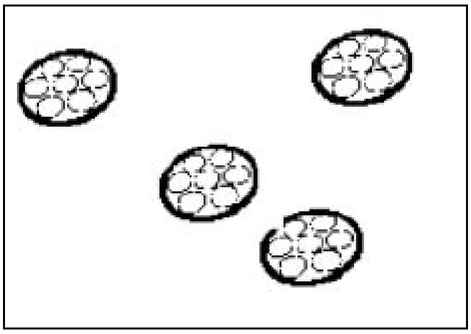

Figure 1.2 Structure of RS type 1 (RS1)

Resistant Starch Type 2 (RS2): This starch present in the raw starch granules is present in a radially tightly packed and relatively dehydrated form. RS2 are starch granules that are uncooked in nature, such as raw potato or banana starch. RS2 describes native starch granules that are protected from digestion by the conformation or structure of the starch granule. This compact structure limits the accessibility of digestive enzymes (α-amylase) to starch and describes the resistant structure of RS2, such as non-gelatinized starch (Fuentes-Zaragoza et al., 2010). Figure 1.3 shows the RS granules that is, raw potato and unripe banana.

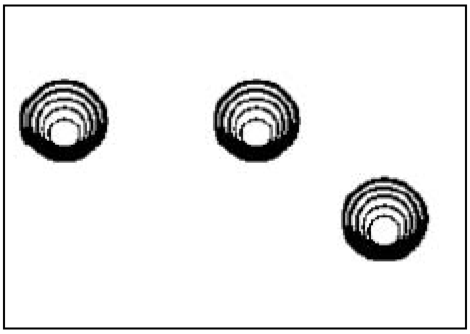

Figure 1.3 Structure of RS type 2 (RS2)

Resistant Starch Type 3 (RS3): RS3 refers to non-granular retrograded starch. RS3 is the most RS fraction to digestive enzymes. RS3 forms usually occur during retrogradation of starch granules RS3 is retrograde starches that may occur in cooked foods maintained at room temperature or below (Hernández et al., 2008). Therefore, most moist heated foods contain some amounts of RS3 (Sajilata et al., 2006). RS3 is of particular interest due to its thermal stability. Its thermal stability ensures that it remains unchanged in most of the normal cooking processes, i.e. it is stable during the cooking process, and has therefore been used as a component in a wide variety of foods (Fuentes-Zaragoza et al., 2010). RS3 preserves its nutritional functionality during baking processes, it may be used as a food ingredient. RS3 is produce in two steps (Shamai et al., 2003). To obtain RS3 from natural starch granules (crude starch), the starch must be gelatinized and subsequently retrograded (Escarpa, 1997). As mentioned above, when the starch granules are heated in the presence of water, the amylose passes into the water as a random coil, known as gelatinization. Upon cooling, the polymer chains are strengthened by hydrogen bonds and re-associate as a double-stranded structure. So, starch is recrystallized, known as retrogradation. The crystalline structure formed by regulating the double helical structure of the starch, resists the diffusion of the enzymes to hydrolyze the starch. And the resulting structure becomes resistant to enzyme hydrolysis (Serinyel, 2013). Figure 1.4 schematically, the process of retrogradation.

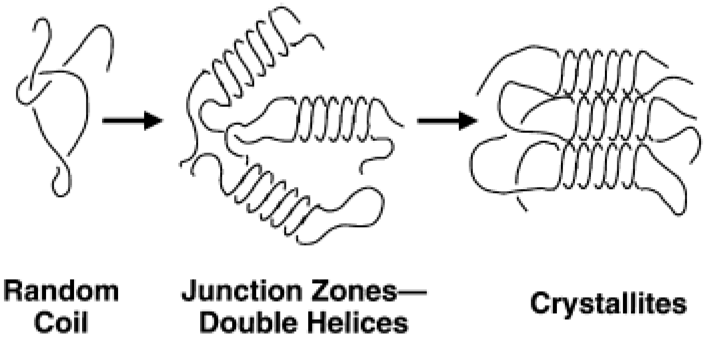

Figure 1.4 Schematic of amylose retrogradation.

Resistant Starch Type 4 (RS4): RS4 describes a group of chemically modified starches and includes starches etherified, esterified, acid modified, bleached, oxidized, or chemically cross-linked to reduce their digestibility (Sofi, 2017). Starch with a high level of cross-linking loses the ability to swell during baking. And after baking the high cross-linked starch remains in granular form with less enzymatic sensitivity and cannot be hydrolyzed by amylases or fermented by microorganisms. When a chemical derivative such as octenylsuccinate groups or acyl groups is added to starch, it changes the structure of the starch and partially limits the enzymatic hydrolysis of the starch molecule, resulting in RS4 (Birt et al., 2013). Figure 1.5 shows to preparation of cross-bonded starch.

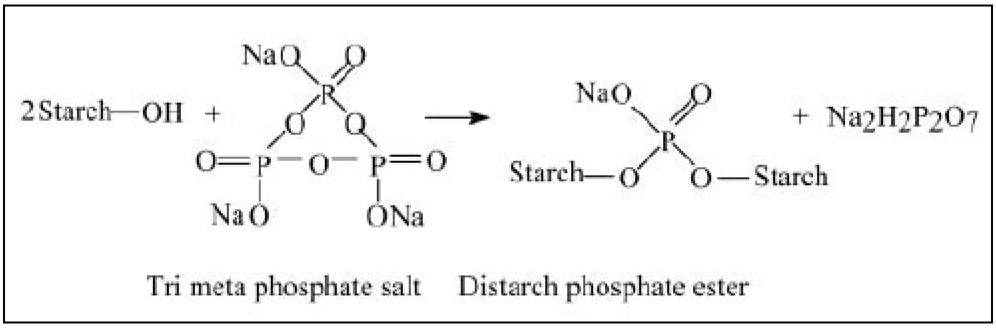

Figure 1.5 Structure of RS type 4 (RS4)

Resistant Starch Type 5 (RS5): When starch interacts with lipids, amylose and long branch chains of amylopectin form single-helical complexes with fatty acids and fatty alcohols. When the linear starch chain is in a helical-complex structure with the complex fatty acid in the cavity of the helix, starch binding and cleavage by amylase are prevented. In addition, the amylose-lipid complex also entangles amylopectin molecules, restricting the swelling of starch granules and enzyme hydrolysis. Because the amylose-lipid complex formation is an instant reaction and the complex can reform after baking, RS5 is considered thermally stable (Birt et al., 2013).

Table 1.2 RS types, description and sources (Khoozani et al., 2019; Birt et al., 2013; Nugent, 2005)

Type of RS	Description	Source
RS1	Physically inaccessible in cell wall	Whole or partly milled grains and seeds, legumes
RS2	Non-gelatinized resistant granules which is native starch	Raw starchy foods (potato, high-amylose corn, unripe banana)
RS3	Retrograded starch	Retrograded starchy foods (Baked and cooled potatoes, bread)
RS4	Chemically modified starch	Esterified or Cross-linked starch (Some fiber-drinks, foods in which modified starches have been used)
RS5	Amylose-lipid complex	Fatty acid treatment of debranched starch

1.4.2 Usage Areas and Advantages of Resistant Starch

RS due to the feature of dietary fiber, it can used easily as a source of dietary fiber in many foods. Naturally sourced, white appearance, fine particle size that affects the product texture positively, bland flavor; this mentioned features are seen as the usage advantage of RS in foods. It has desirable physicochemical properties such as swelling, viscosity increase, gel formation and low water-binding capacity, making it useful in a variety of foods. This physicochemical properties make it possible to use RS without significantly affecting dough handling or rheology. They have high gelatinization temperature, good extrusion and film-forming qualities, and lower water-holding properties. RS added foods compared with high fiber; it provides advantages that affect food positively; improved texture, appearance, and mouth feel in the final product. The energy value of RS has been calculated as approximately 8 kJ/g (2 kcal/g). This is considerably lower than the energy value for completely digestible starch 15 kJ/g (4.2 kcal/g). Reducing energy intake due to its low caloric value that resulting in less appetite, can prevent obesity (Amini et al., 2016). It is a functional food component because decreases the calorific value of foods and it can be used in foods produced for celiac patients (Sajilata et al., 2006; Nugent, 2005; Kotancılar et al., 2009).

1.4.3 Health Effects of Resistant Starch

In the food industry, RS has received great interest both in terms of its potential health benefits and its functional properties (Sajilata et al., 2006). Some physiological properties proven to be beneficial to human health have been attributed to RS. These physiological properties of RS (and hence the potential health benefit) may vary depending on the design of the scientific study, the source, type and amount of consumption of RS consumed (Tabibloghmany and Ehsandoost, 2014).

Hypoglycemic Effects

According to FAO/WHO (1997), the glycemic index (GI) is defined as “the incremental area under the blood glucose response curve of a 50g carbohydrate portion of a test food expressed as a percent of response to the same amount of carbohydrate from a standard food taken by the same subject”. The reference food can be white bread or glucose. Every nutrient raises blood sugar level, basically glycemic index is the name given to the rate of increasing blood sugar level of carbohydrates contained in foods. The rise of blood glucose rate can be measured according to the standard blood glucose curve after eating glucose or white bread in the same subject. The glycemic index of starchy foods may vary depending on various factors such as the amylose/amylopectin ratio, particle size of the starch granule, the gelatinization of the starch, the water content and the baking temperature of the processed foods. Therefore, factors affecting GI values will also affect RS formation. Food products containing RS reduce the rate of digestion. The slow digestion of RS is effective for controlled or slow release of glucose levels in the blood. RS-rich foods secrete glucose slowly and, therefore, have a low insulin response and increase the use of stored fat in the body. The metabolism of RS occurs 5 to 7 hours after consumption, in contrast to normally baked starch, which is digested almost immediately. Digestion over a 5 to 7 hours period reduces postprandial glycaemia and abnormally high concentration of insulin and has the potential for increasing the period of satiety. A study was using 10 healthy normal weight males fed test meals containing either 50 g starch free of RS (0% RS) and 50 g starch containing a high level of RS (54% RS) together with 500 g artificially sweetened syrup. It was proved the ability of high RS meals to significantly lower the postprandial concentration of blood glucose, insulin and epinephrine (Raben et al., 1994). Similarly in a human study, reported that the consumption of RS3 resulted in lower serum glucose and insulin levels than obtained with other carbohydrates (simple sugars, oligosaccharides, and common starch). The RS3 containing bar showed that food containing RS decreased postprandial blood glucose and might play a role in providing improved metabolic control in type II diabetes (Reader et al., 1997).

As a Prebiotic Agent

Gibson & Roberfroid were defined prebiotics as ‘‘growth substrates directly specifically towards potentially beneficial bacteria already resident in the colon’’. Prebiotics are non-digestible food ingredients that stimulate the growth and activity of bacteria in the colon, thereby improving host health (Gibson and Roberfroid, 1995). Probiotics refer to cultures of live micro-organisms which when applied to man or animal may beneficially improve the properties of local flora. Probiotic organisms commonly used in commercial foods are strains of the lactic acid bacteria *lactobacillus* and *bifidobacterium. Streptococcus thermophilus* and *L. delbrueckii subsp. bulgaricus* are used in yoghurt manufacture, although some yeast is also found in probiotic products (Bird et al., 2000). Synbiotics refer to a mixture of prebiotics and probiotics where there is a synergistic interaction between the specific probiotic and a particular prebiotic (Topping et al., 2003). RS appears to function as a prebiotic and symbiotic. Prebiotic effects associated with the consumption of RS, such as growth of beneficial microbial populations and a lowered activity of certain bacterial enzymes (e.g., β-glucuronidase), would be expected to have beneficial impacts on colonic bacterial activity. The various experimental studies on pigs and humans have revealed a time dependent shift in fecal and large bowel short chain fatty acid profile suggesting the possible interaction of RS with the ingested bacteria (Nugent, 2005). A further study demonstrated the capacity for the prebiotic RS3, to reduce the incidence of colon carcinogenesis in rats (Bauer et al., 2006). Consumption of high amylose RS as compared with conventional starch in pigs has resulted in higher fecal concentrations and excretion of *Bifidobacterium longum* ingested orally (Brown et al., 1997). It is also worth noting that RS appears to function differently than more well-known prebiotics (e.g. fructooligosaccharides); when the RS and fructooligosaccharides were fed together, the increase in fecal bacteria was greater than the individual increases observed when these two ingredients were fed separately. These studies concluded that RS qualifies both as prebiotic and synbiotic (Brown et al., 1998).

Absorption of Minerals

In rats and humans, RS increased the absorption of certain minerals in the illeal which is the last part of the small intestine (Sharma et al., 2008). Studies have shown increased calcium, magnesium, zinc, iron and copper absorption in rats fed diets rich in RS, (Younes et al., 1995; Lopez et al., 2001) while increasing only calcium absorption in humans (Trinidad et al., 1996; Coudray et al., 1997). RS could have a positive effect on intestinal calcium and iron absorption. A study to compare the absorption of intestinal calcium, phosphorus, iron, and zinc in the presence of digestible starch showed that a food containing 16.4% RS leads to a more pronounced absorption of calcium and iron compared to fully digestible starch. In view of these studies, RS may have a positive effect on intestinal calcium and iron absorption (Morita et al., 1999).

Reduction of Gall Stone Formation

Digestible starch contributes to the formation of gallstones by further secretion of insulin, and the released insulin also stimulates the synthesis of cholesterol, thus reducing the formation of gallstones since RS does not have these effects. In northern India gallstones cases are less common than in southern India. Because in North India, whole grains are consumed instead of flour. In the United States, Europe and Australia, RS 'dietary intake is 2 to 4 times lower than in populations consuming high starch diets such as India and China. This situation was reflected in the difference in the number of gallstone cases (Tabibloghmany and Ehsandoost, 2014).

Inhibition of Fat Accumulation

Some authors have examined the potential of RS to alter fat oxidation, and although the results are still unclear, several studies have examined its potential as satiety agent and also as well as a component of body weight management. Eating a diet rich in RS is thought to increase the mobilization and use of fat stores as a direct result of a decrease in insulin secretion (Tapsell, 2004). Replacement of 5.4% of total dietary carbohydrates with RS in a food could significantly increase lipid oxidation suggesting reduction in fat accumulation in the long term after eating a meal (Higgins et al., 2004).

Hypocholesterolemic Effects

RS seems to affect lipid metabolism as shown in studies in rats a reduction in a number of lipid metabolism measurements were observed. These include total lipids, total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), triglycerides and triglyceride-rich lipoproteins (Nugent, 2005). Hypocholesterolemic effects of RS have been widely demonstrated. In rats, RS diets (25% raw potato) markedly raised the cecum size and the cecum pool of short chain fatty acids (SCFA), as well as SCFA absorption and lowered plasma cholesterol and triglyceride levels. In addition, all lipoprotein fractions, in particular HDL, have a lower cholesterol concentration and a decreased triglyceride concentration in the triglyceride-rich lipoprotein fraction (Sajilata et al., 2006). Bean starch, rich in resistant starch, decreased serum total cholesterol and VLDL + IDL + LDL cholesterol levels and increased the cecum concentration of SCFA (especially butyric acid concentration).

In a study on hamsters, it was found that when they eat foods containing cassava starch extruded with 9.7% RS or 9.9% oat fiber, both have the potential to be used in foods to improve cardiovascular health as their hypocholesterolemic properties (Martinez-Flores et al., 2004).

According to various studies, RS intake may lower serum cholesterol levels in rats fed a cholesterol-free diet (De Deckere et al., 1993; Hashimoto et al., 2006). Some previous studies in humans have reported the beneficial effect of RS on fasting plasma triglyceride and cholesterol levels. However, some other studies show that RS consumption does not affect total cholesterol measurements in humans. Therefore, it is clear that more research is needed to help us better understand the effects of RS on lipid metabolism in humans (Nugent, 2005).

Prevention of Colonic Cancer

RS is not digested in the small intestine, but reaches the large intestine, where it serves as a substrate for fermentation of the micro flora that encapsulates the intestinal tract (Rahman et al., 2007). It is fermented in the large intestine and results in the production of fermentation end products carbon dioxide, methane, hydrogen, organic acids (e.g. lactic acid) and SCFA (Nugent, 2005). SCFA are the metabolic products of anaerobic bacterial fermentation of polysaccharides, oligosaccharides, proteins, peptides and glycoprotein precursors in the large intestine including those derived from dietary fiber and RS. The dominant SCFA consists of acetate, butyrate and propionate, which account for 90% of total production (Sharma et al., 2008). SCFA are the source of energy of the cells lining the colon. They increase colonic blood flow, lower luminal pH and help prevent the development of abnormal colonic cell populations. SCFA are mainly found in the beginning colon where fermentation is greatest, and the amount present mirrors supply of carbohydrate in the diet. SCFA levels decrease during digestion passing through the column due to the uptake and use by colonocytes and bacteria. In humans, the abundance of SCFA is normally acetate > propionate > butyrate. Total SCFA concentrations are generally between 70 and 140 mm in the beginning colon and between 20 and 70 mm in the center of the colon; therefore, SCFA is found in much lower amounts in the colon center where many colon diseases and colon cancer in most people. As butyrate is one of the main energy substrates for large intestinal epithelial cells. Dietary fiber and RS, as fermented in the large intestine, produce high levels of butyric acid or its salts. According to studies, butyrate production during colonic fermentation is higher in RS than most dietary fibers (Tabibloghmany and Ehsandoost, 2014).

RS can increase the production of SCFA and therefore may help improve colonic health. Animal studies in pigs and rats have reported that feeding RS increased the cecum and fecal production of total SCFA and also the individual concentrations of propionate, butyrate and acetate. In most human studies, increased fecal excretion and/or fecal concentrations of SCFA were reported following supplementation with

RS. However, discrepancies have been observed with respect to effects on the individual SCFA. These differences are most likely due to the experimental method used, the source, type and amount of RS, interindividual variations in length of transit time and on the duration of feeding (Nugent, 2005). Some health advantages of RS summarized in figure 1.6 (Amini et al., 2016).

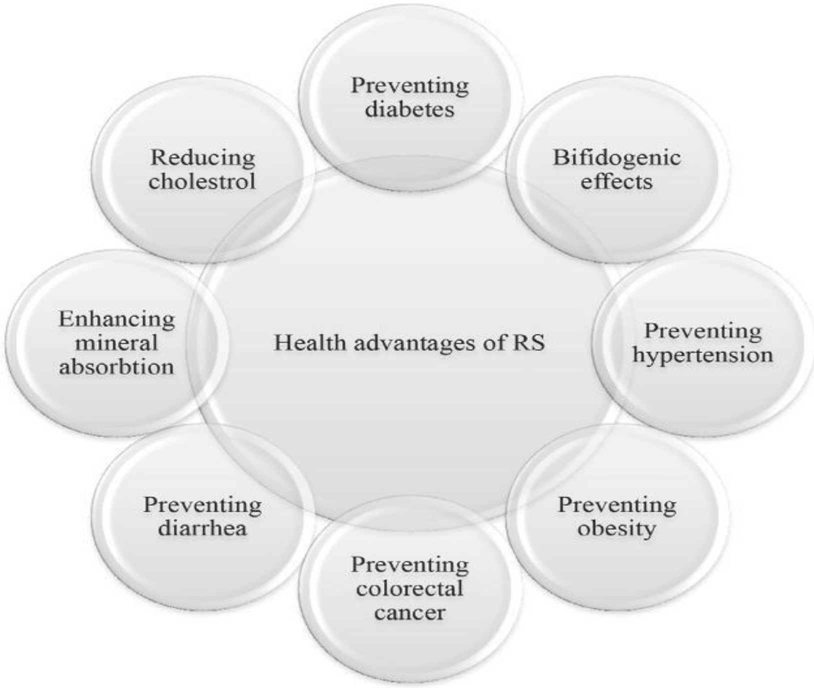

Figure 1.6 Health advantages of RS

1.5 Celiac Disease

Although nutritional habits have changed, cereal and cereal products are gaining value in the nutrition of the world population with the increasing importance given to healthy and balanced nutrition. Grain products are the most important food source of the societies. Grain and grain products have an important place in our nutrition and daily life and can cause discomfort in some people. Celiac disease (CD) is one of these disorders (Türksoy and Özkaya, 2006).

CD is known as gluten-sensitive intestinal disease in genetically sensitive individuals. Today, celiac is the most common genetic disease in all people of the world and it is the food intolerances that continues throughout life. This intolerance, which results in damage to the small intestinal mucosa, prevents the absorption of nutrients known as malabsorption. In celiac patients, the effect of gluten is on the small intestine. When gluten intake, the protrusions (villous) that provide absorption on the inner surface of the small intestine are shortened. Even the inner surface of the intestine is flattened. Single-row "crypt" cells on the surface of villous thickened. Thus, the absorption surface is reduced and food nutrition intake becomes difficult (Türksoy and Özkaya, 2006). Celiac patients cannot tolerate gluten and gluten-like proteins (prolamin) found in cereals throughout their lives. CD is caused by peptide chains containing specific amino acid sequences found in gluten. Gluten is a general term used to describe of wheat storage proteins (prolamin and glutenin). The gluten protein consists of two fractions as prolamin and glutenin. The main toxic effect for CD patients is prolamin fraction, toxic prolamin from different cereals are termed gliadins from wheat, secalin from rye, hordein from barley, avenin from oats and zein from coeliac non-toxic maize (Ciclitira, 2005; Saturni, 2010).

In individuals with CD, iron, folic acid, calcium, minerals and vitamin B_{12} deficiency are seen and bone density decreases (Yalçın, 2005). CD is an adverse immune response to gluten. Celiac patients take small amounts of gluten to their body; symptoms of CD vary, including constipation or loose stools, weight loss, and often fatigue and generally poor health (O'Neill, 2010). Currently, the only scientifically proven treatment for celiac disease is to maintain a gluten-free diet that does not contain wheat, rye, barley or oats for a lifetime (Niewinski, 2008).

In cereal products, biscuits has an important place in the extra-meal nutrition of people of all ages as snacks. Since celiac patients have difficulty in finding safe food other than the food they prepare, they pass their diet with gluten-free products like biscuits, crackers etc. Therefore, snacks are much more important for celiac patients (Dursun, 2015).

According to the research, the formulation used for celiac patients should provide both the desired baking quality and sensory quality. In both respects, the acceptability of quality can be determined by groups consuming these products. At least one of the ingredients in the formula should improve the quality of the baking by providing a viscoelastic structure to the dough, and at least one of the components should provide sensory quality (Singh-Meneghini, 2007). Since the RS provides these two properties at the same time, its use in the biscuit formulation may be the new alternative for celiac patients.

Nowadays, it is stated that gluten-free products contain high amounts of fat and sugar (Hoseney, 1998; Green, 2005; Chugh et al., 2015). One of the nutritional problems in society is the excessive consumption of fat and sugar. With the increasing rate of diseases such as obesity, diabetes and cardiovascular disease, the interest in low-calorie foods is also increasing. In a study, it was determined that the energy intake and carbohydrate consumption of the patients who applied gluten-free diet were less than the patients without CD and the protein and fat consumption were higher (Smecuol et al., 1997; Mariani et al., 1998; Saturni, 2010). RS energy value is lower than the energy value digestible starch and due to its use fat-replacer reduce the amount of fat intake to body.

Around 1% of world population is affected by CD (Cairano et al., 2018). In our country, the incidence of this disease is around 0.2% (Yenice et al., 2005). Generally, the presence of celiac patients in the world at a high rate and the limited number of food they can consume, necessitates increasing the variety of products for these people. For the following reasons more importance should be paid to studies on gluten-free products. There is little variety of gluten-free products in the market. In many gluten-free products (pasta, bread, biscuits, etc.) the quality, consumption and taste are not at the desired level. Gluten-free products are not available in every region. The prices are highly imported from many gluten-free products. With the removal of the glutenin product formulation, major problems occur in the bakery products (Yıldız, 2010). In order to develop new product formulas and improve the quality, research still in progress on the components or mixtures which may be an alternative to gluten. As well as increasing the fiber value, its physical and chemical properties of RS can be an alternative to gluten-free flour.

1.6 Some Components Used in the Production of Gluten-Free Biscuits

The effect of raw materials used in biscuit production on the final product is quite high. The quality of the raw materials used will increase the quality of biscuits. The largest percentage in biscuit formulations is wheat flour. Gluten is often termed the ‘structural’ protein. The gluten in wheat flour is generally responsible for the elastic characteristics of dough, good gas holding properties and contributes to the appearance and crumb structure of many baked products. The absence of gluten network often results in a liquid batter and absence of gluten network can result in baked bread with a crumbling texture, poor color and other quality defects post-baking. Considering the quality parameters such problems are rarely encountered during the manufacture of gluten-free biscuits. Because the development of the gluten network in biscuit doughs is minimal and undesirable except for some semi-sweet biscuits. Generally, biscuit dough is low in water and excess in fat and the desired amount of water for gluten network growth is not present in the dough. The texture of the baked biscuits can be mainly attributed to starch gelatinization and supercooled sugar rather than a protein/starch structure (Gallagher et al., 2004). In this sense, RS can be a new alternative.

Generally in the preparation of gluten-free baked products rice, corn, soya, millet, buckwheat flour are widely used instead of wheat flour. Also corn and potato starch, rice starch, dairy products, gum/hydrocolloids, using in the gluten-free biscuit formula; improve the structure, volume expansion, water absorption, enhance the handling properties of the batter, mouth-feel, acceptability and shelf-life of gluten-free bakery products (Gobbetti et al., 2007; Doğan et al., 2016).

1.6.1 Rice Flour

In the production of gluten-free biscuits, rice flour is used instead of wheat flour (Cairano et al., 2018). Rice flour is the most suitable grain type for gluten-free products because of its white color, flavorless taste, its hypoallergenic properties and its digestibility.

Due to rice flour contains hydrophobic protein, it cannot form a viscoelastic dough. Rice flour products compared to against wheat flour products; rice flour products, more crumbly and have drier structure. And this products during storage is more prone to retrogradation. Therefore, the need for new additives to prevent staling in rice products has become more important (Pastuszka et al., 2009). Many studies are focused on investigating methods that can delay the staling of foods produced from rice. The most successful studies have been the use of additives such as hydrocolloids, different enzymes and emulsifiers (Ji et al., 2010).

1.6.2 Potato and Corn Starch

Most of the studies conducted to produce gluten-free products have used cereal starches. Starches except wheat starch are more preferred. Because some celiac patients may not be able to tolerate even the wheat starch. This is due to the possibility

that the gliadin may be miscible, even if it is small in size. This small amount may disturb the celiac patients when taken for a long time (Chartrand et al., 1997; Horvath and Mehta, 2000; Lohiniemi et al., 2000).

Starches and hydrocolloid can mimic the rheological sensation of fat in mouth due to the binding and orientation of water in the molecule. Therefore, starch and hydrocolloids are widely used to improve the texture and appearance properties of cereal based foods. The most preferred starch types are corn and potato starches (Sharoba, 2014; Ertekin and Dirim, 2015; Doğan, 2016).

1.6.3 Hydrocolloids

Hydrocolloids or gums comprise a number of water-soluble polysaccharides with varied chemical structures providing a range of functional properties that make them suitable for different applications in the food industry. Gums are also called hydrocolloids because colloidal in nature and they are feature hydrophilic colloid (Anton and Artfield, 2008).

Hydrocolloids have been used in foods to improve texture, to increase dough viscosity, to facilitate the processing of the product, to slow down the starch retrogradation, to increase moisture retention, and to extend the overall quality of the product during time (Anton and Artfield, 2008). In the bakery industry there is an increasing demand for hydrocolloids which it is used for various purposes. Guar gum, xanthan gum and locust bean gum are widely used due to their water binding and stabilizing properties (Rojas et al., 1999).

Guar gum (GG) which is derived from the seed of a leguminous plant *Cyamopsis tetragonolobus*, is used as a thickening and stabilizing agent in a wide variety of foods due to the high viscosity of its aqueous solutions even at low concentrations. In baked goods, GG is used to improve the mouth feel, change their rheological properties, mixing and recipe tolerance and to enhance the shelf life through moisture retention

(Kohajdova and Karovicova, 2009). In the study, incorporation of GG significantly increased specific volume, leading to lower crumb hardness on the baking day compared with control of gluten-free bread (Mohammadi et al., 2015). In another study found that the addition of GG into frozen dough produced bread with a higher volume and a more open crumb structure with higher percentage of gas cells as compared to those prepared without GG (Ribotta et al., 2001).

Xanthan gum (XG) is an exocellular heteropolysaccharide produced by the microorganism *Xanthomonas campestris* by a fermentation process. The most important properties of XG are its high low-shear viscosity and strong shear-thinning character. The relatively low viscosity at high shear rates makes it easy to mix, pour, and swallow. Its high viscosity at low shear rates gives it good suspension properties and stability to colloidal suspensions (Kohajdova and Karovicova, 2009). XG increases dough stability, water absorption and gas maintenance. The pseudo plastic property of XG is important during dough preparation, i.e. pumping, kneading and rolling (Mohammadi et al., 2014). In the study, discussed the improvement of viscoelastic properties of gluten-free dough by incorporation of different hydrocolloids, where xanthan exhibited the best performance (Lazaridou et al., 2007).

Locust bean gum (LGB), is a natural hydrocolloid extracted from the seeds of carob tree (*Ceratonia siliqua L.*) after the removal of testa (seed coat). LBG is commonly used in food and industrial applications due to its ability to produce very viscous solutions at relatively low concentrations, which are almost unaffected by pH, salts, or heat processing. LBG is applied in the food industry as a thickener or viscosity modifier, free water binder, or as a suspending agent or stabilizer in cheeses, frozen confections, bakery products. Application of this hydrocolloid for bakery purposes results in higher baked product yields; it improves the final texture and adds viscosity to the dough (Kohajdova and Karovicova, 2009).

1.7 Problem Statement and Aim of Study

Biscuit is one of the most popular bakery products which is consumed frequently in almost every society. The fact that the nutritional quality of biscuit is good, satisfying and cheap is the main reasons for this. Furthermore, biscuit consumption is increasing day by day because it can be stored without stale for long periods and can be presented to the consumer in different tastes. Biscuit which is snacks has an important place in non-meal nutrition. In the content of cereals such as wheat, rye, barley and oats used in biscuit making, gluten which is included as protein group it is responsible for the net structure formed during mixing and fermentation of the dough. Gluten which is the most important component of flour used in biscuit making, affects the workability, elasticity and extensibility of the biscuit dough and affects eating quality, appearance and texture of final baked product. Grain and grain products containing gluten protein that is an important part of our nutrition cause discomforts in some people. Celiac which is one of the cereal diseases, as a result of consumption of wheat, rye, barley and oat products is a disease that occurs in the intestine. Due to celiac patients find it difficult to find safe food outside their homes, they have to diet with gluten-free snacks (biscuit, cracker etc.). Therefore, snacks products are very important for celiac patients. For celiac patients, due to the lack of snacks variety and high prices gluten-free snacks production and variation should be increased. In addition, nutritional specification of gluten-free products should be enriched in terms of adequate, balanced and healthy nutrition. The majority of studies on gluten-free products examine the use of alternative protein sources in the production of these products and the effects of texture regulating additives such as hydrocolloids on the product. The studies related to enrichment of gluten-free products with components of functional importance have not reached the desired level. However, in recent years, studies on gluten-free products with increased functional properties have been increasing. In recent years, differences in the nutritional habits of the society, the development of healthy eating awareness, due to increase obesity and cardiovascular diseases, the consumers expect to provide healthy wounds in addition to their nutritious properties. Depending on these demands of the consumer, functional food production and consumption has increased.

Therefore, the objectives of this work were;

- to produce functional biscuits that are beneficial for health by using resistant starch which is functional component and dietary fiber source in the gluten-free biscuits. The other aim is to contribute to the expansion of the gluten-free product range by developing healthy biscuit formula by increasing the amount of dietary fiber in gluten-free biscuits and to obtain a functional product for celiac patients.

- to determine the optimum baking temperature and resistant starch adding rate for the production process and the final product quality and to investigate physical and chemical properties of the gluten-free biscuits produced.
- to determine of dietary fiber percent of gluten-free biscuits at different resistant starch adding level at the optimum baking temperature.

CHAPTER 2

MATERIAL AND METHODS

2.1 Materials

Gluten-free flour used in the production of gluten-free biscuit were purchased from company Söke Değirmencilik San. ve Tic. A.Ş. (Aydın, Turkey). Resistant starch (Novelose 330, resistant starch type 3) used instead of gluten-free flour in this study were purchased from company of Ingredion Incorporated (Westchester, USA). Also, some raw materials used in biscuit making; sugar (Türkiye Şeker Fabrikaları A.Ş., Ankara), fructose syrup (Sunar Mısır Entegre Tesisleri San. ve Tic. A.Ş., Adana), vegetable oil (Marsa Yağ San. Ve Tic. A.Ş., Adana), ammonium bicarbonate (Beşel Endüstriyel Ürünler Gıda ve Ambalaj San.Tic. A.Ş., Konya), sodium bicarbonate (BRP Kimya Taah. Tic. Ltd. Şti, İstanbul), sodium acid pyrophosphate (Azelis Türkiye Kimya Endüstriyel Ürünler İthalat İhracat San. ve Tic. A.Ş., İstanbul), whole milk powder (Enka Süt Ve Gida Mamülleri Sanayi Ve Ticaret A.Ş., Konya), vanillin (Solvay Kimya Tic. Ltd. Şti., İstanbul).

2.2 Methods

2.2.1 Experimental Biscuit Production

4 different formulations were prepared in this study. One biscuit formulations were prepared with gluten-free flour used instead of wheat flour as a control. Three biscuit formulations were prepared with RS3 type RS (Novelose 330) used instead of gluten-free flour. As a control group biscuits, gluten-free biscuit formulation prepared with gluten-free flour is given in the table 2.1. RS added biscuit formulation prepared with RS instead of 10, 20 and 30% gluten-free flour is given in the table 2.1.

Table 2.1 Biscuit formulation containing gluten-free flour and containing RS instead of gluten-free flour

Components	Control (%)	Trial 1 (%)	Trial 2 (%)	Trial 3 (%)
Gluten-free flour	56.07	50.44	44.86	39.25
Sugar	16.20	16.20	16.20	16.20
Vegetable oil	16.02	16.02	16.02	16.02
Water	6.00	6.00	6.00	6.00
Resistant Starch	0.00	5.63	11.21	16.82
Fructose Syrup	3.20	3.20	3.20	3.20
Whole milk powder	1.00	1.00	1.00	1.00
Ammonium bicarbonate	0.40	0.40	0.40	0.40
Salt	0.34	0.34	0.34	0.34
Sodium bicarbonate	0.30	0.30	0.30	0.30
Emulsifier (lecithin)	0.25	0.25	0.25	0.25
Sapp*	0.20	0.20	0.20	0.20
Vanillin	0.02	0.02	0.02	0.02

*Sapp; Sodium acid pyrophosphate

Biscuit dough was prepared with laboratory type mixer (Kenwood, KMX80 Mixer) was prepared by modifying the AACC method (10-53.1). In the first stage, sugar, vegetable oil, emulsifier, whole milk powder and fructose syrup were added to the mixer and the added mixture was stirred for 5 minutes at cycle 3 (~ 45 rpm). On the substances added in the first stage; water, leavening agents (sodium bicarbonate, ammonium bicarbonate) and salt was added and stirred for 5 minutes at cycle 3 (~ 45 rpm). Subsequently, on the substances added in the second stage; wheat or gluten-free flour, RS, sodium acid pyrophosphate, vanillin was added and stirred for 5 minutes at cycle 2 (~ 30 rpm) and dough preparation process is completed. The same method was used making biscuit dough for 4 different formulations. And standard biscuit mould was used to shape all biscuit dough. The moulded biscuit dough was placed in standard baking trays for baking. The placed biscuit dough in the trays, the baking time was kept constant (7 minutes) and baked at 175^{o}C, 200^{o}C, 225^{o}C different three baking temperatures by using electric stone oven (Wiesheu, Germany). In the conventional oven, the gluten-free biscuits were baked for 3.5 minute at each baking temperatures, after 3.5 minutes the baking trays were turned 180ºC, bringing the rear side of baking tray to the front of the oven to ensure homogenous cooking, and again gluten-free biscuits were baked for a further 3.5 min at the each temperature. Baked biscuits were kept until reach room temperature (after the oven approximately 20 minute). For analysis of biscuit samples which was kept in air-tight and heat-sealed packages at 18ºC (±2).

All raw materials used in the study were kept at 18ºC (±2) in the Şölen Çikolata Gıda San. ve Tic. A.Ş. research and development laboratory.

2.2.2 Analysis of Samples

The analyses were carried out for biscuit samples. The experiments were performed as duplicate and the results were presented as average.

Moisture Contents and Water Activity

The % moisture content of prepared biscuit samples was determined duplicate of each formulation according to approved method 44-15.02 (AACC International, 2009). Baked biscuit samples were ground and 2-3 grams were placed in the moisture device (Sartorius MA-100, Germany) and the results were recorded.

Water activity (aw) of prepared biscuit samples was determined duplicate of each formulation, using Novasina Lab Master (Switzerland) calibrated with a saturated potassium acetate solution (aw=0.22) (Laguna et al., 2011).

pH Analysis

pH meter is based on the principle of measuring the potential difference between the liquid in the meter electrode and the liquid prepared from the biscuit sample.

The sample was homogenized with distilled water at 1:9 ratio and analyzed by using pH-meter (Thermo Scientific Orion 4 Star Plus) which is approved method 02-52.01 (AACC International, 1999).

Color of Biscuits

The color of the biscuit was measured using a Hunter's Lab color analyzer (Color flex Hunter Lab, USA). In the Hunter's lab colorimeter, the color of a sample is shown in the three dimensions, L*, a* and b*. As the L value approaches zero, the color of the samples is blacker. As the L value approaches the hundred, the color of the samples is whiter. Minus a* value indicates that the sample color is greenish, plus a* value indicates that the sample color is reddish. Minus b* value indicates that the sample color is bluish, plus b* value indicates that the sample color is yellowish. The L*, a* and b* readings data were recorded on the analyzed device. In each formulation baked two biscuits were ground and the color values was read on screen.

Texture Analysis

Approved 74-09.01 (AACC International, 1999) method was used to determine the texture properties of biscuit samples and it was determined according to the three point bend rig apparatus (HDP/M3PB blade) using TA.XT plus Texture Analyzer (TA-XT plus, Stable Microsystems Ltd., England). The crusher blade was attached to the cross head of the device. And each gluten-free biscuit sample was placed on the platform such that it was supported from two points and the program was run. The force required to break the biscuit (the hardness of the biscuit, N) and the fracturability (mm) were determined. The TA setting was, load cell: 5 kg, pre-test speed: 1.0 mm/s, test speed: 5.0 mm/s, post-test speed: 10.0 mm/s, distance: 10 mm, trigger force: 50 g. This test simulates the consumer's hardness assessment by holding the biscuit in hand and breaking it in the same way by bending. The absolute peak force, so that was maximum value, from the resulting curve is considered to be the biscuit breaking force (Bukya et al., 2013).

Diameter, Thickness

With approved 10-54.01 method (AACC International, 1999) was used to determine the diameter and thickness of baked biscuits which are randomly selected were measured by using digital caliper (0.01 mm Mitutoyo, Japan).

Total Dietary Fiber

The total dietary fiber amount calculated according to approved 985.29 method (AOAC, 1985). To calculate the amount of dietary fiber in biscuits produced; we were weigh duplicate 1 gram biscuit samples into 400mL tall-form beakers. Added 50 ml pH 6.0 phosphate buffer to each beaker and checked pH with pH meter. The pH of the solution should approximately 6.0. And we were add 0.2 ml heat-stable α-amylase solution. Beaker covered with aluminum foil and placed in boiling water bath. Beaker must be incubated at 98-100°C for 15 min for the starch gelatinization and hydrolysis to dextrin. And cooled solutions to room temperature. After, adjust to pH 7.5 by adding 10 mL 0.275 N NaOH solution and checked pH with pH meter. Added 5 mg protease, again beaker covered with aluminum foil and incubate at 60° with continuous agitation for 30 min for the protein hydrolysis. Cooled solutions to room temperature. And added 10 ml 0.325N HCl solution to adjust pH to 4.5 and checked pH with pH meter. Added 0.3 ml amyloglucosidase, covered with aluminum foil, and incubate 20 min at 60° with continuous agitation for starch dextrin hydrolysis. For precipitation we were added 280 ml 95% EtOH preheated to 60° C and precipitated form at room temperature for 60 min. And that precipitation washed with 78% EtOH, 10-ml portions of 95% EtOH and 10-ml portions of acetone and filtrated. Analysed residue from one sample of set of duplicates for protein, the second residue sample of duplicate incinerated for 5 h at 525°C for ash. To evaluate dietary fiber can be calculated by subtracting, amount of ash and protein according to calculated result from residues.

Statistical Analysis

All measurements and experiments were done in duplicate and mean values of them were reported. Analysis of variance was performed by the SPSS Statistics (SPSS; Statistical package for social sciences, IBM, version 25, New York, USA). Tukey multiple range test was used to obtain comparisons among sample means. The confidence interval was chosen as 95% for the model and all other tests (α=0.05). The fit of the model was assessed by coefficient of determination (R^2) and One-way ANOVA results. Sigma Plot 14.0 (Systat Software GmbH, Erkrath, Germany) software program was also used to analyze the data for the regression coefficients used to form the mathematical models that explained the relationship between the independent variable: temperature (X_1), RS concentration (X_2). The response variables (Y); moisture content, water activity, pH, diameter, height, hardness, fracturability and total dietary fiber of the gluten-free biscuit samples. The model search was started with the quadratic equation as shown in equation 2.1 below:

$$Y= \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_1X_2 + \beta_4X_1^2 + \beta_5X_2^2 \qquad (2.1)$$

As mentioned above where Y is the predicted responses, β is the parameter estimate (coefficient) for each linear and cross product term for the prediction model, X_1, X_2

and X_1X_2 are the linear terms for temperature and RS concentration and their cross product terms.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Moisture Content and Water Activity

Table 3.1 Moisture content and water activity values of gluten-free biscuits according to baking temperature at different RS concentration

No	Temperature (ºC)	RS (%)	Moisture Content (%)	Water Activity
1	175	0	5.33 ± 0.18^{a}	0.321 ± 0.009^{a}
2	175	10	5.51 ± 0.05^{ab}	0.327 ± 0.085^{a}
3	175	20	5.78 ± 0.05^{b}	0.341 ± 0.005^{a}
4	175	30	5.90 ± 0.01^{b}	0.375 ± 0.006^{b}
5	200	0	3.63 ± 0.07^{a}	0.282 ± 0.006^{a}
6	200	10	3.84 ± 0.05^{ab}	0.299 ± 0.003^{b}
7	200	20	4.04 ± 0.11^{bc}	0.303 ± 0.003^{b}
8	200	30	4.27 ± 0.03^{c}	0.310 ± 0.002^{b}
9	225	0	1.92 ± 0.05^{a}	0.138 ± 0.002^{a}
10	225	10	2.04 ± 0.08^{ab}	0.206 ± 0.007^{b}
11	225	20	2.21 ± 0.05^{bc}	0.218 ± 0.001^{b}
12	225	30	2.35 ± 0.18^{c}	0.238 ± 0.004^{c}

RS: Resistant starch. Values are the Mean ± SD from duplicate determinations, the same letter shows non-significant difference at α=0.05 level at the each baking temperature according to the Tukey test.

Moisture content results of gluten-free biscuits, which are baked at different temperatures (175, 200 and 225ºC) and added in different proportions RS (0, 10, 20, and 30%) are shown in the table 3.1. The moisture content of the biscuits was determined between 1.92 - 5.78%. Gluten-free biscuits which were baked at 175ºC and were added 0% RS it has the lowest moisture content (5.33%) and also biscuits which were added 30% RS has the highest moisture content at the same temperature (5.78%).

Biscuits which were baked at 200ºC and were added 0% RS it has the lowest moisture content (3.63%) and also biscuits which were added 30% RS has the highest moisture content at the same temperature (4.27%). Biscuits which were baked at 225ºC and were added 0% RS it has the lowest moisture content (1.92%) and also biscuits which were added 30% RS has the highest moisture content at the same temperature (2.35%). The moisture content of gluten-free biscuits have increased with respect to the control value as the rate of RS was increased at all baking temperatures. For each baking temperature, variance analysis were made according to the level of RS addition. As a result of the analysis of variance, it has determined that the different RS addition that was baked at 175, 200 and 225ºC on the moisture values of gluten-free biscuits was significant ($\alpha<0.05$). ANOVA results of moisture contents values are given in tables A.1 to A.6 in the appendix.

The overall quality of bakery products is dependent on chemical, biochemical, physical, rheological and structural changes (volume expansion, evaporation of water, starch gelatinization, protein denaturation, aroma compound formation) which occur during baking, which are highly influenced by changes of moisture content as a function of process parameters (Saric et al., 2014). For the reasons mentioned above, another important factor affecting the end consumer in terms of eating quality is the amount of moisture content in the product. Although the processing of biscuits types depending on the type of biscuit, the moisture content is generally between 1% and 5%. Moisture content can be increased up to 7% by considering water activity in biscuits special type that eating quality and texture are softer (Manley, 2000). The granular starch can hold about 30% of its dry weight as moisture below its gelatinization temperature (Hoseney, 1994). Starch granules are insoluble in water and swell up starch granules by absorbing water so, water holding capacity increases. If the temperature is increased, the starch granules loses its regular structure after a certain temperature (gelatinization temperature). In the study, they have investigated three different RS samples into the bread formulation. In the farinogram measurements, they have observed that the water holding capacity of the RS added wheat doughs is higher than the ones not added (Öztürk et al., 2007).

It is thought that the high amount of water content of biscuits is due to the high water holding capacity of the substances in the composition. As the starch addition rate increases, the reason for the increase in moisture value is thought to be RS that has a higher water holding capacity than other raw materials found in biscuit recipe. RS3 has shown a higher water holding capacity than granular starch (Nissar et al., 2017). It was stated that the RS (1.20 g/g) has more water holding capacity than wheat dietary fiber (1.06 g/g). Based on the results obtained, it was thought to increase the moisture content, while it might not the most appropriate additive to meet the requirements of special products such as cookies (Wang et al., 2014). Fiber sources added to formulations increase the water holding capacity of the dough and affect the textural properties (De Simas et al., 2009). According to study, obtained an increase in moisture content for biscuits enriched with a RS containing product extracted from bananas. Moisture content of control and experimental biscuits is 4.49% and 5.70%. This increase in moisture content of biscuits can be explained by the abundance of amylose and non-crystalline amylopectin that is amorphous regions present in the starch (Aparicio-Saguila’n et al., 2007). Another study the related with biscuit and RS, the moisture content of control biscuits which contain fully wheat flour, 20%, 40% and 60% RS instead of wheat flour; 4.37%, 5.51%, 5.09% and 5.21%, respectively (Laguna et al., 2011).

In experiments with gluten-free biscuits enriched with blueberry pomace; different baking temperature and baking time has been applied to gluten-free biscuits. Baking time has kept constant (16 minutes) and biscuits have bake at 160^{o}C and 170^{o}C. Moisture content of biscuits baked at 160^{o}C and 170^{o}C was measured as 6.15 and 5.76%, respectively (Saric et al., 2014). Water evaporates from the biscuits with the temperature applied during the baking process. More water will evaporates as the applied temperature increases.

The equilibrium of the polynomial model explaining the effect of factors on moisture content of gluten-free biscuits was given below (R^2=0.99) (Equation 3.1).

$$\text{Moisture Content} = 13.2557 - 0.0273*T + 0.0389*C - 9.9*10^{-5}*T*C - 1.03*10^{-4}*T^2 - 2.0833*10^{-5}*C^2 \quad (3.1)$$

where T is temperature ^{o}C and C is the concentration %.

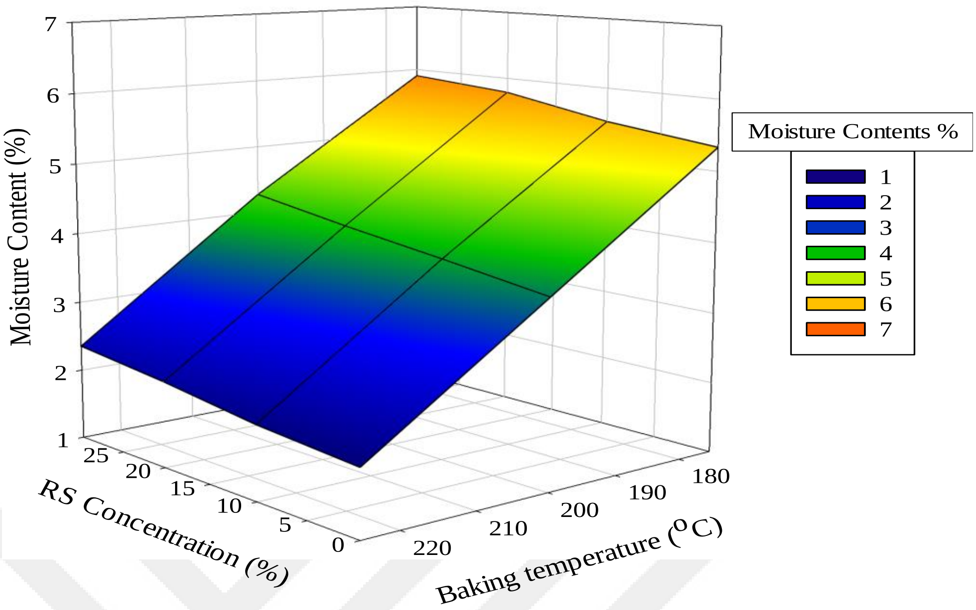

Figure 3.1 3D plot of baking temperature and RS concentration against moisture content values of gluten-free biscuits

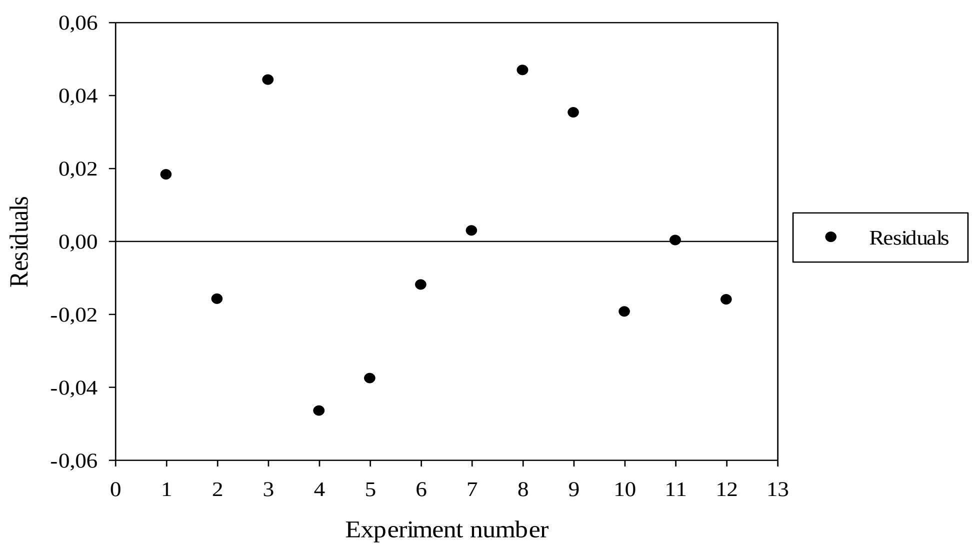

Figure 3.2 Residual plot of moisture content values of gluten-free biscuits

According to the order of experiments baking temperatures and RS concentrations respectively to the polynomial equation obtained as a result of the regression analysis was written. Predicted moisture content of gluten-free biscuits, for each experiment were obtained according to the polynomial equation. After obtaining the predicted moisture contents according to the equation, the residual plot was drawn by subtracting predicted moisture contents from the measured moisture contents. Residual values were calculated for each experiment.

Water activity results of gluten-free biscuits, which are baked at different temperatures (175, 200 and 225ºC) and added in different proportions RS (0, 10, 20, and 30%) are shown in the table 3.1. The water activity of the biscuits was determined between 0.138 - 0.375. Gluten-free biscuits which were baked at 175ºC and were added 0% RS it has the lowest water activity (0.321%) and also biscuits which were added 30% RS has the highest water activity at the same temperature (0.375%). Biscuits which were baked at 200ºC and were added 0% RS it has the lowest water activity (0.282%) and also biscuits which were added 30% RS has the highest water activity at the same temperature (0.310%). Biscuits which were baked at 225ºC and were added 0% RS it has the lowest water activity (0.138%) and also biscuits which were added 30% RS has the highest water activity at the same temperature (0.238%).

In all experiment water activity were higher in the gluten-free biscuits added RS than from the control (Table 3.1). The increase in water holding capacity of gluten-free biscuits can be explained by the presence and increase of the amount of resistant starch in the formulations.

Knowledge of a product's moisture content gives no indication of whether that product will lose or gain water if left exposed in an atmosphere of given humidity. The relation between a product and its environment is defined by a property known as the water activity. The relative humidity of an atmosphere in equilibrium with a product defines its water activity. Water activity scale is 0 - 1.0 (Manley, 2000). It has become the basic controlling factor in the preservation of foods against microbiological, chemical and physical deterioration (Chowdhury et al., 2012). And microbial activity occurs only water activity above 0.65 (Manley, 2000).

According to the study, obtained an increase in water activity for short dough biscuits replacing part of the wheat flour with a RS. The water activity of control, 20%, 40% and 60% RS instead of wheat flour; 0.50, 0.59, 0.56 and 0.55, respectively (Laguna et al., 2011).

In experiments with gluten-free biscuits enriched with blueberry pomace; different baking temperature and baking time has been applied to gluten-free biscuits. Baking time has kept constant (16 minutes) and biscuits have bake at 160ºC and 170ºC. Water activity of biscuits baked at 160ºC and 170ºC was measured as 0.41 and 0.36, respectively (Saric et al., 2014). As in our results, in the experiment a decrease in water activity is observed with the effect of temperature. As it is worth the moisture content, the water evaporates from the biscuits with the temperature applied during the baking process. More water will evaporates as the applied temperature increases and the amount of water trapped by the biscuit decreases.

The water activity of gluten-free biscuits increased with respect to the control value as the rate of RS was increased at all baking temperatures. As a result of the analysis of variance, it was found that the different RS addition that was baked at 175, 200 and 225ºC on the water activity of gluten-free biscuits was significant ($\alpha<0.05$). ANOVA results of water activity values are given in tables A.7 to A.12 in the appendix.

The equilibrium of the polynomial model explaining the effect of factors on water activity of gluten-free biscuits was given below ($R^2=0.96$) (Equation 3.2).

$$\text{Water activity} = -0.8792 + 0.0147*T - 0.0028*C + 2.72*10^{-5}*T*C - 4.48*10^{5}*T^{2} - 2.50*10^{-5}*C^{2} \quad (3.2)$$

where T is temperature ºC and C is the concentration %

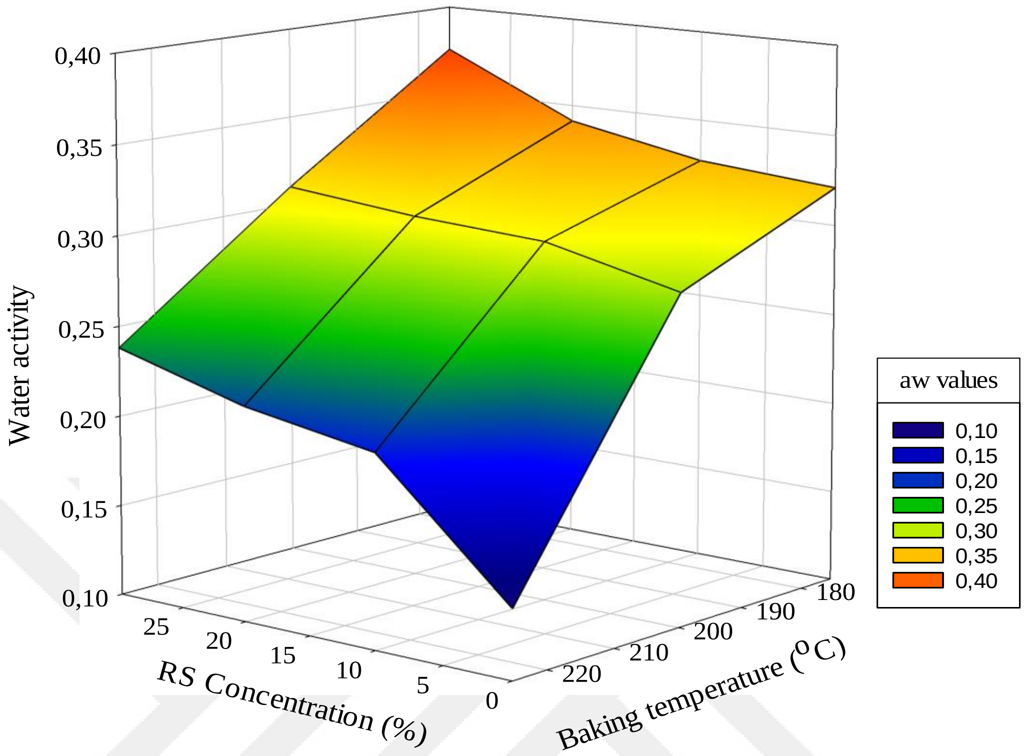

Figure 3.3 3D plot of baking temperature and RS concentration against water activity values of gluten-free biscuits

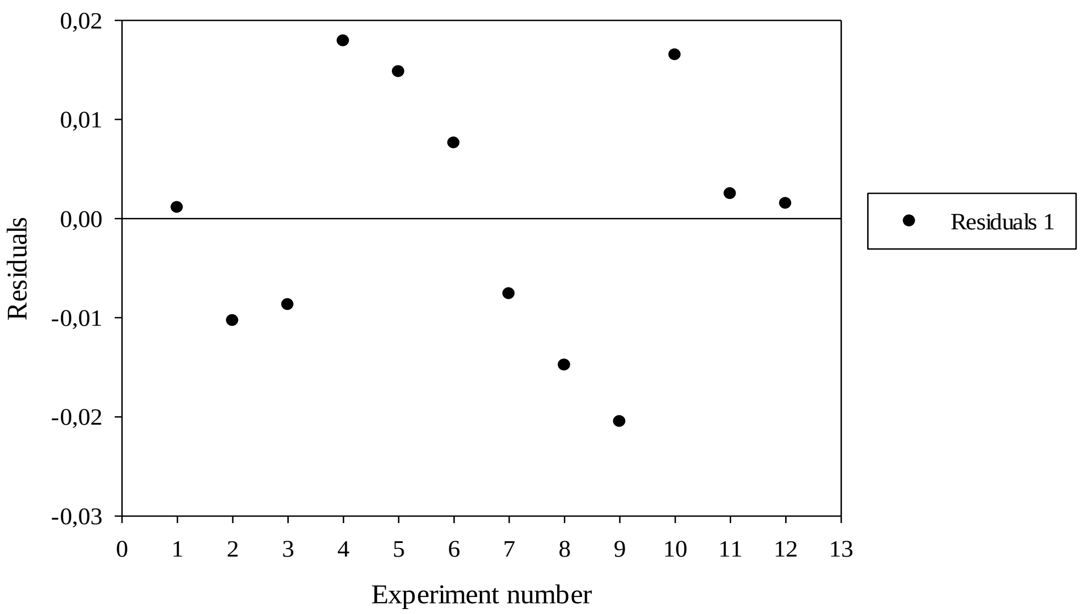

Figure 3.4 Residual plot of water activity values of gluten-free biscuits

According to the order of experiments baking temperatures and RS concentrations respectively to the polynomial equation obtained as a result of the regression analysis was written. Predicted water activity values of gluten-free biscuits, for each experiment were obtained according to the polynomial equation. After obtaining the predicted water activity values according to the equation, the residual plot was drawn by subtracting predicted water activity values from the measured water activity values. Residual values were calculated for each experiment.

3.2 pH

Table 3.2 pH values of gluten-free biscuits according to baking temperature at different RS concentration

No	Temperature (^oC)	RS (%)	pH
1	175	0	7.37±0.24^a
2	175	10	7.30±0.03^a
3	175	20	7.28±0.23^a
4	175	30	7.19±0.02^a
5	200	0	7.15±0.20^a
6	200	10	7.07±0.03^a
7	200	20	7.04±0.01^a
8	200	30	7.01±0.03^a
9	225	0	6.92±0.18^a
10	225	10	6.83±0.03^a
11	225	20	6.67±0.06^a
12	225	30	6.61±0.01^a

RS: Resistant starch. Values are the Mean ± SD from duplicate determinations, the same letter shows non-significant difference at α=0.05 level at the each baking temperature according to the Tukey test.

pH results of gluten-free biscuits, which are baked at different temperatures (175, 200 and 225^oC) and added in different proportions RS (0, 10, 20, and 30%) are shown in the table 3.2. The pH value of the biscuits was determined between 6.61 - 7.37. Gluten-free biscuits which were baked at 175^oC and were added 0% RS it has the highest pH value (7.37) and also biscuits which were added 30% RS has the lowest pH value at the same temperature (7.19). Biscuits which were baked at 200^oC and were added 0%

RS it has the highest pH value (7.15) and also biscuits which were added 30% RS has the lowest pH value at the same temperature (7.01). Biscuits which were baked at 225^{o}C and were added 0% RS it has the highest pH value (6.92) and also biscuits which were added 30% RS has the lowest pH value at the same temperature (6.61).

The pH value of gluten-free biscuits decreased with respect to the control value as the rate of RS was increased at all baking temperatures. For each baking temperature, variance analysis were made according to the level of RS addition. As a result of the analysis of variance, it was found that the different RS addition that was baked at 175, 200 and 225^{o}C on the pH values of gluten-free biscuits was not significant ($\alpha > 0.05$). ANOVA results of pH values are given in tables B.1 to B.6 in the appendix.

It has been determined, the pH value has decreased as the amount of RS addition increased at each baking temperature. The reason for this could be pH value of the RS. The pH value of the RS (Novelose 330, RS3 type) ranges between from 4 to 7. Due to the pH value of the RS, produced gluten-free biscuits pH value also decreased. In one study, the pH value of the RS3 type that was stated 5.33 (Sanz et al., 2008). In a study conducted by Doğan, *Rheum ribes* (ışgın) plant that is functional component was added to 1 and 2% in gluten-free biscuit formulation. The pH of the gluten-free biscuit that was control group has measured as 7.74. The pH values of gluten-free biscuits with added 1 and 2% *Rheum ribes* have measured as 7.45 and 7.27. It is stated that Rheum has low pH due to its acidic properties and produced biscuits also decrease the pH value (Doğan, 2016). In experiments, it was found that the pH value decreased as the baking temperature increased.

The equilibrium of the polynomial model explaining the effect of factors on pH value of gluten-free biscuits was given below (R^2=0.98) (Equation 3.3).

$$pH = 5.9363 + 0.0214*T + 0.0129*C - 1.00^{-4}*T*C - 7.6*10^{-5}*T^2 + 5.83*10^{5}*C^2 \quad (3.3)$$

where T is temperature ^{o}C and C is the concentration %.

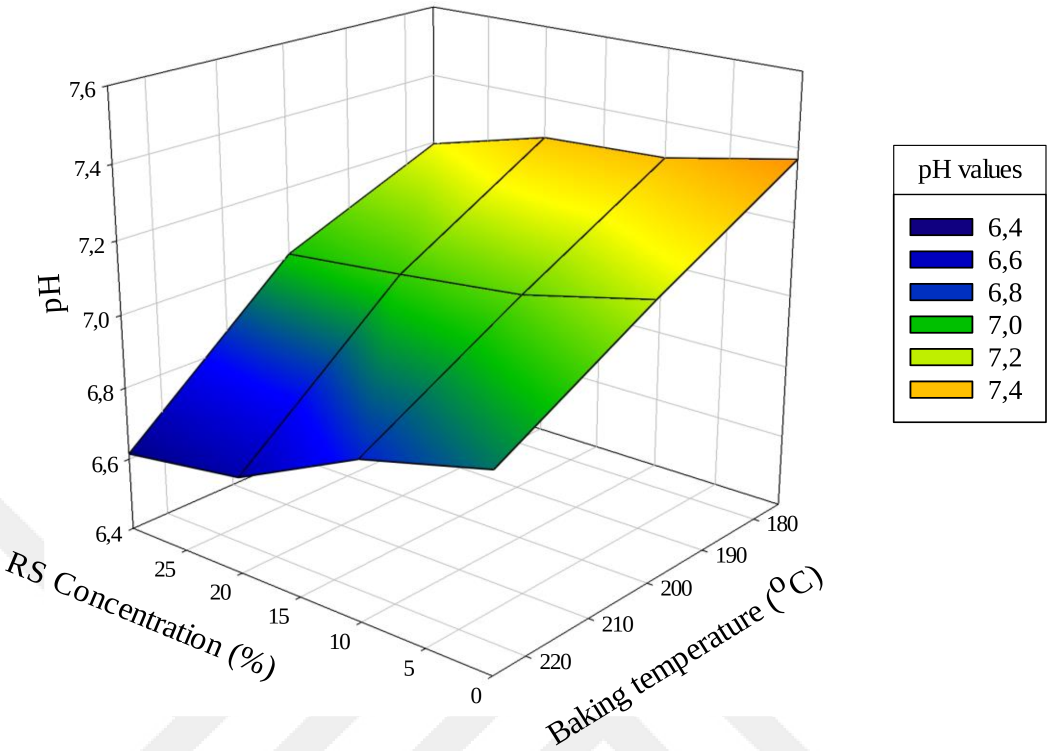

Figure 3.5 3D plot of baking temperature and RS concentration against pH values of gluten-free biscuits

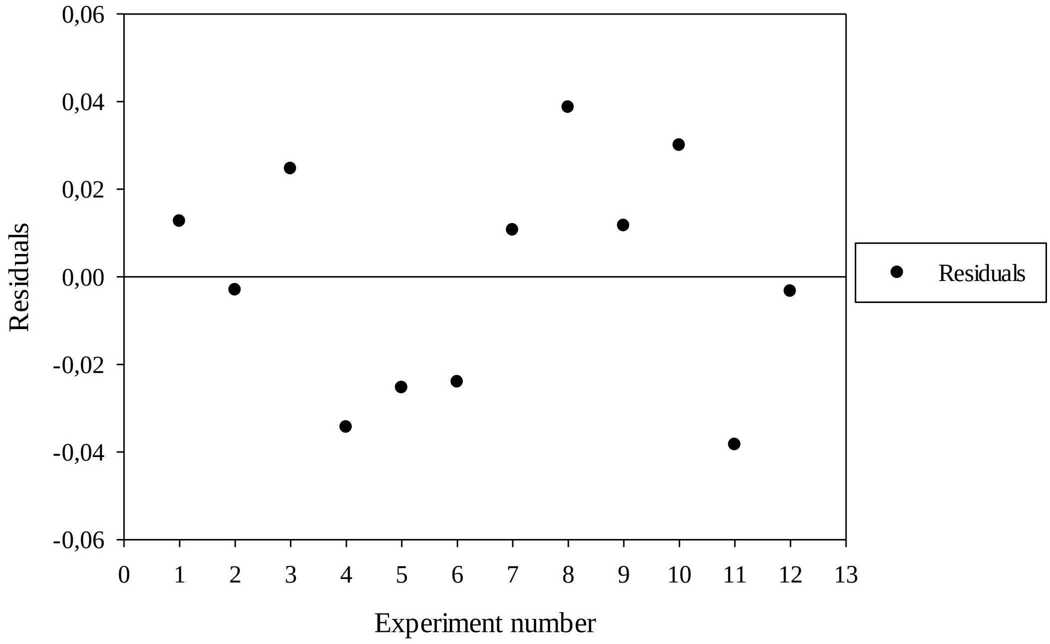

Figure 3.6 Residual plot of pH values of gluten-free biscuits

According to the order of experiments baking temperatures and RS concentrations respectively to the polynomial equation obtained as a result of the regression analysis was written. Predicted pH values of gluten-free biscuits, for each experiment were obtained according to the polynomial equation. After obtaining the predicted pH values according to the equation, the residual plot was drawn by subtracting predicted pH values from the measured pH values. Residual values were calculated for each experiment.

3.3 Color Analysis

Table 3.3 Color parameters of gluten-free biscuits according to baking temperature at different RS concentration

No	Temperature (°C)	RS (%)	L*	a*	b*
1	175	0	81.32±1.25^{a}	7.16±0.19^{a}	32.74±0.51^{a}
2	175	10	83.74±2.63^{ab}	3.11±014^{b}	25.84±0.80^{b}
3	175	20	85.45±1.77^{ab}	1.98±0.01^{c}	19.82±0.29^{c}
4	175	30	88.73±0.03^{b}	0.84±0.02^{d}	16.79±0.15^{d}
5	200	0	80.68±1.25^{a}	8.16±0.17^{a}	33.96±0.29^{a}
6	200	10	81.61±0.44^{ab}	4.74±0.10^{b}	28.06±0.82^{b}
7	200	20	84.26±0.99^{b}	3.28±0.09^{c}	24.23±1.06^{c}
8	200	30	84.90±0.02^{b}	2.74±0.00^{d}	23.89±0.08^{c}
9	225	0	70.81±0.38^{a}	16.47±0.36^{a}	36.19±0.59^{a}
10	225	10	72.27±1.31^{ab}	12.75±0.14^{b}	35.99±0.14^{a}
11	225	20	72.88±0.48^{ab}	10.86±0.35^{c}	34.06±0.07^{b}
12	225	30	74.56±0.71^{b}	7.47±0.12^{d}	32.55±0.64^{b}

RS: Resistant starch. L*: Lightness a*: redness; b*: yellowness. Values are the Mean ± SD from duplicate determinations, the same letter shows non-significant difference at α=0.05 level at the each RS concentration according to the Tukey test.

Color parameters (L*, a*, b*) results of gluten-free biscuits, which are baked at different temperatures (175, 200 and 225°C) and added in different proportions RS (0, 10, 20, and 30%) are shown in the table 3.3. The L* value of the biscuits was determined between 70.81 - 88.73. Gluten-free biscuits which were baked at 175°C and were added 0% RS it has the lowest L* value (81.32) and also biscuits which were added 30% RS has the highest L* value at the same temperature (88.73). Biscuits which were baked at 200^{o}C and were added 0% RS it has the lowest L* value (80.68) and also biscuits which were added 30% RS has the highest L* value at the same temperature (84.90). Biscuits which were baked at 225^{o}C and were added 0% RS it has the lowest L* value (70.81) and also biscuits which were added 30% RS has the highest L* value at the same temperature (74.56).

The a* value of the biscuits was determined between 0.84 - 16.47. Gluten-free biscuits which were baked at 175^{o}C and were added 0% RS it has the highest a* value (7.16) and also biscuits which were added 30% RS has the lowest a* value at the same temperature (0.84). Biscuits which were baked at 200^{o}C and were added 0% RS it has the highest a* value (8.16) and also biscuits which were added 30% RS has the lowest a* value at the same temperature (2.74). Biscuits which were baked at 225^{o}C and were added 0% RS it has the highest a* value (16.47) and also biscuits which were added 30% RS has the lowest a* value at the same temperature (7.47).

The b* value of the biscuits was determined between 16.79 - 36.19. Gluten-free biscuits which were baked at 175^{o}C and were added 0% RS it has the highest b* value (32.74) and also biscuits which were added 30% RS has the lowest b* value at the same temperature (16.79). Biscuits which were baked at 200^{o}C and were added 0% RS it has the highest b* value (33.96) and also biscuits which were added 30% RS has the lowest b* value at the same temperature (23.89). Biscuits which were baked at 225^{o}C and were added 0% RS it has the highest b* value (36.19) and also biscuits which were added 30% RS has the lowest b* value at the same temperature (32.55).

For each baking temperature, variance analysis were made according to the level of RS addition. As a result of the analysis of variance, it was found that the different RS addition that was baked at 175, 200 and 225^{o}C on the L*, a* and b* value of gluten-free biscuits was significant ($\alpha<0.05$). ANOVA results of L*, a* and b* values are given in tables C.1 to C.18 in the appendix.

Similar results were obtained in the literature compared to similar studies. As the rate of RS increased at each baking temperatures, the L* value of gluten-free biscuits increased, a* and b* values of gluten-free biscuits decreased with respect to the control gluten-free biscuits. As the RS addition rate increased, lighter colored biscuits have obtained compared to control biscuits. The L* value gives a measure of the lightness of the product color from 100 for perfect white to 0 for black, as the eye would evaluate it. The redness/greenness and yellowness/ blueness are denoted by the a* and b* values, respectively (Navneet and Shitij, 2011).

In a study conducted by Bayrakçı and Bilgiçli, wheat flour used in tarhana formulation was reduced by 15, 30 and 45% and two different RS3 were used instead of wheat flour. L* value has increased linearly compared to control group as the rate of RS addition increased. L* value of control group was determined 81.01 and the L* value was determined 84.24, 86.09 and 86.92 with addition of RS replacement to wheat flour at 15, 30 and 45%, respectively. a* value has decreased compared to control group as the rate of RS addition increased. a* value of control group was determined 5.92 and the a* value was determined 4.90, 4.77 and 4.81 with addition of RS replacement to wheat flour at 15, 30 and 45%, respectively. And b* value has decreased compared to control group as the rate of RS addition increased. b* value of control group was determined 27.31 and the b* value was determined 25.16, 24.22 and 23.85 with addition of RS replacement to wheat flour at 15, 30 and 45%, respectively (Bayrakçı and Bilgiçli, 2015).

Öztürk et al., also found that also found that crust color values decreased at 30% addition in several third type RS (Novelose 330) supplemented breads (Öztürk et al., 2007). In the study by Laguna, RS was added instead of wheat flour in certain proportions. As a result of this study, the value of L* and a* value have increased but b* value has decreased with increasing RS (Laguna et al., 2011).

Some studies were carried out using the fat replacer feature of the RS. In this study, the amount of fat used in the formulation of the biscuits produced was reduced by 25 and 50% and replaced with five different RS. Parallel to the results we found in our experiments, it was stated that in all made experiments as the rate of RS addition increased, L* value has increased and there was a decrease in a* and b* values (Serinyel, 2013).

The maillard reaction is a complex series of browning reactions (color development) between amino acids and reducing sugars, usually caused by the action of high temperature. It is a kind of non-enzymatic browning, such as caramelization. During the cooking process formation of biscuit golden-brown color, from non-enzymatic browning (maillard reactions) which forms brown polymers or melanoidins between reducing sugars and amino acids, from starch dextrinization and sugar caramelisation. In particular, it has stated that the a* value increased with the maillard reaction. In this study, three different gluten-free biscuit formulations based on rice and wheat flour (90/10, 80/20 and 70/30) were tested. And at all recipes as color parameters it has stated that L* value has increased, a* and b* values have decreased. As the reason for this, that the raw materials used in place of wheat flour are thought to be caused by sugar and amino acid amounts, so that is maillard reaction (Torbica et al., 2012).

As mentioned above, high temperature and low moisture content in the surface layers cause caramelisation of sugars and oxidation of fatty acids to aldehydes, lactones, ketones, alcohols and esters which cause a deeper brown color. In the table 3.3, all RS addition rate when the temperature value increases, L* value was decreased, a* and b* value was increased. So, the color in gluten-free biscuits has turned to intense red and yellow and dark when baked at 200ºC and 225ºC according to 175ºC.

The equilibrium of the polynomial model explaining the effect of factors on L* value (R^2=0.99) (Equation 3.5), a* value (R^2=0.99) (Equation 3.6), and b* value (R^2=0.99) (Equation 3.7), of gluten-free biscuits was given below.

$$L^* \text{ value} = -143.9642 + 2.4519*T + 0.6247*C - 2.4*10^{-3}*T*C - 6.7*10^{-3}*T^2 + 6*10^{-4}*C^2 \quad (3.5)$$

$$a^* \text{ value} = 151.441 - 1.6269*T - 0.0254834*C - 1.745*10^{-3}*T*C + 4.563*10^{-3}*T^2 + 5.05833*10^{-3}*C^2 \quad (3.6)$$

$$b^* \text{ value} = 124.0319 - 1.0027*T - 2.1802*C + 8.208*10^{-3}*T*C + 2.744*10^{-3}*T^2 + 6.7583*10^{-3}*C^2 \quad (3.7)$$

where T is temperature ºC and C is the concentration %.

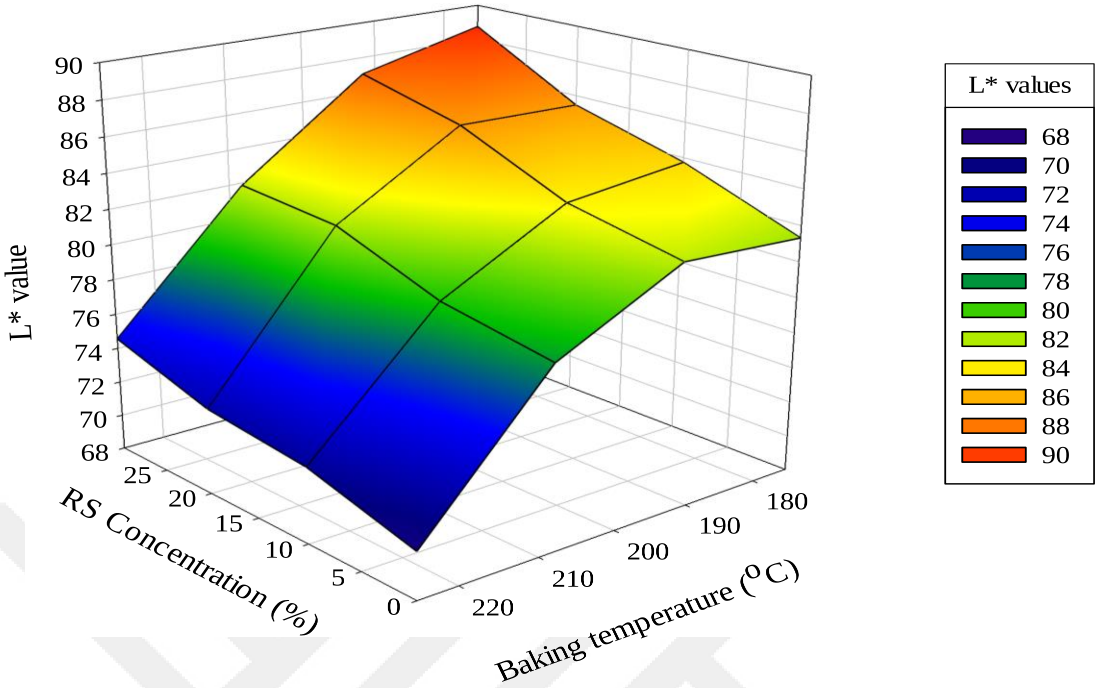

Figure 3.7 3D plot of baking temperature and RS concentration against L* values of gluten-free biscuits

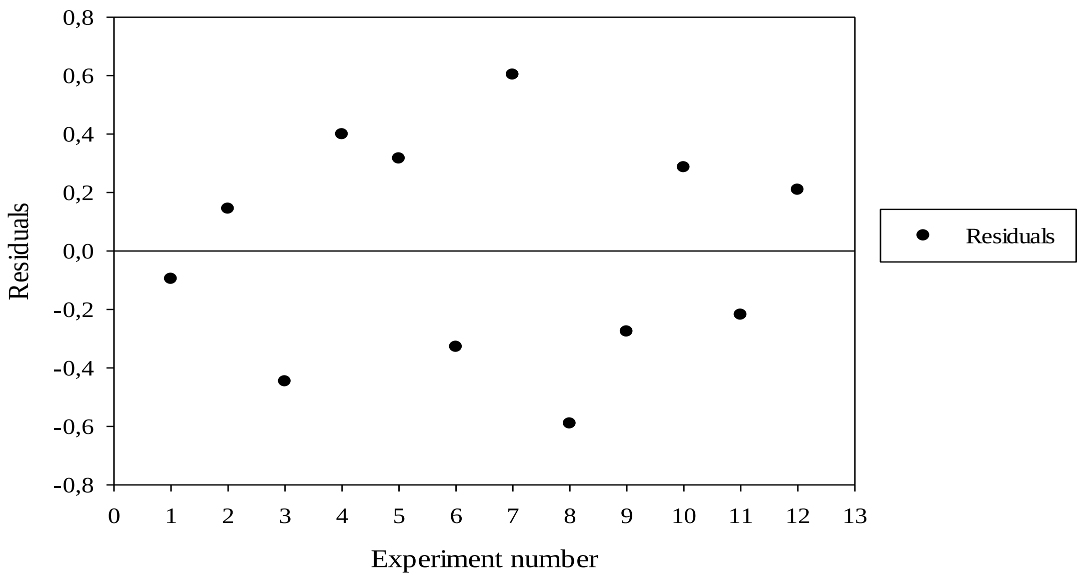

Figure 3.8 Residual plot of L* values of gluten-free biscuits

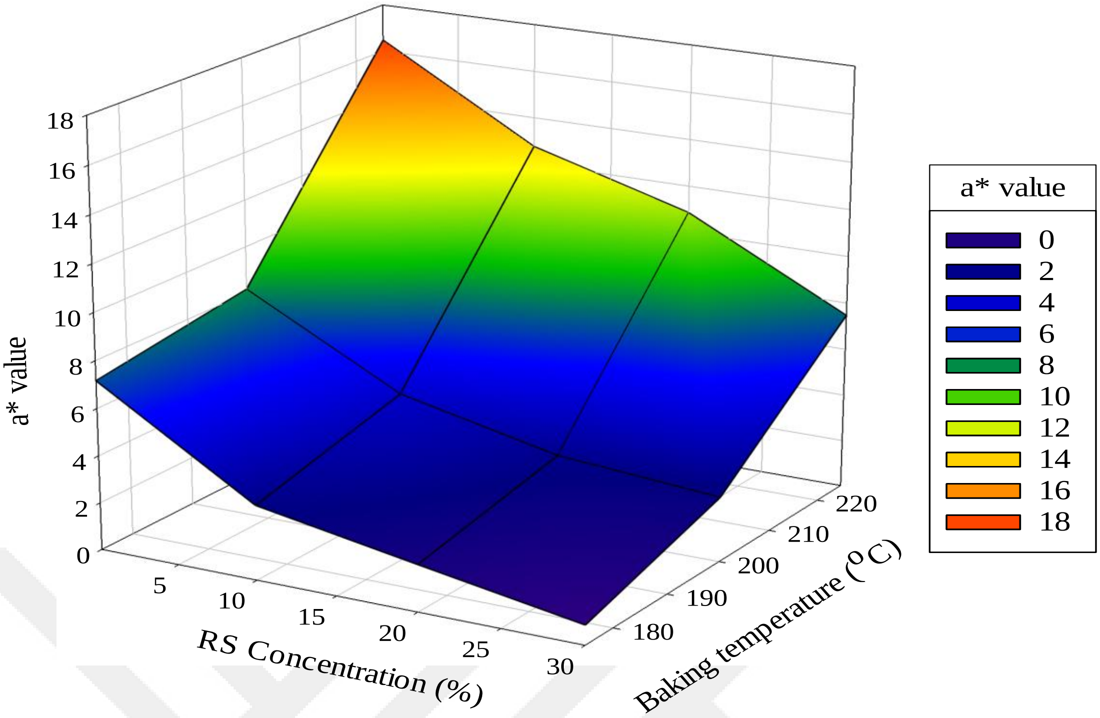

Figure 3.9 3D plot of baking temperature and RS concentration against a* values of gluten-free biscuits

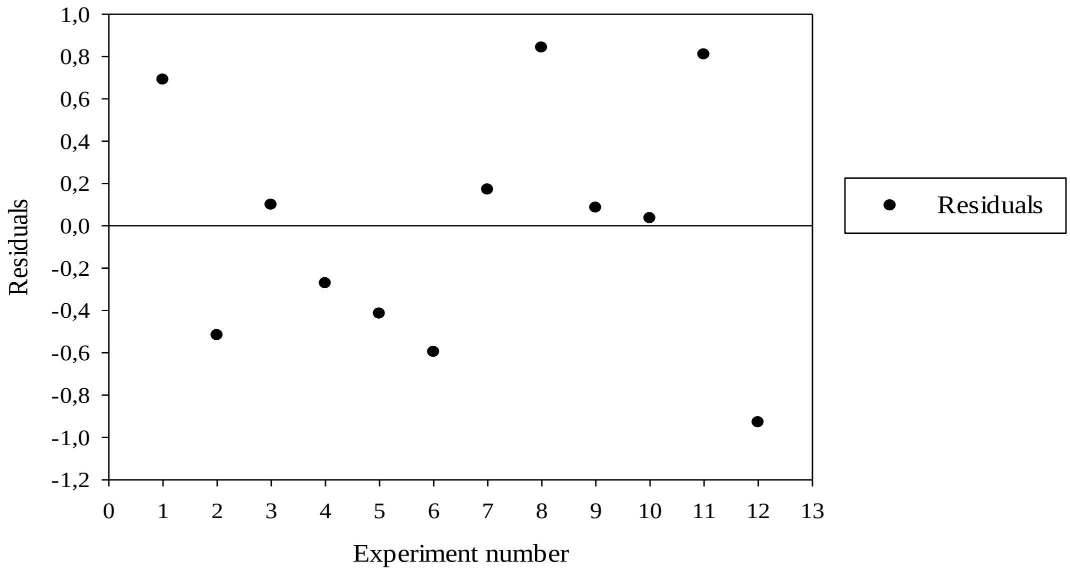

Figure 3.10 Residual plot of a* values of gluten-free biscuits

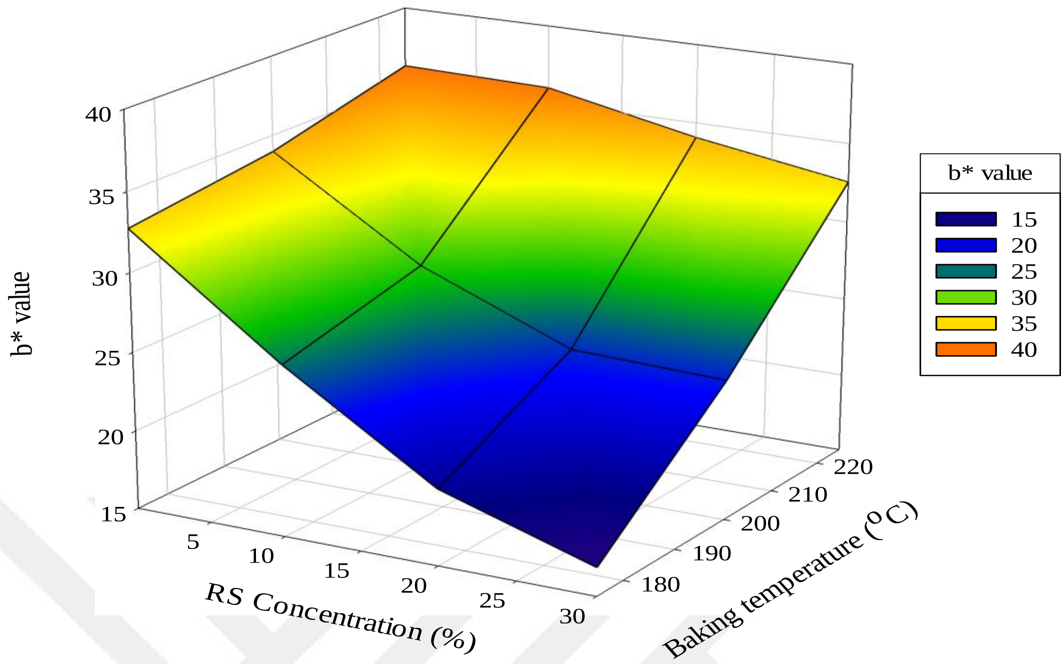

Figure 3.11 3D plot of baking temperature and RS concentration against b* values of gluten-free biscuits

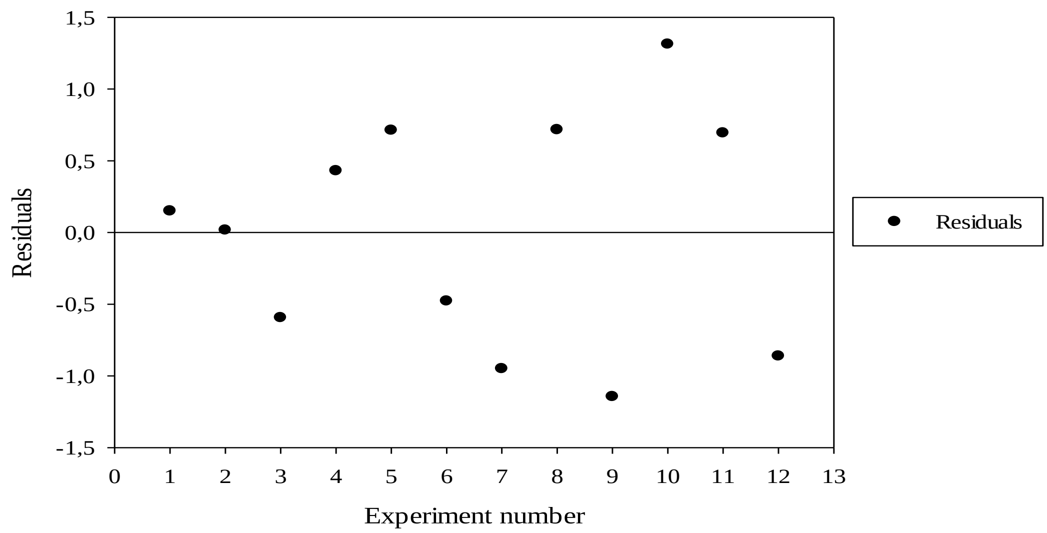

Figure 3.12 Residual plot of b* values of gluten-free biscuits

According to the order of experiments baking temperatures and RS concentrations respectively to the polynomial equation obtained as a result of the regression analysis was written. Predicted L*, a*, b* values of gluten-free biscuits, for each experiment were obtained according to the polynomial equation. After obtaining the predicted L*, a*, b* values according to the equation, the residual plot was drawn by subtracting predicted L*, a*, b* values from the measured L*, a*, b* values. Residual values were calculated for each experiment.

3.4 Diameter and Height Value

Table 3.4 Diameter and height value of gluten-free biscuits according to baking temperature at different RS concentration

No	Temperature (°C)	RS (%)	Diameter value (mm)	Height value (mm)
1	175	0	45.05±0.70[a]	7.52±0.02[a]
2	175	10	43.82±0.03[b]	7.22±0.03[b]
3	175	20	42.34±0.14[c]	6.61±0.14[c]
4	175	30	42.17±0.03[c]	7.52±0.03[a]
5	200	0	44.79±0.06[a]	7.82±0.03[a]
6	200	10	43.27±0.05[b]	8.11±0.03[b]
7	200	20	42.12±0.01[c]	6.82±0.04[c]
8	200	30	41.96±0.02[c]	7.79±0.04[d]
9	225	0	44.52±0.08[a]	8.24±0.02[a]
10	225	10	43.23±0.04[b]	8.67±0.03[b]
11	225	20	42.00±0.03[c]	7.00±0.04[c]
12	225	30	42.82±0.06[d]	8.45±0.01[d]

RS: Resistant starch. Values are the Mean ± SD from duplicate determinations, the same letter shows non-significant difference at α=0.05 level at the each baking temperature according to the Tukey test.

Diameter and height value results of gluten-free biscuits, which are baked at different temperatures (175, 200 and 225°C) and added in different proportions RS (0, 10, 20, and 30%) are shown in the table 3.4. The diameter value of the biscuits was determined between 41.96 - 45.05 mm. Gluten-free biscuits which were baked at 175°C and were added 0% RS it has the highest diameter value (45.05 mm) and also biscuits which were added 30% RS has the lowest diameter value at the same temperature (42.17 mm). Biscuits which were baked at 200°C and were added 0% RS it has the highest diameter value (44.79 mm) and also biscuits which were added 30% RS has the lowest diameter value at the same temperature (41.96 mm). Biscuits which were baked at 225°C and were added 0% RS it has the highest diameter value (44.52 mm) and also biscuits which were added 30% RS has the lowest diameter value at the same temperature (42.82 mm).

The height value of the biscuits was determined between 6.61 - 8.67 mm. Gluten-free biscuits which were baked at 175°C and were added 0% RS it has the highest height value (7.52 mm) and also biscuits which were added 20% RS has the lowest height value at the same temperature (6.61). Biscuits which were baked at 200°C and were added 10% RS it has the highest height value (8.11 mm) and also biscuits which were added 20% RS has the lowest height value at the same temperature (6.82 mm). Biscuits which were baked at 225°C and were added 10% RS it has the highest height value (8.67 mm) and also biscuits which were added 20% RS has the lowest height value at the same temperature (7.00 mm).

The diameter value of gluten-free biscuits decreased with respect to the control value as the rate of RS was increased at all baking temperatures. There is an imbalance in the height values of gluten-free biscuits. It is thought that this is due to biscuit recipe and mould. The raising agents which have a significant effect on the biscuit eating quality, used in the biscuit recipes, are active with temperature. With high temperature water and raising agents (sodium acid pyrophosphate, ammonium bicarbonate and sodium bicarbonate) generated carbon dioxide gas and water vapor. So, air and CO_2 bubbles were trapped inside the biscuits because there were no small holes in the biscuit mould to allow air bubbles and carbon dioxide gas to escape. That can cause unstable raising in gluten-free biscuits.

In the study by Laguna, RS was added instead of wheat flour in 0 (control), 20, 40 and 60%. As the RS addition rate increased, the diameter and height values have decreased compared to the control biscuits. The reason for this was that the use of RS instead of wheat flour in the formulation had a diluting property on wheat proteins (Laguna et al., 2011). In a study made with corn starch which containing 15.3% RS, in increasing amounts of RS was added to biscuits, the biscuit diameter decreased and the thickness increased with the increase in the rate of addition (Şeker et al., 2006). As Kissell and Yamazaki mentioned, during biscuit dough mixing, any raw material (wheat-free high protein flour, starch and other raw materials) that will absorb water will reduce the spread ratio (diameter/thickness) of biscuits. In such a system, the water in the prescription will be absorbed by the protein and starch and the amount of water required to dissolve the sugar will not remain in the system and will be insufficient to increase the viscosity. As a result, the biscuit spread ratio will remain lower rate (Kissell and Yamazaki, 1975). Since the protein content of the RS used was maximum 0.75%. Due to low protein content the protein was not very effective, water retention capacity was of great importance as mentioned earlier.

For each baking temperature, variance analysis were made according to the level of RS addition. As a result of the analysis of variance, it was found that the different RS addition that was baked at 175, 200 and 225ºC on the diameter and height value of gluten-free biscuits was significant ($\alpha<0.05$). ANOVA results of diameter and height values are given in tables D.1 to D.12 in the appendix. In the control group and gluten-free biscuits with RS, when the baking temperature have increased, diameter values have decreased and the height values have increased.

The equilibrium of the polynomial model explaining the effect of factors on diameter ($R^2=0.98$) (Equation 3.8) and height values ($R^2=0.50$) (Equation 3.9) of gluten-free biscuits was given below.

$$\text{Diameter value} = 52.3679 - 0.0643*T - 0.2177*C + 2*10^{-4}*T*C + 1*10^{-4}*T^2 + 3*10^{-3}*C^2 \quad (3.8)$$

$$\text{Height value} = 5.4109 + 7.52*10^{-3}*T - 0.0655*C - 8.80^{-5}*T*C + 2.79*10^{-5}*T^2 + 2.4292*10^{-3}*C^2 \quad (3.9)$$

where T is temperature ºC and C is the concentration %.

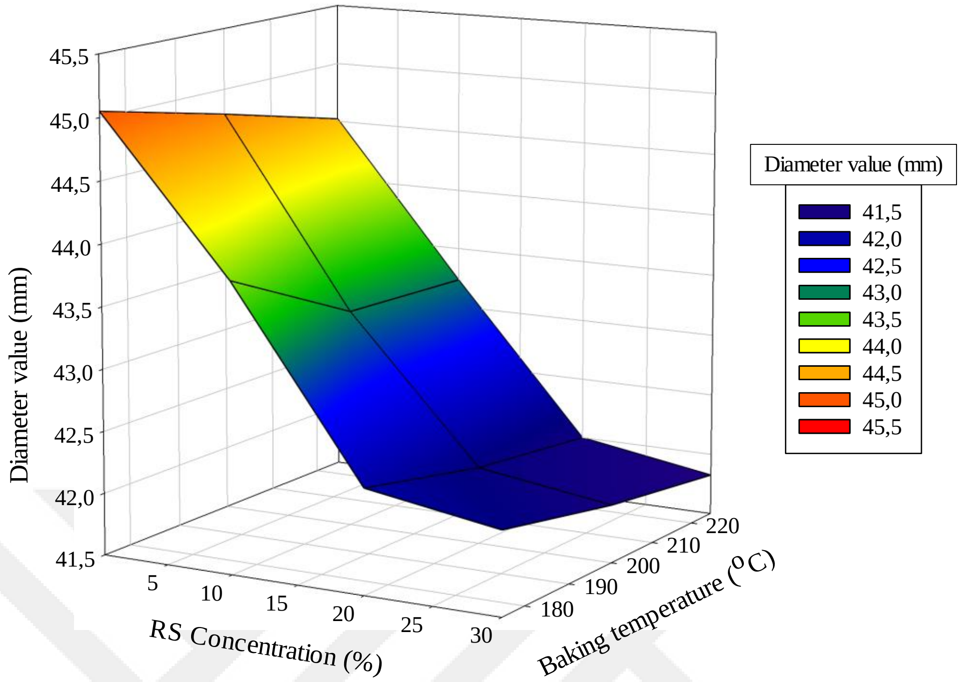

Figure 3.13 3D plot of baking temperature and RS concentration against diameter values of gluten-free biscuits

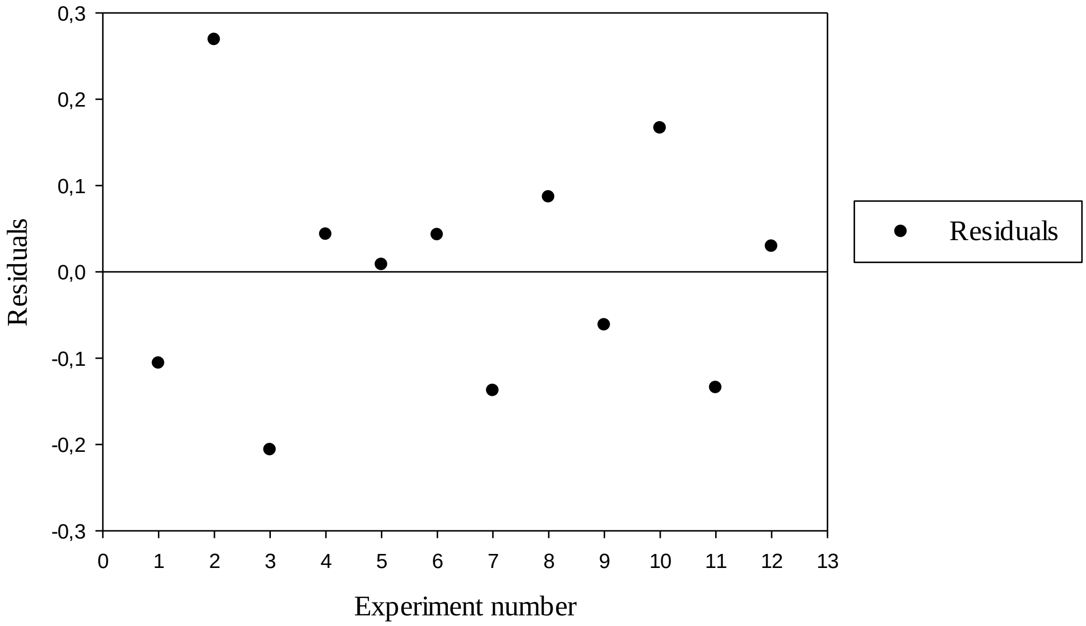

Figure 3.14 Residual plot of diameter values of gluten-free biscuits

According to the order of experiments baking temperatures and RS concentrations respectively to the polynomial equation obtained as a result of the regression analysis was written. Predicted diameter values of gluten-free biscuits, for each experiment were obtained according to the polynomial equation. After obtaining the predicted diameter values according to the equation, the residual plot was drawn by subtracting predicted diameter values from the measured diameter values. Residual values were calculated for each experiment.

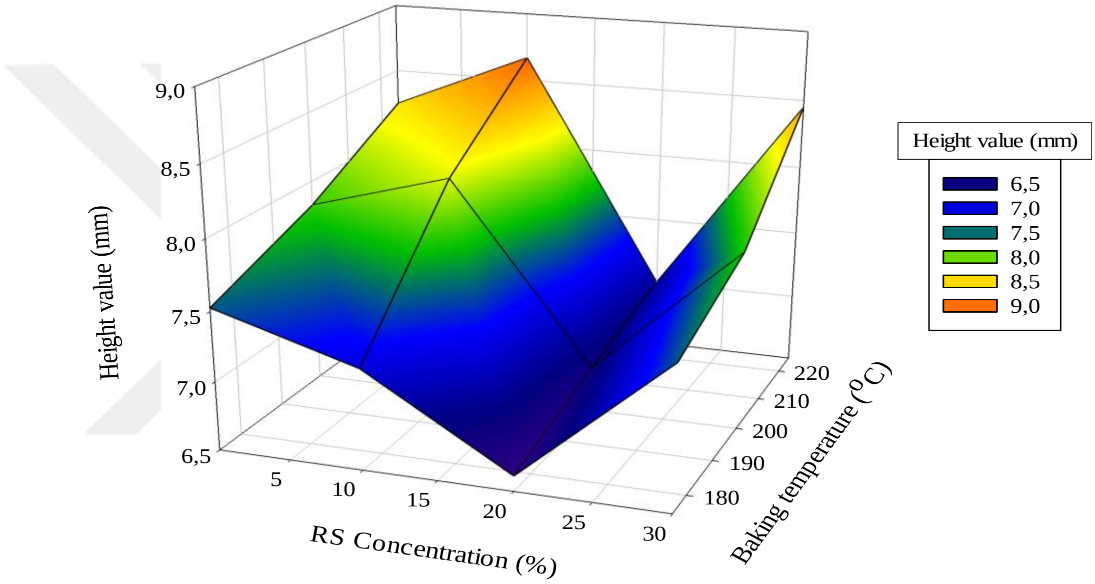

Figure 3.15 3D plot of baking temperature and RS concentration against height values of gluten-free biscuits

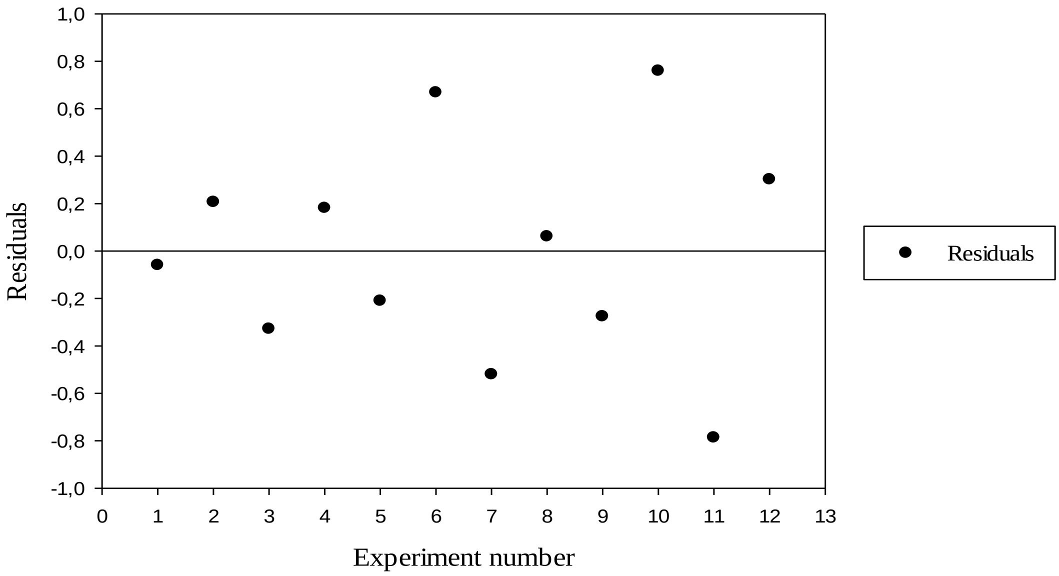

Figure 3.16 Residual plot of height values of gluten-free biscuits

According to the order of experiments baking temperatures and RS concentrations respectively to the polynomial equation obtained as a result of the regression analysis was written. Predicted height values of gluten-free biscuits, for each experiment were obtained according to the polynomial equation. After obtaining the predicted height values according to the equation, the residual plot was drawn by subtracting predicted height values from the measured height values. Residual values were calculated for each experiment.

3.5 Hardness and Fracturability

Table 3.5 Hardness and fracturability of gluten-free biscuits according to baking temperature at different RS concentration

No	Temperature (ºC)	RS (%)	Hardness (F (g))	Fracturability (mm)
1	175	0	1464.57±45.57[a]	30.99±0.13[a]
2	175	10	1907.64±16.97[b]	30.72±0.08[bc]
3	175	20	1884.96±20.50[b]	30.60±0.07[c]
4	175	30	1864.39±28.28[b]	30.50±0.07[c]
5	200	0	1121.91±27.20[a]	30.24±0.07[a]
6	200	10	1305.82±9.75[c]	29.81±0.09[b]
7	200	20	1222.26±14.15[b]	29.59±0.04[ac]
8	200	30	1155.64±21.70[ab]	29.43±0.06[c]
9	225	0	796.95±8.56[a]	29.30±0.14[a]
10	225	10	876.32±19.61[b]	29.15±0.01[bc]
11	225	20	845.39±7.26[a]	28.88±0.04[cd]
12	225	30	820.21±14.08[a]	28.68±0.06[d]

RS: Resistant starch. Values are the Mean ± SD from duplicate determinations, the same letter shows non-significant difference at α=0.05 level at the each baking temperature according to the Tukey test.

Hardness and fracturability value results of gluten-free biscuits, which are baked at different temperatures (175, 200 and 225ºC) and added in different proportions RS (0, 10, 20, and 30%) are shown in the table 3.5. The hardness of the biscuits was determined between 796.95 - 1907.64 F (g).Gluten-free biscuits which were baked at 175ºC and were added 0% RS it has the lowest hardness value (1464.57 F (g)) and also biscuits which were added 10% RS has the highest hardness value at the same temperature (1907.64 F (g)). Biscuits which were baked at 200ºC and were added 0% RS it has the lowest hardness value (1121.91 F (g)) and also biscuits which were added 10% RS has the highest hardness value at the same temperature (1305.82 F (g)). Biscuits which were baked at 225ºC and were added 0% RS it has the lowest hardness value (796.95 F (g)) and also biscuits which were added 10% RS has the highest hardness value at the same temperature (876.32 F (g)).

The fracturability of the biscuits was determined between 28.68 - 30.99 mm. Gluten-free biscuits which were baked at 175ºC and were added 0% RS it has the highest fracturability value (30.99 mm) and also biscuits which were added 30% RS has the lowest fracturability value at the same temperature (30.50 mm). Biscuits which were baked at 200ºC and were added 0% RS it has the highest fracturability value (30.24 mm) and also biscuits which were added 30% RS has the lowest fracturability value at the same temperature (29.43 mm). Biscuits which were baked at 225ºC and were added 0% RS it has the highest fracturability value (29.30 mm) and also biscuits which were added 30% RS has the lowest fracturability value at the same temperature (28.68 mm).

In general, when the texture samples are measured, the soft sample exhibits a slight resistance to deformation, while a hard product is rather resistant to deformation. Hardness describes a product that exhibits significant resistance to deformation (Stable Micro Systems, 2018). The instrumentally measured biscuit hardness is expressed as the maximum force required to break the biscuits. As sensory, hardness is defined as a force required to bite biscuits. The textural properties of biscuits are one of the most important quality parameters for the end consumer to accept the product or to buy the same product again. It was determined that the hardness values of gluten-free biscuits with added RS increased compared to the control group at each temperature. In other words, RS added gluten-free biscuits were harder than the control group. When look at biscuits with added RS, except the control group, hardness value was decreases as the rate of RS addition was increases. However, despite this reduction, gluten-free biscuits with 30% RS were harder than the control group. The fracturability of a product includes crumbliness, crispiness, crunchiness and brittleness. When a product is exposed to external force, if it is prone to breakage or breaks, it is referred to as fragile. That is, there is little tendency to deform before breaking, and it usually makes a breaking sound (Stable Micro Systems, 2018). At each temperature the most fragile gluten-free biscuits belong to the control group. As the rate of RS to gluten-free biscuits increases, the rate of fragility has was decreases.

In a study carried out by adding different RS samples produced from different starch sources into biscuits at different rates, the biscuit hardness value changed according to the RS source and in parallel with our results, the hardness value of biscuits decreased as the RS addition rate increased (Wang et al., 2014).

In the study by Laguna, RS was added instead of wheat flour in 0 (control), 20, 40 and 60%. The hardness value of biscuits decreased as the RS addition rate increased. The reason for this, was evaluated in two points. One of these is due to the increasing presence of RS, because wheat flour has a higher moisture level than RS. Therefore, the dry matter content of the dough increased, so the dough moisture level is reduced. RS has reduced the strength of wheat proteins by trapping the water that wheat proteins should take (Laguna et al., 2011). Because, gluten protein provide to form the structure and improve the texture during cooking in the products produced with wheat flour (Dizlek, 2011). In addition, as mentioned earlier the increase moisture content of biscuits with the increased RS addition rate is thought to be effective on decreasing the hardness and brittleness values.

With increasing temperature, starch gelatinization and the recrystallization of the starch (retrogradation) occurs, so that it can trap moisture in the tissue during the shelf life. Moisture release from the biscuit surface can be prevented as the shell forms on the biscuit surface due to the plastic structure of proteins and starch due to the increased temperature during the baking of the biscuits. For these reasons, more force may be required to break the biscuit, so the biscuit may be harder and brittle (Ashok et al., 2012).

For each baking temperature, variance analysis were made according to the level of RS addition. As a result of the analysis of variance, it was found that the different RS addition that was baked at 175, 200 and 225^oC on the hardness value and fracturability of gluten-free biscuits were significant ($\alpha<0.05$). ANOVA results of hardness and fracturability values are given in tables E.1 to E.12 in the appendix. At the rate of addition each RS, the hardness and fracturability of the gluten-free biscuits were decreases and as the temperature was increases. In other words, as temperature increases, gluten-free biscuits were less hard and less brittle.

The equilibrium of the polynomial model explaining the effect of factors on hardness value (R^2=0.97) (Equation 3.10) and fracturability (R^2=0.99) (Equation 3.11) of gluten-free biscuits was given below.

$$\text{Hardness value} = 10964.7288 - 83.4324{*}T + 70.0962{*}C\,(\%) - 0.2276{*}T{*}C + 0.1698{*}T^2 - 0.6823{*}C^2 \quad (3.10)$$

$$\text{Fracturability} = 42.2171 - 0.0885{*}T - 0.0105{*}C - 1.041{*}10^{-4}{*}T{*}C - 1.403{*}10^{-4}{*}T^2 - 3.3417{*}10^{-4}{*}C^2 \quad (3.11)$$

where T is temperature °C and C is the concentration %.

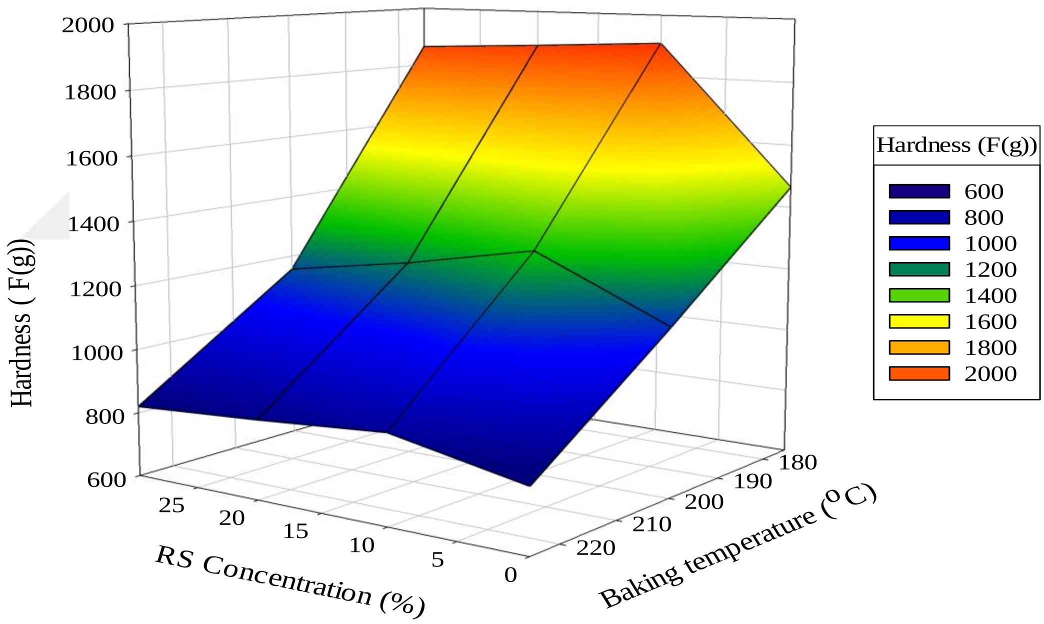

Figure 3.17 3D plot of baking temperature and RS concentration against hardness values of gluten-free biscuits

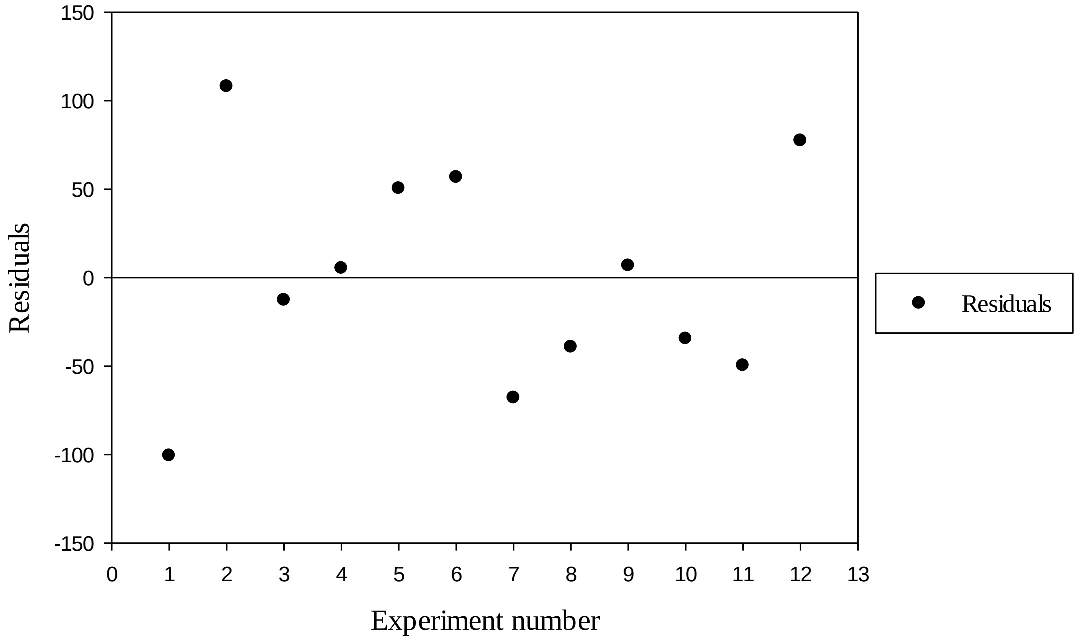

Figure 3.18 Residual plot of hardness values of gluten-free biscuits

According to the order of experiments baking temperatures and RS concentrations respectively to the polynomial equation obtained as a result of the regression analysis was written. Predicted hardness values of gluten-free biscuits, for each experiment were obtained according to the polynomial equation. After obtaining the predicted hardness values according to the equation, the residual plot was drawn by subtracting predicted hardness values from the measured hardness values. Residual values were calculated for each experiment.

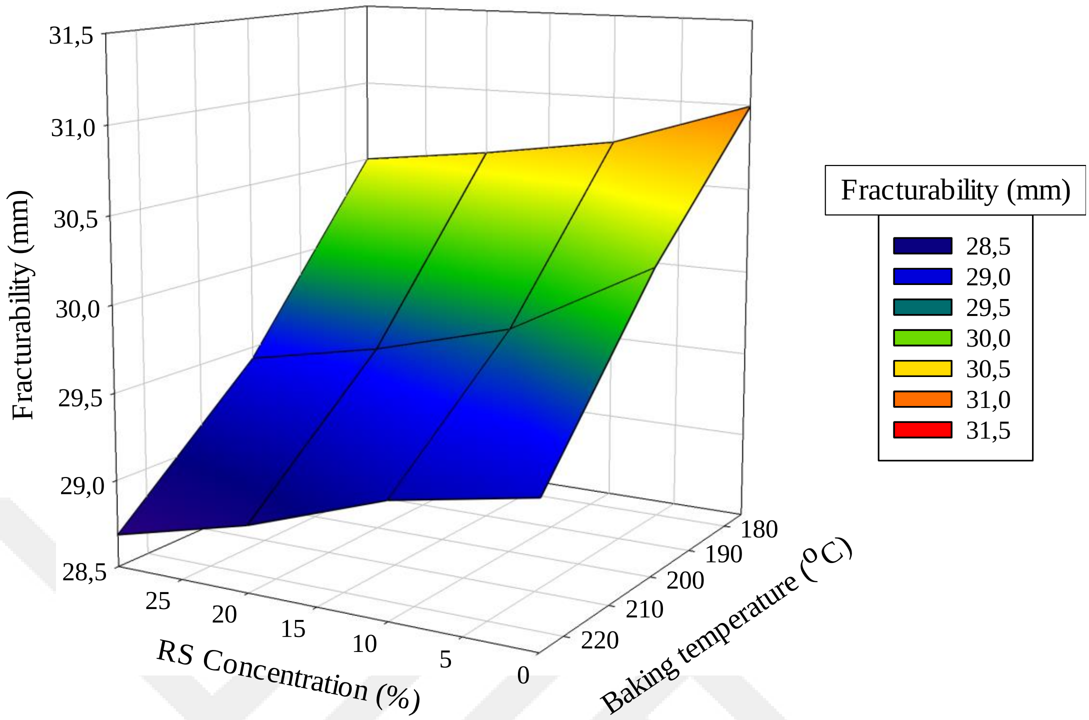

Figure 3.19 3D plot of baking temperature and RS concentration against fracturability values of gluten-free biscuits

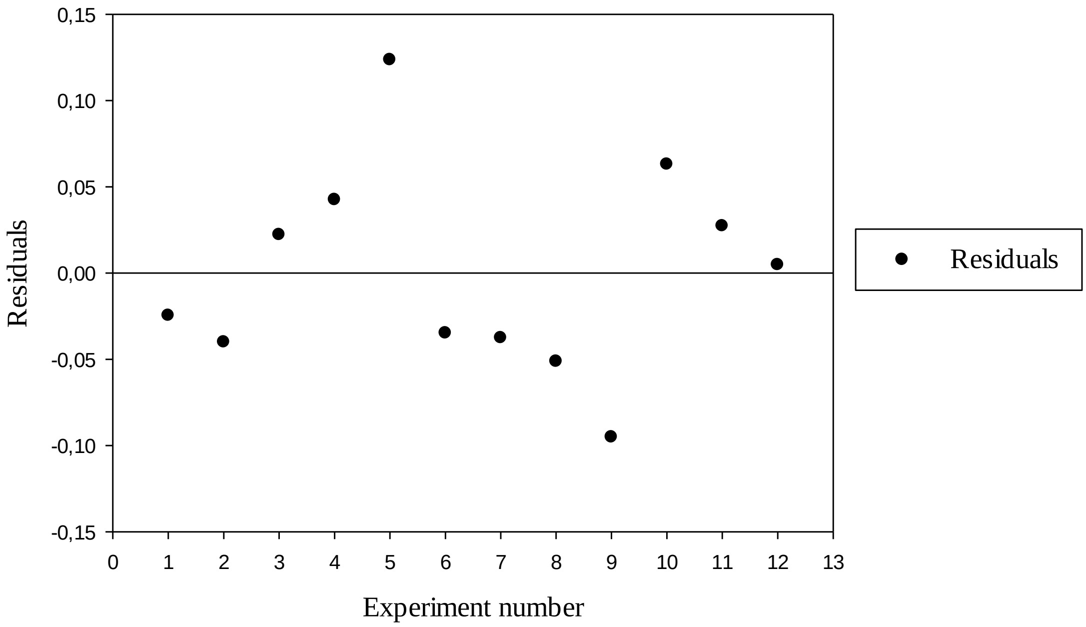

Figure 3.20 Residual plot of fracturability values of gluten-free biscuits

According to the order of experiments baking temperatures and RS concentrations respectively to the polynomial equation obtained as a result of the regression analysis was written. Predicted fracturability values of gluten-free biscuits, for each experiment were obtained according to the polynomial equation. After obtaining the predicted fracturability values according to the equation, the residual plot was drawn by subtracting predicted fracturability values from the measured fracturability values. Residual values were calculated for each experiment.

3.6 Total Dietary Fiber

Table 3.6 Total dietary fiber of gluten-free biscuits according to baking temperature at different RS concentration

No	Temperature (°C)	RS (%)	Total Dietary fiber (%)
5	200	0	1.51±0.70[a]
6	200	10	2.79±0.03[b]
7	200	20	5.35±0.14[c]
8	200	30	5.84±0.03[d]

RS: Resistant starch. Values are the Mean ± SD from duplicate determinations, the same letter shows non-significant difference at $\alpha=0.05$ level at the baking temperature according to the Tukey test.

Total dietary fiber (TDF) results of gluten-free biscuits, which are baked at 200°C and added in different proportions RS (0, 10, 20, and 30%) are shown in the table 3.6. The dietary fiber of the biscuits was determined between 1.51 - 5.84%. Biscuits which were added 0% RS it has the lowest TDF (1.51%). Biscuits which were added 30% RS it has the TDF (5.84%). The dietary fiber percent value of gluten-free biscuits have increased with respect to the control value as the rate of RS was increased. The total fiber content was higher in the 10, 20 and 30% replacement instead of gluten-free flour than the gluten-free biscuits (as control) due to some ingredients having a high fiber content, such as RS. Dietary fiber levels of gluten-free biscuits are related to the composition of the ingredients RS that include high fiber levels. Dietary fiber in RS (RS3, Novelose 330), is between 33 and 43%. As shown in Table 3.6, use of RS increase levels of dietary fiber in gluten-free biscuits.

This agrees with studies of Nugraheni et al., Maranta arundinacea flour rich in RS3 was used in 8, 10, 12% different ratios instead of gluten-free flour and as a control, biscuit was made with wheat flour. The TDF content was measured higher in the 8, 10 and 12% Maranta arundinacea flour added biscuits than the wheat flour biscuits (as control). TDF content was 25.96, 25.36 and 26.14 in biscuit made with Maranta arundinacea flour, respectively. In control biscuits, the amount of TDF was measured 21.09. As the cause of these results, Maranta arundinacea flour rich in RS3 It is thought to be effective dietary fiber levels (Nugraheni et al., 2017). Biscuits developed from RS rich in dietary fiber can be used as functional food because they have a low glycemic index. While the glycemic index of white bread varied between 87 and 69, it was observed that this ratio decreased to 41% when biscuits were made with flour which is the source of dietary fiber (Marangoni and Poli, 2008).

For 200^{o}C baking temperature, variance analysis were made according to the level of RS addition. As a result of the analysis of variance, it has determined that the different RS addition that was baked at 200^{o}C on the dietary fiber percent of gluten-free biscuits was significant (α<0.05). ANOVA results of total dietary fiber values are given in tables F.1 and F.2 in the appendix.

The equilibrium of the linear model explaining the effect of factors on moisture content of gluten-free biscuits was given below (R^2=0.94) (Equation 3.12).

$$\text{Dietary fiber} = 1.535 + 0.156*C \qquad (3.12)$$

where C is the concentration %.

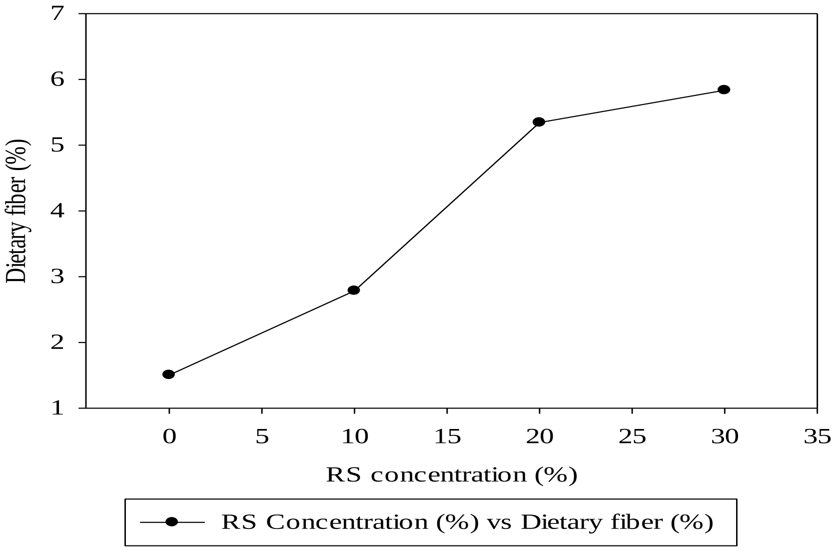

Figure 3.21 Dietary fiber curve of gluten-free biscuits baked at 200^{o}C

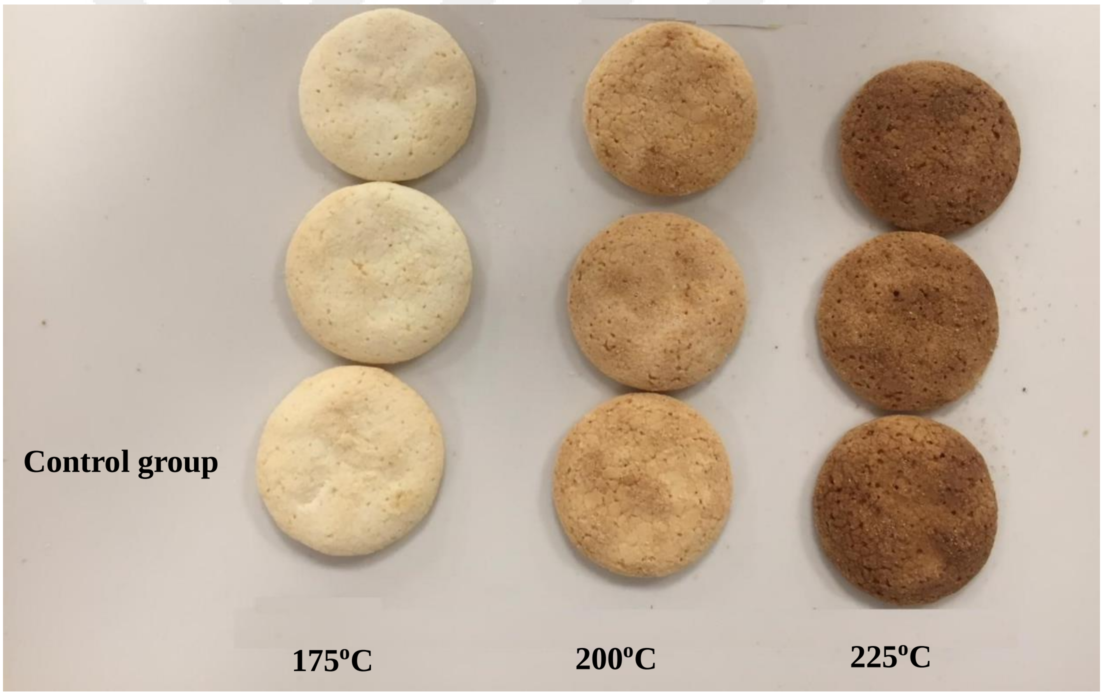

Figure 3.22 Gluten-free biscuits with no RS (Control group)

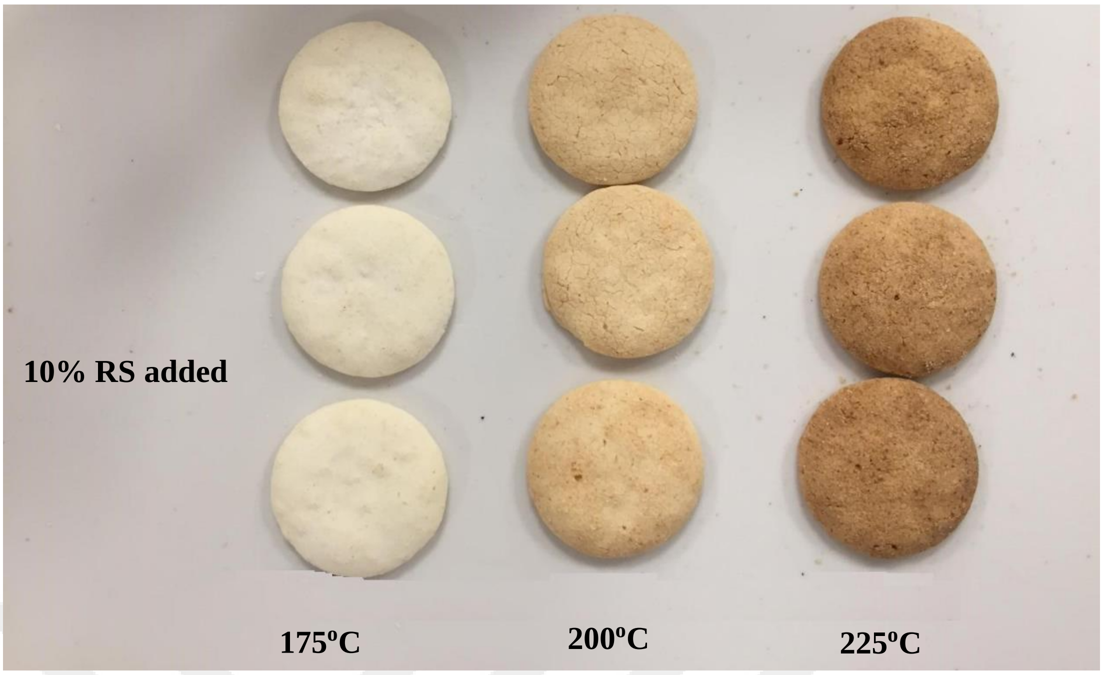

Figure 3.23 Gluten-free biscuits with 10% RS instead of to gluten-free flour

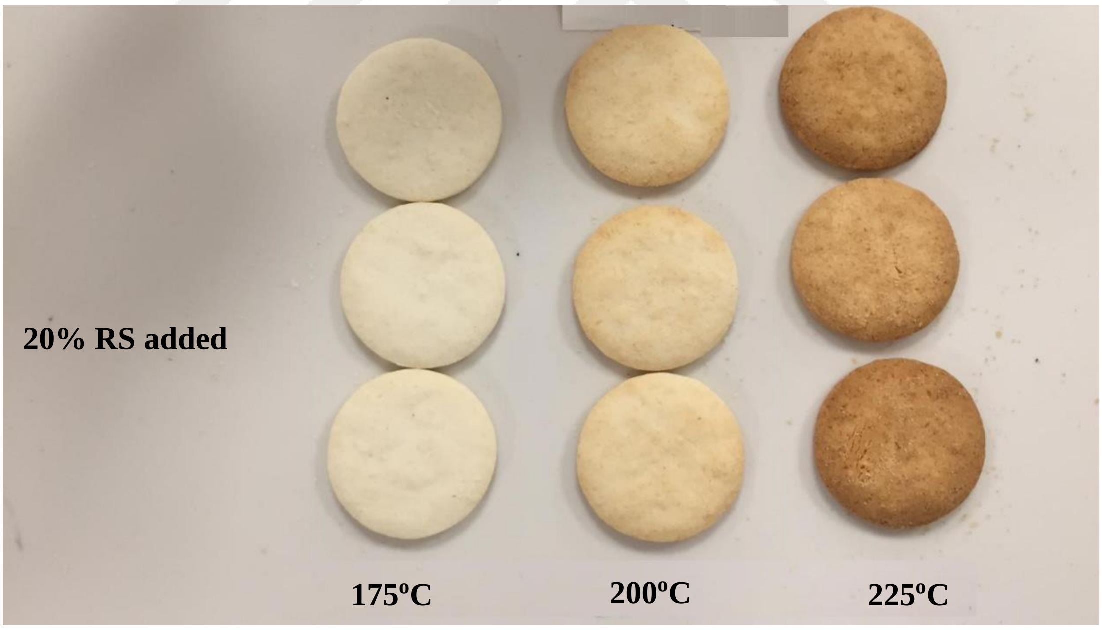

Figure 3.24 Gluten-free biscuits with 20% RS instead of to gluten-free flour

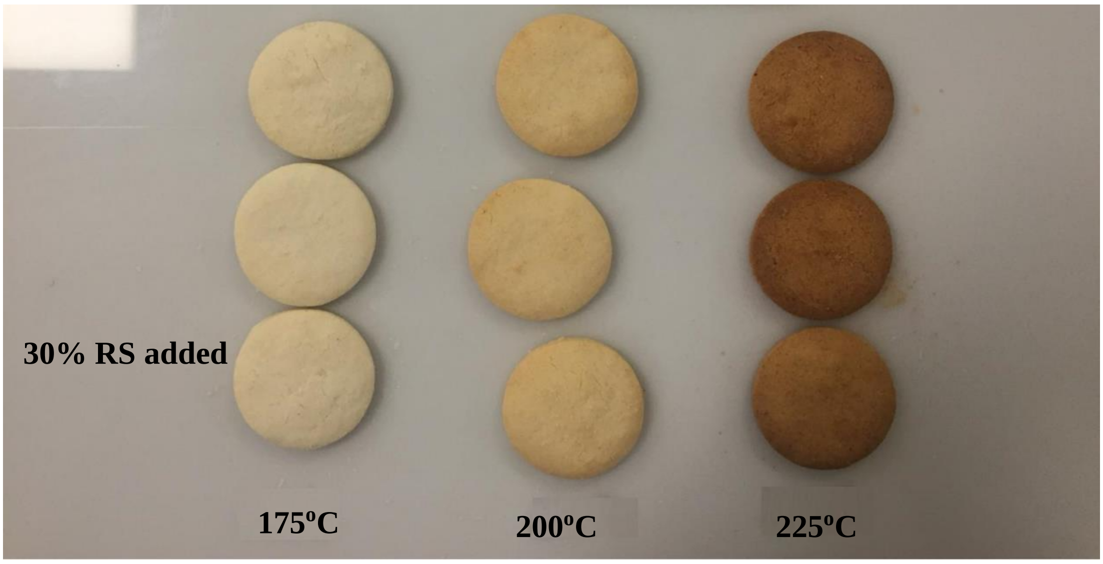

Figure 3.25 Gluten-free biscuits with 30% RS instead of to gluten-free flour

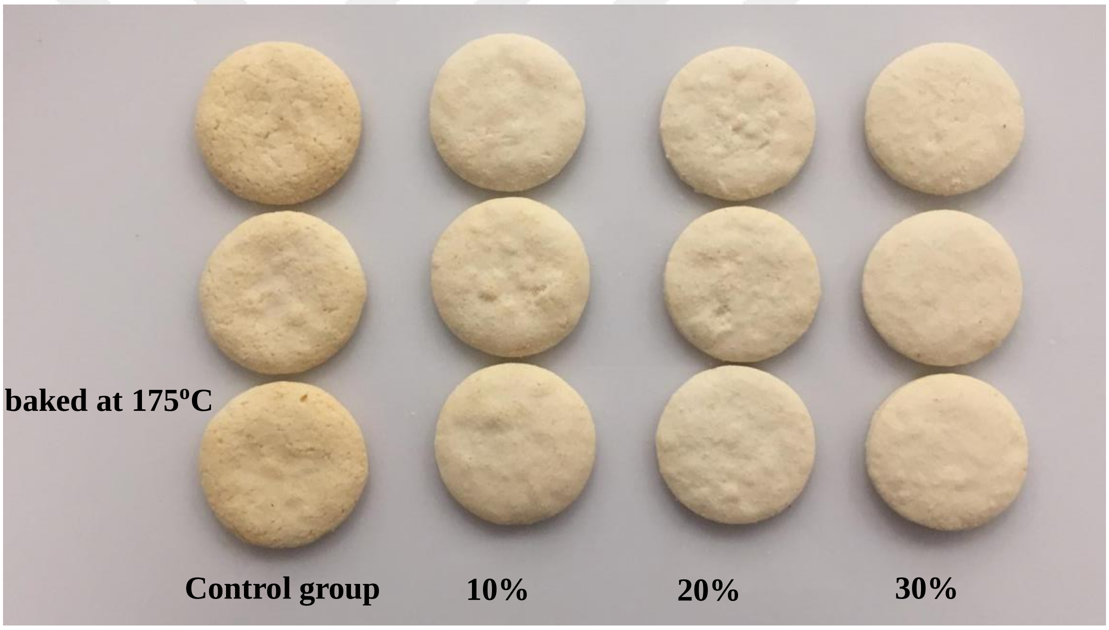

Figure 3.26 Gluten-free biscuits with different concentration of RS baked at 175ºC

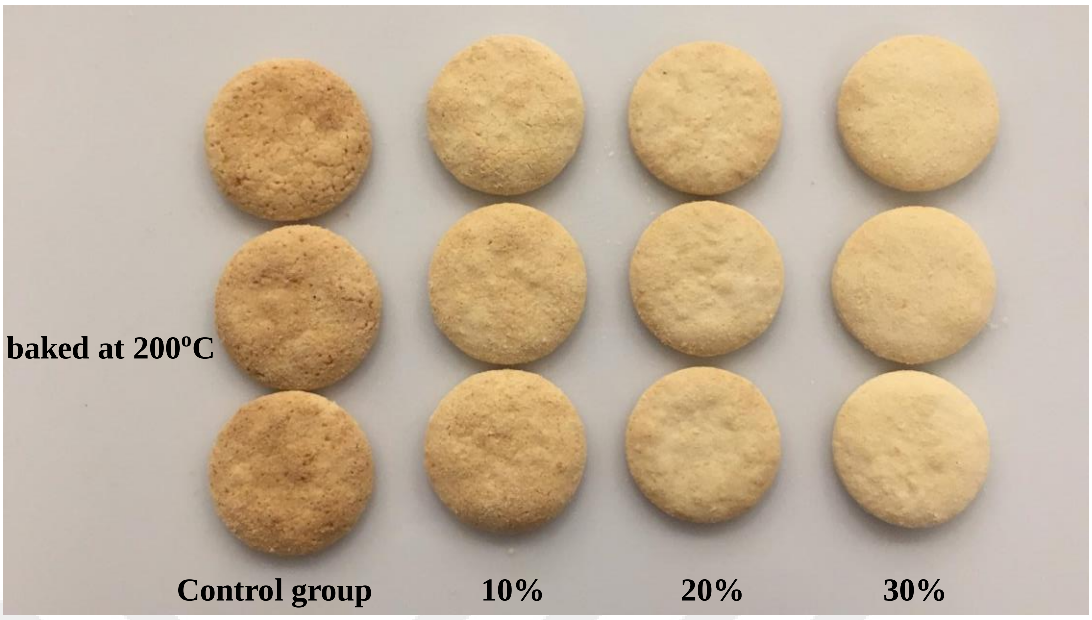

Figure 3.27 Gluten-free biscuits with different concentration of RS baked at 200ºC

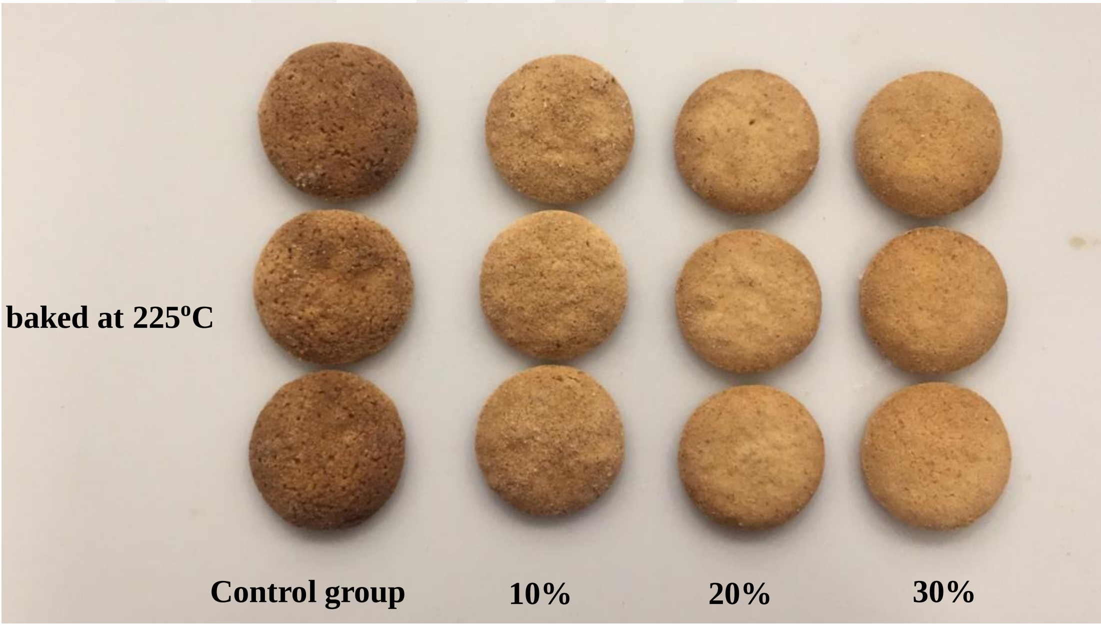

Figure 3.28 Gluten-free biscuits with different concentration of RS baked at 225ºC

CHAPTER 4

CONCLUSION

In this study, dietary fiber-rich gluten-free biscuits were produced as an alternative to gluten-free biscuits on the market. For this purpose, resistant starch (RS) were used as a functional component and a source of dietary fiber in the production of gluten-free biscuits. The amount of gluten-free flour in the gluten-free biscuit formulation was reduced by 10, 20 and 30% and resistant starch was used instead of gluten-free flour, the baking time of the biscuits was kept constant, and they were baked at 175, 200 and 225^{o}C temperatures. The control group biscuits was completely made with gluten-free flour (with no RS). Chemical and physical properties were investigated produced biscuits. Biscuits were compared by measuring the quality parameters in terms of moisture, water activity, pH, color, diameter, height, texture and dietary fiber.

When moisture content and water activity of gluten-free biscuits were examined, it was determined that the different percentages RS addition that was baked at 175, 200 and 225^{o}C on the moisture contents and water activity of gluten-free biscuits was significant ($\alpha<0.05$). At all baking temperatures, it was determined that the control groups (with no RS) has the lowest moisture contents and water activity. Moisture contents and water activity of gluten-free biscuits increased with increasing RS addition rate at each baking temperature. At each RS addition rates, it was found that the effect of baking temperatures on moisture content and water activity values was significant ($\alpha<0.05$). At all RS addition rates, gluten-free biscuits baked at 175^{o}C has been determined highest moisture and water activity contents. As predicted, gluten-free biscuits baked at 225^{o}C has been determined lowest moisture and water activity contents. Therefore, it was found that moisture contents and water activity of gluten-free biscuits decreased with increasing baking temperature.

As mentioned before, RS used in the study has a lower pH value than other raw materials in formula. When pH of gluten-free biscuits were examined, it was found that the different percentages RS addition that was baked at 175, 200 and 225^{o}C on the pH values of gluten-free biscuits was not significant. (α>0.05). At all baking temperatures, it was determined that the control groups (with no RS) has the highest pH value. It has been determined, the pH value has decreased as the amount of RS addition increased at each baking temperature. At each RS addition rates, it was determined that the baking temperature did not have an effect on the pH of the gluten-free biscuits in the control group. In control group, it was found that the effect of baking temperatures on pH values was not significant. (α>0.05). Gluten-free biscuits baked at 175^{o}C has been determined highest pH value, gluten-free biscuits baked at 225^{o}C has been determined lowest pH value. Therefore, the pH value decreased as the baking temperature increased. But, it was found that the effect of each baking temperature on pH values was significant on gluten-free biscuits with RS (α<0.05). The same assessment is also valid for these results, pH value decreased as the baking temperature increased.

When color parameters (L*, a* and b*) of gluten-free biscuits were examined, it was determined that the different percentages RS addition that was baked at 175, 200 and 225^{o}C on the color parameters (L*, a* and b*) of gluten-free biscuits was significant (α<0.05). At all baking temperatures, it was determined that the control groups (with no RS) has the lowest L* values and gluten-free biscuits containing 30% RS have the highest L* values. L* values of gluten-free biscuits increased with increasing RS addition rate at each baking temperature. At each RS addition rates, it was found that the effect of baking temperatures on color parameters (L*, a* and b*) was significant (α<0.05). At all RS addition rates, gluten-free biscuits baked at 175^{o}C has been determined highest L* value. As predicted, gluten-free biscuits baked at 225^{o}C has been determined lowest L* value. Therefore, it was found that L* values of gluten-free biscuits decreased with increasing baking temperature. At all baking temperatures, it was determined that the control groups (with no RS) has the highest a* and b* values and gluten-free biscuits containing 30% RS have the lowest a* and b* values. a* and b* values of gluten-free biscuits decreased with increasing RS addition rate at each baking temperature. At all RS addition rates, gluten-free biscuits baked at 175^{o}C has been determined lowest a* and b* value. As predicted, gluten-free biscuits baked at 225^{o}C has been determined highest a* and b* value. Therefore, it was found that a* and b* values of gluten-free biscuits increased with increasing baking temperature.

When the diameter and height values of gluten-free biscuits produced with different percentages of RS are examined, it was found that the different RS addition that was baked at 175, 200 and 225°C on the diameter and height value of gluten-free biscuits were significant. ($\alpha<0.05$). At all baking temperatures, it was determined that the control groups (with no RS) has the highest diameter value and gluten-free biscuits containing 30% RS have the lowest diameter value. Diameter value of gluten-free biscuits decreased with increasing RS addition rate at each baking temperature. Although statistically significant, there was imbalance in height value of gluten-free biscuits at all baking temperatures. Different height values were measured as RS addition rate increased at each cooking temperature. There was no linear increase or decrease was observed. The reason for this is thought to be due to the raising agents in the biscuit recipe or used biscuit mold. At each RS addition rates, it was found that the effect of baking temperatures on diameter and height value of gluten-free biscuits were significant ($\alpha<0.05$). In the control group and gluten-free biscuits with RS, when the baking temperature have increased, diameter values of gluten-free biscuits have decreased and the height values have increased.

When the hardness value and fracturability of gluten-free biscuits produced with different percentages of RS are examined, it was found that the different RS addition that was baked at 175, 200 and 225°C on the hardness value and fracturability of gluten-free biscuits were significant. ($\alpha<0.05$). At all baking temperatures, it was determined that the control groups (with no RS) has the lowest hardness values and highest fracturability. Hardness value of gluten-free biscuits increased with increasing RS addition rate at each baking temperature compared to the control group. In other words, RS added gluten-free biscuits were harder than the control group. When look at biscuits with added RS, except the control group, hardness value was decreases as the rate of RS addition was increases. However, despite this reduction, gluten-free biscuits with 30% RS were harder than the control group. Fracturability of gluten-free biscuits decreased with increasing RS addition rate at each baking temperature compared to the control group. At each RS addition rates, it was found that the effect of baking temperatures on hardness value and fracturability of gluten-free biscuits were significant ($\alpha<0.05$). At all RS addition rates, gluten-free biscuits baked at 175°C has been determined highest hardness value and fracturability. As predicted, gluten-free biscuits baked at 225°C has been determined lowest hardness value and fracturability. In other words, as temperature increases, gluten-free biscuits were less hard and less brittle.

When the total dietary fiber (TDF) of gluten-free biscuits produced with different percentages of RS are examined, only baked at 200^{o}C gluten-free biscuits dietary fiber amounts of were measured. It was determined that the different percentages RS addition that was baked at 200^{o}C on the TDF of gluten-free biscuits was significant ($\alpha<0.05$). It was determined that the control group gluten-free biscuits (with no RS) has the lowest TDF and gluten-free biscuits containing 30% RS have the highest TDF. Therefore, TDF of gluten-free biscuits increased with increasing RS addition rate at 200^{o}C.

As a result, it has been determined that the use of RS in the production of gluten-free biscuits in order to increase the total dietary fiber properties of biscuits gives positive results and that RS can be added to the biscuits in terms of the parameters examined.

Since celiac patients do not get enough benefit from the food they take, the foods they will consume should be more nutritious and healthy. Most of the gluten-free products sold in the market are imported from abroad and their prices are high, the variety of products is low and generally these products are starch based. For these reasons, it is necessary to develop new gluten-free products healthy and enriched with functional properties in our country and researching and increasing the production possibilities of these products.

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APPENDICES

Table A.1 ANOVA for moisture content values of gluten-free biscuits containing different level RS baked at 175ºC

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.394	3	0.131	14.461	0.013
Within Groups	0.036	4	0.009		
Total	0.431	7			

Table A.2 Tukey's multiple range test table for moisture content values of gluten-free biscuits containing different level RS baked at 175ºC

RS (%) Concentration	N	Subset for alpha = 0.05	
		1	2
00.00	2	5.33500	
10.00	2	5.51500	5.51500
20.00	2		5.78500
30.00	2		5.90000
Sig.		0.358	0.051

Table A.3 ANOVA for moisture content values of gluten-free biscuits containing different level RS baked at 200ºC

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.450	3	0.150	30.745	0.003
Within Groups	0.019	4	0.005		
Total	0.469	7			

Table A.4 Tukey's multiple range test table for moisture content values of gluten-free biscuits containing different level RS baked at 200°C

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
00.00	2	3.6300		
10.00	2	3.8450	3.8450	
20.00	2		4.0450	4.0450
30.00	2			4.2700
Sig.		0.116	0.141	0.102

Table A.5 ANOVA for moisture content values of gluten-free biscuits containing different level RS baked at 225°C

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.218	3	0.073	23.798	0.005
Within Groups	0.012	4	0.003		
Total	0.230	7			

Table A.6 Tukey's multiple range test table for moisture content values of gluten-free biscuits containing different level RS baked at 225°C

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
00.00	2	1.9250		
10.00	2	2.0350	2.0350	
20.00	2		2.2150	2.2150
30.00	2			2.3550
Sig.		0.324	0.099	0.192

Table A.7 ANOVA for water activity values of gluten-free biscuits containing different level RS baked at 175°C

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.004	3	0.001	20.270	0.007
Within Groups	0.000	4	0.000		
Total	0.004	7			

Table A.8 Tukey's multiple range test table for water activity values of gluten-free biscuits containing different level RS baked at 175ºC

RS (%) Concentration	N	Subset for alpha = 0.05	
		1	2
00.00	2	0.3210	
10.00	2	0.3270	
20.00	2	0.3415	
30.00	2		0.3755
Sig.		0.168	1.000

Table A.9 ANOVA for water activity values of gluten-free biscuits containing different level RS baked at 200ºC

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.001	3	0.000	20.421	0.007
Within Groups	0.000	4	0.000		
Total	0.001	7			

Table A.10 Tukey's multiple range test table for water activity values of gluten-free biscuits containing different level RS baked at 200ºC

RS (%) Concentration	N	Subset for alpha = 0.05	
		1	2
00.00	2	0.2820	
10.00	2		0.2985
20.00	2		0.3030
30.00	2		0.3105
Sig.		1.000	1.000

Table A.11 ANOVA for water activity values of gluten-free biscuits containing different level RS baked at 225ºC

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.011	3	0.004	204.721	0.000
Within Groups	0.000	4	0.000		
Total	0.011	7			

Table A.12 Tukey's multiple range test table for water activity values of gluten-free biscuits containing different level RS baked at 225ºC

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
00.00	2	0.1385		
10.00	2		0.2060	
20.00	2		0.2185	
30.00	2			0.2380
Sig.		1.000	0.133	1.000

Table B.1 ANOVA for pH values of gluten-free biscuits containing different level RS baked at 175ºC

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.031	3	0.010	0.365	0.783
Within Groups	0.113	4	0.028		
Total	0.145	7			

Table B.2 Tukey's multiple range test table for pH values of gluten-free biscuits containing different level RS baked at 175ºC

RS (%) Concentration	N	Subset for alpha = 0.05
		1
30.00	2	7.1950
2000	2	7.2850
10.00	2	7.3000
,00	2	7.3700
Sig.		0.739

Table B.3 ANOVA for pH values of gluten-free biscuits containing different level RS baked at 200ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.022	3	0.007	0.666	0.616
Within Groups	0.045	4	0.011		
Total	0.067	7			

Table B.4 Tukey's multiple range test table for pH values of gluten-free biscuits containing different level RS baked at 200^{o}C.

RS (%) Concentration	N	Subset for alpha = 0.05
		1
30.00	2	7.0150
20.00	2	7.0400
10.00	2	7.0750
0.00	2	7.1550
Sig.		0.739

Table B.5 ANOVA for pH values of gluten-free biscuits containing different level RS baked at 225^{o}C.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.121	3	0.040	4.171	0.101
Within Groups	0.039	4	0.010		
Total	0.160	7			

Table B.6 Tukey's multiple range test table for pH values of gluten-free biscuits containing different level RS baked at 225^{o}C.

RS (%) Concentration	N	Subset for alpha = 0.05
		1
30.00	2	6.6150
20.00	2	6.6650
10.00	2	6.8300
0.00	2	6.9200
Sig.		0.114

Table C.1 ANOVA for L* values of gluten-free biscuits containing different level RS baked at 175^{o}C.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	58.058	3	19.353	6.671	0.049
Within Groups	11.604	4	2.901		
Total	69.663	7			

Table C.2 Tukey's multiple range test table for L* values of gluten-free biscuits containing different level RS baked at 175ºC.

RS (%) Concentration	N	Subset for alpha = 0.05	
		1	2
00.00	2	81.3250	
10.00	2	83.7390	83.7390
20.00	2	85.4500	84.4500
30.00	2		88.7250
Sig.		0.214	0.133

Table C.3 ANOVA for L* values of gluten-free biscuits containing different level RS baked at 200ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24.876	3	8.292	11.990	0.018
Within Groups	2.766	4	0.692		
Total	27.642	7			

Table C.4 Tukey's multiple range test table for L* values of gluten-free biscuits containing different level RS baked at 200ºC.

RS (%) Concentration	N	Subset for alpha = 0.05	
		1	2
00.00	2	80.6800	
10.00	2	81.6175	81.6175
20.00	2		84.2600
30.00	2		84.9050
Sig.		0.695	0.055

Table C.5 ANOVA for L* values of gluten-free biscuits containing different level RS baked at 225ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.464	3	4.821	7.333	0.042
Within Groups	2.630	4	0.657		
Total	17.094	7			

Table C.6 Tukey's multiple range test table for L* values of gluten-free biscuits containing different level RS baked at 225^{o}C.

RS (%) Concentration	N	Subset for alpha = 0.05	
		1	2
00.00	2	70.8100	
10.00	2	72.2700	72.2700
20.00	2	72.8850	72.8850
30.00	2		74.5600
Sig.		0.188	0.146

Table C.7 ANOVA for a* values of gluten-free biscuits containing different level RS baked at 175^{o}C.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45.483	3	15.161	1062.055	0.000
Within Groups	0.057	4	0.014		
Total	45.540	7			

Table C.8 Tukey's multiple range test table for a* values of gluten-free biscuits containing different level RS baked at 175^{o}C.

RS (%) Concentration	N	Subset for alpha = 0.05			
		1	2	3	4
30.00	2	0.8450			
20.00	2		1.9800		
10.00	2			3.1100	
0.00	2				7.1650
Sig.		1,000	1,000	1,000	1,000

Table C.9 ANOVA for a* values of gluten-free biscuits containing different level RS baked at 200^{o}C.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35.695	3	11.898	934.119	0.000
Within Groups	0.051	4	0.013		
Total	35.746	7			

Table C.10 Tukey's multiple range test table for a* values of gluten-free biscuits containing different level RS baked at 200°C.

RS (%) Concentration	N	Subset for alpha = 0.05			
		1	2	3	4
30.00	2	2.7400			
20.00	2		3.2850		
10.00	2			4.7450	
0.00	2				8.165
Sig.		1,000	1,000	1,000	1,000

Table C.11 ANOVA for a* values of gluten-free biscuits containing different level RS baked at 225°C.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	84.178	3	28.239	387.837	0.000
Within Groups	0.291	4	0.073		
Total	85.009	7			

Table C.12 Tukey's multiple range test table for a* values of gluten-free biscuits containing different level RS baked at 225°C.

RS (%) Concentration	N	Subset for alpha = 0.05			
		1	2	3	4
30.00	2	7.4700			
20.00	2		10.8600		
10.00	2			12.7500	
0.00	2				16.4750
Sig.		1,000	1,000	1,000	1,000

Table C.13 ANOVA for b* values of gluten-free biscuits containing different level RS baked at 175°C.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	298.093	3	99.364	395.126	0.000
Within Groups	1.006	4	0.251		
Total	299.099	7			

Table C.14 Tukey's multiple range test table for b* values of gluten-free biscuits containing different level RS baked at 175^{o}C.

RS (%) Concentration	N	Subset for alpha = 0.05			
		1	2	3	4
30.00	2	16.7900			
20.00	2		19.8250		
10.00	2			25.8450	
0.00	2				32.7400
Sig.		1,000	1,000	1,000	1,000

Table C.15 ANOVA for b* values of gluten-free biscuits containing different level RS baked at 200^{o}C.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	131.531	3	43.844	92.634	0.000
Within Groups	1.893	4	0.473		
Total	133.424	7			

Table C.16 Tukey's multiple range test table for b* values of gluten-free biscuits containing different level RS baked at 200^{o}C.

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
30.00	2	23.8900		
20.00	2	24.2300		
10.00	2		28.0600	
0.00	2			33.9600
Sig.		0.956	1.000	1.000

Table C.17 ANOVA for b* values of gluten-free biscuits containing different level RS baked at 225^{o}C.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17.777	3	5.926	29.893	0.003
Within Groups	0.793	4	0.198		
Total	18.570	7			

Table C.18 Tukey's multiple range test table for b* values of gluten-free biscuits containing different level RS baked at 225ºC.

RS (%) Concentration	N	Subset for alpha = 0.05	
		1	2
30.00	2	32.5550	
20.00	2	34.0650	
10.00	2		35.9900
0.00	2		36.1900
Sig.		0.088	0.966

Table D.1 ANOVA for diameter values of gluten-free biscuits containing different level RS baked at 175ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.056	3	3.685	2033.313	0.000
Within Groups	0.007	4	0.002		
Total	11.063	7			

Table D.2 Tukey's multiple range test table for diameter values of gluten-free biscuits containing different level RS baked at 175ºC.

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
30.00	2	42.1700		
20.00	2	42.3400		
10.00	2		43.8250	
0.00	2			45.0500
Sig.		0.053	1.000	1.000

Table D.3 ANOVA for diameter values of gluten-free biscuits containing different level RS baked at 200ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.270	3	3.423	1733.308	0.000
Within Groups	0.008	4	0.002		
Total	10.278	7			

Table D.4 Tukey's multiple range test table for diameter values of gluten-free biscuits containing different level RS baked at 200ºC.

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
30.00	2	41.9650		
20.00	2	42.1200		
10.00	2		43.2700	
0.00	2			44.7950
Sig.		0.081	1.000	1.000

Table D.5 ANOVA for diameter values of gluten-free biscuits containing different level RS baked at 225ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.404	3	3.135	921.152	0.000
Within Groups	0.014	4	0.003		
Total	9.418	7			

Table D.6 Tukey's multiple range test table for diameter values of gluten-free biscuits containing different level RS baked at 225ºC.

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
30.00	2	41.8250		
20.00	2	42.0000		
10.00	2		43.2300	
0.00	2			44.5210
Sig.		0.124	1.000	1.000

Table D.7 ANOVA for height values of gluten-free biscuits containing different level RS baked at 175ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.116	3	0.372	472.524	0.000
Within Groups	0.003	4	0.001		
Total	1.119	7			

Table D.8 Tukey's multiple range test table for height values of gluten-free biscuits containing different level RS baked at 175°C.

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
20.00	2	6.6100		
10.00	2		7.2250	
30.00	2			7.5250
0.00	2			7.5250
Sig.		1.000	1.000	1.000

Table D.9 ANOVA for height values of gluten-free biscuits containing different level RS baked at 200°C.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.893	3	0.631	373.953	0.000
Within Groups	0.007	4	0.002		
Total	1.900	7			

Table D.10 Tukey's multiple range test table for height values of gluten-free biscuits containing different level RS baked at 200°C.

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
20.00	2	6.8250		
10.00	2		7.7900	
30.00	2		7.8250	
0.00	2			8.1150
Sig.		1.000	0.829	1.000

Table D.11 ANOVA for height values of gluten-free biscuits containing different level RS baked at 225°C.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.356	3	1.119	1376.918	0.000
Within Groups	0.003	4	0.001		
Total	3.359	7			

Table D.12 Tukey's multiple range test table for height values of gluten-free biscuits containing different level RS baked at 225ºC.

RS (%) Concentration	N	Subset for alpha = 0.05			
		1	2	3	4
20.00	2	7.0000			
00.00	2		8.2450		
30.00	2			8.4500	
10.00	2				8.6700
Sig.		1,000	1,000	1,000	1,000

Table E.1 ANOVA for hardness values of gluten-free biscuits containing different level RS baked at 175ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	267852.644	3	89284.215	99.611	0.000
Within Groups	3585.309	4	896.327		
Total	271437.953	7			

Table E.2 Tukey's multiple range test table for hardness values of gluten-free biscuits containing different level RS baked at 175ºC.

RS (%) Concentration	N	Subset for alpha = 0.05	
		1	2
00.00	2	1464.5700	
30.00	2		1864.3885
20.00	2		1864.9580
10.00	2		1907.6450
Sig.		1.000	0.538

Table E.3 ANOVA for hardness values of gluten-free biscuits containing different level RS baked at 200ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	39499.757	3	13166.586	34.960	0.002
Within Groups	1506.466	4	376.617		
Total	41006.223	7			

Table E.4 Tukey's multiple range test table for hardness values of gluten-free biscuits containing different level RS baked at 200ºC.

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
00.00	2	1121.9120		
30.00	2	1155.6465	1155.6465	
20.00	2		1222.2600	
10.00	2			1305.8175
Sig.		0.413	0.085	1.000

Table E.5 ANOVA for hardness values of gluten-free biscuits containing different level RS baked at 225ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6963.962	3	2321.321	13.094	0.016
Within Groups	709.139	4	177.285		
Total	7673.101	7			

Table E.6 Tukey's multiple range test table for hardness values of gluten-free biscuits containing different level RS baked at 225ºC.

RS (%) Concentration	N	Subset for alpha = 0.05	
		1	2
00.00	2	796.9505	
30.00	2	820.2075	
20.00	2	845.3865	845.3865
10.00	2		876.3260
Sig.		0.071	0.235

Table E.7 ANOVA for fracturability values of gluten-free biscuits containing different level RS baked at 175ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.275	3	0.092	10.794	0.022
Within Groups	0.034	4	0.008		
Total	0.308	7			

Table E.8 Tukey's multiple range test table for fracturability values of gluten-free biscuits containing different level RS baked at 175ºC.

RS (%) Concentration	N	Subset for alpha = 0.05	
		1	2
30.00	2	30.5000	
20.00	2	30.6000	
10.00	2	30.7250	30.7250
00.00	2		30.9945
Sig.		0.210	0.133

Table E.9 ANOVA for fracturability values of gluten-free biscuits containing different level RS baked at 200ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.749	3	0.250	50.173	0.001
Within Groups	0.020	4	0.005		
Total	0.769	7			

Table E.10 Tukey's multiple range test table for fracturability values of gluten-free biscuits containing different level RS baked at 200ºC.

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
30.00	2	29.4300		
20.00	2	29.5900	29.5900	
10.00	2		29.8060	
00.00	2			30.2445
Sig.		0.248	0.118	1.000

Table E.11 ANOVA for fracturability values of gluten-free biscuits containing different level RS baked at 225ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.459	3	0.153	22.856	0.006
Within Groups	0.027	4	0.007		
Total	0.485	7			

Table E.12 Tukey's multiple range test table for fracturability values of gluten-free biscuits containing different level RS baked at 225ºC.

RS (%) Concentration	N	Subset for alpha = 0.05		
		1	2	3
30.00	2	28.6850		
20.00	2	28.8800	28.8800	
10.00	2		29.1550	29.1550
00.00	2			29.3030
Sig.		0.222	0.090	0.386

Table F.1 ANOVA for total dietary fiber values of gluten-free biscuits containing different level RS baked at 200ºC.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25.615	3	8.538	923.047	0.000
Within Groups	0.037	4	0.009		
Total	25.652	7			

Table F.2 Tukey's multiple range test total dietary fiber values of gluten-free biscuits containing different level RS baked at 200ºC.

RS (%) Concentration	N	Subset for alpha = 0.05			
		1	2	3	4
00.00	2	1.5050			
10.00	2		2.7850		
20.00	2			5.3450	
30.00	2				5.8350
Sig.		1,000	1,000	1,000	1,000