PAPER

THE EFFECTS OF LOCUST BEAN GUM AND RHUBARB ON THE PHYSICAL AND FUNCTIONAL PROPERTIES OF THE GLUTEN-FREE BISCUITS

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ABSTRACT

In this study, rhubarb plant, which is known to have functional properties, was added to gluten-free biscuit formulations and the effects of rhubarb were examined on the physical and antioxidant properties of biscuits. From the examination, it was observed that the spread ratio of the biscuits varied between 51.70 and 63.80. Also, the spread ratios of the biscuits decreased as the rhubarb and gum levels increased. Firmness values of the gluten-free biscuits were 29.71-54.02 N. The increase in gum levels of biscuit formulas that did not contain rhubarb led to an increase in the firmness values. Total phenolic content (TPC) of the biscuits varied between 2.00 and 3.18 μg GAE/g. The lowest TPC value was determined in the biscuit formula 2 which did not contain rhubarb and contained the highest level of gum, while the highest TPC value was determined in the biscuit formula 1 which did not contain gum, but contained the highest level of rhubarb. The DPPH scavenging values of the gluten-free biscuits were 42.60% and 57.51%.The addition of rhubarb alone in the formula increased antioxidant activity. However, the addition of rhubarb with gum in the formula decreased antioxidant activity. The aim of this study was to produce biscuits with high nutritional value using functional food components for the consumption of patients with celiac disease. The addition of rhubarb increased the TPC of the biscuits as well as the functional properties of the biscuits by inhibiting free radicals.

Keywords: antioxidant, biscuit, gluten-free, rhubarb, RSM

1. INTRODUCTION

Celiac disease has been identified as an inflammatory intestinal disease induced by gluten in wheat, barley, rye etc. (JNAWALI *et al.*, 2016). Currently, the only treatment for celiac disease is a gluten-free diet. However, gluten-free products have low nutritional quality and are more expensive than gluten-containing food products. Therefore, there is a strong need to develop gluten-free products that have high nutritional and economic value (JNAWALI *et al.*, 2016). The production of gluten-free products will increase with increase in technological developments. Flours from pseudocereals such as buckwheat, quinoa or amaranth, alternative cereals such as white sorghum or pigmented cereal grains and non-cereals sources such as legumes, seeds or nuts are emerging as alternatives to common gluten free flours and they have been used as components in the production of different gluten free cereal based foods (CAPRILES *et al.*, 2016; PADALINO *et al.*, 2016; GIUBERTI *et al.*, 2018). In addition, by-products from fruit and vegetable processing have been incorporated into gluten free food formulations (BOUREKOUA *et al.*, 2017; DRABINSKA *et al.*, 2017). Also, chestnut flour has been incorporated into gluten free biscuit formula by PACIULLI *et al.* (2018) and it was observed that chestnut flour improved the technological and organoleptic quality of gluten-free biscuits. Although, in recent years, the enhancement of common gluten-free cereal-based foods with ingredients rich in phytochemicals such as polyphenols is gaining a growing interest, the number of studies on the enrichment of gluten-free products with components of functional properties is still limited (ROCHETTI *et al.*, 2018).

Functional food is defined as food that possesses health benefits beyond its basic nutritional properties (YETIM *et al.*, 2010). In recent years, the changes in the dietary habits and increasing awareness of healthy eating have increased the production and consumption of functional foods. Functional foods contain physiologically active ingredients that have positive health benefits beyond their basic nutritional value (MERAL and DOGAN, 2009). The enrichment of the gluten-free biscuit formulations with functional components is of great importance as these functional components are also ingested along with the product.

There are various studies investigating the effects of the natural additives on the quality and nutritional properties of the biscuits. However, the number of studies involving the addition of natural components to enrich gluten-free biscuit are limited. Natural components are rich in antioxidant activity and help to increase the antioxidant activity of the foods. Furthermore, these components are found widely in nature and are used in the treatment of various diseases.

Rhubarb (*Rheum ribes*) is a perennial plant from the Polygonaceae family. It grows in rocky areas of mountainous regions. Rhubarb is rich in Vitamin C and grows during spring (ANDİÇ *et al.*, 2009). MERAL (2011) reported that rhubarb had a high phenolic compound content and inhibited the free radicals. It could be used as a functional food component due to its antioxidant properties. In spite of these functional properties, there are limited number of studies on its usage as a functional food component. The aim of this study was to produce functional biscuits that are rich in components such as phenolic and antioxidants. Thus, the study aimed to develop a healthy gluten-free biscuit formula. In this study, rhubarb and locust bean gum were used to produce the gluten-free biscuits. The levels of rhubarb and gum were selected based on preliminary experiments; the optimal amount of rhubarb and gum was obtained by means of preliminary baking tests combined with a consumer test with 10 untrained panelists to evaluate consumers' acceptance of the improved biscuits. While choosing the levels of rhubarb and gum, the firmness of dough and the tastes of the biscuits were taken into account by the untrained panelists.

2. MATERIAL AND METHODS

2.1. Material

Rice flour was obtained from Makeks (İstanbul, Turkey), potato starch was obtained from Smart Chemistry (İzmir, Turkey), locust bean gum was obtained from A&D (İstanbul, Turkey), and rhubarb plant used in the formulations were obtained from a market in Van, Turkey. Chemical substances at analytical grade used in the study were obtained from Merck (Darmstadt, Germany). Rhubarb was manually separated from the bark and dried at 40°C. Then, the dried rhubarb was ground with a laboratory mill (Perten LM 120, Hägersten, Sweden) just before usage.

2.2. Pasting properties of flours

The viscosity properties of starch used in formulations were determined using the Rapid Viscosity Analyser (RVA) (RVA-4500, Perten, Switzerland) depending on temperature and time. The viscosity values were given as cP. For RVA analysis, 3 g samples were taken (corrected according to 14% moisture) and 25 g of deionized water were added. The heating and cooling cycles of the device were adjusted as follows: The sample was kept at 50°C for 1 min, then the temperature was increased to 95°C in 3.5 min. The samples were kept for 2.5 min at 95°C and cooled to 50°C in 3.5 min, and then kept at 50°C for 2 min (SAKIYAN *et al.*, 2009). Values obtained from RVA graph were expressed as peak viscosity, trough viscosity, breakdown viscosity, setback viscosity and final viscosity.

2.3. Preparation, shaping and baking of the dough

The optimization of the formula to produce gluten-free biscuits was conducted using the Response Surface Methodology (RSM) and biscuit doughs were prepared following the formation of design patterns. The formulations of gluten-free biscuits were presented in Table 1.

Table 1. Gluten-free biscuit formula.

Ingredients	(g)
Rice flour	80
Potato starch	20
Sugar	42
Shortening	40
Water	22
Sodium bicarbonate	1.0
Amonium bicarbonate	0.5
Corn syrup	1.5
Salt	1.2
Vanilla	0.3
Rhubarb	0, 1 and 2
Locust bean gum	0, 1.5 and 3

The biscuit dough was prepared according to the AACC (1999) method with some modifications using the KitchenAid Mixer (Model 5KSM45). After the addition of shortening, sugar, salt, vanilla and sodium bicarbonate, the mixture was stirred for 3 minutes at approximately ~60 rpm and then the specific gravity of the obtained cream was determined. Subsequently, high-fructose corn syrup, ammonium bicarbonate and water were added and stirred again for 1 minute at ~90 rpm. Then, flour was added to the formula and the dough preparation process was completed by stirring for another two minutes at ~60 rpm. The AACC (10-50.05) (AACC, 1999) method was used to shape the biscuit dough. The doughs were cooked in the electric oven (Öztiryakiler, İstanbul-Turkey) at 85% humidity and at 185˚C for 12±1 min.

2.4. Determination of baking loss and spread ratio of biscuits

The weight of the biscuit was determined both before and after the cooking of the biscuits and the baking loss was determined. The width (W) and height (H) values of the biscuit samples were determined in mm using the scaled wooden apparatus according to the AACC (10-53.01) method (AACC, 1999). To obtain the average value, four measurements were made by changing the position and location of the biscuits and then the spread ratio of the biscuit was calculated using the formula (W/H)*10.

2.5. Determination of firmness

The firmness of the biscuit samples were determined using the TA.XT plus Texture Analyzer (TA.TX2. Stable Micro Systems Ltd. Godalming Surrey, UK) according to three-point bend rig technique. (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).

2.6. Extraction of the phenolic compounds

The method proposed by MERAL and DOĞAN (2013) was used to extract the phenolic compounds. The 5 g sample collected from the milled samples was placed in centrifuge tubes and, then, 15 mL of methanol was added to the tubes. Using a shaking incubator at 35˚C, the tubes were kept for 22 hours in a shaking incubator at 35˚C and at a rotational speed of 3500 rpm. Thereafter, the tubes were centrifuged at 12,000 *g* for 15 minutes. After centrifugation, the supernatant was transferred to amber bottles and the process continued until the volume of the final supernatant reached 50 mL. Each sample was transferred to amber vials, and kept at -20˚C until analysis.

2.7. Determination of total phenolic content (TPC)

TPC were determined using the Folin-Ciocalteu method. For this purpose, 300 μL sample and 3 mL Na_2CO_3 (2%) were added to test tubes and about 2 minutes later, 150 μL of the Folin-Ciocalteu reagent that was 1:1 diluted with ultra-pure water was added to the tubes. The mixture was vortex mixed and kept for 45 minutes at room temperature in darkness and, then, the absorbance of the mixture was read on a spectrophotometer at 765 nm (T80 UV/VIS, PG Instrument, UK). TPC was calculated using the calibration graph plotted using gallic acid and the results were expressed as gallic acid equivalent (GAE) (MERAL and DOĞAN, 2013).

2.8. Determination of DPPH radical scavenging effects

The extracts containing antioxidant substances were transferred to a test tube and the final volume was brought to 1 mL with methanol. For the samples, 3 mL of the 0.004% DPPH solution that was prepared with methanol on a daily basis was added and the samples were rapidly vortex-mixed. Upon mixing, the test tubes were incubated in darkness and their absorbance was read against a control sample (the sample containing 1 mL methanol and 3 mL DPPH solution) on a spectrophotometer at 517-nm wavelength using a quartz cuvette. The measurements were performed in two repetitions and the radical scavenging property of each sample was calculated using the following equation (MERAL and DOĞAN, 2013).

$$\% \text{ Inhibition} = A_{control} - A_{sample} / A_{control}$$

A control: Absorbance of control at 517 nm

A sample: Absorbance of sample at 517 nm

2.9. Experimental design

The experimental design for the production of gluten free biscuits containing rhubarb was carried out using response surface methodology (RSM) (StatGraphics Centurion 15.19) which gave 9 experimental runs as shown in Table 2. Three-level factorial design patterns were used to determine the effects of the factors and interactions on the properties of the biscuits. The variables, A was determined as the gum and the level of gum varied between 0 and 3%. B was determined as the rhubarb and the level of rhubarb varied between 0 and 2%. Significant difference between group averages was determined using Duncan multiple comparison tests at the *P<0.05* level of significance. The appropriate response values for each formulation were determined.

3. RESULTS AND DISCUSSION

3.1. Pasting properties of biscuit flours

The viscosity analysis results were reported in Table 2.

Table 2. Pasting properties of different biscuit dough formulations.

Trail	Gum (%) (A)	Rhubarb (%) (B)	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown Viscosity (cP)	Setback Viscosity (cP)	Final Viscosity (cP)
1	0	2	3022±50.9	1503±61.0	1519±16.2	912±30.6	2415±91.1
2	3	0	4611±55.8	2204±60.8	2406±172.8	1099±62.7	3304±117.8
3	1.5	2	3502±127.4	1665±110.0	1836±52.9	906±25.3	2572±135.0
4	3	1	4199±33.2	1924±151.8	2274±118.9	975±49.7	2899±110.2
5	3	2	4155±37.5	2010±101.6	2145±137.4	874±52.8	2884±48.9
6	1.5	1	3583±42.6	1893±94.5	1690±132.9	926±46.1	2819±56.8
7	0	1	3000±17.8	1503±150.5	1496±133.1	1014±33.4	2517±160.5
8	1.5	0	4053±110.4	2093±33. 6	1960±89.3	1049±38.0	3142±37.8
9	0	0	3660±10.7	1717±123.4	1942±128.2	1192±45.4	2910±89.9

Peak viscosity

The peak viscosity values of the biscuit formulations ranged from 3000 to 4611 cP. The lowest viscosity value was obtained in the biscuit formula 7 (0% gum, 1% rhubarb) and the highest viscosity value was determined in the biscuit formula 2 (3% gum, 0% rhubarb). The model, which explained the relationship between the peak viscosity of the dough and the independent factors (A and B), is outlined in Equation 1:

$$Peak\ viscosity = 3634{,}07 + 224{,}93 \times A - 789{,}94 \times B + 36{,}47 \times A^2 + 30{,}4444 \times A \times B + 230{,}06 \times B^2$$
$$R^2=0.99 \qquad (1)$$

The total difference in terms of the peak viscosity values can be explained with the linear and quadratic effects of the components in the model and the interaction between the components. Peak viscosity values were significantly affected by the linear effects of gum and rhubarb. Furthermore, the interaction effect of the factors was not significant (*P>0.05*). Fig. 1A showed the response surface for peak viscosity. The effects of gum and rhubarb on the peak viscosity values were found to be significant (*P<0.05*). Peak viscosity values increased as the level of gum in formulation increased, while the peak viscosity values decreased with increasing levels of rhubarb (Fig. 1A). High viscosity value indicates that starch granules and starch-gum mixture have higher swelling powers (JYOTHI *et al.*, 2005). Various studies have reported that gum influenced the gelatinization and rheological properties of starch. Gum is widely used in foods containing starch to improve the textural and rheological properties, the water holding capacity as well as the quality of the product (SHI and MILLER, 2002; CHAISIWANG and SUPHANTHARIKA, 2006). In gluten-free products, it is difficult to maintain the desired viscoelastic structure, gas holding capacity and textural properties due to the lack of the gluten protein. Different starch sources, gums and dairy products can be added to the gluten-free formulation to eliminate the problems arising from the lack of gluten in the food. Previous studies have shown that gums increased the peak viscosity due to their water binding properties (CHAISIWANG and SUPHANTHARIKA, 2006). Rhubarb has high water binding capacity due to its high nutritional fiber content (MERAL, 2011). However, in this study, it was determined that rhubarb reduced the peak viscosity. This result was associated with the low pH value of rhubarb. Acids, due to their hydrolyzing effects, form cracks on the starch granules and, as a result, the viscosity decreases (JYOTHI *et al.*, 2005).

Trough viscosity

Trough viscosity varied between 1503 and 2204 cP. The lowest trough viscosity values were determined in the flour formulations containing 0% gum and 1% rhubarb, and containing 0% gum and 2% rhubarb, whereas, the highest trough viscosity value was determined in the formula containing 3% gum and 0% rhubarb (Fig. 1B). Results revealed that gum and rhubarb content had significant effects on the trough viscosity of flours (*P<0.05*). Gums increased the viscosity by binding water, there by making water release from starch-gum systems difficult. The decrease in viscosity of mixtures containing gum was lower than that in mixtures that do not contain gum. Rhubarb, probably due to its hydrolyzing effect, decreased the viscosity value at a higher level compared to gum.
The final equation for the trough viscosity of the dough is shown in equation 2 below.

$$Trough\ viscosity = 1754.8 + 232.56 \times A - 321.5 \times B - 26.27 \times A^2 + 3{,}4444 \times A \times B + 84.89 \times B^2$$
$$R^2=0.83 \qquad (2)$$

Breakdown viscosity

Breakdown viscosity is one of the important gelatinization characteristics of starch gel (YILDIZ *et al.*, 2013; YILMAZ *et al.*, 2015). The breakdown viscosity values of the flours ranged from 1496 to 2406 cP (Fig. 1C). The model, which explained the relationship between the breakdown viscosity of the dough and the independent factors (A and B), is shown in Equation 3:

$$Breakdown\ viscosity = 1879.28 - 7.62963 \times A - 468.444 \times B + 62.7407 \times A^2 + 27 \times A \times B + 145.17 \times B^2 \quad R^2=0.87 \tag{3}$$

It was determined that the effects of gum and rhubarb had significant effects on trough viscosity ($P<0.05$). The decomposition of starch due to high temperature and mechanical stress resulted to a decrease in viscosity. Breakdown viscosity is the difference between the peak viscosity and trough viscosity. Low breakdown viscosity value indicates a final product with good stability (YILDIZ *et al.*, 2013; YILMAZ *et al.*, 2015).

Setback viscosity

The setback viscosity of the flours ranged from 906 to 1192cP. Rhubarb had a significant linear effect, while gum had both significant quadratic and linear effects on the setback viscosity of the flours ($P<0.05$). Equation 4 showed the model, which explained the relationship between the setback viscosity of the dough and the independent factors (A and B).

$$Setback\ viscosity = 117393 - 100.407 \times A - 187.78 \times B + 24.124 \times A^2 + 9{,}11 \times A \times B + 32.11 \times B^2 \quad R^2=0.85 \tag{4}$$

Fig. 1D showed the effects of gum and rhubarb on the setback viscosity values of the flours. With increase in the gum, viscosity values of the flours initially decreased for a while, followed by an increase. Setback viscosity is related to the retrogradation that occurs during the cooling of the starch gel. Low setback viscosity value indicates low retrogradation and syneresis rates (YILMAZ *et al.*, 2015). YILDIZ *et al.* (2013) stated that fiber type and concentrations significantly affected gelatinization properties in starch-fiber mixtures. It was determined that, with the increase in the fiber content of starch-fiber mixtures, peak viscosity, trough viscosity, breakdown viscosity, setback viscosity and final viscosity values increased, while peak viscosity values decreased.

Final viscosity

Final viscosity of the flours varied between 2415 and 3304 cP. The suggested model including linear, quadratic and interaction effects of gum and rhubarb levels in the formula explains 90% of changes in the final viscosity. The final equation for the final viscosity was given in Equation 5.

$$Final\ viscosity = 2928.72 + 132.148 \times A - 509.278 \times B - 2.14815 \times A^2 + 12.56 \times A \times B + 117 \times B^2 \quad R^2=0.90 \tag{5}$$

It was determined that while the linear effects of gum and rhubarb and quadratic effect of rhubarb on the final viscosity values were significant ($P<0.05$), interaction effects of gum level and rhubarb level were not also significant (*P>0.05*). Final viscosity values decreased as the rhubarb level increased (Fig. 1E).

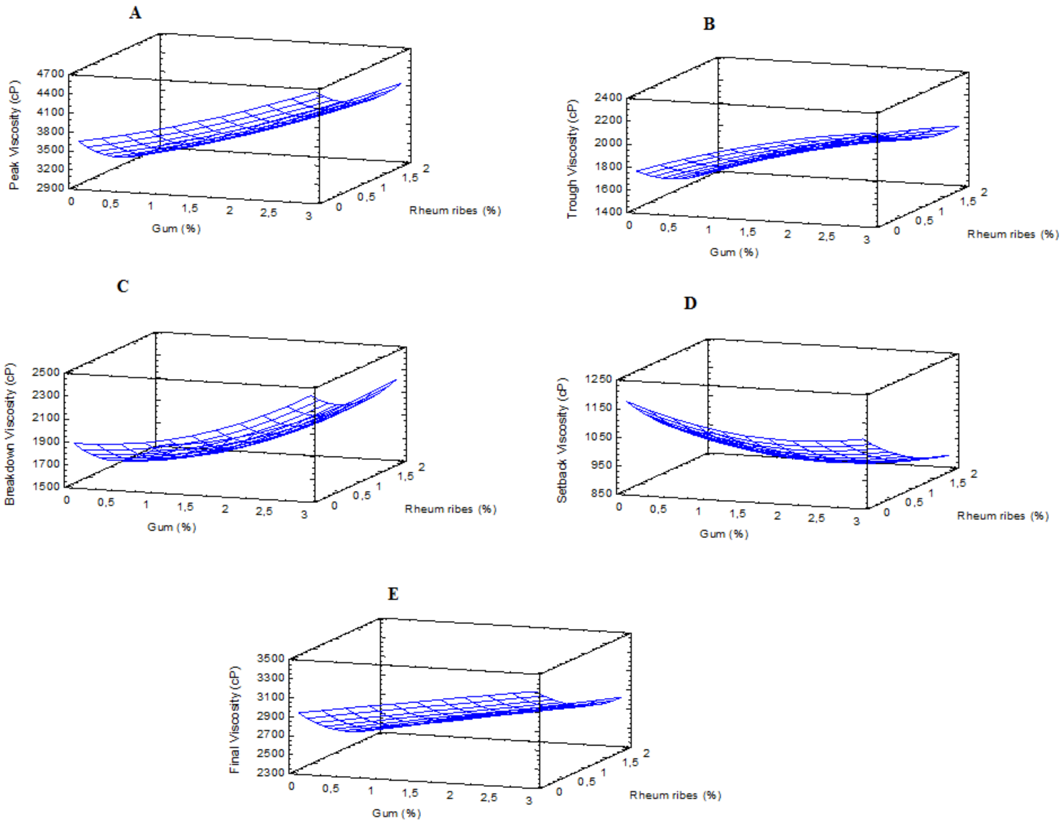

Figure 1. Response surface of viscosity properties of gluten-free biscuit doughs.
A: Peak viscosity, B: Trough viscosity, C: Breakdown viscosity, D: Setback viscosity, E: Final viscosity.

According to YILMAZ *et al*. (2015), low breakdown viscosity value indicates a final product with good stability and low setback viscosity value indicates low retrogradation and syneresis rates. In this study, it was observed that gum and rhubarb affected the pasting properties of flour. While increasing level of gum increased the peak, trough breakdown and final viscosity values of flour, increasing level of rhubarb decreased the peak through breakdown and final viscosity values of flour. The obtained results indicated that rhubarb delayed retrogradation and syneresis.

3.2. Baking loss and spread ratio of biscuits

The physical and functional properties of biscuits were reported in Table 3.

3.2.1. Baking loss

The baking loss values of the biscuits varied between 11.90% and 15.68%. High baking loss values in the biscuits results in the lack of consistency in the actual weight of packaging, subsequent increase in brittleness in the following stages and decrease in appeal. In a study, it has been reported that baking loss values of biscuits varied between 13.3% and 21.4% (DOĞAN and UĞUR, 2005). The quadratic effects of gum and rhubarb and the

interactions of gum and rhubarb on baking loss values of biscuits were insignificant (*P>0.05*).
Equation 6 showed the model, which explained the relationship between the baking loss of the biscuits and the independent factors (A and B).

$$Baking\ loss = 15.2783 - 1.04 \times A - 1.14667 \times B + 0.197778 \times A^2 + 0.0133333 \times A \times B + 0.47 \times B^2$$
$$R^2=0.21 \quad (6)$$

It was reported that baking loss did not change significantly when the level of gum and rhubarb was increased. A decrease in baking loss indicated a decrease in the shrinkage. The changes in baking loss values were associated with the water binding effects of gum and rhubarb. As known, baking loss occurs due to the evaporation of moisture during the baking process (MALKANTHI and UMADEVI, 2018). RODRIGUEZ-GARCIA *et al.* (2012) stated that lower baking loss is desirable for retaining its components and shape.

Table 3. Physical and functional properties of biscuits.

Trail	Gum (%) (A)	Rhubarb (%) (B)	Baking loss (%)	Spread ratio	Firmness (N)	TPC (µg GAE/g)	DPPH Scavenging (%)
1	0	2	14.83±0.0	52.94±0.1	35.07±5.9	3.18±0.1	57.51±2.4
2	3	0	13.18±0.1	51.70±0.1	54.02±0.7	2.00±0.4	47.74±2.2
3	1.5	2	13.88±0.1	56.00±0.1	40.52±2.2	2.96±0.1	56.67±2.8
4	3	1	14.12±0.5	53.32±0.2	53.35±2.9	2.95±0.3	49.06±2.1
5	3	2	13.56±0.4	52.74±0.0	36.20±3.8	3.10±0.4	42.60±2.3
6	1.5	1	11.90±3.0	54.78±0.7	41.66±0.7	2.91±0.1	52.05±1.9
7	0	1	15.40±1.0	60.17±0.3	41.34±6.5	3.11±0.2	50.26±3.5
8	1.5	0	15.68±1.7	56.99±0.2	29.71±1.6	2.76±0.1	43.23±1.6
9	0	0	14.53±1.0	63.80±0.4	32.12±2.8	2.69±0.1	52.68±4.0

3.2.2. Spread ratio

Spread ratio of the products varied between 51.70 and 63.80. The effect of gum and rhubarb and the interactions between these components on the spread ratio of biscuits were found to be significant (*P<0.05*). The spread ratios of the biscuits decreased as the rhubarb and gum levels increased. The spread ratio values of the biscuits that do not contain rhubarb were higher than those that contained rhubarb and the spread ratio values of the biscuits increased as the gum level decreased. DE SIMAS *et al*. (2009), in their study on gluten-free biscuits, associated the decrease in the spread ratio with the absorption of water added to the dough due to the functional components added to the formula and the consequent lack of water for the dissolution of sugar left in the system during baking, leading to an increase in viscosity.
The final equation for the spread ratio was given in Equation 7.

$$Spread\ ratio = 63.6083 - 3.91389 \times A - 3.98083 \times B - 0.0655556 \times A^2 + 1.98333 \times A \times B - 0.3975 \times B^2$$
$$R^2=0.92 \quad (7)$$

3.3. Firmness of biscuits

Firmness values of the gluten-free biscuits varied between 29.71 and 54.02 N. The lowest firmness value was obtained in the biscuit formula 8 (1.5% gum, 0% rhubarb) and the highest firmness value was determined in the biscuit formula 2 (3% gum, 0% rhubarb). Equation 8 showed the model which explained the relationship between the hardness of the biscuits and the independent factors (A and B).

$$Firmness = 2973.32 + 108.512\times A + 1991.38\times B + 213.805\times A^2 - 352.943\times A\times B - 765.401\times B^2$$
$$R^2=0.72 \qquad (8)$$

Gum, gum-rhubarb interaction and quadratic effects of gum and rhubarb had significant effects on the firmness values (*P<0.05*). The effect of rhubarb on the firmness values was not significant *(P>0.05*). Fig. 2C showed the effects of gum and rhubarb on the firmness values. It was previously mentioned that setback viscosity decreased with the increase in rhubarb levels and the decrease in the setback viscosity value may indicate that the retrogradation may occur at lower levels. The addition of rhubarb alone did not change the firmness values, however, the interaction between rhubarb and gum significantly changed the firmness value. The increase in gum levels in biscuit formulas that did not contain rhubarb led to an increase in the firmness values. As a result of the water binding by gum, free water in the medium decreased, leading to an increase in the firmness values. In biscuits containing 2% rhubarb, with the increase in gum levels, firmness values initially decreased for a while, followed by an increase. HOSENEY and ROGERS (1994) associated the firmness in biscuits with the development of dough and the interaction of starch and proteins during baking. PACIULLI *et al* (2018) demonstrated the fact that a direct link existed between fibre content and firmness of wheat and gluten-free biscuits.

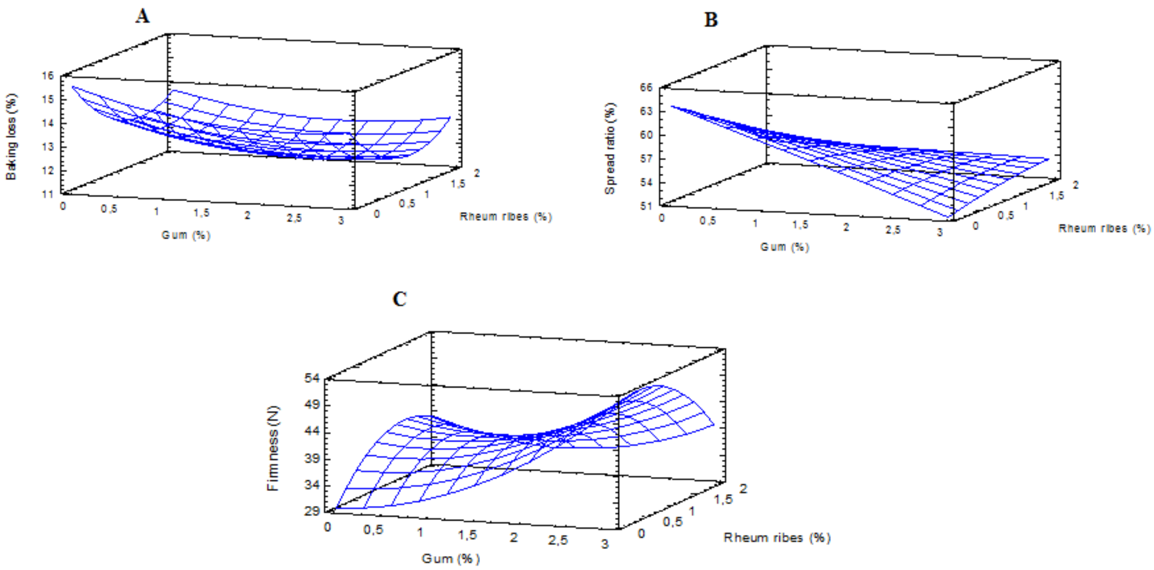

Figure 2. Response surface of physical properties of gluten-free biscuit doughs.
A: Baking loss, B: Spread ratio, C: Firmness.

3.9. Total Phenolic Content

Phenolic compounds possess antioxidant effects. Numerous studies have reported the antioxidant activity of phenolic compounds and the determination of TPC was emphasized as one of the measurement methods for antioxidant activity (MICHALSKA *et al.,* 2008). The TPC contents of the biscuits varied between 2.00 and 3.18 μg GAE/g. The lowest TPC value was determined in the biscuit formula 2 which did not contain rhubarb and contained the highest level of gum, while the highest TPC value was determined in the biscuit formula 1 which did not contain gum but contained the highest level of rhubarb. The model comprising the linear and quadratic effects of the components and their interactions explained 63% of the total changes in the TPC. The increase in the rhubarb contents significantly increased the TPC values. In a study in which rhubarb was used as a functional compound, it was stated that rhubarb contained high levels of phenolic compounds (MERAL, 2011). Furthermore, gum supplementation had a significant effect on the TPC contents of the biscuits ($P<0.05$). TPC decreased with the increase in gum levels (Fig. 3A). Numerous studies were carried out to determine the TPC values of foods and the results obtained in these studies have revealed that composition of foods, the ratio of the food components and the interaction between these components significantly changed the TPC and antioxidant properties of the foods (MERAL, 2011). The decreasing effect of gum on TPC can be associated with the decrease in the TPC dissolved in water due to the binding of the free water by gum. However, further studies should be conducted to verify this hypothesis. The results regarding the TPC obtained in the present study were verified using the results reported by SAKAČ *et al*. (2015) in their study on biscuits produced using rice starch and buckwheat flour at different ratios. The researchers reported that the TPC values increased as the level of buckwheat increased. The final equation for TPC was given in Equation 9.

$$TPC = 2.76468 - 0.145345\times A + 0.57325\times B - 0.0179496\times A^2 + 0.0977287\times A\times B - 0.20917\times B^2 \quad R^2=0.63 \quad (9)$$

3.10. DPPH radical scavenging effect of the biscuits

The DPPH scavenging values of the gluten-free biscuits varied between 42.60% and 57.51%.

$$\%\,scavenging = 48.6729 + 0.221667\times A + 5.45125\times B - 0.299444\times A^2 - 1.6625\times A\times B - 0.385\times B^2 \quad R^2=0.50 \quad (10)$$

Equation 10 showed the model that explained the relationship between the baking loss of the biscuits and the independent factors (A and B).

The highest antioxidant activity was found in the biscuits prepared from the biscuit formula 1 which did not contain gum and contained 2% rhubarb, while the lowest antioxidant activity was found in the biscuits prepared from the biscuit formula 5 which contained the highest levels of gum and rhubarb. The effect of rhubarb and gum used in the formula and gum-rhubarb interaction on inhibition was significant. Fig. 3B showed the response surface the effects of gum and rhubarb have on the antioxidant activity. The DPPH radical scavenging effect increased with increasing rhubarb levels, while the scavenging effect decreased with increasing gum levels. According to the comparisons of the biscuits with the highest and the lowest antioxidant activities, the increase in the antioxidant activity was determined to be 26%. The scavenging effect increased with increasing rhubarb levels and decreasing gum levels. Previous studies have revealed the antioxidant activity of rhubarb and its use as a functional component in food formulas

(ÖZTÜRK *et al.*, 2007; MERAL, 2011). Therefore, the increase in the antioxidant activity due to rhubarb supplementation is an expected result. However, the biscuit formula 5, which was one of the formulas with the highest rhubarb content, had the lowest antioxidant activity. This result was attributed to the lack of dissolved antioxidant agents due to the binding of the free water and/or the binding of some functional groups with antioxidant activity by the gum. However, further studies should be conducted to better understand the reason for this result and the gum-antioxidant relationship.

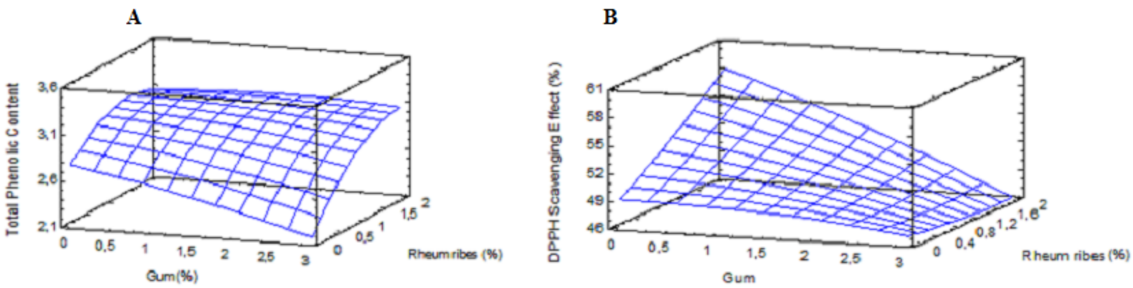

Figure 3. Response surface of functional properties of gluten-free biscuit doughs.
A:TPC, B: DPPH Scavenging.

4. CONCLUSIONS

In this study, rhubarb, which has an antioxidant activity, and is rich in nutritional fiber content, was added to the biscuit composition to increase the functional properties of gluten-free biscuits. Rhubarb was added at two different ratios to the formulas and the effects of this functional component on the biscuit properties, and antioxidant properties of the biscuits were investigated. The peak viscosity, trough viscosity and final viscosity values decreased in biscuits with the addition of rhubarb and increased with the addition of gum. Although, an increase was expected in the spread ratio values with the addition of components containing fiber (with the increase in the rhubarb ratio of the biscuits), there were no significant changes in the spread ratio values of the biscuits. Spread ratio is one of the important quality criteria for biscuits and high spread ratio rate indicates dryer, firmer biscuits with smoother upper surface appearance, pore structure and pore distribution. Therefore, we concluded that the quality was preserved, since rhubarb did not affect the spread ratio. It was determined that rhubarb had no effect on the firmness values of the gluten-free biscuits, while gum had an increasing effect on the firmness. Rhubarb supplementation positively affected the TPC of the gluten-free biscuits. Gum supplementation decreased the TPC of the gluten-free biscuits. Our study showed that by adding natural components to gluten-free biscuit formulations, functional properties can be obtained in biscuits without sacrificing quality and biscuits with positive effects on health can be produced by improving the antioxidant properties.

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