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Research Paper / Araştırma Makalesi

Quality Characteristics of Biscuits Fortified with Pomegranate Peel

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ABSTRACT

In this study, some chemical, physical and sensory properties of biscuits prepared with various substitution ratios (0, 6, 12 and 18%) of pomegranate peel were determined. To this end, pomegranate peel substitution did not cause a significant alteration in the protein, fat and ash contents of the biscuits. Results show that the antioxidant activity (from 5.06 μmol TE/100g to 288.38 μmol TE/100g), total phenolic content (from 56.49 mg GAE/100g to 1108.35 mg GAE/100g), soluble, insoluble and total dietary fiber contents (from 1.93% to 9.31%) of the biscuits increased significantly by increase of the substitution ratio of pomegranate peel powder (PPP) in the formulation. Hardness decreased significantly with PPP addition in biscuits. It was observed a decrease in *L* and *b* color values and an increase in *a* values of biscuits by increase in the ratio of pomegranate peel substitution. The PPP added samples revealed bigger air cells compared to the control samples in the internal SEM micrographs. In sensory analyses, no significant differences in sensory parameters except taste and overall acceptance of biscuits were observed. Moreover, the panelists confirmed that they felt a more sour and bitter taste in biscuits prepared with 18% pomegranate peel powder and it was considered to be the reason of the decrease in sensory scores. Therefore, it is advised not to exceed 12% pomegranate peel powder substitution in biscuits.

Keywords: Antioxidant, Biscuits, Dietary fiber, Pomegranate peel, Sensory analysis

Nar Kabuğu ile Zenginleştirilmiş Bisküvilerin Bazı Kalite Karakteristikleri

ÖZ

Bu çalışmada çeşitli oranlarda (%0, 6, 12, 18) nar kabuğu ikame edilmiş bisküvilerin bazı kimyasal, fiziksel ve duyusal özellikleri belirlenmiştir. Nar kabuğu ikamesinin, bisküvilerin protein, yağ ve kül değerlerinde önemli bir değişikliğe neden olmadığı görülmüştür. Formülasyondaki nar kabuğu ikame oranının artmasıyla bisküvilerin antioksidan aktivite (5.06 μmol TE/100g'dan 288.38 μmol TE/100g'a), toplam fenolik madde miktarı (56.49 mg GAE/100g'dan 1108.35 mg GAE/100g'a), suda çözünür, suda çözünmez ve toplam diyet lifi miktarlarının (%1.93'ten %9.31'e) arttığı, sertlik değerlerinin ise nar kabuğu ikamesiyle azaldığı tespit edilmiştir. Nar kabuğu ikame oranının artmasıyla bisküvilerin *L* ve *b* değerlerinde azalma olurken *a* değerinin ise arttığı görülmüştür. SEM analizi sonucunda, nar kabuklu bisküvilerde, kontrol bisküvilere göre daha büyük hava boşlukları olduğu saptanmıştır. Duyusal analizler sonucunda ise, tat ve genel beğeni parametreleri dışındaki diğer parametrelerde nar kabuğu ilavesinin önemli bir değişime neden olmadığı belirlenmiştir. Ayrıca, panelistler %18 nar kabuğu içeren bisküvilerde ekşi ve acı bir tat hissettiklerini bildirmişlerdir, bu durumun duyusal değerlendirme skorlarındaki azalmanın nedeni olabileceği düşünülmektedir. Bu nedenle bisküvi üretiminde nar kabuğu ikame oranının %12'nin üzerine çıkılmaması gerektiği önerilmektedir.

Anahtar Kelimeler: Antioksidan, Bisküvi, Diyet lifi, Nar kabuğu, Duyusal analiz

INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the family of *Punicaceae* and is a widely grown horticulture crop in tropical and subtropical countries [1]. Over 1000 cultivars of pomegranate exist and are originated in Iran, and spread to the Mediterranean countries, eastward to China and India, then onto Middle and South America [2]. Pomegranate is also native to Turkey, and it is one of the biggest pomegranate producers in the World with 537,847 tons/year produced in 2018 only [3].

In addition to fresh consumption of the pomegranate, it is also processed to further goods such as juice, wine, jam and sour sauce [1]. During the pomegranate processing, 50% of the fruit is discarded as waste [4]. Due to the kind of the pomegranate, 26-40% of the whole fruit is peel [5]. Pomegranate peel is used as fertilizer, or in the best case, as animal feed with no significant added value. However, pomegranate peel is considered one of the most valuable by-products of the entire food industry [4].

The high level of antioxidant activity of pomegranate peel was proven in many studies [5-8]. Pomegranate peel is rich in phenolic compounds that show antioxidant activity, especially flavonoids (anthocyanins, catechin and other complex flavonoids) and tannins (punicalin, punicalagin, gallic acid and ellagic acid) [9]. Several studies showed that intake of phenolics in pomegranate peel is effective on to reduce cardiovascular diseases, neurological disorders, cancer varieties, dermatological disorders and cataract risks [10-14].

In addition to its high level of antioxidant activity, pomegranate peel is also a great source of dietary fiber (with 33.10–62.09 g/100g) and rich in some minerals (Ca, K, and Mg) [4, 15]. There are lots of research on to produce bakery products rich in antioxidants and dietary fiber with the use of by-products of fruit process [16-21]. Pomegranate peel received great interest by researchers due to its bioactive components rich composition. Topkaya and Isik [5] used pomegranate peel in muffins, Cam et al. [22] in ice cream preparation, Ismail et al. [15] and Prithwa and Sauryya [23] used the peels in cookies production.

Baked goods are either ready-to-eat or pre-processed and can be consumed with some additional further processes products that are obtained from cereal flours. Biscuits are the most consumed baked goods worldwide thanks to its nutritional value, ready-to-it packages, being relatively cheap and the ability to be produced in various flavors and aromas. The main ingredients used for biscuits making are wheat flour (WF), oil, sugar, water and baking powder. In addition, various additives such as flavoring and texturizing agents, antioxidants, milk and milk powder, colorants and enzymes can be added to the biscuit formulation [24].

With this background, it was aimed to investigate the use of pomegranate peel in biscuit production and to determine the changes of chemical, physical, microstructural and sensory properties of biscuits.

MATERIAL and METHODS

Materials

WF (Söke Un, Aydin, Turkey), margarine (Orkide, İzmir, Turkey), sugar (Torku, Konya, Turkey), salt (Horoz Tuz, Denizli, Turkey) and baking powder (sodium bicarbonate and sodium acid pyrophosphate) (Dr. Oetker, İzmir, Turkey) used in this study were purchased from local markets in Denizli, Turkey. Pomegranate (*Punica granatum L. cv*.) was collected from local producers.

Preparation of Pomegranate Peel Powder (PPP)

Pomegranates were washed in tab water and they were peeled by a knife and the peels were dried in a cabinet dryer (Yucebas Machine Analytical Equipment Industry, Izmir, Turkey) at 50°C until the peels reached below 10% moisture content. The dried peels were ground in a blender (Waring Commercial Products, Stamford, USA) and sieved in a 500 μm particle size sieve (Mesh No. 35, Retsch, Haan, Germany). Ground peels were stored in LDPE bags (Koroplast, Istanbul, Turkey) at -18°C (Hotpoint Ariston, UPS1711, Turkey) for further use.

Production of Biscuits

The biscuits were prepared according to the procedure of Turan et al. [25] with modifications. The PPP ratios in the formulation were decided in the light of preliminary experiments and the PPP addition was kept under 20% due to the bitter taste. The formulations that followed in the biscuit production were given in Table 1. Biscuits contain PPP were prepared by substituting 6, 12 and 18% of WF in the formulation. All the ingredients were mixed for 5 min. at a medium speed in a kitchen robot (Kenwood KMM060, UK) and the obtained dough was rolled out approximately 2 cm thick with a dough roller (Kitchenaid KSMPRA, USA) and cut into 5x5 cm squares. The shaped biscuit doughs were baked at 200°C for 10 min. in an oven (Ozkoseoglu Oven, Turkey). After cooling in room temperature, samples were packed in LDPE bags for further analyses. Photograph of the biscuits after baking was shown in Figure 1.

Proximate Composition

The moisture, ash, crude protein and crude fat contents of samples were measured according to the methods of AOAC [26]. Dietary fiber content of the samples was determined with using a fiber assay kit (Megazyme K-TDFR, Wicklow, Ireland) as given in Ozgoren et al. [27].

Mineral Matter Composition

The minerals (P, K, Ca, Mg, Fe, Zn and Mn) of biscuits and raw materials were determined by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Perkin Elmer, Optima 2100 DV model, Massachusetts, USA) with following the method that Kacar and Inal [28] and Boss and Freeden [29] reported.

Table 1. Formulations of biscuits

Ingredients (g)	C	PP6	PP12	PP18
Wheat flour (WF)	100.00	94.00	88.00	82.00
Pomegranate peel powder (PPP)	0.00	6.00	12.00	18.00
Margarine	35.00	35.00	35.00	35.00
Sugar	35.00	35.00	35.00	35.00
Salt	0.70	0.70	0.70	0.70
Baking Powder	0.50	0.50	0.50	0.50
Water	50.00	55.00	60.00	65.00

C: Control biscuit, PP6: 6% of WF was substituted with PPP, PP12: 12% of WF was substituted with PPP, PP18: 18% of WF was substituted with PPP.

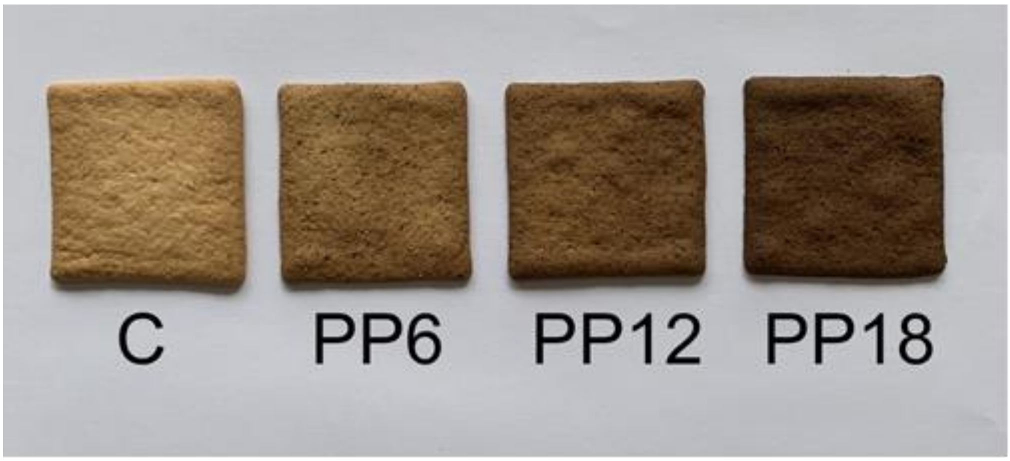

Figure 1. Photograph of biscuits after baking (C: Control biscuit, PP6: 6% of wheat flour (WF) was substituted with pomegranate peel powder (PPP), PP12: 12% of WF was substituted with PPP, PP18: 18% of WF was substituted with PPP)

Total Phenolic Content and Antioxidant Activity

For the extraction of phenolics the method used in Ozgoren et al. [27] was used. Total phenolic content of the samples was measured with using Folin-Ciocalteu method [30]. The absorbances of the reaction mixtures were read at 760 nm by T80 UV/VIS Spectrometer (PG Instruments Ltd., Leicestershire, UK), and the results were expressed in milligrams gallic acid equivalent (GAE)/100 g dry basis.

Total antioxidant activity of samples was determined with using 2.2-Diphenil-1-picrylhydrazyl (DPPH) method [31]. The absorbances were measured at 515 nm, the results were given in µmol Trolox equivalent (TE)/100 g dry basis.

Physical Properties

The color values of raw material and biscuit samples were measured by using Hunter Lab Miniscan XE colorimeter (Hunter Associates Laboratory, Reston, VA) [32]. The total difference in color (ΔE) was calculated with using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

$\Delta L = L_{sample} - L_{control}$, $\Delta a = a_{sample} - a_{control}$, $\Delta b = b_{sample} - b_{control}$.

Yamauchi [33] described the ΔE values indicates visual color differences as noticeable (1.5–3.0, detectable by trained people), appreciable (3.0–6.0, detectable by ordinary people), large (6.0–12.0, large difference in the same color group) and extreme (over 12, another color group).

Textural properties of biscuits were evaluated with using three-point-bending test (TA-TPB) in a texture analyzer (Brookfield, CT3-4500, Massachusetts, USA). The maximum force recorded was referred to as the hardness of the biscuits. In the analysis, a whole biscuit was used, and the analyzer performed at 1.00 N trigger load, 2.00 mm/s test speed and 31.00 mm distance.

Microstructure Properties

In order to monitor the internal microstructure, biscuit samples were cracked and freeze-dried (Thermo Savant ModulyoD-230, USA) for 12 h. The freeze-dried samples were sputter-coated with gold in order to render them thermoelectrically conductive with using a high-vacuum coating machine (Q 150R ES, Quorum Technologies Ltd., UK) for 120 s. The internal microstructure of the biscuits was visualized with a field emission scanning electron microscope (FE-SEM) (Zeiss Supra 40 VP, Carl Zeiss GmbH, Germany) at 10 kV filament voltage. The micrographs were taken at magnification of 200x for the crumb of the biscuits.

Sensory Evaluation

The sensory characteristics of the biscuits were evaluated as color, odor, hardness, taste, chewiness and overall acceptance by 48 untrained panelists from Pamukkale University (62% female, 38% male, aged from 18 to 51). The panelists scored the samples in a 7-point hedonic scale which a score of 7 = like extremely and 1 = dislike extremely. Each sample was labeled with randomly selected 3-digit numerical code. Before tasting each sample, the panelists were asked to rinse their mouths with water and eat a bite of unsalted cracker to refresh the taste sense.

Statistical Analysis

All data was analyzed with using "Minitab 13 Statistical Software". In order to determine statistical differences at = 0.05, ANOVA (one-way analysis of variance) with Tukey's multiple comparison test was performed.

RESULTS and DISCUSSION

Proximate Composition of the Raw Materials

As seen from Table 1, WF and PPP are the raw materials which exist in different concentrations in the formulations of biscuits and could affect the compositions of biscuits in dry basis. So, the proximate compositions of WF and PPP were determined and are given in Table 2.

It was seen that PPP had higher amounts of fat, ash, total acidity, soluble, insoluble and total dietary fiber, antioxidant activity, total phenolic content and K, Ca, Mg minerals while WF had higher protein, pH and P, Fe, Zn, Mn minerals.

Table 2. Some properties of wheat flour and pomegranate peel powder

Parameters	Wheat Flour	Pomegranate Peel Powder
Protein (%)*	11.93±0.34	2.63±0.11
Fat (%)*	1.66±0.14	3.48±0.07
Ash (%)*	0.50±0.01	4.12±0.10
Total acidity (%)* (anhydrous citric acid)	0.19±0.01	7.55±0.16
pH	6.08±0.54	3.77±0.09
Soluble dietary fiber (%)*	1.39±0.02	8.15±0.12
Insoluble dietary fiber (%)*	1.50±.0.22	35.34±0.40
Total dietary fiber (%)*	2.89±0.24	43.49±0.52
Antioxidant activity (µmol TE/100 g)*	2.31±1.04	4340.11±9.40
Total phenolic content (mg GAE/100 g)*	104.54±2.79	16160.19±124.45
P (ppm)*	1329.80±22.50	245.57±13.02
K (ppm)*	1785.60±24.30	12931.00±45.00
Ca (ppm)*	360.50±14.00	3160.50±80.50
Mg (ppm)*	442.30±13.30	772.00±30.05
Fe (ppm)*	111.36±3.31	86.11±4.49
Zn (ppm)*	10.86±0.90	4.54±0.60
Mn (ppm)*	9.01±1.10	5.07±0.52
Hunter color values		
L	89.00±0.03	53.08±0.04
a	-0.84±0.04	9.78±0.01
b	7.48±0.03	17.38±0.02

*: In dry matter basis

In previous studies on the chemical composition of pomegranate peel, it was reported that pomegranate peel contains 0.70-8.72% protein, 0.40-9.40% fat, 0.50-6.07% ash, 17.53-62.09% total dietary fiber and 8560-22656 mg GAE/100g total phenolic content [5, 34-40]. Mineral composition of PPP was determined in previous studies and they were ranged as K (2749.46-16237.41 ppm), P (33.96–330.30 ppm), Ca (645.70–11240.10 ppm), Mg (105.00-1644.47 ppm), Fe (18.33-210.3 ppm), Zn (4.0-16.20 ppm), Mn (4.50-16.40 ppm) minerals [37, 39-42]. The results of the pomegranate peel powder in the present study concur the previous findings.

As mentioned above, PPP contains substantial number of polyphenols such as tannins; ellagic acid and gallic acid as well as citric acid that effectuate most of the total acidity in PPP [43]. Linked to its acid composition, PPP showed higher amount of total acidity, and parallel to this, had a lower pH value compared to WF. Ullah et al. [40] reported that PPP had 4.86 total acidity and 3.75 pH in their study.

It was determined that the PPP had higher *a* and *b* values and lower *L* value than the WF. Color *a* results of the raw material were thought to be linked to the water-soluble anthocyanins provide natural pink, red and purple color present in PPP. Also, higher *b* results could be due to the carotenoids in the PPP which cannot be eliminated by washing. In previous studies color values of PPP were found as *L* (53.85-90.68), *a* (7.23-41.12) and *b* (16.26-22.28) [5, 44, 45].

Proximate Composition of the Biscuits

Table 3 indicates some chemical properties of the biscuit samples. Although there were differences in the crude protein, crude fat and crude ash results of the biscuits, these differences were not significantly ($p>0.05$) important. It was determined a great increase ($p<0.05$) in soluble and insoluble dietary fiber of the biscuits by increasing PPP ratio in the biscuit formulation, thus the total dietary fiber also increased directly. This result may be attributable to the higher level of dietary fiber content of PPP compared to the WF (Table 2). Dietary fiber consumption offers health benefits including lower risk of cancer, coronary hearth diseases, obesity, and gastrointestinal diseases [46, 47]. According to the U.S. Food and Drug Administration (FDA), recommended dietary fiber intake is 25 g per day for adults on a 2000 kcal diet. Hence, a portion (30 g) of PP18, PP12 and PP6 biscuits can deliver up to 11.17%, 10.60%, and 8.32% respectively of an adult's daily dietary fiber need, while it is 2.32% for C biscuit.

Table 3. Proximate composition of the biscuits

Parameters	C	PP6	PP12	PP18
Protein (%)*	6.66±1.02a**	6.46±0.44a	6.11±0.52a	5.76±0.89a
Fat (%)*	17.90±0.80a	18.22±1.20a	18.94±0.32a	19.23±1.20a
Ash (%)*	1.98±0.14a	2.02±0.08a	2.09±0.18a	2.16±0.10a
Total acidity (%)*	0.26±0.03d	0.67±0.13c	0.99±0.03b	1.35±0.03a
pH	6.26±0.02a	5.03±0.01b	4.62±0.04c	4.39±0.02d
Soluble dietary fiber (%)*	0.59±0.14c	1.17±0.24b	1.69±0.16ab	2.19±0.17a
Insoluble dietary fiber (%)*	1.35±0.32b	5.77±0.59a	6.63±0.43a	7.13±0.18a
Total dietary fiber (%)*	1.93±0.26c	6.93±0.52b	8.83±0.40ab	9.31±0.33a

*: Dry matter basis. **: Different letters within the column across the table show significant differences at $p<0.05$. C: Control biscuit, PP6: 6% of wheat flour (WF) was substituted with pomegranate peel powder (PPP), PP12: 12% of WF was substituted with PPP, PP18: 18% of WF was substituted with PPP

In the study performed for determination some chemical properties of cookies, Ismail et al. [15] enriched cookies with up to 7.5% of PPP and found a significant increase in crude dietary fiber of the cookies by PPP addition. The cookies with 7.5% PPP addition had over 6 times more crude dietary fiber than control biscuits. Omar and Mehder [48] added PPP up to 5% in flat bread formulation. The researchers found bread samples with 5% of PPP had 3.5 times more dietary fiber than the control breads. In another study, Topkaya and Isik [5] substituted WF with PPP up to 15% in muffin making, they evaluated the dietary fiber in muffins with 15% had almost 3 times more than control muffins.

Total acidity of the biscuits increased significantly ($p<0.05$) and corollary to this increase, pH decreased significantly ($p<0.05$). These changes were thought to be related with the total acidity and pH values of raw material PPP (Table 2). Similar results were found by El-Batawy et al. [49] who fortified yoghurt with PPP and Cam et al. [50] who fortified ice cream with PPP that were resulted an increase in acidity and a decrease in pH of the yoghurts and ice creams.

Mineral Matter of the Biscuits

K and Ca content of the biscuits increased significantly ($p<0.05$) and P content decreased significantly ($p<0.05$) by increasing PPP level in the biscuit formulation (Table 4). Nevertheless, there was no significant difference ($p>0.05$) in Mg, Mn, Zn and Fe content of the biscuits with PPP addition. The increase of K and Ca minerals in biscuits were considered to be related to the high level of K and Ca minerals present in PPP (Table 2).

Table 4. Mineral matter composition of the biscuits (mg/100 g)*

Parameters	C	PP6	PP12	PP18
P	131.13±3.76a**	133.13±3.90a	125.78±0.21ab	122.24±0.82b
K	125.22±2.09d	176.01±4.24c	224.51±2.61b	269.60±7.73a
Ca	62.39±1.56d	72.32±3.54c	86.40±0.39b	100.91±0.29a
Mg	26.04±0.74a	27.41±1.95a	28.71±0.13a	29.99±0.28a
Fe	4.76±0.39a	4.73±0.24a	4.26±0.13a	4.04±0.35a
Zn	0.52±0.14a	0.51±0.14a	0.49±0.00a	0.49±0.00a
Mn	0.74±0.14a	0.73±0.10a	0.70±0.12a	0.68±0.14a

*: Dry matter basis. **: Different letters within the column across the table show significant differences at $p<0.05$. (C: Control biscuit, PP6: 6% of wheat flour (WF) was substituted with pomegranate peel powder (PPP), PP12: 12% of WF was substituted with PPP, PP18: 18% of WF was substituted with PPP)

Minerals are known to be essential to support biochemical process in human body. Minerals play crucial role in regulation the functions of the circulation, nerve, muscle and skeletal systems. An adult need to take 3000 mg K and 1000 mg Ca daily [51]. According to our calculations, consuming 1 portion (30 g) of PP18 biscuit can deliver 2.16 times more of K and 1.62 times more of Ca daily intake need, than consuming 1 portion of C biscuits. Topkaya and Isik [5] determined that K, Ca and Mg content of the muffin cakes increased significantly with PPP addition.

Antioxidant Activity and Total Phenolic Content Results

Antioxidant activity and total phenolic content values of the biscuits are given in Figure 2 and Figure 3.

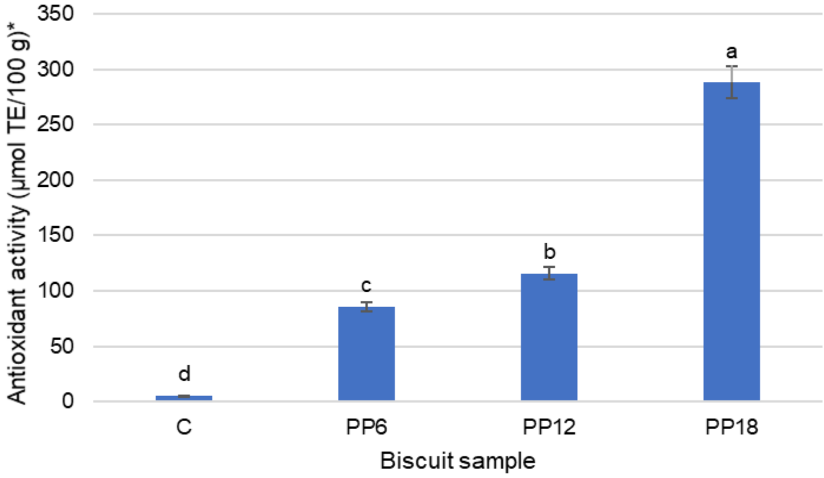

Figure 2. Antioxidant activity values of biscuits
*: In dry matter basis. Different letters (a, b, c and d) indicate statistical differences ($p < 0.05$). (C: Control biscuit, PP6: 6% of wheat flour (WF) was substituted with pomegranate peel powder (PPP), PP12: 12% of WF was substituted with PPP, PP18: 18% of WF was substituted with PPP.

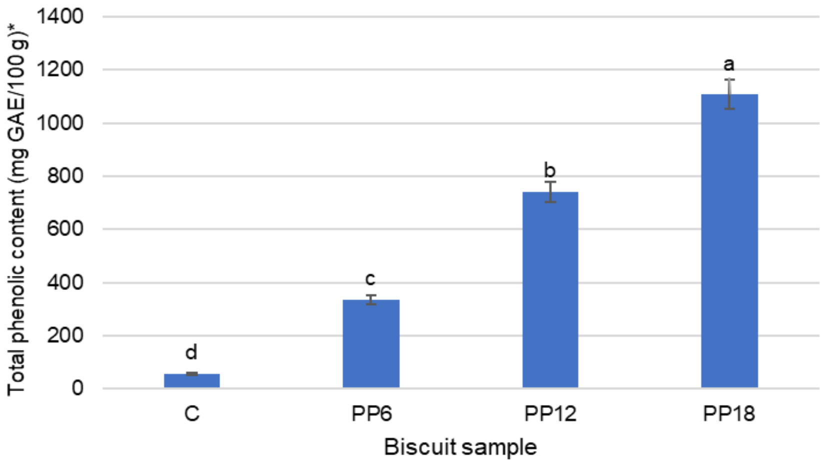

Figure 3. Total phenolic contents of biscuits
*: In dry matter basis. Different letters (a, b, c and d) indicate statistical differences ($p < 0.05$). C: Control biscuit, PP6: 6% of wheat flour (WF) was substituted with pomegranate peel powder (PPP), PP12: 12% of WF was substituted with PPP, PP18: 18% of WF was substituted with PPP.

The total phenolic content and antioxidant activity of biscuit samples were increased significantly ($p<0.05$) by increasing of PPP ratio in the biscuit formulation. Phytochemicals are plants derived bioactive components and polyphenols are the main phytochemicals in PPP. The major class of these polyphenols are mainly flavonoids (flavanols, flavonols and anthocyanins), condensed tannins and hydrolysable tannins (ellagitannins and gallotannins). The main group from ellagitannins in PPP is punicalagin that produces ellagic acid, punicalin and gallic acid by both enzymatic and non-enzymatic hydrolysis. In addition to phytochemicals, PPP contains organic acids, alkaloids, phenolic acids, triterpenoids and sterols. It was reported the share of punicalagin is to be 80-85% (w/w) and followed by ellagic acid with 1.3% of the total phenolics in PPP. 92% of the antioxidant activity of PPP is provided by these ellagitannins [52, 53]. In this aspect, it is considered that higher level of antioxidant activity and total phenolic content of biscuit includes PPP were associated with high phenolic content of PPP (Table 2). These results occur with the findings of Prithwa and Sauryya [23], who reported that enriching cookies with 2.5, 5, 7.5 and 10% of PPP caused significant increase in total phenolic content and antioxidant activity values.

Biscuit Hardness

Hardness analysis results of the biscuits were given in Figure 4. Gluten network presents in WF is the mainly responsible component in hardness of biscuits [54]. The hardness value which is related the force necessary to break the biscuits decreased by addition of PPP. While the hardness value of control sample was 9.62±1.52 N, PP18 biscuits had 4.12±0.98 N hardness results. Silva et al. [55] reported that the decrease in network created by gluten can cause a weak and fragile structure. It is considered that these results were related to decrease of total gluten content of biscuits as a result of PPP substitution. Similar conclusions were reached by researchers who replaced WF with tomato seed and Jerusalem artichoke powders in crackers [5, 27].

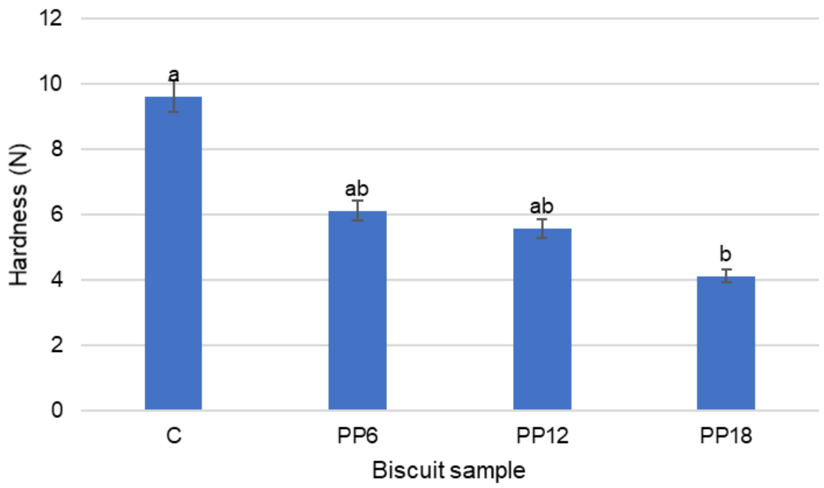

Figure 4. Hardness results of biscuits*
*: Different letters (a and b) indicate statistical differences ($p< 0.05$). C: Control biscuit, PP6: 6% of wheat flour (WF) was substituted with pomegranate peel powder (PPP), PP12: 12% of WF was substituted with PPP, PP18: 18% of WF was substituted with PPP.

Color Results of Biscuits

The applied PPP substitution had also a significant effect upon the color of biscuits samples (Table 5.). It was inevitable that color values of raw material change the color of final product (Table 2). The color of biscuits became significantly darker with PPP substitution, the lowest *L* value was assayed in PP18 (39.31) while C biscuits was the brightest (77.21). PPP addition also caused an increase in redness in biscuits as expected due to the anthocyanidin content of PPP, the highest *a* value was evaluated in PPP18 (9.58) while C biscuits had 2.41. In contrary, *b* values (yellowness/blueness) of biscuits decreased significantly (14.58 for PP18, 21.04 for C biscuits). These changes were also observed in the appearance of biscuits, control biscuits were bright yellowish while PP18 biscuits were dark red-brownish. It is known that anthocyanidins change color in different pH and temperatures [56, 57]. Hence it was thought that the changes in *b* values occurred duo to the changes in anthocyanidins. An increase in *a* value and a decrease in *L* and *b* values in crumb color were also observed by Topkaya and Isik [5] after replacing 5-15% of WF with PPP. However, in the same study, the researchers found a decrease in *a* value of the crust color. They reported that the redness might be masked by Maillard reactions occurred during baking.

Table 5. Color results of the biscuits

Biscuit sample	*L*	*a*	*b*	Δ*E*
C	77.21±1.20a*	2.41±0.98b	21.04±1.20a	
PP6	57.10±1.04b	5.30±0.79ab	19.06±0.84ab	18.26±0.49c
PP12	49.83±0.87c	8.87±1.20a	17.69±0.99ab	31.90±1.14b
PP18	39.31±0.98d	9.59±1.04a	14.58±0.90b	40.66±1.10a

*: Different letters within the column across the table show significant differences at $p<0.05$. C: Control biscuit, PP6: 6% of wheat flour (WF) was substituted with pomegranate peel powder (PPP), PP12: 12% of WF was substituted with PPP, PP18: 18% of WF was substituted with PPP

The ΔE results indicate the color differences between the C biscuits and biscuits with PPP. It was determined that PPP addition caused a significant ($p<0.05$) increase in ΔE results. According to the Handbook of Colour cience [33], all biscuit samples with PPP enrichment can be classified as “extreme, another color group”. These findings concur with the results of Essa and Mohamed [44] who enriched spaghetti with 3.0, 5.0 and 7.0% PPP and calculated ΔE values 17.15, 21.67 and 25.99 respectively.

SEM Results

SEM annexes a unique approach to research of food materials and allows to examine the surface and the internal structure at low magnification in food systems, especially baked goods [58]. In this study, microstructural analyses using SEM were carried out to examine and characterize the biscuit microstructure formed using different ratios of PPP in the formulation. Figure 5 indicates the SEM micrographs of the biscuits.

According to the SEM micrographs, internal microstructure was changed with PPP addition. It was seen that C biscuits revealed relatively greater uniform and compact structure while PP18 biscuits had big pores in the crumb. The internal microstructure of PPP incorporated biscuits showed a severe disrupted gluten protein matrix due to the replacement of WF with high dietary fiber. Thus, the biscuit samples showed an increasing open structure consisting of gaps in between layers with increasing PPP which in turn resulted a poor texture. This was also indicated in the texture analysis (Figure 4), as the hardness of biscuits decreased due to the PPP ratio in the formulation. Similar conclusions were reached by Jia et al. [59] who enriched biscuits with high dietary fiber rice bran up to 10.8%. The researchers reported that defatted rice bran addition caused torn and loose biscuit microstructure which also resulted a decrease in biscuit hardness.

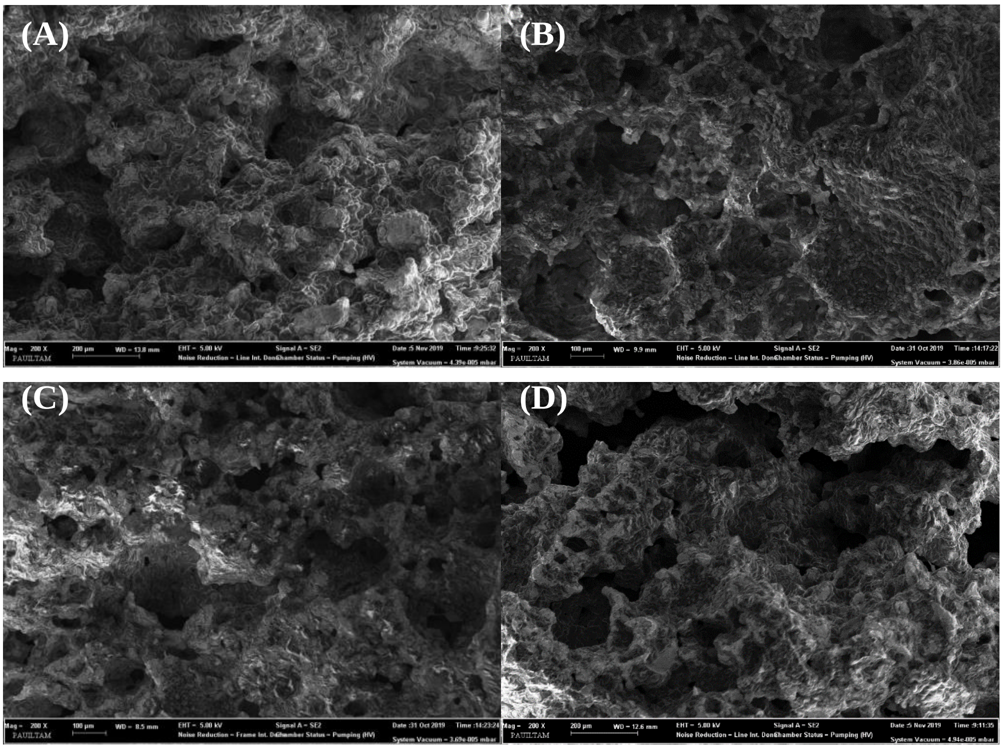

Figure 5. SEM micrographs of biscuits (200x). (A): Control, (B): 6% of wheat flour (WF) was substituted with pomegranate peel powder (PPP), (C): 12% of WF was substituted with PPP, (D): 18% of WF was substituted with PPP.

Sensory Evaluation Results

The sensory scores of biscuits were given in Figure 6. Even though the darkness (Figure 6) of biscuits increased significantly ($p<0.05$) with PPP substitution, the decrease in sensorial color scores of samples were not statistically ($p>0.05$) different. Similar results were seen in sensorial hardness of biscuits. Although the three-point-bending hardness results of the biscuits showed that the PPP substitution caused a decrease in biscuit hardness (Figure 4), there was no significant ($p>0.05$) differences in sensorial hardness scores. Additionally, all biscuits samples received statistically similar ($p>0.05$) odor and chewiness scores. Taste is one of the most important characteristics in biscuit acceptability. However, PP18 biscuits received significantly lower ($p<0.05$) scores than C and PP6 biscuits. It was considered that the phenolics in PPP occurred an acidic taste in PP18 biscuits and it affected the taste scores. Thus, the panelists expressed that there was a slight bitterness and sourness in PP18 biscuits. In previous studies, it was expressed that high citric acid [60] and low pH can cause sourness in foods [61]; hence, the panelist opinions about sourness can be related to the citric acid content and low pH value of the PPP (Table 2). The overall acceptability scores of the biscuits were also parallel with taste.

CONCLUSION

In this study, it was aimed to determine the chemical, physical and sensorial properties of widely consumed biscuits substituted with PPP, which is edible but considered as waste, as well as to gain added value to the peels. PPP substitution did not cause any differences ($p>0.05$) in crude protein, crude ash and crude fat of the biscuits. With the increase of PPP rate in formulation, dietary fiber, total phenolics and antioxidant capacity increased significantly ($p<0.05$). As a result, enriching biscuits with PPP in terms of making functional biscuits goal was met. In sensory analysis, some panelists expressed that there was a slight sourness and bitterness in PP18 biscuits and PP18 received significantly ($p<0.05$) lower taste and overall acceptance scores. Therefore, it is recommended not to increase PPP substitution rate up to 18% in the formulation. In conclusion, in the light of success in sensorial acceptance of biscuits PP6 and PP12, the possibility to use of PPP in biscuits to increase nutritional values was proven. Moreover, PPP will be promoted as human feed and will gain added value more than as a waste. Furthermore, the utilization of PPP will help to decrease environmental pollution. The authors believe that PPP can be used in other foods, especially in sweet baked goods as well as sweeter products such as jams and juices that can mask the bitter taste. More studies are needed to investigate other possible usages of PPP.

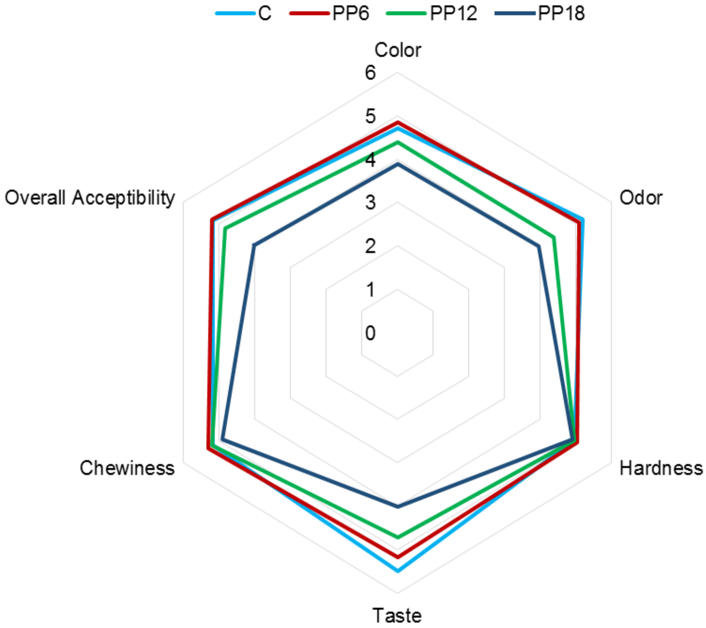

Figure 6. Sensory scores of biscuits
C: Control biscuit, PP6: 6% of wheat flour (WF) was substituted with pomegranate peel powder (PPP), PP12: 12% of WF was substituted with PPP, PP18: 18% of WF was substituted with PPP

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