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Research Article

Paliurus spina-christi Mill.: A New Alternative Flour on Gluten-Free Bread

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Abstract: Gluten is an essential protein in bakery products made from wheat flour. Gluten fraction forms skeletal structures in bakery products, especially bread. However, some people can cause problems called gluten intolerance or celiac disease. For this reason, research on products that are alternatives to gluten-free foods continues daily. In this study, fruits of Paliurus spina-christi Mill. species were used. This plant has been studied as an alternative to gluten-free bread (less than 5 ppm gluten). The bread was produced from the flour obtained by grinding the ripe fruits of the *P. spina-christi.* Quality parameters and heavy metal contents of these flour and bread samples were investigated. Comparisons were made with commercially available gluten-free breads. Energy, protein, moisture, ash, dietary fiber, carbohydrate, gluten, fat, heavy metal contents in bread of P. spina-christi were investigated. The results showed that the ash, protein, and dietary fiber content of the bread obtained from *P. spina-christi* were higher than the other gluten-free breads. It has been shown that the amounts of heavy metals are lower than the amounts tolerated by humans. The study revealed that bread made from flour obtained from *P. spina-christi* could be an alternative to gluten-free products. In this study, a method of flour and bread production and quality parameters of *P. spina-christi* was tried for the first time. In addition, it will likely be an alternative to the studies on gluten-free bakery products.

Keywords: *Paliurus spina-christi*; gluten; flour; bread; ethnobotany.

1. Introduction

Many diseases, such as diabetes, cholesterol, high blood pressure, and cardiovascular diseases, can occur in humans due to many reasons such as malnutrition, improper lifestyle, and old age. This is similar to suffering from illnesses; people are obliged to pay attention to their diet. Studies on the production of special foods for the prevention or treatment of diseases are increasing day by day. Celiac disease (Gluten intolerance) is one of the diseases. It refers to a lifelong chronic sensitivity of the small intestine to the protein called gluten. It is defined as a disease triggered by the consumption of gluten in genetically susceptible individuals (Hayıt and Gül, 2017). Gluten, the basic protein, is essential in the quality of bakery products, while gluten intolerance is present in approximately 1% of the world's population. (Novotni et al., 2012). The demand for gluten-free foods is increasing day by day with the increase of gluten intolerance-based diseases. The demand for gluten-free foods is increasing day by day with the increase of gluten intolerance-based diseases (Hopman et al., 2008; Beck, 2011; Juhász et al., 2020).

The many methods of feeding and for disease studies found in the literature (Polat et al., 2012; Selvi et al., 2013; Guandalini and Discepolo, 2016; Jnawali et al., 2016; Bascunan 2017; Churruca et al., 2017; Habel, 2017; Hosseini et al., 2018; Syage et al., 2018; Adıgüzel, 2019; Fernández et al., 2019; Yıldırım, 2020; Norambuena et al., 2021; Tahir et al., 2021). Studies on gluten-free alternative plants and foods have been studied before. Sarı and Tiryaki (2018) investigated the gluten-free teff (Eragrostis tef (Zucc.) Trotter) in their study and stated that it can be used as an alternative food in human and animal nutrition. As an alternative to gluten-containing foods, brown rice, rice bran, rice flour, rice-added gluten-free products have shown good results in many studies (Juliano and Hicks, 1996; Gallagher et al., 2004; Torbica et al., 2010; Blanco et al., 2011; Phimolsiripol et al., 2012; Moongngarm et al., 2014; Udachan and Sahoo, 2017; Kahraman et al., 2018; Paz et al., 2020).

The study aimed to offer an alternative raw material for gluten-free foods, increase the variety of gluten-free foods, meet the increasing demands of consumers for these products in the market and reduce production costs. For this reason, an economical and easily available alternative plant has been investigated. As a result of the research, it was determined that the plant known as an ethnobotanical alternative, which is found in large quantities in nature, named as *Paliurus spina-christi* Mill. from the Rhamnaceae family in the literature, and also known as Çaltı or Karaçalı plant in the colloquial language could be used. The ripe fruits of the plant were ground to make Çaltı Flour (ÇF), and the qualitative parameters were determined in the ÇF bread made from the obtained flour, and it was evaluated whether it was suitable for gluten-free bread production. In addition, the areas where the plant to be breaded grows were evaluated in two regions. These regions are agricultural areas and areas of urbanization. The amounts of some heavy metals that may pose a risk to human health, according to these regions, were examined.

2. Materials and Methods

2.1. Plant Material and specimen collection

P. spina-christi is a shrub-like species with a cosmopolitan distribution in Türkiye. In this study, the ripe fruits of the species were collected from Mount Ida in Edremit district of Balıkesir province. The gathering locality of the species is given below. The study area is also shown in Figure 1. The general appearance and organs (leaves, flowers, and fruit) of the plant are given in Figure 2.

Türkiye, B1 Balıkesir: Edremit, Ida Mount, Mehmetalan village, roadsides, 39.636115° N, 26.957029°, 169 m, 05.03.2021., HIA18.

P. spina-christi is popularly known as "Çaltı" in Türkiye. Therefore, in this paper, the flour obtained from *its* fruits is named "Çaltı Flour.

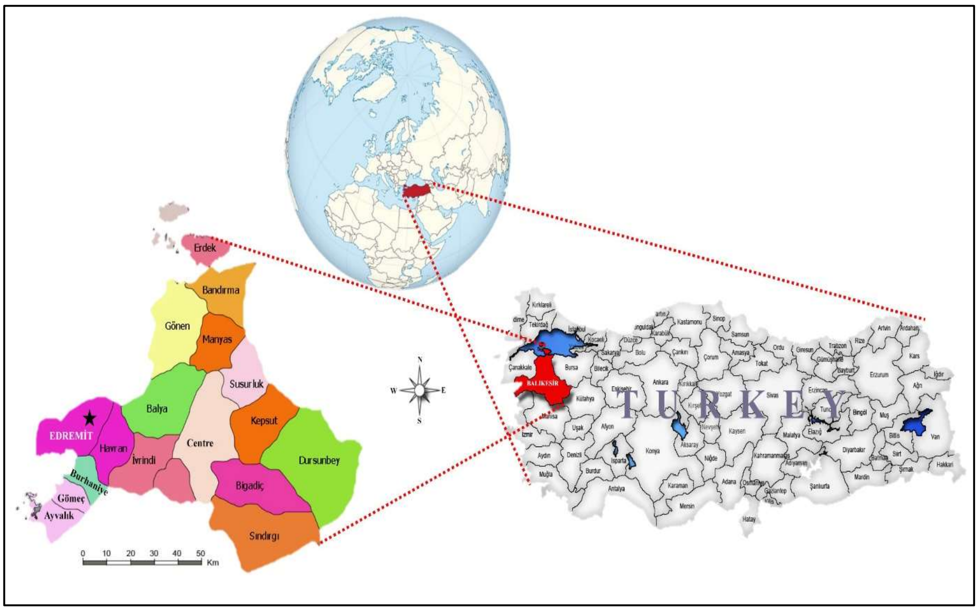

Figure 1. Map of the geographical area where the plant was collected.

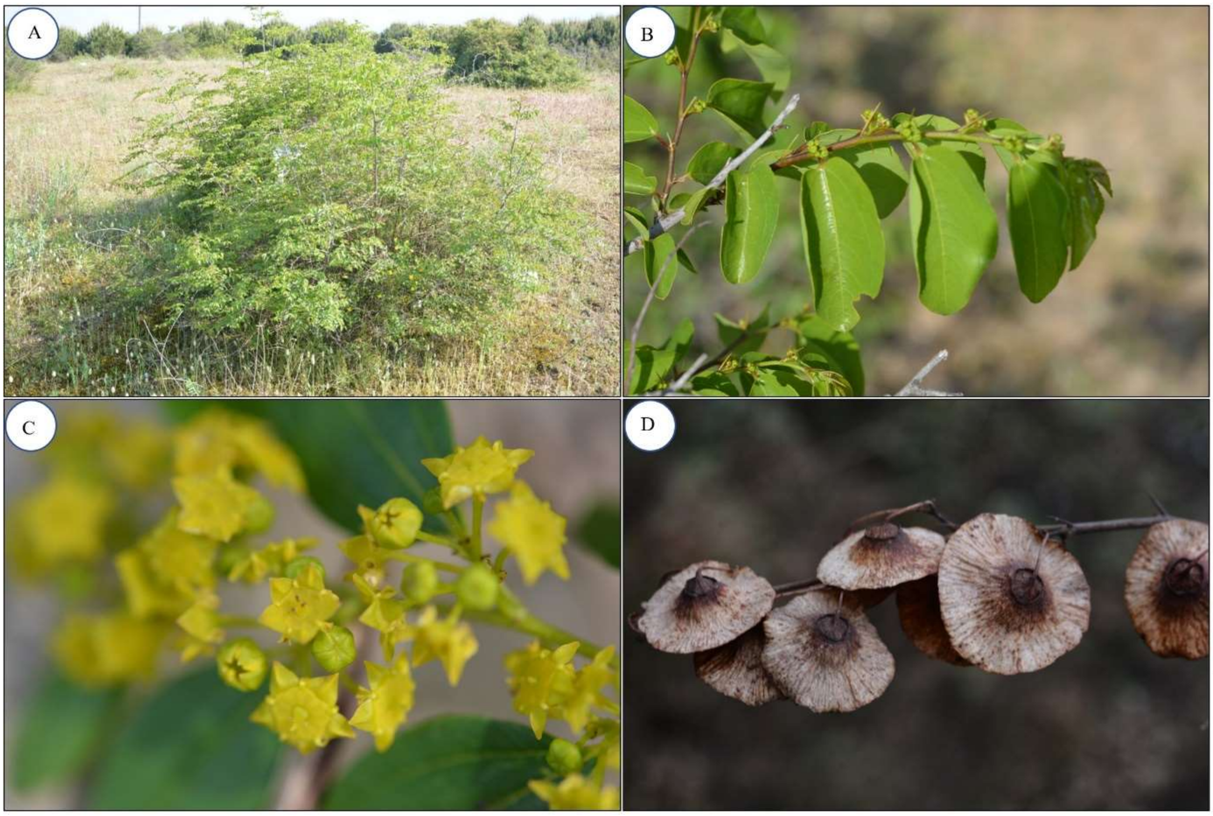

Figure 2. *Paliurus spina-christi*: A) general view B) leaves C) flowers D) fruit.

2.2. Preparation of çaltı flour (ÇF)

1000 g of çaltı fruit, which was dried for 24 hours on the shelves in the drying room and, was crushed by pulverized with a grinder. The crushed drugs were passed through a 0.5 mm sieve and 500 gr "Çaltı" Flour" was obtained.

2.3. Preparation of çaltı bread

In the bread-making process, 200 ml of milk, 500 g of çaltı flour, 5 g of salt, and 1 egg were used. After the dough obtained from the mixture rested for 30 minutes, it was shaped into bread and baked at 180 °C for 45 min (Figure 3).

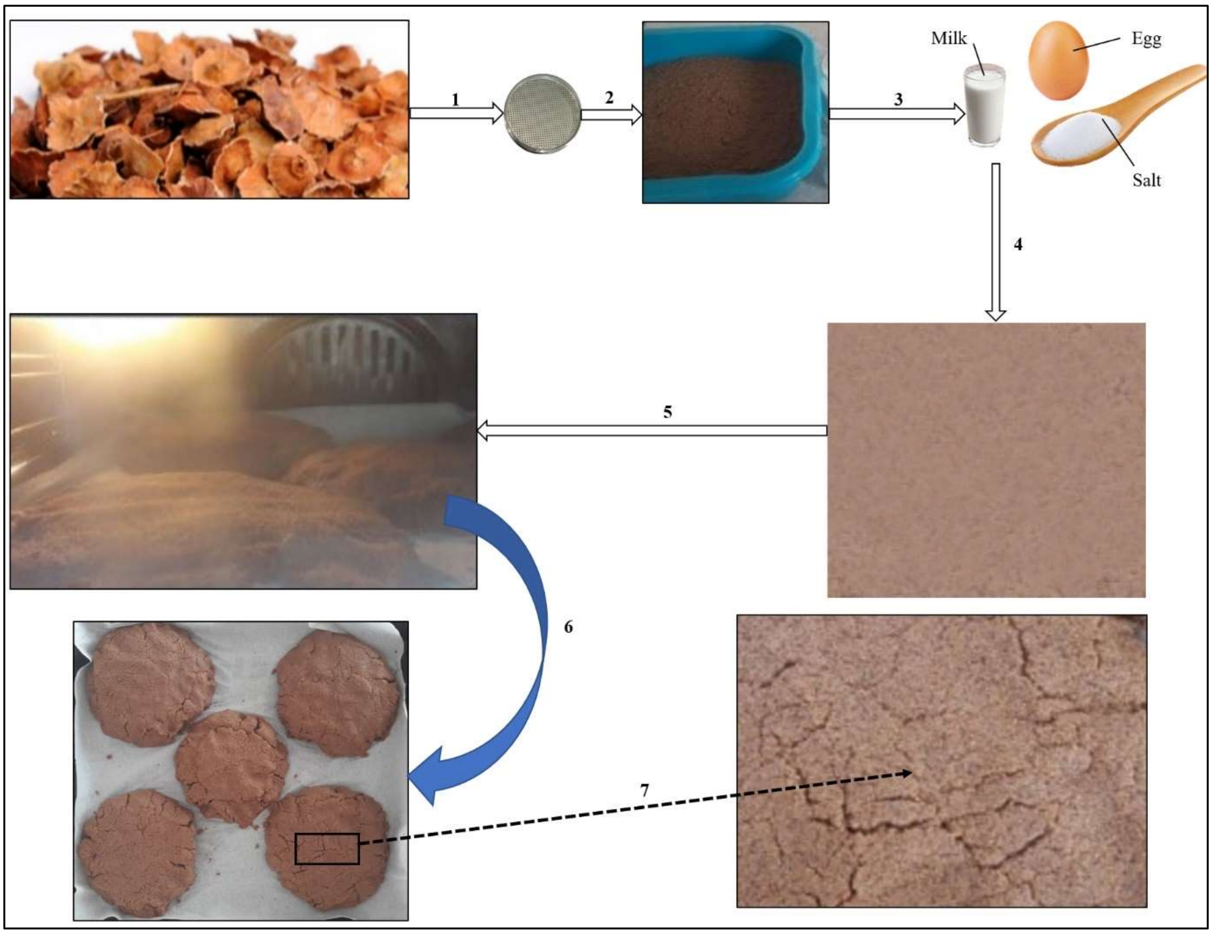

Figure 3. The stages of making bread from Çaltı flour (ÇF): 1) Grinding and pulverizing the fruits 2) Passing it through a metal sieve (0.5 mm) 3) Mixing Çaltı flour with milk, egg and salt 4) Çaltı dough 5) Turning Çaltı dough into 100 gr round bread and putting it in the oven 6) Baked Çaltı breads 7) surface of Çaltı bread.

2.4. Chemical and physicochemical analyses

Some chemical and physicochemical properties of breads made from the flour of *P. spina-christi* fruit were investigated. In addition, analyzes of heavy metals were made in ÇF. Chemical and physicochemical analyzes of ÇF bread sample; energy and carbohydrate analysis Atwater method (Falch et al., 2010), moisture analyzer AOAC Official Method 925.10 (AOAC, 2010) ash analysis AOAC Official Method 923.03 (Chemists, 1990) , protein analysis, AOAC Official Method 960.52 (AOAC, 1995) dietary fiber analysis of the AOAC Official Method 991.43 (AOAC, 1998), oil analysis AOAC Official Method 960.39 (AOAC, 2000) and for gluten analysis AOAC Official Method 991.19 (AOAC, 1995) was made using methods. The methods specified in Protein Tecator App. Notes (Anonymous, 1995) were used for protein analysis in ÇF samples, TS EN ISO 2171 (TSE, 2010) for ash analysis, and TS EN 712 (TSE, 2012) for moisture (moisture) analysis.

2.5. Heavy metal analyzes

Heavy metal analyzes at ÇF were performed with the Perkin Elmer Optima 7300 DV inductively coupled plasma optical emission spectrometer (ICP-OES). Samples were collected separately from agricultural areas in the village and from roadsides where heavy metal risk is likely to be high. By adding 5 mL of concentrated HNO 3 to the samples, solubilization was carried out with the CEM brand MARS 6 model microwave system. Then, the solubilized sample was diluted with 25 ml of pure water was made available to the target analysis and inductively coupled plasma optical determination of analytes specified emission spectrometry (ICP-OES) and is made. In this way, heavy metal amounts have been determined in the flours of the *P. spina-christi* plant fruit grown in the natural environment and in areas close to human habitats.

3. Results

Percent protein, moisture and ash values of ÇF are given in Table 1. Moisture rate in flour is an important factor in terms of the durability of flour during storage. Many grain flour u in a variety of critical moisture content 14.5% respectively. When the moisture rate in flour rises above 14%, undesirable conditions such as bitterness, mold, bacterial spoilage, and pest damage may occur in flour (Kurşunel, 1997). In the CF analysis results, the moisture content was below the critical moisture level (8.82%). This value indicates that the shelf life of ÇF will be longer and the spoilage risk will be lower than other grain flours in storage.

Table 1. ÇF chemical analysis results.

Analysis	Amount (%)
Crude protein*	10.98
Ash*	2.97
Moisture (%)	8.82

*: It was determined in % dry matter.

The crude protein ratio in *P. spina-christi* can vary between 10-18% seasonally (Alatürk et al., 2014). The same is true for wheat. The amount of protein in wheat and flour is one of the important factors in determining quality and cost (Elgün and Ertugay, 1995; Bonfil et al., 2004; Kihlberg et al., 2004; Mader et al., 2007; Bilgiçli and Soylu, 2016). According to the protein content of wheat flour, it is low (10% ≥), medium (10-12%), and high quality (12% ≤) (Zanetti et al., 2001). ÇF protein content was found close to the protein content of medium quality (10-12%) bread flour (Table 1). The protein ratio should be a minimum of 10.5% in dry matter in bread flour and a minimum of 7% in special-purpose flour (Anonymous, 2013). When the protein contents of the gluten-free bread mixes used in the study by Yılmaz and Doğan (2015) are examined, it is observed that the ÇF crude protein content (10.98%) is significantly higher than the protein content of the mixes (1.5-2.5%) (Table 1 and Table 2). This indicates that ÇF is nutritionally superior to other gluten-free bread formulas. When compared with other gluten-free bread samples obtained from the market, it is seen that protein superiority is again in ÇF (Table 3).

The ash ratio in flour gives information about the inorganic substance or mineral content. It varies depending on the soil and climate. As seen in Table 1, the ÇF ash rate is 2.97%. This ratio indicates that ÇF is rich in mineral matter and bran content. Compared to gluten-free bread mixes, ÇF mineral content appears to be higher (Table 2). Since the bran and endosperm are separated during milling in bread flour, the mineral loss is quite high (Özkaya et al.,1984). Therefore, our body cannot benefit from many mineral substances in our diet with white bread made from bread flour. Bread made from ÇF provides an advantage over white bread because of its mineral rich content.

Table 2. Quality and components of witness flour and gluten-free bread mixes (Yılmaz and Doğan, 2015).

Sample	Main Component	Emulgator/ Thickener	Other Components	Moisture (%)	Ash (%)	Protein (%)
Witness flour	Wheat flour		L- Ascorbic acid	14.27±0.05a	0.57±0.01e	12.64±0.08aa
Mixture-1	Potato starch, corn flour and starch, rice flour, buckwheat flour	Guar gum, hydroxyl propyl methyl cellulose	Sugar, salt, sunflower oil, silicon dioxide (anti-caking)	8.05±0.05f	1.93±0.02a	1.99±0.09bc
Mixture -2	Corn Starch, Potato Starch, Rice Flour, Gluten Free Wheat Starch	Guar gum, hydroxyl propyl methyl cellulose	Glucose, dietary fiber, baking soda, glucano delta lactone (acidity regulator)	12.94±0.02b	1.38±0.04c	1.58±0.05cd
Mixture -3	Gluten free wheat starch, corn starch	Guar gum, lemon pectin	Glucano delta lactone, sodium bicarbonate	10.52±0.03d	0.67±0.01e	0.99±0.03de

Mixture -4	Glutensiz buğday nişastası, mısır nişastası	Guar gum, hydroxyl propyl methyl cellulose	Glucose, dietary fiber, baking soda, glucano delta lactone	11.77±0.01c	0.44±0.05f	0.67±0.06ee
Mixture -5	Gluten free wheat starch, corn starch	Hydroxyl propyl methyl cellulose	Dextrose, vegetable protein, vegetable fiber, salt	10.81±0.04d	1.29±0.03c	2.32±0.07bb
Mixture -6	Corn starch, rice flour	Xanthan gum, pectin	Granulated sugar, sodium bicarbonate, sodium acid pyrophosphate	10.53±0.20d	1.06±0.03d	1.84±0.09bc
Mixture -7	Rice flour, cornmeal and starch	Thickener	Salt, leavening agent	11.49±0.20c	1.71±0.01b	1.79±0.09bc
Mixture -8	Modified cornstarch	Xanthan gum, pectin, DATEM (Diacetyl tartaric acid esters of monoglycerides	Sucrose, cooking salt, baking soda,,$CaCl_2$, ascorbic acid	9.25±0.12e	1.71±0.02b	1.71±0.02bb

When Table 1 and Table 2 are compared, PDF's 8.82% moisture value is the best after mixture-1. The low moisture content of flour under storage conditions provides an advantage in terms of microbial spoilage. Therefore, since the mold and bacterial growth rate is high at a moisture value of 14.5%, gorse flour will be more resistant to mold and bacterial growth compared to wheat flour and gluten-free mixed flours (except for mix-1).

In their study, Yılmaz and Doğan (2015) determined the gluten content of gluten-free flour mixtures given in Table 2 as 109.33 mg/kg in Mix-7, 5.7 mg/kg in Mix-3, Mixture-1, 2, 4, 5, 6 and 8 were found to be below 3 mg/kg. It has been determined that the gluten amounts of the mixtures other than Mix-7 are by the "Food suitable for individuals with gluten intolerance" communiqué (TS 13143). To sell products for celiac patients, the amount of gluten must be less than 20 ppm by law (Anonymous, 2012). The value obtained as a result of the analysis (<5 ppm) reveals that it may be an ideal alternative for celiac patients (Table 3).

Table 3. Comparison of nutritional and energy values of two different gluten-free bread brands and ÇF bread.

Analysis	ÇF Bread	A Bread	B Bread
Energy	123 kcal / 100 g	223 kcal / 100 g	215 kcal / 100 g
Moisture	60.95 g /100 g	-----	-----
Ash	1.20 g / 100 g	-----	-----
Protein	4.73 g / 100 g (Nx5,70)	4.03 g / 100 g	0.7 g / 100 g
Carbohydrate	6.79 g/ 100 g	49.07 g / 100 g	48.9 g / 100 g
Dietary Fiber	22.88 g / 100 g	1.66 g / 100 g	-----
Oil	3.45 g / 100 g	1.19 g / 100 g	1.9 g / 100 g
Gluten	<5 ppm	Suitable for gluten free diet	Suitable for gluten free diet

The nutritional and energy values of two different gluten-free bread brands produced for individuals with gluten intolerance (Anonymous, 2012) and offered for sale in the market, and the nutritional and energy values of bread made from blackthorn flour were compared (Table 3).

Flour mixtures consisting of corn starch, rice flour, potato starch, and tapioca starch-soy flour were used in the gluten-free bread ingredients sold on the market. The difference of ÇF from gluten-free flours offered for sale in the market is that it does not consist of flour in the form of a mixture and is only obtained by grinding the dry form of the fruits of the *P. spina-christi.*

In the gluten-free bread content of brand A, corn starch, potato starch, rice flour, water, tapioca starch, soy flour, thickener (hydroxypropyl methylcellulose), vegetable fiber, yeast, salt, sugar, vegetable oil (sunflower), acidity regulator (citric acid), antioxidant (ascorbic acid), preservative (calcium propionate), and emulsifier (diacetyl tartaric acid esters of mono and diglycerides) were used.

In the content of the bread we make from the flour obtained from the *P. spina-christi*; flour, water, salt, sugar, lactose-free milk, fresh yeast, and egg whites were used, and no preservatives or thickeners were used.

As seen in Table 3, while the energy values of the A and B brands are 223 kcal and 215 kcal, respectively, the energy value of ÇF is 123 kcal. According to these values, it seems possible to use ÇF bread with a very good dietary fiber ratio (22.88 g/100 g) as a dietary product. Ash gives information about the mineral content of the flour. It varies depending on the soil and climate. For example, wheat flour's bran is indicative of the mixture. Mineral distribution varies within the grain but is most concentrated in the bran content. In Table 1, while the ash value of ÇF was 2.97%, this value decreased to 1.2% with the effect of other mixtures in bread form.

4. Discussion

Moisture content in bread gives an idea about the freshness and staling time of the product. Breads with high moisture content take longer to stale. In their study, Tümer and Özer determined that the moisture content of gluten-free bread products was between 44.66% and 48.74% (Tümer and Özer, 2018). Sabanis et al., (2009) determined the moisture content in the range of 47.42% to 53.34% in parallel with the results of our thesis study in their study on fiber-added gluten-free breads. Similar results have been observed in other studies of gluten-free breads (Bernardi et al., 2010; Sabanis et al., 2008; Gallagher et al., 2003). The rate of 60.95 % (Table 3) seen in ÇF bread was higher than the results of studies on gluten-free bread. This is thought to be due to the high fiber content it contains. Therefore, ÇF bread has a longer staling time and is superior to other gluten-free breads as fresh bread. It has been determined that the amount of protein (4.73 g/100 g) in ÇF bread is higher than the A and B brand gluten-free breads offered in the market (Table 3). The carbohydrate rate in ÇF bread is quite low compared to A-brand and B-brand bread (Table 3). This result is also reflected in the energy value of bread. While the dietary fiber ratio of brand A is very low at 1.66 g/100g in gluten-free bread in the market, the dietary fiber value of gluten-free bread of brand B could not be determined. Dietary fiber has both a satiating effect and a lowering effect on the glycemic index in nutrition. The glycemic index levels in foods vary according to the amount of carbohydrates and fiber they contain (Jenkins et al., 2002; Wong et al., 2010). Adding dietary fiber to meals with high carbohydrates reduces the glycemic response (Çatak, 2019). ÇF bread provides an advantage in terms of glycemic index value due to its high fiber and low carbohydrate content.

Oils have roles in improving the qualitative properties of bread crumbs and delaying staling (Ertugay et al., 1988). The fat value of ÇF bread was determined to be 3.45g/100g. This value is lower in A and B brand gluten-free bread (1.9 g/100g). Therefore, it is concluded that the bread properties of ÇF bread are better than the others.

The amounts of some heavy metals determined in ÇF are presented in Table 4. Accordingly, sample no-1 were analyzed from the areas close to the highway, and samples no-2 were analyzed from the flour samples of the *P. spina-christi* collected from the agricultural areas. When samples 1 and 2 were compared, it was observed that the heavy metal ratios in sample 2 were generally lower. When heavy metals exceed the tolerated amounts, they can cause various types of cancer, organ failure, neurological diseases, and skeletal system diseases (Serencam et al., 2018). Heavy metals are chemical pollutants that can contaminate foodstuffs with various environmental effects. There can be more than 60 metals, mainly Cr, Se, Pb, Cd, Fe, Co, Cu, As, Sn, Al, Ni, Hg, and Zn (Duffus, 2002; Serencam et al., 2018). In particular, as Cd, Cr, Ni, and metals such as Pb, humans have potential severe cancer risks and can form them (Szefler and Grembeck, 2007).

The safe limit of chromium (Cr), which is involved in fat metabolism and insulin hormone production, for adults is between 0.05-0.20 mg/day (Nabrzyski, 2007). Manganese (Mn), on the other hand, is a trace mineral that plays a role in the realization of various enzymes, protein metabolism and bone formation (Zafar and Fatima, 2018). Iron (Fe) is a mineral that causes poisoning in living cells when taken in high amounts but must be taken in certain concentrations for human health (Ayar et al., 2009). Nickel (Ni) ratio of 0.05-0.3 mg /day has been reported to be safe and sufficient for adults (Nabrzyski, 2007). In copper (Cu), the recommended intake amount has been determined as 1.5-3 mg /day according to gender (Demirci, 2011). However, JECFA (2009) reported the tolerable daily intake rate as 0.5 mg/kg body weight in adults. Zinc (Zn) is an essential mineral that is important in the growth and development of humans (Gharibzahedi and Jafari, 2017). The tolerable weekly limit for lead (Pb) is 0.025 mg/kg body weight and for cadmium (Cd) this rate is 0.007 mg/kg body weight (JECFA, 2009). Aluminum (Al) is toxic to humans if taken in excess of the tolerated amount and is a heavy metal often associated with Alzheimer's disease (Reilly, 2007). The tolerable weekly intake is 2 mg/kg body weight (JECFA, 2009). Çığ et al., (2020) investigated the presence of heavy metals Ni, Pb, Cd and Cr in wheat samples in their study in Van. They found heavy metal values as 981-541.3 ppb for nickel, 25.6- 907.4 ppb for chrome, 0.9-504 ppb for cadmium and 19.6-350.9 ppb for lead in wheat grain samples. The results showed that heavy metals such as lead, chromium and cadmium in the wheat grains were also within the permissible limits. In nickel, the amounts were found below the limit values in some locations and above the limit values in some locations. According to the Turkish Food Codex Communiqué on Maximum Limits of Contaminants in Foodstuffs, ‘‘the maximum limits of heavy metals that cereals (including buckwheat) and legumes can contain are Pb 0.20 mg/kg, Cadmium 0.10 mg/kg’’ (Anonymous, 2008). When the literature research and the limits given according to the Turkish Food Codex are examined, the analysis results in Table 4 show that the heavy metal values in PDF are well below the limits. In addition, it has been determined that valuable minerals such as Fe, Zn and Mn, which are important for human health, are in sufficient quantities.

Table 4. Some heavy metal analysis results in ÇF.

Minerals	Amount (ppb)	
	1	2
Cr	3.466	2.715
Mn	123.1	112.7
Fe	263.5	242
Ni	6.318	4.369
Cu	51.260	43.79
Zn	122.2	92.55
Pb	4.358	4.198
Cd	0.671	0.475
Al	58.370	46.47

In summary, the study showed that ÇF bread is suitable as an alternative product to gluten-free foods. The results of ÇF bread showed that there are advantages such as richness of dietary fiber ratio, lower carbohydrate ratio compared to other gluten-free products, rich content of minerals, the superiority of bread properties compared to gluten-free mixtures, and the amount of gluten in its content within limits determined in the TGK for celiac patients. Since the dietary fiber content of ÇF bread will have a positive effect on the glycemic index, it will also allow its consumption as a diet product. It was determined that the presence of heavy metals in ÇF was below the tolerated limits and it was concluded that there was no risk in terms of health. With the production of ÇF bread, the variety of gluten-free products in the market for celiac patients will be increased. It is thought that this study will shed light on the gluten-free production of not only bread but also different bakery products such as biscuits, cakes and crackers by using ÇF for celiac patients.

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted. Authors can combine results and discussion if they wish.

5. Conclusions

P. spina-christi (Çaltı) is an important plant that grows cosmopolitan in Türkiye, but its use as food is unknown. Medicinally, its fruits are widely used among people to reduce kidney stones. Celiac disease and Irritable Bowel Syndrome (IBS) occur with the consumption of foods containing gluten. People suffering from this disease cannot consume gluten-containing foods. With this study, a study of the quality parameters of flour and bread production of Çaltı plant was tried for the first time. According to the results obtained, gorse flour is low in gluten and rich in fiber. These successful results It is thought to be an alternative to the studies on gluten-free bakery products.

Conflicts of Interests
Authors declare that there is no conflict of interests

Financial Disclosure
Author declare no financial support.

Statement contribution of the authors
This study's experimentation, analysis and writing, etc. all steps were made by the authors.

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