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# Effects of pseudocereal flours addition on chemical and physical properties of gluten-free crackers

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# Abstract

In this study, potato flour used in gluten free cracker formulation was substituded with pseudocereal (amaranth, buckwheat, quinoa) flours, and the effects of pseudocereals on some chemical, physical and sensory characteristics of gluten-free crackers were determined. Results show that the crackers having pseudocereals had significantly higher (p < 0.05) fat, protein, insoluble and total dietary fiber, essential and non essential amino acid, and Mg contents than control sample which was produced with potato flour and potato starch. In addition, crackers having amaranth had higher (p < 0.05) Ca and P contents than control. Pseudocereal containing crackers had lower  $L^*$  and higher  $a^*$  values than control, and according to  $\Delta E$  values, they were evaluated as extreme (another) color group than control crackers. In SEM micrographs, more cracks and pores were observed with the addition of pseudocereal flours to crackers. Therefore, hardness decreased significantly with pseudocereal flours addition in crackers. Flavor, chewiness, crispiness and overall acceptability scores of crackers having pseudocereal flours were higher than control in sensory analysis.

Keywords: gluten-free cracker; pseudocereals; dietary fiber; amino acid; structural.

**Practical Application:** Crackers having pseudocereal flours instead of potato flour were produced for celiac patients in the study. The chemical composition of products containing pseudocereal flours were found to be richer than those containing potato flour. In addition they liked by celiac patients in sensory evaluation. These results show that pseudocereal flours are feasible ingredients in the manufacture of good-quality and healthy gluten-free crackers.

# **1** Introduction

In botanical terms, amaranth, quinoa and buckwheat are not true cereals, they are dicotyledonous plants as opposed to most cereals (e.g. wheat, rice, barley) which are monocotyledonous. They are referred to as pseudocereals, as their seeds resemble in function and composition those of the true cereals (Alvarez-Jubete et al., 2010a). These pseudocereals are excellent fiber, protein and mineral (Ca, Mg, Fe) sources and are becoming a current trend in human diet as gluten-free grains have an excellent nutritional and nutraceutical value (Machado Alencar et al., 2015; Martínez-Villaluenga et al., 2020). Pseudocereals are currently emerging as healthy alternatives to gluten-containing grains in gluten-free diet (Alvarez-Jubete et al., 2010a).

Celiac disease, otherwise known as gluten-sensitive enteropathy, treats with a strict lifelong adhesion to a gluten-free diet (Murray et al., 2004; Alvarez-Jubete et al., 2009; Göncü & Çelik, 2020). With a gluten-free diet, patients have substantial and rapid improvement of symptoms, including symptoms other than the typical ones of diarrhea, steatorrhea, and weight loss (Murray et al., 2004). It is very difficult for celiac patients to find "safe" meal outside home. So they demand the diet with consistent amounts of packaged gluten-free products, such as snacks, biscuits and crackers (Caponio et al., 2008). Therefore, more supply of gluten-free products is needed in the field of bakery products. This has led to the development of new bakery products with the addition of different gluten-free alternative raw materials like pseudocereals. But, as known, gluten forms the main structure of the flour and gives the dough its elasticity. Gluten deficiency causes changes in the shape, texture, smell and taste of baked goods. Due to the absence of gluten, many gluten-free products on the market are of low quality, contain poor texture and flavor. Due to this situation, there is a need for research and development of gluten-free bakery products (Caponio et al., 2008; Sedej et al., 2011; Tavares et al., 2016; Franco et al., 2020).

Crackers are popular snack foods in human diet (Sedej et al., 2011). They are dry, thin and crisp bakery products and the low level of moisture, decreased even further with baking, left no medium for mold growth (Han et al., 2010). The basic ingredients in cracker production are soft white wheat flour, fat (or shortening), salt, leavening agents (yeast, chemical leaveners, or combinations), whey powder, and sugar and/or glycose syrup (Ozgoren et al., 2019). In practise today, gluten free flours (potato, rice or corn flours) and starches are used in bakery foods for celiac patients instead of wheat flour. But, many gluten-free cereal foods indicate an unbalanced intake of carbohydrates, protein, and fat, as well as limited intake of

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certain essential nutrients in celiac subjects compared to gluten containing ones. In addition, many gluten-free cereal foods do not contain the same levels of calcium, iron, and fiber as their gluten-containing counterparts (Alvarez-Jubete et al., 2010b).

Pseudocereals amaranth, quinoa, and buckwheat are alternatives to common gluten-containing grains (Kupper, 2005). These grains are also nutrient-dense and thus, incorporation of these gluten-free grains in the gluten free diet could not only add variety but also improve its nutritional quality. In a few previous studies (Sedej et al., 2011; Gebreil et al., 2020; Cannas et al., 2020), they were used in some gluten free cracker or biscuit formulations and their some properties were compared with the control samples containing rice or corn flours. However, there is no study in the literature comparing the properties of crackers containing pseudocereals with the properties of crackers prepared with potato flour. The objective of this study was to formulate gluten-free crackers with acceptable taste and appearance by substuting potato flour with pseudocereals (amaranth, buckwheat and quinoa) flour, and to compare their chemical, physical and sensory properties with each other.

#### 2 Materials and methods

#### 2.1 Materials

Amaranth (*Amaranthus hypochondriacus*) (Ala Çiftçi), buckwheat (*Fagopyrum esculentum*) (Duru), white quinoa (*Chenopodium quinoa*) (Delizia), potato flour (PF) (Tito), potato starch (PS) (Migros), margarine (Sana), powdered sugar (Dr. Oethker), salt (Billur), xanthan gum (Tito) and baking powder (sodium bicarbonate, sodium acid pyrophosphate) (Dr. Oethker) were purchased from local markets in Denizli, Turkey.

#### 2.2 Methods

### Preparation of the pseudocereal flours

For the production of amaranth flour (AF), buckwheat flour (BF) and quinoa flour (QF), pseudocereals were ground to particle size  $\leq 1$  mm using the grinder (Felix wave, Felix Household Supplies, Istanbul, Turkey). The ground particles were passed through a 1.00 mm sieve (Mesh No. 18, Retsch, Haan, Germany).

#### Production of crackers

The whole manufacture was repeated twice and two seperate measurements were taken for each manufacture. The formulations presented in Table 1 were used for the production of the crackers. At the beginning of production, firstly raw materials except water were stirred for 1 minute, then, the water was added and the dough was kneaded for 5 minutes to form. The dough was allowed to stand at room temperature for 10 minutes. After resting, the dough was thinned to 2 mm and cut into suitable shapes. Shaped sheets were baked for 11 minutes at 210 °C in turbo-flow ovens. The baked crackers were packed in plastic bags (PE/PA/EVOH/PA/PE) and stored under room conditions until analysis.

Ingredients (g)	С	AC	BC	QC
Pseudocereal flour	-	40.0	40.0	40.0
Potato flour	50.0	30.0	30.0	30.0
Potato starch	50.0	30.0	30.0	30.0
Water	95.0	90.0	90.0	80.0
Margarine	20.0	20.0	20.0	20.0
Salt	1.5	1.5	1.5	1.5
Powder sugar	3.0	3.0	3.0	3.0
Baking powder	1.0	1.0	1.0	1.0
Xanthan gum	1.8	1.8	1.8	1.8

C: Control, AC: Crackers having amaranth flour, BC: Crackers having buckwheat flour, QC: Crackers having quinoa flour.

#### Chemical analyses

Moisture, fat, ash, and protein contents of crackers and raw materials were determined following the AOAC methods (AOAC, 1990). For protein content analysis, factor used to convert nitrogen to protein was 5.70 for crackers and raws. Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) amounts of crackers and raw samples were determined according to the method of AOAC 991.43 (AOAC, 1995) and AACC 32-07 (AACC, 1995) with using total dietary fiber analysis kit (Megazyme International Ireland Ltd, Wicklow, Ireland). Total dietary fiber content was calculated as the sum of insoluble and soluble dietary fiber contents. All dietary fiber contents were corrected for residual protein, ash, and blank.

The mineral composition (P, K, Ca, Mg, Mn, Zn, Fe) of the cracker samples and raw materials were determined by a inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin Elmer, Optima 2100 DV, Massachusetts, US). Nitric acid and hydrogen peroxide were added in the samples that were weighted 0.5 g. The mixture was left to stand for 30 minutes and in microwave method of wet decomposition was fulfilled. Samples burnt in microwave were filtered through filter paper. The mineral elements in filtrate were analyzed by an ICP-OES. Standard curves were used for the determination of analyzed elements. The mineral concentration was expressed in mg/100 g.

Amino acid compositions of the samples were determined by the method of Bilgin et al. (2019). LC-MS/MS device (Agilent Infinity 1260 HPLC system, Agilent Technologies, Santa Clara, CA, USA) and Jasem LC-MS/MS amino acid kits (Sem Laboratuvar Cihazları Pazarlama San. ve Tic. Inc. Istanbul/TURKEY) were used for the determination of amino acid compositions. Firstly 0.5 g of sample was hydrolyzed with 4 mL of acidic hydrolysis reagent in a screw capped glass tube for 24 hours at 110 °C. HPLC system was operated to inject 3  $\mu$ L of prepared sample into the Jasem analytical column specified for amino acid analysis (depending on the analysis kit) maintained at 30 °C. Chromatographic separation was carried out using Jasem's mobile phase A and B with gradient elution at a flow rate of 0.7 mL/min. The optimal MS detector settings were as follows: drying gas temperature 150 °C, drying gas flow 10 L/min, nebulizer pressure 40 psi and capillary voltage of 2000 V. A total of 19 amino acids were identified as essential and non-essential amino acids. The results are expressed in mg/100 g dry weight.

### Physical analysis

Color values were determined by a Hunter Lab Mini Scan XE model colorimeter (Hunter Associates Laboratory, Reston, VA) (Reston, VA, USA) (Hunterlab, 1995). The total color difference ( $\Delta$ E) between the control and the pseudocereal flours containing crackers was calculated using the following Equation 1:

Total color difference 
$$(\Delta E) = \sqrt{(\Delta L^*)^2 + (\Delta a)^2 + \Delta (\Delta b)^2}$$
 (1)

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

According to Yamauchi & Kanazawa (1989)  $\Delta E$  values describe visual color differences as follows; (0–0.5, trace difference); (0.5–1.5, slightly discernible; hard to detect with the human eye); (1.5–3.0, noticeable; detectable by trained people); (3.0–6.0, appreciable; detectable by ordinary people); (6.0–12.0, large; large difference in the same color group); (Larger than 12; extreme; another color group).

Texture analysis was performed using the Brookfield CT3 texture analyzer (Brookfield Engineering Laboratories Inc., USA). A three-point bending test (TA-TPB) was used to determine the hardness characteristics of the cracker samples. The pre-test, test and post-test speed were adjusted to 1 mm/s, 2 mm/s and 2 mm/s respectively. Trigger load was 102 g.

#### Scanning Electron Microscopy (SEM)

Before the images were taken with scanning electron microscopy of the crackers, freeze-drying was applied using lyophilizer (Savant Modulyod-230, Thermo Electron Corporation,

Table 2. Chemical compositions and physical properties of raw materials.

USA). After the freze-dried cracker samples were mounted on aluminum stubs and sputter-coated with gold layer (Quorum, Q150R ES, UK), the inner and exterior surface images of cracker samples were taken at 200 X magnifications with scanning electron microscope (FEI Quanta 250 FEG brand, Hillsboro, Oregon, USA) at a voltage of 10 kV and 5–6 mbar pressure.

#### Sensory analysis

The sensory characteristics of the cracker samples were evaluated for color, odor, flavor, chewiness, crispiness and overall acceptability by 36 untrained panelists with celiac disease from Denizli (20 females and 16 males; aged 8-50). The panelists scored the cracker samples using seven point hedonic scales in which a score of 7=like extremely, 6=like very much, 5=like, 4=neither like nor dislike, 3=dislike, 2=dislike very much, 1=dislike extremely. The samples were labeled with randomly selected three-digit numerical codes.

#### Statistical analysis

The results were compared using analysis of variance (ANOVA) at 5% significance level using Minitab 16.0 software statistical program (LEAD Technologies, USA). The means were tested for significant differences using Tukey's test (multiple comparison test).

# 3 Results and discussion

#### 3.1 Chemical and physical properties of raw materials

The results obtained for the chemical compositions and physical properties of potato starch (PS), potato flour (PF) and the pseudocereal flours of amaranth (AF), quinoa (QF) and buckwheat (BF) are summarised in Table 2.

Parameters	PS	PF	AF	BF	QF
Fat (%) <sup>§</sup>	$0.75 \pm 0.40^{c^*}$	$0.77\pm0.28^{\circ}$	$6.69 \pm 0.13^{a}$	$3.61\pm0.06^{\rm b}$	$7.05\pm0.93^{\rm a}$
Ash (%) <sup>§</sup>	$0.35\pm0.19^{\rm c}$	$2.98\pm0.10^{\rm a}$	$2.37\pm0.02^{\rm b}$	$2.33\pm0.01^{\rm b}$	$2.56\pm0.11^{\rm b}$
Protein (%) <sup>§</sup>	nd	$7.68 \pm 0.30^{\circ}$	$15.08\pm0.34^{\rm a}$	$11.78 \pm 0.55^{\rm b}$	$14.93\pm0.54^{\rm a}$
Soluble dietary fiber (%) <sup>§</sup>	$0.25\pm0.19^{\rm b}$	$3.45 \pm 1.31^{\text{a}}$	$2.79 \pm 1.27^{a}$	$3.29\pm0.54^{\rm a}$	$2.64\pm0.49^{\rm a}$
Insoluble dietary fiber (%) <sup>§</sup>	$0.03\pm0.06^{\rm d}$	$3.80\pm0.26^{\circ}$	$13.20 \pm 2.29^{a}$	$6.97 \pm 1.16^{\rm b}$	$13.49\pm0.61^{\text{a}}$
Total dietary fiber (%) <sup>§</sup>	$0.28\pm0.20^{\rm d}$	$7.25 \pm 1.17^{\circ}$	$15.99 \pm 2.71^{a}$	$10.26 \pm 1.65^{b}$	$16.13 \pm 0.96^{a}$
Minerals (mg/100 g) <sup>§</sup>					
Р	$77.0 \pm 8.5^{\circ}$	$262.3\pm41.7^{\rm bc}$	$634.7 \pm 131.9^{a}$	$615.6 \pm 166.6^{a}$	$400.3\pm41.4^{ab}$
К	$47.4 \pm 13.0^{\circ}$	$1575.5 \pm 35.7^{a}$	$527.5\pm78.7^{\rm d}$	$687.1 \pm 38.5^{\circ}$	$962.2 \pm 63.5^{\mathrm{b}}$
Ca	$16.5 \pm 9.2^{\circ}$	$34.3 \pm 1.1^{bc}$	$171.7 \pm 22.0^{a}$	$37.3 \pm 3.2^{bc}$	$64.7\pm8.5^{\mathrm{b}}$
Mg	$5.6 \pm 2.4^{\circ}$	$88.8 \pm 8.5^{\circ}$	$443.4\pm65.8^{\text{a}}$	$337.3\pm37.6^{ab}$	$239.8\pm54.8^{\mathrm{b}}$
Mn	$0.0\pm0.0^{\mathrm{d}}$	$0.2 \pm 0.1^{\circ}$	$0.9\pm0.1^{a}$	$1.1 \pm 0.0^{a}$	$0.6 \pm 0.1^{\mathrm{b}}$
Zn	$0.1\pm0.1^{ m b}$	$0.4\pm0.2^{\mathrm{ab}}$	$1.1 \pm 0.2^{a}$	$1.2 \pm 0.6^{a}$	$1.0 \pm 0.3^{a}$
Fe	$3.7\pm0.9^{\mathrm{a}}$	$3.2 \pm 1.2^{a}$	$5.5 \pm 1.1^{a}$	$3.9 \pm 1.6^{a}$	$4.0\pm0.7^{a}$
Hunter color values					
$L^*$	$94.51\pm0.08^{\rm a}$	$84.14\pm0.21^{\rm b}$	$74.29 \pm 0.20^{\circ}$	$63.68 \pm 0.09^{\circ}$	$69.77\pm0.16^{\rm d}$
a*	$3.60\pm0.02^{\rm b}$	$2.85\pm0.06^{\rm b}$	$7.07\pm0.06^{\text{a}}$	$7.93 \pm 2.07^{a}$	$7.94\pm3.87^{\rm a}$
$b^*$	$-3.29\pm0.07^{\rm d}$	$14.17 \pm 0.14^{\circ}$	$16.05 \pm 0.24^{\text{a}}$	$14.20 \pm 0.53^{\circ}$	$14.94\pm0.39^{\mathrm{b}}$

\*Different superscript within the same row differ significantly (p < 0.05) using Tukey multiple range test. nd: not determined, PS: Potato starch, PF: Potato flour, AF: Amaranth flour, BF: Buckwheat flour, QF: Quinoa flour. <sup>6</sup>In dry basis.

As can be understood from Table 2, QF and AF had significantly (p < 0.05) higher, PF and PS had significantly (p < 0.05) lower fat, protein, insoluble dietary fiber, and total dietary fiber contents. Additionally, PF had significantly (p < 0.05) higher ash content while PS had lower (p < 0.05) ash content than others.

In previous studies, AF was reported to have a protein content of 12.00-18.13%, fat content of 5.70-8.82%, dietary fiber content of 11.15-20.06% and ash content of 2.00-3.11%, BF was reported to have a protein content of 6.82-15.02%, fat content of 1.22-5.39%, dietary fiber content of 2.70-9.52% and ash content of 1.33-3.11%, QF was reported to have a protein content of 10.01-16.90%, fat content of 4.40-8.36%, dietary fiber content of 8.70-19.70% and ash content of 1.20-8.47% (Bonafaccia et al., 2003; Ogungbenle, 2003; Skrabanja et al., 2004; Qin et al., 2010; Torbica et al., 2012; Fiorda et al., 2013; Chauhan et al., 2015; Nowak et al., 2016; Diaz-Valencia et al., 2018; Kurek et al., 2018; Miranda-Ramos et al., 2019). Our results concur with those reported in literature.

According to our results, the PF has the highest K (1575.5  $\pm$  35.7 mg/100 g) content; the AF has the highest P (634.7  $\pm$  131.9 mg/100 g), Ca (171.7  $\pm$  22.0 mg/100 g), Mg (443.4  $\pm$  65.8 mg/100 g) and Fe (5.5  $\pm$  1.1 mg/100 g) contents; the BF has the highest Mn (1.1  $\pm$  0.0 mg/100 g) and Zn (1.2  $\pm$  0.6 mg/100 g) contents. There is no mineral where PS and QF have the highest content.

In previous studies, AF, BF and QF were reported to have Ca content of 93.5-206.0 mg/100 g, 3.2-72.7 mg/100 g, 28.0-149.0 mg/100 g; Fe content of 1.8-12.0 mg/100 g, 2.2-6.0 mg/100 g, 1.8-15.0 mg/100 g; Mg content of 205.0-328.0 mg/100 g, 89.8-302.0 mg/100 g, 196.0-502.0 mg/100 g; P content of 381.0-663.0 mg/100 g, 414.0-554.0 mg/100 g, 350.0-482.0 mg/100 g; K content of 400.0-552.0 mg/100 g, 156.0-530.0 mg/100 g, 559.0-1475.0 mg/100 g; Zn content of 1.6-5.6 mg/100 g, 0.7-4.5 mg/100 g, 0.8-4.0 mg/100 g; Mn content of 1.5-4.4 mg/100 g, 0.5-3.3 mg/100 g, 1.9-2.0 mg/100 g, respectively (Ikeda & Yamashita, 1994; Ikeda et al., 2001; Bilgiçli, 2009; Alvarez-Jubete et al., 2010b; Sanz-Penella et al., 2013; Nascimento et al., 2014; Nowak et al., 2016; Mota et al.,

Table 3. Chemical	properties of c	racker samples.
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2016; Rybicka & Gliszczyńska-Świgło, 2017). Our results were concomitant to literature findings.

 $L^*$  (lightness) values of PS and PF were higher (p < 0.05) and  $a^*$  values of PS and PF were lower (p < 0.05) than the values of pseudocereals flours (Table 2). PS, what  $L^*$  value was the highest, gave the lowest  $b^*$  (yellowness) value. The difference in the color characteristics may be attributed to the differences in colored pigment in the potato and pseudocereals which in turn depends on the biological origin of the plant (Singh et al., 2003).

A total of 19 amino acids were detected in the pseudocereals, PS and PF (Table 2). The essential amino acids (EAA) constituted approximately 29.3% in PF, 35.9% in AF, 40.8% in BF and 40.0% in QF of total amino acids. The EAA which are essential for human nutrition had not been detected in PS. The results of amino acid composition analysis showed that the AF had significantly (p < 0.05) higher value of EAA than others followed by QF, BF and PF. In addition, AF had also significantly (p < 0.05) higher value of non-essential amino acids (NEAA) than others followed by QF, BF, PF, PS (Figure 1).

Amino acid results of our ingredients were mostly similar to those reported in the literature (Bremer et al., 1996; Kim et al., 2004; Lockwood & King, 2008; Zhu et al., 2010; Palombini et al., 2013; Akin Idowu et al., 2013; Nowak et al., 2016; Mota et al., 2016; Vilcacundo & Hernández-Ledesma, 2017; Sytar et al., 2018; Motta et al., 2019). The small differences can be due to several factors including climate, geography, geochemistry, agricultural practices like fertilization and the genetic composition (Isik &Yapar, 2017).

# 3.2 Effect of pseudocereals addition on the proximate chemical composition of crackers

The incorporation of amaranth, buckwheat and quinoa flours to the formulation significantly (p < 0.05) increased protein, fat, insoluble and total dietary fiber contents compared to the control sample (Table 3). These results were connected with the higher protein, fat, insoluble and total dietary fiber contents of

Parameters	С	AC	BC	QC
Fat (%) <sup>§</sup>	$11.01 \pm 0.35$ <sup>b,*</sup>	$13.46 \pm 0.92^{a}$	$12.89 \pm 0.74^{a}$	$14.42 \pm 1.30^{a}$
Ash (%)§	$2.36\pm0.50^{\rm a}$	$2.85\pm0.44^{\rm a}$	$2.16\pm0.33^{\rm a}$	$2.56 \pm 0.65^{a}$
Protein (%) <sup>§</sup>	$3.22 \pm 0.22^{\circ}$	$6.57\pm0.22^{a}$	$5.33\pm0.27^{\rm b}$	$6.41 \pm 0.08^{a}$
Soluble dietary fiber (%) <sup>§</sup>	$4.02 \pm 1.52^{a}$	$5.34 \pm 1.24^{\rm a}$	$3.65\pm0.31^{a}$	$3.55 \pm 0.16^{a}$
Insoluble dietary fiber (%)§	$7.09 \pm 1.87^{\mathrm{b}}$	$11.69 \pm 0.56^{a}$	$9.96\pm2.16^{\rm a}$	$10.76 \pm 1.62^{a}$
Total dietary fiber (%) <sup>§</sup>	$11.11 \pm 2.51^{b}$	$17.03 \pm 1.66^{a}$	$13.61 \pm 1.85^{a}$	$14.31 \pm 1.61^{a}$
Minerals(mg/100g) <sup>§</sup>				
Р	$262.7\pm28.8^{\mathrm{b}}$	$394.9 \pm 17.2^{a}$	$354.4\pm12.2^{ab}$	$294.6\pm74.4^{\rm ab}$
K	$770.3 \pm 13.7^{a}$	$590.4\pm5.8^{\circ}$	563.2 ± 3.9°	$700.3 \pm 47.1^{b}$
Ca	$81.0\pm3.4^{\rm b}$	$128.1\pm1.6^{\rm a}$	$68.7 \pm 1.6^{\circ}$	$79.9 \pm 1.9^{\mathrm{b}}$
Mg	$52.7 \pm 9.7^{\circ}$	$116.3 \pm 1.9^{a}$	$90.3 \pm 0.1^{\mathrm{b}}$	$79.5\pm6.7^{\mathrm{b}}$
Mn	$0.1\pm0.0^{a}$	$0.5\pm0.2^{a}$	$0.3\pm0.2^{a}$	$0.3 \pm 0.1^{a}$
Zn	$0.2\pm0.1^{a}$	$0.5\pm0.1^{a}$	$0.4\pm0.1^{a}$	$0.4 \pm 0.2^{a}$
Fe	$1.8\pm0.1^{a}$	$2.5 \pm 0.3^{a}$	$1.7 \pm 0.7^{a}$	$2.2 \pm 0.5^{a}$

\*Different superscript within the same row differ significantly (p < 0.05) using Tukey multiple range test. C: Control, AC: Crackers having amaranth flour, BC: Crackers having buckwheat flour, QC: Crackers having quinoa flour. <sup>5</sup>In dry basis.

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**Figure 1**. Non-essential (A), essential (B) and total amino acids values (C) of the raw materials Different superscript letters (a, b, c, ...) indicate statistical differences (p<0.05). §: in dry basis, PS: Potato starch, PF: Potato flour, AF: Amaranth flour, BF: Buckwheat flour, QF: Quinoa flour.

pseudocereal flours than PF and PS (Table 2). All the gluten-free cracker samples had similar ash contents.

Protein is the most fundamental component of tissues in animals and humans, and they are essential components of the diet (Wu, 2016). Their function in nutrition is to supply adequate amounts of needed amino acids for the metabolism (Abdel-Aal & Hucl, 2002). So, consumption of the bakery goods having higher protein content is useful for health and the crackers having pseudocereals had higher protein contents than C sample. AC, BC and QC samples produced in this study had protein contents of 2.08, 1.72 and 1.98 times that of the C respectively. It was found that the gluten-free products which contain AF, BF or QF in the formulation had higher protein contents than the other gluten-free products produced with rice flour, corn flour, potato starch and corn starch in Krupa-Kozak et al. (2011), Demir (2014), Machado Alencar et al. (2015), Kaur et al. (2018), Gebreil et al. (2020), and Cannas et al. (2020).

The common feature of pseudocereals is that they are rich in fiber. The consumption of food naturally rich in dietary fiber is beneficial to the maintenance of health (Alvarez-Jubete et al., 2010a). High-fiber foods have been shown to be highly satiating (Berti et al., 2005). High-fiber diet reduces bleeding and pain in patients with hemorrhoids and the risk of colorectal cancer, obesity, pre-diabetes, diabetes-releted complications (Moesgaard et al., 1982; Schatzkin et al., 2000; Martínez-Villaluenga et al., 2020).

Dietary fiber intake recommendations for adults generally fall in the range of 20 to 35 g/day or 10 to 13 g per 1,000 kcal energy intake (Marlett et al., 2002). According to the calculations made assuming that an individual should take 27.5 g of dietary fiber a day, a person consuming one portion (40 g) AC, BC and QC crackers can satisfy 21.28%, 19.40% or 19.48% of his daily dietary fiber needs, respectively, while a person consuming one portion of C cracker can satisfy 15.20% of these needs. Nutrient deficiencies, particularly low levels of fibers, are seen in glutenfree diets (Vici et al., 2016). So producing fiber-enriched goods will provide extra benefits for celiac patients.

Pseudocereal crackers contained significantly higher amounts of phosphorus and magnesium contents than C. C sample had significantly higher potassium content than pseudocereal crackers and AC had significantly higher calcium content than others. Manganese, zinc and iron contents of all cracker samples were similar (p > 0.05).

Minerals are essential for a wide variety of metabolic and physiologic processes in human body. They are useful for many actions in the body like muscle contraction, normal heart rhythm, nerve impulse conduction, oxygen transport, oxidative phosphorylation, enzyme activation, immune functions, antioxidant activity, bone health, and acid base balance of the blood (Williams, 2005; Saldamlı & Sağlam, 2007). An adult needs nearly 800 mg P, 2000 mg K, 1000 mg Ca, 370 mg Mg, 2 mg Mn, 10 mg Zn and 9 mg Fe intake a day (Metin, 2001; Baysal, 2006).

According to our calculations, an adult can take about 18.9, 17.4, 17.3% of P, 11.3, 11.0, 13.1% of K, 4.9, 2.7, 3.0% of Ca, 12.1, 9.6, 8.0% of Mg, 9.8, 6.0, 5.8% of Mn, 1.7, 1.6, 1.4% of Zn and 10.6, 7.6, 9.0% of Fe daily requirements by the consumption of 1 portion of AC, BC and QC respectively. On the other

hand, he or she can take about 14.0% of P, 14.5% of K, 3.7% of Ca, 5.4% of Mg, 2.4% of Mn, 0.7% of Zn and 7.6% of Fe daily requirements by the consumption of 1 portion of C cracker. Based on the results, all the crackers having pseudocereals may satisfy Mg and P needs, in addition AC may satisfy Ca needs of the consumers better. Minerals such as Mg, Ca, Zn, and Fe are found to be poor in gluten-free diet (Vici et al., 2016). For this reason, the significant increase in the Mg ratio of gluten-free crackers produced with PF by using pseudocereals and the additional increase in Ca ratio with the use of AF is a pleasing result in terms of producing nutritionally enriched gluten-free crackers.

In Vitali et al. (2009), it was determined that the biscuits that includes amaranth in the formulation had higher Ca and Mg contents than biscuits having only wheat flour and biscuits including oat fiber and apple fiber. In the study, it was also found that the biscuit having amaranth had also higher Mg content than the biscuits having carob and soy flour. In the study by Sakač et al. (2015), it was determined that the substitution of gluten-free cookie formulation with light buckwheat flour contributed to the significantly higher mineral content, especially magnesium, potassium, iron and copper, in comparison with the control rice cookies.

The results of amino acid composition analysis showed that pseudocereal crackers contained significantly (p < 0.05) higher amounts of the EAA and the NEAA contents than C sample (Figure 2). As known, amino acids are cell signaling molecules, regulators of gene expression, protein phosphorylation cascade, key precursors for syntheses of hormones and low-molecular weight nitrogenous substances with each having enormous biological importance (Wu, 2009). In addition, essential amino acids are the amino acids for which humans do not have the required enzymes for their biosynthesis (Aristoy & Toldra, 2012), and having higher EAA increases the protein quality in a product. In this case, our results indicate that pseudocereals could be used in cracker formulations for fortification of total and essential amino acid compositions of gluten free crackers prepared with potato flour and starch.

Gambuś et al. (2009) studied the effects of amaranth flour addition on gluten free sponge cakes produced with corn flour and potato starch, and an increase was observed in the protein and EAA contents of cakes with the addition of amaranth flour. In the study, the effects of amaranth and buckwheat flours on gluten free biscuits were also studied and significant increases were determined in protein and EAA contents of biscuits by these additions.

In the study of Gebreil et al. (2020), the crackers prepared from 75% amaranth and 25% corn flour was analyzed for its amino acids and its results were compared with the results of crackers prepared from 100% corn flour (control). The results of the study showed that the crackers prepared from 75% amaranth and 25% corn flour contained higher percentage of the essential amino acids which including leucine, lysine and valine as compared to control sample. It was also found that the cracker having amaranth flour had higher contents of glutamic acid, aspartic acid, arginine, glycine, and serine as non-essential amino acids. The results of Gebreil et al. (2020), Gambuś et al. (2009) and our study show that amaranth flour could be used Aslan; Isik



Non Essential Amino Acids
C AC BC QC





С



**Figure 2**. Non-essential (A), essential (B) and total amino acids values (C) of the crackers. Different superscript letters (a, b, c, ...) indicate statistical differences (p<0.05). <sup>§</sup>: in dry basis, nd: not determined, C: Control, AC: Crackers having amaranth flour, BC: Crackers having buckwheat flour, QC: Crackers having quinoa flour.

Parameters	С	AC	BC	QC
Hunter color values				
$L^*$	$74.27\pm3.56^{\text{a}}$	$60.71 \pm 4,03^{\rm b}$	$54.15 \pm 1.02^{\circ}$	$63.21 \pm 5.81^{b}$
$a^*$	$9.35 \pm 2.20^{\circ}$	$14.14 \pm 1.55^{a}$	$14.70 \pm 0.21^{a}$	$12.11 \pm 2.91^{b}$
$b^*$	$23.88\pm3.27^{\mathrm{b}}$	$26.41\pm0.82^{\rm ab}$	$27.21 \pm 0.71^{a}$	$25.49\pm2.18^{ab}$
$\Delta E$	nd	$14.61 \pm 4.38^{\mathrm{ab}}$	$21.10\pm0.92^{\text{a}}$	$11.70 \pm 6.48^{b}$
Hardness (g)	$2902.50 \pm 50.21^{a}$	$1926.50 \pm 106.77^{\rm b}$	$1551.00 \pm 90.51^{\circ}$	$1981.50 \pm 38.89^{\mathrm{b}}$

Table 4. Physical properties of cracker san	nples.
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Different superscript within the same row differ significantly (p < 0.05) using Tukey multiple range test. C: Control, AC: Crackers having amaranth flour, BC: Crackers having buckwheat flour, QC: Crackers having quinoa flour.

in bakery foods for fortification of total and essential amino acid compositions of gluten free crackers prepared with corn flour and potato flour.

# **3.3** Effect of pseudocereals addition on color and texture properties of crackers

Color is one of the most important sensory characteristics determining the acceptability of food products by the consumer (Machado Alencar et al., 2015). The hunter color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) of crackers are shown in Table 4.  $L^*$  (lightness) values of PS and PF were higher than the values of pseudocereal flours (Table 2). So, C cracker had higher L\* value than crackers with pseudocereal flours. Similar reflections of the color values of the raw materials were also observed on the  $a^*$  and  $b^*$  values of the crackers. While the addition of pseudocereal flours were resulted in significant (p < 0.05) decreases in  $L^*$  value,  $b^*$  and  $a^*$  values increased. The buckwheat cracker had the lowest  $L^*$  value and the highest  $b^*$  and  $a^*$  values. So, the darkest, the reddest and the yellowest cracker was the buckwheat cracker and the lightest one was the C cracker (Figure 3). In a study published by Alvarez-Jubete et al. (2010c), it was found that the pseudocereal-containing gluten-free breads were significantly darker (lower  $L^*$  values) compared to the gluten-free control produced from rice flour and potato starch.

The  $\Delta E$  values indicate the color differences between the control and the crackers added pseudocereal flours. Buckwheat cracker had the highest color differences (21.10) followed by amaranth cracker (14.61) and quinoa cracker (11.70). Accorging to these results, AC and BC that had  $\Delta E$  values greater than 12.00 were evaluated as in extreme (another) color group, and QC that had  $\Delta E$  value between 6.00-12.00 was evaluated as in large different in the same color group (Yamauchi & Kanazawa, 1989). These results show that use of pseudocereals instead of potato flour caused important changes in color of crackers.

The results of texture profile analysis showed that the control had higher value of hardness than others followed by quinoa cracker, amaranth cracker and buckwheat cracker. The high hardness values indicate high resistance of product and require high force to break (Noorakmar et al., 2012). The lower hardness values of crackers having pseudocereals appeared to be related to their lower potato starch contents and higher dietary fiber contents. The content of potato flour as well as potato starch decreased with the increasing amount of pseudocereals flour; and the changes in the proportions may have an influence on the thermal and gelatinisation properties of the gluten-free products (Alvarez-Jubete et al., 2010c). In addition, the reduction in hardness values of crackers having pseudocereal flours may be related to the dilution effects of fibers on the starch-protein matrix of the crackers. This is likely to disrupt the formation of a homogeneous matrix and hence led to a weakening in cracker structure (Mais & Brennan, 2008).

Our results concur with the findings of Wronkowska et al. (2013) who reported that increasing amount of buckwheat flour in gluten free bread produced with corn starch caused significant decrease in hardness values. In de la Barca et al. (2010), the gluten free cookies having amaranth flour in the formulation were also less hard than the controls.

#### 3.4 Scanning Electron Microscopy (SEM)

SEM micrographs (200x) for surface and inner structures of cracker samples are given in Figure 3. According to the surface and inner SEM micrographs, the structure of the control sample seems to be more uniform than the pseudocereal flours added samples. Because of the higher dietary fibers (Table 2 and 3), different compositions and gelatinization characteristics of raw materials (Wronkowska et al., 2013), the more cracks and pores had occured with adding the pseudocereal flours to crackers. Therefore, a more heterogeneous image was obtained than the C cracker. These images are also related to the lower hardness value of the pseudocereal flours had the less hardness (Table 3). These large pores of pseudocereal added crackers having pseudocereals.

#### 3.5 Sensory analysis

Sensory characteristics are one of the most important factors in consumers' product preference. The consumers are looking for new foods or familiar foods with new ingredients that show new sensory characteristics and quality assurance (Delorme et al., 2021; da Silva et al., 2021). In the study, the sensory scores of produced crackers were defined based on evaluation of color, odor, flavor, chewiness, crispiness, and overall acceptability. The obtained results are shown in Figure 4. The scores of all the evaluation except the odor of the crackers with adding pseudocereal flours were higher than the C cracker in sensory evaluation. The odor scores of the crackers were statistically (p > 0.05) similar. BC and AC had the highest flavor, chewiness, crispiness, and overall acceptability scores by celiac patients,



**Figure 3**. SEM micrographs (200x) for surface structure of C (A), AC (C), BC (E), QC (G). SEM micrographs (200x) for inner structure of C (B), AC (D), BC (F), QC (H). C: Control, AC: Crackers having amaranth flour, BC: Crackers having buckwheat flour, QC: Crackers having quinoa flour.



Figure 4. The sensory scores of gluten-free crackers adding pseudocereal flours.

followed by QC and C. These results of sensory evaluation show that crackers with pseudocereals could be good alternatives for the consumption of celiac disease patients. In the study of Alvarez-Jubete et al. (2010a), no significant differences were obtained in the acceptability of the pseudocereal-containing gluten-free breads in comparison with the control having rice flour and potato starch.

It hasn't met any study in the literature comparing the sensory properties of pseudocereal added crackers or biscuits with gluten-free products obtained from potato flour. There are only a few articles of which sensory properties of the products prepared with pseudocereals were compared with the sensory characteristics of products prepared with wheat flour. In Sedej et al. (2011), crackers prepared with refined buckwheat flour got higher appearance, texture and chewiness scores than crackers prepared with refined wheat flour. Taste and odor scores were similar. In Chauhan et al. (2015), cookies having amaranth flour had statistically similar color- appearance, aroma and texture scores with cookies having wheat flour. In Filipčev et al. (2011), the biscuits made with buckwheat flour were also scored significantly higher than the control made with wheat flour. In the study, the highest score given to the formulation at 40% level buckwheat flour.

In Filipčev et al. (2011), biscuits supplemented with buckwheat flour rated higher for chewiness compared to biscuits prepared with wheat flour. This result was associated with softer texture character of buckwheat supplemented biscuit. In our study, it was concluded that the higher crispiness and chewiness scores of crackers having pseudocereals were also associated with the lower hardness values (Table 4) of these crackers.

### **4** Conclusions

In this research, potential use of pseudocereals instead of potato flour and starch in crackers was studied and successful results were obtained. Formulations with amaranth, buckwheat and quinoa flours were viable to obtain good technological and nutritional quality gluten-free crackers for celiac patients. Adding pseudocereal flours to cracker formulations caused significant (p < 0.05) increases in protein, fat, insoluble dietary fiber, total dietary fiber, EAA, P and Mg contents. The use of pseudocereal flours caused decrease in lightness and increase in redness and yellowness values in color of crackers. All the pseudocereal-containing gluten-free crackers gave significantly lower hardness values in texture analysis, and showed better chewiness characteristics in sensory analysis. In addition, in the sensory evaluation test, buckwheat and amaranth containing crackers had the highest overall acceptability scores by celiac patients, followed by quinoa and control crackers. The results show that pseudocereal flours represent feasible ingredients in the manufacture of good-quality and healthy gluten-free crackers.

# **Conflict of interest**

The author declares no conflicts of interest.

# Author contributions

Sinem Turk Aslan was responsible for analyzing the samples, writing the manuscript. Fatma Isik was responsible for writing the project and manuscript, organization of the study.

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