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RESEARCH ARTICLE

Chemical and nutritional characteristics of crackers substituted with *Cucurbita pepo* L. seed flour

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ABSTRACT

The aim of the study was determined the suitability of *Cucurbita pepo* L. (pumpkin) seed flour (PSF) supplement, which is limited for use in the food industry, to increase some chemical and functional properties of crackers. PSFs were used to replace wheat flour in cracker formulation at the levels of 5, 10, 20 and 30%. Particularly, the total dietary fiber, protein and ash content of crackers with PSF increased. The highest increase in mineral contents of crackers were determined in P> K> Mg> Ca> Zn> Fe> Cu. The predominant organic acid of crackers was tartaric acid and the dominant fatty acid was linoleic acid. Crackers prepared with Nevşehir variety PSF yielded better results than other crackers due to their nutritious properties. As a result, with the addition of PSF, a functional food product with health benefits was obtained and a contribution was made to the product range.

Keywords: *Cucurbita pepo* L. flour; Functional product; Dietary fiber; Minerals; Fatty acid composition

INTRODUCTION

Today, food is consumed not only to satisfy hunger but also to obtain sufficient and necessary nutrients and to be physically and mentally healthy individuals by preventing diseases (Siro et al., 2008). With a better understanding of the relationship between health and nutrition, the interest in functional foods has increased (Menrad, 2003). As an integral part of the diet, functional snack foods have a wide variety of commercial products (Mir et al., 2017; Sedej et al. 2011). Crackers are among the product groups with high consumer demand due to their delicious taste, long shelf-life, and easy accessibility among these snack products (Maneerote et al., 2009; Venkatachalam and Nagarajan, 2017; Lekjing and Venkatachalam, 2019).

In the search for alternative products, different additives have been used to improve the nutritional and functional properties of the cracker. There are many studies in the literature on crackers prepared with gluten-free crackers for celiac patients, snacks with reduced-fat to protect heart health, oat whole meal crackers for intestinal problems, crackers produced by adding tropical fruit flours to wheat flour, fried rice crackers with fish powder, crackers with green banana flour, alternative grains (brown rice, amaranth, quinoa, banana, buckwheat, green gram etc.) (Heller, 2009; Omobuwajo, 2003; Maneerote et al. 2009; Sedej et al. 2011; Wang et al., 2012; Mir et al., 2017; Venkatachalam et al., 2017; Lekjing and Venkatachalam, 2019). Flours obtained from legumes with high dietary fiber and protein content and flour obtained by drying fruit and vegetables were also added to these products in the studies in recent years (Heller, 2009).

Pumpkin (*C. pepo* L.) is an important vegetable for health due to its high protein and mineral content, as well as its high content of dietary fiber. İn Turkey, pumpkin cultivation is carried out widely and used in snack, food, pharmaceutical, and cosmetic industries as bark, fruit, meat, and beans (Yoshida et al., 2004). Although pumpkin seeds are widely produced, they have limited use in the food industry. Its important features include affordability and easy access, high nutritional properties, and health benefits. In the literature, there are studies on cakes and biscuits produced using pumpkin flour (Baltacıoğlu and Ülker 2017; Baltacıoğlu and Uyar 2017; Çat and Yardımcı 2015;

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Karaca and Aytaç 2006). However, there are no studies on the use of flour (PSF) obtained from the seeds of different pumpkin varieties in the production of crackers.

Although pumpkin seeds are consumed as snacks in Turkey, their use in food industry is limited. The Edirne (PSF1) from Trakya Region, Nevşehir Sivrisi (PSF2) and Ürgüp Sivrisi (PSF3) varieties from Central Anatolia Region were ground into flour and substituted with cracker wheat flour in cracker dough at 0, 5, 10, 20 or 30% ratios and the crackers were produced from these dough. The present study investigated the potential use of three different pumpkin seeds flour (PSF) obtained from three different *C. pepo* varieties are commonly produced in two different regions in Turkey at different substitution rates in cracker production. The nutritional and functional properties of crackers were determined.

MATERIALS AND METHODS

Materials

Pumpkin seeds (*Cucurbita pepo* L.) to be used in this study were Edirne variety (PSF1) from Trakya Region, Nevşehir Hanım Tırnağı (PSF2) and Ürgüp Sivrisi (PSF3) varieties from Central Anatolia Region, where cultivation is widely carried out. All pumpkin seeds provided from Yayla Kuruyemiş Iç. Dış. Tic. Ltd. in vacuum packages. Pumpkin seeds roasted for 10-15 minutes at 140-180°C and they were ground in a coffee grinder and sieved through a standard sieve (mesh size 60), and pumpkin seed flours (PSF) were obtained. The flour samples were kept in glass jars were kept at +4°C until their use.

The obtained three different PSF flours were used based on replace wheat flour and in the development of functional cracker formulas. Wheat flour used in the production of crackers was provided from Bandırma Has Un (Toru Un) Ltd Şti. Other ingredients in the cracker formulas were purchased from local markets. The crackers prepared with flour obtained from Edirne variety (PSF1), Nevşehir Hanım Tırnağı (PSF2), and Ürgüp Sivrisi (PSF3) varieties were coded as C1, C2, and C3 respectively. All the chemicals used in the experiments were at analytical grade.

Methods

Production of crackers

For cracker production, the one-step fermentation method proposed by Lee et al. (2002) was modified and adopted. Pumpkin seed flour (PSF1, PSF2 and PSF3) were used to replace wheat flour in the formulation at the levels of 5, 10, 20, and 30% (w/w). Control sample were also produced without the addition of pumpkin seed flour. The modified cracker formula is given in Table 1. Cracker production steps are given in Fig. 1. The prepared crackers were cooked in a convection oven (Inoksan FKE 006, TR) at 180 ± 2 ° C for 6-7 minutes. After the crackers were taken out of the oven, they were (C1, C2 and C3) left to be cool, wrapped and allowed to stand at room temperature prior to analyses.

Table 1: Cracker formula

Ingredients[1]	Ratio (%)
Flour[2]	100, 95, 90, 80, 70
Pumpkin Seed Flour (PSF)	0, 5, 10, 20, 30
Water[3]	Variable (mean value; 40-57 ml)
Fat[4] (Shortening)	13 – control, others variable (11-0)
Sodium bicarbonate	0.5
Ammonium bicarbonate	2.0
Salt[5]	Variable (0-1.25)
Yeast	0.5

[1]Ingredients 21±1°C, [2]Based on 14% moisture [3]40-57 ml of water was used for 100 g flour mixture at the rate determined by the farinograph pre-trials. [4]The amount of fat used varied in the range of 11-0%. Pumpkin seed flour additive at the rate of 30% and above is the fat substitute. [5]There is no added salt at a PSF additive at a rate of 20% or above, the natural salt content from pumpkin seed flour was calculated and salt was reduced in the formulation.

Analytical methods

Moisture, ash, protein, and fat values in cracker samples were determined according to AOAC (Association of Official Analytical Chemists) standard methods (Method No:925.40, 950.49, 950.48, and 948.22, respectively) (AOAC, 1990). Carbohydrate and energy values were calculated using the Atwater general factor system. According to this system, conversion factors were taken as 4.0 kcal/g for proteins and carbohydrates, and 9.0 kcal/g for fats (Anonymous, 2003). Total dietary fibers were determined enzymatically according to AOAC Method No. 985.29 (with alpha-amylase, amyloglucosidase, and protease enzymes) (AOAC, 2007). The tests were performed in triplicate and the mean values are reported. The analyses represented % results as g/100 g dry weight.

Determination of organic acids

Oxalic acid, tartaric acid, malic acid, acetic acid, citric acid, ascorbic acid, lactic acidand benzoic acid contents of cracker samples were determined using UHPLC Dionex Ultimate 3000 Model liquid chromatography (CA, USA) with an Acclaim 4x250 mm organic acid column, which is a specific organic acid column, at 210 nm with the ICS-VWD UV detector. The operating conditions; mobile phase: 100 mM Na_2SO_4 (pH adjusted with 2.65), flow rate: 0.60 mL/min, temperature 30 °C, detector 210 nm, injection volume 5 µl (Qiu and Jin, 2002).

Determination of fatty acid composition

The fatty acids of the samples were separated by the Soxhlet Extraction Method (Daglıoglu et al., 2000). Fatty acids were

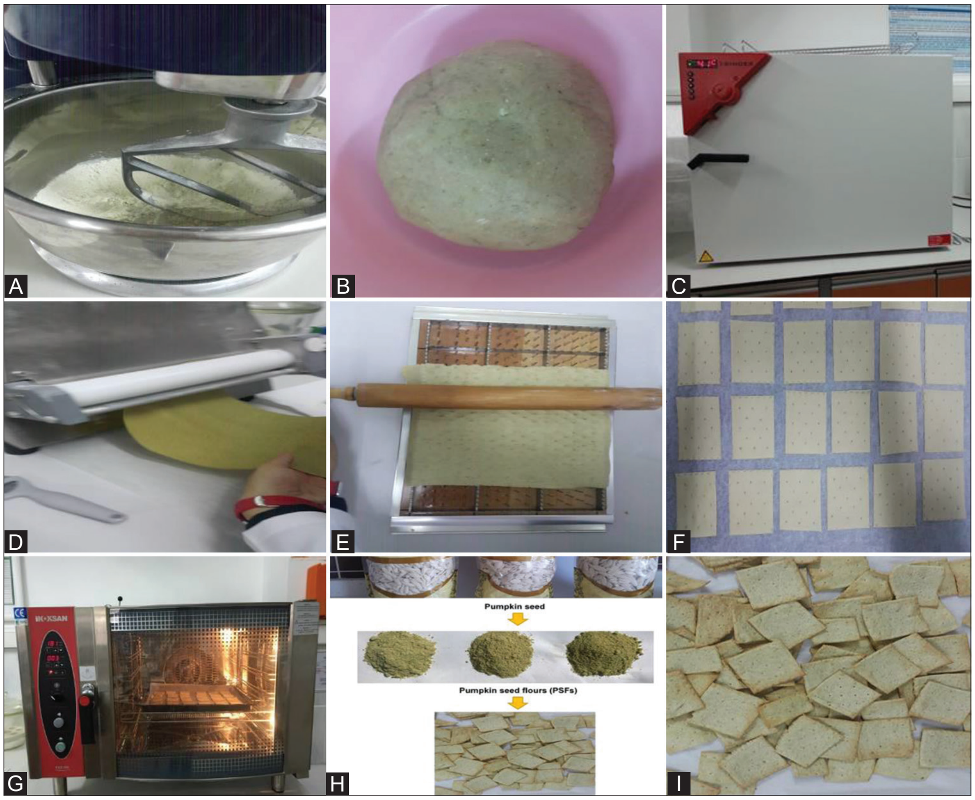

Fig 1. Cracker production stages- (A) Mixing, (B) Kneading, (C) Fermentation, (D) Dough Thinning, (E) Docking, (F) Cutting, (G) Cooking-180ºC, (H) Cooling

converted to fatty acid methyl esters before analysis by shaking a solution of 0.6 g of oil and 4 mL of isooctane with 0.2 mL of 2 N methanolic potassium hydroxide. The samples were then analyzed in Gas chromatography with Agilent 6890 N ECD/FID detector with split/splitless block. In GC operating conditions, the injector, detector, and furnace temperature were 250 oC, 280 oC, and 210 oC respectively, carrier gas was Helium, the constant flow rate was 2 mL/min and the column was HP 88 100x 0.25mm; 0.20μm. The analytical methods for the determination of fatty acid composition were described in the regulation standard method (ISO,12966- 4:2015).

Determination of macro and micro minerals

Reagents

All solutions were of analytical purity and prepared using ultrapure water (18 MΩ cm resistant) with TKA Ultra Pacific and Genpura water purification system. The 67% HNO_3 was obtained from Merck (Darmstadt, Germany). Argon (99.9995% purity, Linde, Turkey) was used as the carrier gas. Standard stock solutions (1000 mg/L) were used to prepare Merck (Darmstadt, Germany) calibration standards for each element (Na, K, Ca, Mg, P, Zn, Fe, Cu, Mn, and Se). Standards solutions were prepared daily using 0.3% HNO_3.

Sample preparation process

Anton Paar Multiwave Go microwave digestion system with a rotor with 12 sample chambers and polyethylene teflon cups were used in the digestion process of the samples. Polyethylene teflon containers were disinfected in a 10% HNO3 (67% v/v) bath, then cleaned in ultra-pure water and dried in an oven at 40°C. The samples were homogenized and subsequently around 0.5 g was weighed directly on PTFE flasks after adding6 ml of concentrated HNO_3 + 1 ml of H_2O_2. Then, the sample was digested using an Anton Paar Multiwave Go model microwave burner according to the following program (step-1: Ramp (min), Temperature (ºC), Holding (min) 10: 00-120-5: 00; step-2: 5: 00-200-10: 00) and then diluted to 25 ml with deionized distilled water. It was then analyzed by ICP-MS and ICP-OES.

Instrumentation

ICP-MS measurements were made using Agilent 7500a Series Shield Torch System ICP-MS (Agilent 7500a Series Shield Torch System ICP- MS, USA). The samples were taken from the tubes with CETACASX 520 model autosampler (CETAC, Omaha, Nebraska, USA) with a peristaltic pump. The ^{82}Se isotope was determined according to its analytical mass by the ICP-MS standard mode. The analyses were performed at the following flow rates: (a) plasma gas of 15 L/min, (b) auxiliary gas of 0.9 L/min, and (c) sample of 0.8 mL/min. External calibration was formed using multi-element standards. The isotope was prepared with 8 calibration solutions and replicates in the range of 0- 200 μg/L with 0.3% HNO_3. Calibration curves were drawn linearly with six standard solutions (Sahan et al., 2007).

The Na, Mg, Ca, P, K, Zn, Fe, Cu, and Mn determination process was performed using an inductively coupled plasma optical emission spectrometer (ICP-OES) model Perkin Elmer 2100 with an axial view (USA) (Anonymous, 2007). The emission intensities were obtained for the most sensitive lines free of spectral interference. The analyses were performed at the following flow rates: plasma gas of 15 L/min, auxiliary gas of 1 L/min, and sample of 0.8 mL/min. All chemical analyses were carried out in duplicate on each sample (Sahan et al., 2007).

Statistical analyses

The data obtained as a result of the analysis were statistically analyzed using JMP IN 7.0.0 (Statistical Discovery from SAS 2007. Institue Inc.). LSD (Least Significant Difference) test at the probability level was used to determine statistically different groups (P <0.05) among the obtained mean values. The randomized plots were carried out according to the experimental design in triplicate. A statistical comparison of the different ratios of the same pumpkin seeds and the same ratios of different pumpkin seeds was carried out. Also, for some analyzes, using the "Hierarchical Clustering Method" (Cluster) -Ward's technique "in the JMP program, dendrogram charts were created to identify groups that are close to each other in the analyzes with many variables and groups close to each other were determined. In the color map in the graphs, the analysis results were shown from the smallest to the largest in blue, gray, and red color tones from light to dark.

RESULTS AND DISCUSSION

Chemical compositions

Some chemical analysis results of crackers are given in Table 2. According to the control sample, the moisture content of the crackers was found to be statistically significantly high (P < 0.05). It was thought that the increase in moisture % values of crackers may be caused by the high fiber content and water absorption and holding capacity of PSF. The ash, protein and fat contents of PSF-added crackers varied in the range of 1.44-1.63%, 9.77-17.77% and 11.29-13.71% respectively. It was determined these values of PSF-added crackers were significantly higher (P < 0.05) compared to the control. The high ash content of the PSF additive caused an increase in the mineral content of the crackers. In the literature, it has been reported that the ash content of biscuits produced with additives such as banana starch was lower (Bello-Perez et al., 2004). The fat content of the crackers increased due to the increase in PSF additive with high oil content. Crackers with 30% PSF had the highest fat content (13.09-13.71%) (Table 2).

The total dietary fiber, carbohydrate and energy values are given in Table 3. The total dietary fiber values of PSF-added crackers varied in the range of 4.73-13.37%. As the PSF addition increased, the total dietary fiber (TDF) value of the crackers increased at a statistically significant level (P < 0.05) compared to the control (3.48%). The highest

Table 2: Some chemical analysis results of crackers*[1]

Sample	PSF Ratio (%)	Moisture (%)	Ash* (%)	Fat* (%)	Protein* (%)
	0	3.03±0.25Bc^{de}	1.33±0.04D^{h}	11.16±0.00C^{g}	9.04±0.53D^{h}
	5	5.03±0.06A*A*^{a}	1.47±0.03C*A*^{fg}	11.29±0.27C*A*^{fg}	10.73±0.21C*A*^{fg}
C1	10	4.58±0.11A*A*^{ab}	1.55±0.02BA*A*^{bcd}	11.93±0.15B*A*^{e}	11.82±0.55C*A*^{e}
	20	4.41±0.12A*A*^{ab}	1.54±0.01BC*A*^{cde}	12.28±0.10B*A*^{cd}	14.36±0.47B*AB*^{cd}
	30	3.05±0.95B*A*^{cde}	1.76±0.04A*A*^{a}	13.09±0.17A*B*^{b}	16.42±0.24A*A*^{b}
	0	3.03±0.25B^{cde}	1.33±0.04C^{h}	11.16±0.00E^{g}	9.04±0.53D^{h}
	5	4.89±0.03A*A*^{a}	1.44±0.03B*A*^{g}	11.54±0.08D*A*^{f}	10.58±0.01CD*AB*^{g}
C2	10	3.81±0.79B*A*^{bc}	1.48±0.01B*B*^{efg}	12.04±0.06C*A*^{de}	11.64±0.71C*A*^{ef}
	20	3.79±0.17B*B*^{bc}	1.49±0.01B*B*d^{efg}	12.24±0.02B*A*^{cde}	14.62±0.02C*A*^{c}
	30	3.17±0.19B*A*^{cde}	1.57±0.03A*B*^{bc}	13.71±0.20A*A*^{a}	17.77±1.06A*A*^{a}
	0	3.03±0.25B*C*^{cde}	1.33±0.04C^{h}	11.16±0.00D*A*^{g}	9.04±0.53D^{h}
	5	4.86±.30A*A*^{a}	1.47±0.07B*A*^{fg}	11.40±0.12D*A*^{fg}	9.77±0.26D*B*^{gh}
C3	10	3.47±0.05B*A*^{cd}	1.52±0.03B*AB*^{cdef}	12.00±0.20C*A*^{de}	10.63±0.02C*A*^{g}
	20	2.73±0.00C*D*^{Cde}	1.57±0.01AB*A*^{bc}	12.47±0.32B*A*^{c}	13.50±0.37B*B*^{d}
	30	2.39±0.09D*A*^{e}	1.63±0.02A*B*^{b}	13.49±0.01A*A*^{a}	16.14±0.14A*A*^{b}

The mean values indicated with different letters in the same column are significantly different P < 0.05). The capital letters in the same column indicate a statistically significant difference at P < 0.05 between all samples, the capital letters indicate a statistically significant difference at P < 0.05 between different proportions of the same seed, the italic capital letters indicate a statistically significant difference at P < 0.05 between all samples. [1]The crackers prepared with flour obtained from pumpkin seed flours PSF) PSF1 Edirne), PSF2 Nevşehir Hanım Tırnağı), and PSF3 Ürgüp Sivrisi) varieties were coded as C1, C2, and C3 respectively. % on dry weight basis).

Table 3: The total dietary fiber, total carbohydrates and energy values of crackers

Sample	PSF Ratio (%)	Total Dietary Fiber* (%)	Total Carbohydrates* (%)	Energy (kcal)
C1	0	3.48±0.12D^{g}	75.44±0.74A^{a}	424.41±1.31A^{a}
	5	4.73±0.24D*B*^{g}	71.49±0.09B*B*^{cd}	411.56±0.26B*A*^{b}
	10	8.23±0.24C*A*^{ef}	70.11±0.49B*B*^{ef}	402.21±0.19C*B*^{c}
	20	11.11±0.75B*A*^{cd}	67.40±0.48C*B*^{g}	393.17±3.98D*A*^{d}
	30	13.31±0.96A*A*^{a}	65.69±0.83D*AB*^{h}	392.98±1.04D*A*^{d}
C2	0	3.48±0.12D^{g}	75.44±0.74A^{a}	424.41±1.31*A*^{a}
	5	7.25±0.51C*A*^{f}	71.55±0.01B*B*^{bcd}	403.36±2.67BC*B*^{c}
	10	9.67±1.68BC*A*^{de}	71.03±0.13B*B*^{de}	400.38±3.90BC*A*^{c}
	20	12.06±0.17AB*A*^{abc}	67.87±0.19C*B*^{g}	391.83±1.34C*A*^{d}
	30	12.95±1.82A*A*^{ab}	63.77±0.82D*B*^{i}	397.76±8.11BC*A*^{cd}
C3	0	3.48±0.12E^{g}	75.44±0.74A^{a}	424.41±1.31A^{a}
	5	6.89±0.76D*A*^{f}	72.49±0.48B*A*^{b}	404.13±1.96B*B*^{c}
	10	10.06±0.35C*A*^{d}	72.38±0.26B*A*^{bc}	399.79±0.75C*A*^{c}
	20	11.25±0.26B*A*^{bcd}	69.72±0.03C*A*^{f}	400.14±2.56BC*A*^{c}
	30	13.37±0.10A*A*^{a}	66.36±0.02D*A*^{h}	397.94±0.81C*A*^{cd}

The mean values indicated with different letters in the same column are significantly different $P < 0.05$). The capital letters in the same column indicate a statistically significant difference at $P < 0.05$ between all samples, the capital letters indicate a statistically significant difference at $P < 0.05$ between different proportions of the same seed, the italic capital letters indicate a statistically significant difference at $P < 0.05$ between all samples.

total dietary fiber value was found in crackers with 30% PSF with 12.95-13.37%. The addition of PSF increased the TDF value an average of four times compared to the control sample. Since PSF has a high content of dietary fiber (23.26-25.95%), it was thought that it can be used as a source of dietary fiber in different food products as well as in cracker production. The total dietary fiber results reported for biscuits produced using wheat, rice, oats, and barley bran as dietary fiber source by Sudha et al. (2007) was reported lower than that of our study results.

While the carbohydrate values of the crackers with PSF varied in the range of 63.77-72.49%, their energy values varied in the range of 391.83-411.56 kcal. These values were significantly lower ($P < 0.05$) than the carbohydrate (75.44%) and energy (424.41 kcal) values of the control cracker. It was thought to be associated with PSF, which is substituted for wheat flour, decreased the carbohydrate and energy values by increasing the dietary fiber value of crackers. In this context, it can be argued that PSF has the potential to be used as an additive in energy-reduced cracker production. These results were consistent with the results showing that as dietary fiber ratios of biscuits added with palm flour (Vieira et al., 2008) increased, the energy values of the products decreased. Comparing these results with PSF added crackers, it was seen that the addition of PSF reduced the energy values of crackers at a higher level.

To the best of our knowledge, there are no studies on FSP-added crackers production in the literature. Baltacıoğlu and Ülker (2017), produced biscuits from the flour obtained from pumpkin (*Cucurbita pepo* L.). With the addition of pumpkin flour, the ash content of the biscuits varied in the range of 0.46-1.55% and the moisture content in the range of 5.99-9.49%. It has been reported that all samples increased compared to the control. Kulkarni et al. (2013), in their study by adding 0, 2.5, 5.0, 7.5, 10% pumpkin powder in biscuit production, have reported the mean moisture as 2.47%, ash as 0.40%, protein as 5.48%, fat as 27.11% and the carbohydrates as 64.47% in the control while, in the experimental group which contained 2.5% pumpkin flour that yielded the best results, moisture was found as 2.31%, ash as 0.50%, protein as 5.03%, fat as 27.12%, and carbohydrate as 64.81%. As the water retention absorption capacity of pumpkin flour is less than wheat flour, it was determined that there was a decrease in the moisture value compared to the control sample. Also, it has been reported that in cakes in which pumpkin flour was substituted with wheat flour at 15, 30 and 45% (Baltacıoğlu et al., 2007), in biscuits, cookies, and cakes produced using with gluten-free flours such as rice, corn, sorghum, buckwheat, quinoa, lupine, chickpea, etc. (Xu et al., 2020), values such as ash, fat, protein and fiber increased significantly. Compared with other studies, the nutritional and functional properties of the product were similarly developed in the present study.

Although there are minor differences between the chemical composition of C1, C2 and C3 crackers produced using PSFs obtained from different pumpkin varieties, due to pumpkin seed variety and growing conditions, similar chemical properties were found. According to the cluster analysis, PSF-added crackers were generally divided into two groups according to their chemical properties (Fig. 4A). Although the control group yielded different results from all PSF added crackers, they were in the first group with 5% PSF added crackers and had similar characteristics in terms of chemical analysis results. The second group comprised 10, 20, 30% PSF-added crackers samples. In general, C2 and C3-coded crackers produced with Nevşehir Hanım Tırnağı and Ürgüp Sivrisi PSF yielded similar results, whereas they had different characteristics from C1-coded cracker type prepared with code PSF1 flour (Edirne pumpkin seed variety). Therefore, these two pumpkin seed varieties have the potential to be used interchangeably.

Fatty acids

The results of the fatty acid composition of the crackers are given in Fig. 2A. The predominant fatty acid in PSF-added crackers was linoleic acid (C18: 2), followed by oleic acid (C18: 1). The linoleic acid value was 16.50% in the control sample whereas it increased to 41.34-43.69% in crackers with 30% PSF. On the other hand, palmitic acid value decreased from 38.34% in the control group to 13.78-14.53% in the crackers with 30% PSF. The majority of PSF oil (73-80%) consists of unsaturated fatty acids (Korkmaz, 2011). Therefore, the unsaturated fatty acid values of different PSF-added crackers increased with the ratio of additives. The average saturated fatty acid (SFA), monounsaturated (MUFA) and polyunsaturated fatty acid polyunsaturated FAs (PUFAs) values in the C1-30%, C2-30% and C3-30% added cracker samples were 21.9%, 35.3% and 42.5%, 21.4%, 34.1% and 44.1%, 20.4%, 34.7% and 44.1%, respectively, while it was determined as 48.7%, 34.1% and 16.7% in control crackers. Reducing the consumption of saturated fats and giving the necessary importance to unsaturated fatty acids in the diet have been reported to be very effective in reducing cholesterol, cardiovascular diseases, protecting the immune system, growth, and repair of the body (Alhusseiny and El-Beshbishi 2020). In this respect, crackers with PSF are regarded as healthier. Santos et al. (2015) analyzed the fatty acid composition of 50 commercially produced crackers, biscuits, and wafers collected from national supermarkets, and the most dominant SFAs were determined to be palmitic acid (C16: 0; avg.33.4%, 5.3% -49.5%) and stearic (C18: 0; 2.9% -25.9%) acid, the average MUFA value was 33.7% (5.6% -77.5%), the average PUFA value was 12.2% (1.8% -43.1%), the other predominant fatty acids were

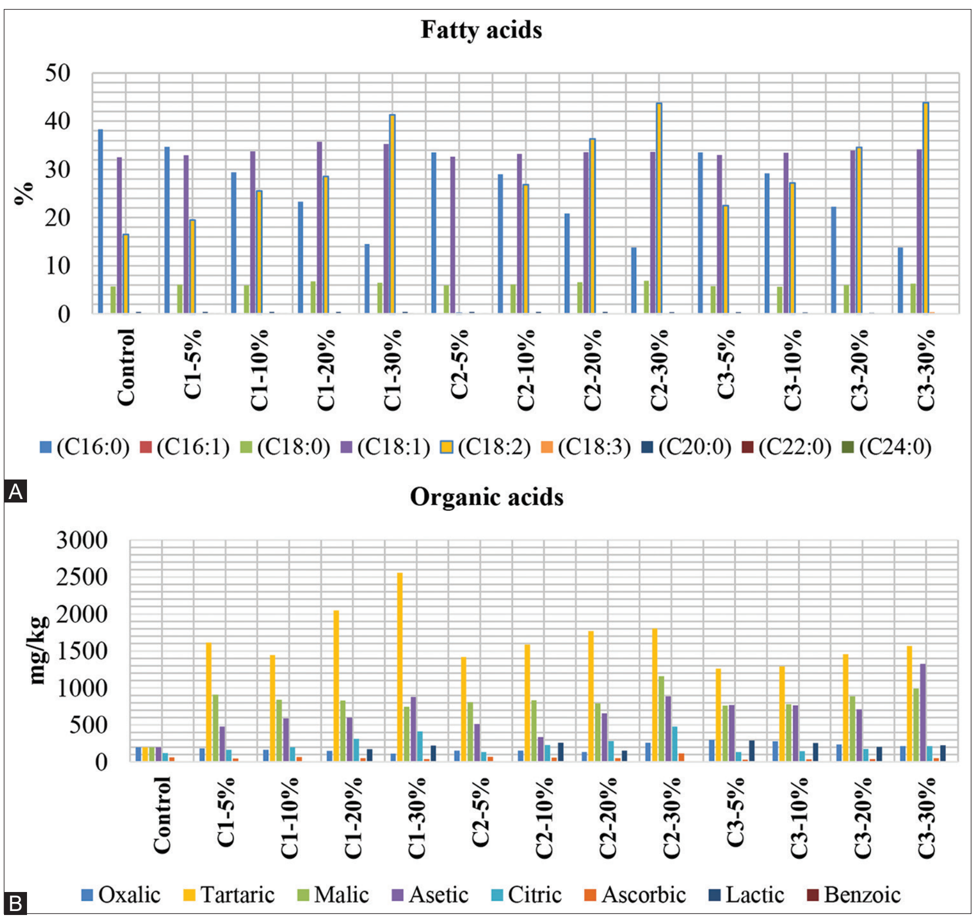

Fig 2. Fatty acid composition % (A) and organic acids (mg/kg) (B) contents of the cracker samples. The crackers prepared with pumpkin seed flours (PSF) PSF1 (Edirne), PSF2 (Nevşehir Hanım Tırnağı), and PSF3 (Ürgüp Sivrisi) varieties were coded as C1, C2, and C3 respectively. (A) Fatty acid compositions: Palmitic (C16:0), Palmitoleic (C16:0), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2), Linolenic (C18:3), Arachidic (C20:0), Behenic (C22:0), Lignoceric (C24:0).

determined to be oleic acid (C18:1, avg. 32.9% (5.3-77.3%), and linoleic acid (C18: 2; avg. 11.4%). Comparing the fatty acid results of the crackers produced in the present study to the literature data, it was seen that they had a rich content of unsaturated fatty acids that are beneficial for health compared to commercial products.

Examining the crackers produced with 30% PSF, palmitic, palmitoleic, oleic and arachidic fatty acid values of the C1 cracker were found to be statistically significantly higher ($P < 0.05$) compared to those of C2 and C3. The stearic acid content of C2 crackers with 30% added and the linoleic acid content of the C3 cracker sample with 30% added were significantly higher ($P < 0.05$) compared to the C1 sample with a 30% additive ratio. Examining different ratios of the same pumpkin seed, oleic acid, linoleic acid, and stearic acid values of C1, C2, and C3 samples were found to be significantly higher ($P < 0.05$) compared to the control sample (Fig. 2A). Evaluating all the samples, according to the results of the dendrogram graph, 5% and 10% PSF-added crackers were found to be similar, while 20% -30% PSF added crackers were found to be close to each other and showed similar characteristics. The control cracker sample was different from the other groups (Fig. 4B).

Organic acids

Organic acids are important food components in improving sensory properties and increasing shelf-life and are effective in protection against diseases due to their antioxidant properties (Su et al., 2019). Results for seven different organic acid contents of crackers are given in Fig. 2B. The average organic acid amounts of the crackers were determined to be tartaric acid>acetic acid> malic acid> citric acid>lactic acid> ascorbic acid and benzoic acid. Compared to the control sample (198.15 mg/kg), the highest increase was observed in the tartaric acid (1292.31-2556.57 mg/kg) values of crackers. This was related to the predominant organic acid tartaric acid in PSF additive. With the increasing PSF concentration, the highest increase in organic acids was observed in acetic acid and citric acid along with tartaric acid. Citric acid and malic acid are organic acids that are abundant in vegetables and fruits (Vicente et al., 2009). There were no changes or decreases in the other acids. This was thought to be due to the low levels of these organic acids in PSF.

According to Fig. 2B oxalic acid, acetic acid, citric acid, and ascorbic acid values of C2 crackers were found to be significantly higher ($P < 0.05$) compared to those of C1 and C3 samples in crackers with 30% PSF. The tartaric acid content of C1 crackers and benzoic acid content of C3 crackers were significantly higher ($P < 0.05$) than those of the 30% C2 crackers. Acetic acid values of 30% crackers were found to be significantly similar ($P < 0.05$).

The control group yielded different results than all PSF crackers. Among the PSF added crackers, the C2-coded Nevşehir Hanım Tırnağı variety showed different characteristics compared to the other varieties with PSF (30% C2). The closest group to the C2 group was the C3 (Ürgüp Sivrisi) group (Fig. 4C). This was thought to be due to different types of use, as well as different geographical conditions, climatic conditions, soil structure, and agricultural practices applied. There are no studies in the literature on the organic acid content of PSF-added crackers.

Macro and micro minerals

Minerals, which constitute the micro group of nutrients in foods, are very important for the regulation of body metabolism, the proper functioning of hormones and enzymes, the formation of muscles and bones, and the maintenance of other vital activities. Since they cannot be synthesized in the body, they must be taken daily from foods or other supplements (Costa-Pinto and Gantner, 2020). Excessive or insufficient intake of minerals, which are important in daily recommended amounts, causes disruptions in body functions and diseases (Nosratpour and Jafari, 2019). The mineral content of fruits and vegetables varies according to different cultures and environmental conditions (Perović et al., 2020).

The changes in mineral analysis results of crackers according to the PSF addition rates are given in Fig. 3A and Fig. 3B. In terms of macro minerals in PSF-added crackers, respectively, the highest values were determined in Na (6150-8809 mg/kg), P (1659-4012 mg/kg), K (1935-3480 mg/kg), Mg (590.78-1815 mg/kg) and Ca (264-486.96 mg/kg) (Fig. 3A). In terms of micro minerals, the highest mineral values were Fe (15.22-33.25 mg/kg), Zn (7.12-20.67 mg/kg), Mn (8.45-18.70 mg/kg) and Cu (2.81-5.86 mg/kg), respectively (Fig. 3B). Selenium contents of crackers remained below the LOD values. The mineral values increased as the PSF additive rates increased in all crackers. Na contents were similar to those of the control sample ($P < 0.05$). The mineral content of C2 with 30% additive and C3 with 30% additive ratio was statistically higher than those of the other samples ($P < 0.05$). Studies have reported that some absorption inhibitors and phytates in wheat flour used in bakery products have the ability to bind minerals and that these products containing wheat flour are insufficient in terms of Ca, Fe, and Zn (Vitali et al., 2008; Agrahar-Murugkar, 2020).

Kaic-Rak and Antonic (1990) in commercially available biscuits and cookies, found the average mineral contents as potassium (230--250 mg/100 g), calcium (30--110 mg/100 g), magnesium (42 mg/100 g) reported as iron (1.0–2.0 mg/100 g), zinc (0.6–0.8 mg/100 g), and

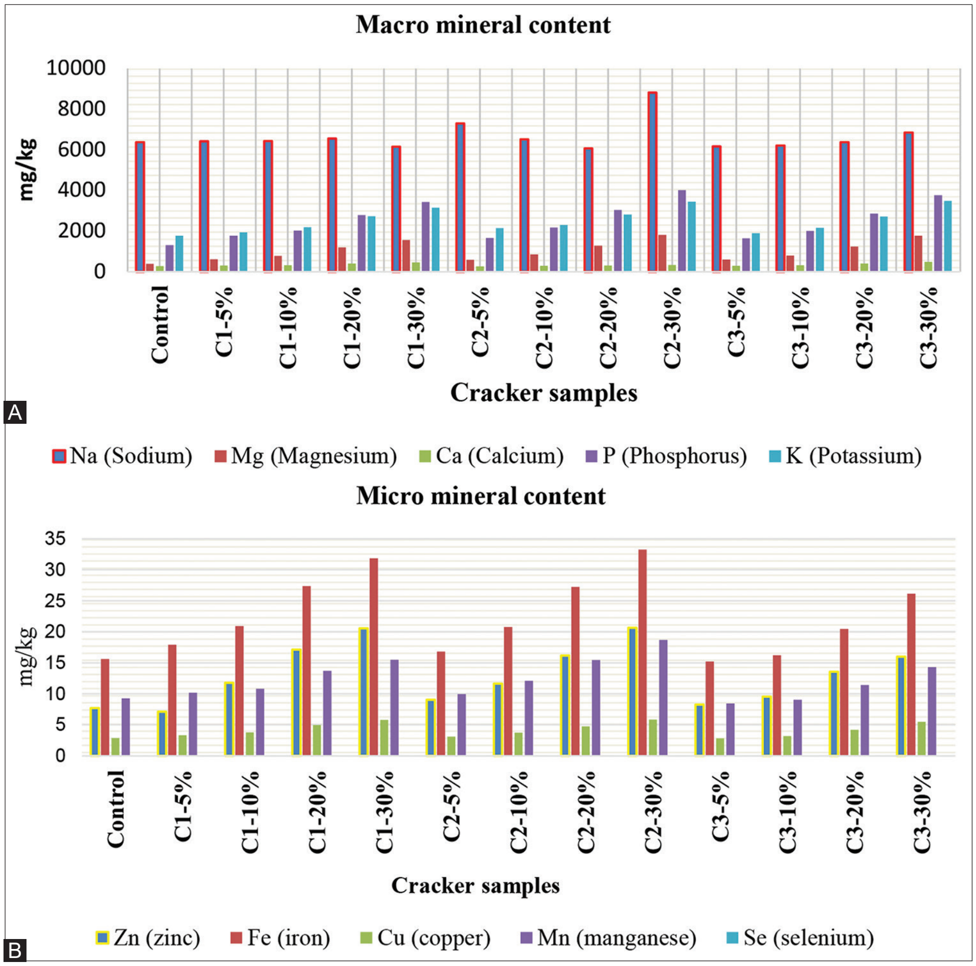

Fig 3. Macro mineral (mg/kg) (C) Micro mineral (mg/kg) (D) contents of the cracker samples. The crackers prepared with pumpkin seed flours (PSF) PSF1 (Edirne), PSF2 (Nevşehir Hanım Tırnağı), and PSF3 (Ürgüp Sivrisi) varieties were coded as C1, C2, and C3 respectively.

copper (0.10–0.25 mg/100 g). Kulkarni and Joshi (2013) have stated that there was an increase in the mineral values calcium (0.17%), phosphorus (0.04%), potassium (0.07%), and iron (21-32 ppm) in the production of biscuits with pumpkin flour compared to the control group. Cookies produced using baru flour is enriched in iron, zinc, and copper (Pineli et al., 2015). Similar results were obtained in PSF-added crackers and the mineral contents increased as the PSF addition ratio increased.

Evaluating the crackers with a 30% PSF added, Na and P values of C2 crackers were found to be significantly higher ($P < 0.05$) compared to those of C1 and C3. There were no significant differences between the Mg and K values of PSF-added crackers obtained from different pumpkin varieties ($P < .05$). C1 and C3 crackers were found to be similar in terms of Ca content and were significantly higher than those of the C2 crackers ($P < 0.05$). The 30% C1 and C2 samples were found to be statistically similar in terms of Zn and Fe content, however, differing from the C3 sample. Examining the mineral contents of crackers in C1, C2, and C3 groups, according to the dendrogram obtained in the cluster analysis in Fig. 4, the cracker sample with 30% PSF (C3) was found to be different from the others in terms of macrominerals and showed the best mineral content (Fig. 4D). In terms of micro mineral content, crackers with 20-30% PSF were in the same group and showed similar characteristics, whereas the control sample and the 5% PSF cracker samples showed similar characteristics (Fig. 4E). The good mineral content of the PSF additive increased the nutritional values of the crackers. Considering the macro and microelements that should be taken daily, crackers with pumpkin seed flour were found to be a good source of Na, P, K, Fe, Zn, Mn, and Cu.

CONCLUSION

Crackers were produced by substituting three different PSFs produced from different pumpkin seeds separated from the shell and milled with wheat flour in the ratio of 0, 5, 10, 20, and 30% to the cracker dough to improve their functional properties. It was determined that the addition

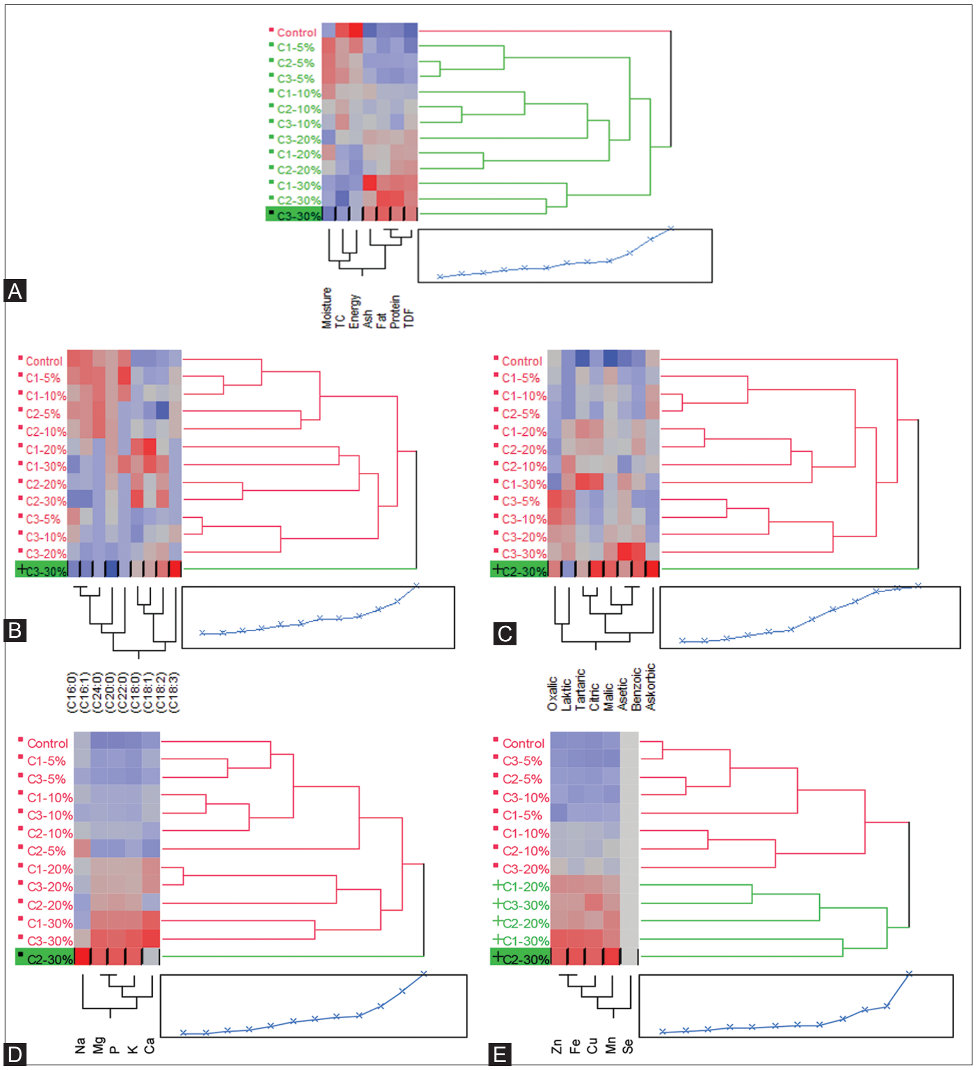

Fig 4. Hierarchical Clustering Analysis-Dendrogram for all cracker samples concerning the content of (A) chemical analyses, (B) fatty acids, organic acids (C), (D) macro mineral, (E) micromineral contents. The crackers prepared with flour obtained from pumpkin seed flours (PSF) PSF1 (Edirne), PSF2 (Nevşehir Hanım Tırnağı), and PSF3 (Ürgüp Sivrisi) varieties were coded as C1, C2, and C3 respectively. TC: Total Carbohydrate, TDF: Total Dietary Fiber.

of PSF improves the functional and nutritional properties of crackers. As a result of the study, crackers were produced rich in unsaturated fatty acids and mineral substances, with increased dietary fiber ratio and decreased energy value. The differences between C1, C2, and C3 were related to the raw material used, geographical conditions, growing conditions, climate, soil structure, and agricultural processes, etc. In general, the crackers prepared with Nevşehir Hanım Tırnağı (C2) and Ürgüp Sivrisi (C3) varieties gave similar results whereas they had different properties from the C1 Edirne variety. Evaluating all the results in general, the C2 cracker prepared with Nevşehir Hanım Tırnağı variety pumpkin seed flour yielded better results but all the crackers had acceptable properties. Today, to prevent health problems and to control weight, it is recommended to produce and consume crackers with PSF due to the increasing demand for products with low fat, high fiber ratio and low calorie.

It was concluded that pumpkin seed flour can be used in different sectors in food industry as a good source of dietary fiber, minerals and antioxidants. With PSF addition, a new and alternative product was obtained with functional properties, a high nutritional and low energy values.

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Authors' contributions

Dilek Dülger Altıner: designed and performed the experiment, analyzed the data, wrote the manuscript.

Merve Sabuncu: performed the experiments and analyzed the data.

Yasemin Şahan: designed, performed the experiment, and wrote the manuscript.

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