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Gluten-Free Crackers Based on Chickpea and Pumpkin Seed Press Cake Flour: Nutritional, Functional and Sensory Properties

Running head: Utilization of Non-Conventional Flours in Formulation of Gluten-Free Crackers

Jelena Tomić[1*], Dubravka Škrobot[1], Ljiljana Popović[2], Tamara Dapčević-Hadnađev[1], Jelena Čakarević[2], Nikola Maravić[1] and Miroslav Hadnađev[1]

[1]University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2]University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

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SUMMARY

Research background. Despite the growing trend of the gluten-free market and the presence of a wide range of gluten-free products, there are still some shortcomings of these products in terms of nutritional and sensory quality. The commercially available gluten-free products are characterised as products of inferior nutritional quality, particularly in terms of protein and dietary fibre content and with high glycemic index. On the other hand, from a sensory point of view, gluten-free products usually show inappropriate textural and mechanical properties, poor mouthfeel and flavour. This is a consequence of the limiting choice of raw materials that mainly possess large share of carbohydrate components.

Experimental approach. Chickpea flour (ChF) and two types of pumpkin seed press cake flour (virgin (VF) and cold pressed (CF)), at two substitution levels (20 and 35 %, *m*/*m*) were blend to produce gluten-free crackers without the presence of conventional gluten-free starch-rich ingredients. This study

*Corresponding author:
Phone: +3814853780
E-mail: jelena.tomic@fins.uns.ac.rs

aims to investigate the suitability of using these non-conventional flours on nutritional and physico-chemical properties, sensory acceptability, antioxidant activity and glycemic index of crackers.

Results and conclusions. All produced crackers can bear a nutritional claims "high fibre", "source of protein", "source of minerals". Replacing ChF with VF and CF increased protein and total phenolic content and enhanced antioxidant activity. The selected combination of raw materials allows the production of gluten-free crackers with a moderate glycemic index. Besides nutrient content, CF addition increased overall sensory acceptability, noticeably improving taste and flavour scores compared to Control and VF containing counterparts.

Novelty and scientific contribution. To the best of our knowledge, there is no study investigating the use of chickpea and pumpkin seed press cake flour blend without using conventional gluten-free flours and starches. The used non-conventional flours represent complementary raw materials in terms of protein quality and valuable alternatives to produce nutrient dense, health promoting gluten-free crackers with reduced glycemic response and acceptable sensory properties.

Keywords: gluten-free; chickpea flour; pumpkin seed press cake flour; glycemic index; total phenolic content; protein content

INTRODUCTION

In the circumstances of constant population growth and modern diet trends, the food industry faces great challenges in its desire to balance market dynamics and sustainable supply of various nutritious foods. The baked products (bread, biscuits, and crackers) as the core of the consumer segment in the food industry are particularly susceptible to modifications and constant development in order to improve their nutritional composition without compromising consumer acceptability. In this regard, the utilization of various food ingredients which will provide adequate nutritional effects becomes the priority for both the food scientist and the food industry.

For the new food ingredients, especially in the case of gluten-free products, it is desirable to provide dual benefit of enhancing nutritional and retaining or improving technological quality of final products. How gluten-free products are recognized as products with inferior nutritional quality, many studies with different technological and compositional approaches were devoted to the fortification of this category of food (*1–4*).

The lower dietary fibre and complex carbohydrates contents along with higher glycemic index (GI) are considered as main drawbacks of gluten-free diet (*5*). In addition, a lower protein content compared to their gluten-containing counterparts has also been reported (*6,7*).

A compromise between the health benefits of a gluten-free diet and its nutritionally balanced profile and technological quality is very difficult to achieve. The reason for this is certainly the limiting choice in terms of raw materials that would provide the functional properties characteristic of gluten. By reviewing the literature data, but also by looking at commercially available gluten-free products, it is evident that rice and corn flour, as well as starches of different origins, are the most common ingredients of gluten-free products (*5,8*). Their selection is based primarily on the fact that they are of neutral taste and acceptable colour, good digestibility and hypoallergenic properties (*9,10*). On the other hand, as a consequence of the large share of carbohydrate components in these raw materials, gluten-free products are usually characterized by poorer sensory quality, especially in terms of textural and mechanical properties (crumbling texture, poor mouthfeel and flavour) (*11*). Taking into consideration all mentioned shortcomings of gluten free products, reformulation of food by introducing wide range of unconventional gluten free flours become a common practice. Evidence about considerable amounts of nutrient-rich ingredients found in chickpea has focused a lot of attention on the matter of redesigning conventional foods containing this raw material, mainly in the form of protein flour (*1,12*). Chickpea is a valuable source of proteins with a good amino acid profile (high lysine), complex carbohydrates (dietary fibres, resistant starch, and oligosaccharides), important vitamins and minerals (B-vitamins, folates, and iron), as well as antioxidants and polyphenols (*13*). Additionally, the benefit of usage of chickpea flour as an alternative raw material in gluten-free product is reflected through the slow release of glucose from starch which contributes to the lower glycemic index (*2*). In the view of the environmental sustainability and minimisation of food waste, agro industrial by-products present a real impetus for the development of additional health promoting ingredients. Pumpkin seed press cake, a by-product of oil production, is food resources that offer various health benefits. In addition to fibre, pumpkin seed press cake is a source of proteins, essential fatty acids, polyunsaturated fatty acids, omega 3, 6 and 9 fatty acids, as well as significant amounts of vitamins and minerals (*14–16*). Regarding the protein quality, this raw material has a suitable amino acid profile. Compared to soya bean meal it contains higher levels of most essential amino acids (except lysine) (*17*).

From nutrition point of view, chickpea and pumpkin seed press cake flours are complementary raw materials in terms of protein quality. Most studies that have investigated these raw materials were related to their valorisation and potential as gluten-free substitutes of conventional gluten-free flours in the production of bakery products. However, there are no published data on the use of chickpea and pumpkin seed press cake flour blend in gluten free bakery products processing without the presence of conventional gluten-free flours and starches. Therefore, the objective of this study was to investigate the possibility of using aforementioned raw materials as base ingredients for production of sensory acceptable and nutritionally improved gluten-free crackers with respect to content of protein and dietary fibre. Additionally, fatty acid content, glycemic index, polyphenolic content and antioxidant activity were determined and discussed. Moreover, the impact of pumpkin seed press cake flour treatment history on the quality of obtained crackers was evaluated.

MATERIALS AND METHODS

Raw materials

Chickpea flour (ChF) was locally produced and obtained from agricultural household "Mirilov", Bačko Gradište, Serbia. Chickpea flour had moisture, protein, fat, ash and carbohydrates (calculated by difference) contents of 9.27, 21.52, 4.78, 3.29 and 61.14 g/100 g, respectively.

Samples of hull-less pumpkin (*Cucurbita pepo* L) seed press cake, a by-product of the pumpkin cold pressed and virgin pressing process, were obtained from a “Suncokret d.o.o”, Hajdukovo, Subotica, that uses traditional mechanical oil pressing technology. The pumpkin seeds used for the production of virgin oil, prior oil extraction went through thermal processing. The obtained press cake samples were grinded to obtain flour. The roasted pumpkin seed press cake flour (VF) had moisture, protein, fat, ash and carbohydrates (calculated by difference) contents of 2.70, 56.16, 13.92, 8.59 and 18.63 g/100 g, respectively while the cold pressed pumpkin seed cake flour (CF) had moisture, protein, fat, ash and carbohydrates (calculated by difference) contents of 8.27, 56.49, 16.79, 7.57 and 10.88 g/100 g, respectively. Vegetable fat (palm oil based, melting range temperature 34-37 °C) was obtained from oil factory “Dijamant” (Zrenjanin, Serbia). Salt, sodium bicarbonate, ammonium bicarbonate and powdered sugar were purchased in a local food store.

Preparation of gluten-free cracker

Five different formulations of gluten-free crackers were produced under the same processing conditions by mixing chickpea flour with virgin (VF) or cold press (CF) pumpkin seed press cake flour in different ratio (Table 1). The other ingredients were used in the same proportions. Chickpea flour, pumpkin seed press cake flour, salt and baking powder were mixed together. The dough was prepared using the following procedure: vegetable fat was mixed with soy lecithin for 2 min, and then water was added and thoroughly blended to obtain homogenous mixture. Finally, all powdery ingredients were added together and mixed for additional 3 min. The dough was laminated immediately after preparation on pilot scale dough laminator (Thiene, Italy) to the desired thickness (*b*=3.0 mm). Cracker samples were shaped using a circular cutter (Ø45 mm) and baked at 190 °C for 11 min in a laboratory oven (MIWE gusto® CS, Arnstein, Germany). The crackers were produced in four batches where each batch yielded 20 crackers. The obtained gluten-free crackers were left to cool down at room temperature for 1 h and then they were hermetically stored for analysis.

Proximate composition

Proximate composition of raw materials and crackers including protein (Method No. 920.87), fat (Method No. 922.06), ash (Method No. 923.03) and moisture contents (Method No. 925.09) was determined by AOAC standard methods of analysis (*18*). Total dietary fibre (TDF) content of the obtained crackers was determined using of the Megazyme International total DF assay kit (adopted from AACC method 32–07 and AOAC method 985.29) (*18,19*). Available carbohydrate was obtained by difference, by subtracting the sum of grams of water, protein, fat, ash and dietary fibre from a 100 g basis mass. The total energy was calculated using the European Regulation No. 1169/2011 (*20*).

Mineral content

Mineral content (Zn, Fe, Mg, K, Na, Ca) was determined by atomic absorption spectrophotometry (Method No. 984.27) on a Varian Spectra AA 10 (Varian Techtron Pty Ltd., Mulgvare Victoria, Australia) (*19*).

Fatty acid composition

The procedure published in Pojić *et al*. (*21*) was used to determine fatty acid profile. Total lipids were extracted with a chloroform-methanol solution (2:1 ratio of chloroform to methanol) and the obtained extracts were dried by vacuum evaporation (40 °C). The residue obtained after evaporation of solvent under steam of nitrogen was weighed. The extracted lipids were transformed in fatty acid methyl esters using 14 % boron (III)-fluoride in methanol. The samples were analysed by GC Agilent 7890A (Flame-ionization detector, auto injection module for liquid samples, fused silica capillary column DB WAX 30 m, 0.25 mm, 0.50 µm) (Agilent Technologies, Santa Clara, USA). As a carrier gas, helium was used with purity over 99.9997 vol. % and flow rate of 1.26 ml/min. Identification of fatty acids was done by comparing of retention times with standards from Supelco 37 Component Fatty Acid Methyl Ester Mix (Sigma-Aldrich, St. Louis, MO, USA). All analyses were performed in duplicates and the results were expressed as a percentage of each fatty acid in total fatty acids.

Total phenolic content

Total phenolics from the samples were extracted by the method of Świeca (*22*). Briefly, crackers were ground (1.0 g) and mixed with 20 mL of PBS (phosphate buffered saline, pH=7.4) for 1 h at room temperature. The procedure was repeated twice. The extracts were centrifuged at 9200 x*g* (Sorvall® RC-5B Refrigerated Superspeed Centrifuge, Du Pont Instruments, Wilmington, USA) for 15 min and the resulting supernatants were pooled and stored at 4 °C.

Total phenolic content of PBS extracts was determined spectrophotometrically by using Folin-Ciocalteu's reagent method described by Singleton *et al.* (*23*). The extract (0.1 mL) was diluted with distilled water (7.9 mL). Folin-Ciocalteu's reagent (0.5 mL) and 20 % sodium carbonate solution (1.5 mL) were added at the reaction mixture. The mixture was allowed to stand for 60 min and the absorbance at 750 nm was measured (T80 UV–Vis Spectrophotometer; PG Instruments, Lutterworth, UK). Gallic acid was used as a standard and results were expressed as Gallic acid equivalents (GAE) (mg GAE per g of sample).

Antioxidant activity

The radical scavenging activity was determined by the ABTS scavenging activity assay described by Popović *et al.* (*24*), with some modifications. Briefly, an aliquot of 30 µL of the PBS extract was mixed with 3 mL of a daily prepared ABTS solution (*A*=0.7±0.02). The absorbance was measured at 734 nm (T80 UV–Vis Spectrophotometer; PG Instruments, Lutterworth, UK), after 10 min. The activity was expressed as Trolox equivalent in mM per g of sample.

In vitro starch digestion rate and predicted glycemic index

Predicted glycemic index of crackers was determined by method of Molinari *et al.* (*25*), with some modifications. The ground crackers (200 mg) were mixed with 20 mL HCl-KCl buffer (0.01 M, pH=1.5) and 0.2 mL of pepsin solution (1 g in 10 mL HCl-KCl buffer). The mixture was incubated for 60 min in a shaking water bath at 40 °C. After pepsin digestion, the total sample volume was adjusted to 60 mL with phosphate buffer (0.2 M, pH=6.9). Then, the 10 mg of α-amylase were added and incubated for 3 h in a shaking water bath at 37 °C. Aliquots (1 mL) at 0, 10, 20, 30, 60, 90,120, 150 and 180 min were obtained from each sample and incubated at 100 °C for 5 min to inactivate the enzyme. After incubation time, 250 µL of each supernatant was taken to a volume of 750 µL with sodium acetate buffer (0.2 M, pH=4.75). Subsequently, 30 µ of amyloglucosidase was added and incubated at 60 °C for 45 min with constant stirring. Glucose content was measured by the GOPOD kit (Megazyme, Wicklow, Ireland).

The hydrolysis curve of samples was expressed by concentration of glucose measured in samples during the time. The hydrolysis index (HI) was calculated as the ratio between the areas under the hydrolysis curve (0–180 min) of the experimental samples and the area of reference sample (white bread). The predicted glycemic index (pGI) was calculated using the equation (Eq. 1) proposed by Goñi *et al.* (*26*):

$$\mathrm{pGI} = 39.71 + 0.549 \cdot \mathrm{HI} \qquad /1/$$

Physical properties

Eccentricity, spread factor and puffiness

Physical parameters including mass (g), diameters of baked cracker (d_1 and d_2, perpendicular to each other), thicknesses of the cracker before (b_1) and after baking (b_2) were measured by using Vernier calliper using 10 crackers taken randomly from the batch of crackers. The eccentricity was calculated as the ratio between d_1 and d_2. Spread factor was calculated as the ratio between average diameter and average thickness (*3*). Sample puffiness (P) was calculated as shown in Eq. 2:

$$\%\,P = \left(\frac{b_2 - b_1}{b_1}\right) \cdot 100 \qquad /2/$$

Textural measurements

The crackers hardness and fracturability were measured by using TA-XT2 Texture Analyser (Stable Micro System, Godalming, United Kingdom) equipped with a 30 kg load cell and three-point bending rig (HDP/3PB). The two adjustable supports of the rig base plate were placed at 30 mm distance apart so as to support the sample. The upper blade was positioned to be equidistant from the two lower supports. During measurements, the upper blade descended at a speed of 1mm/s until a 50 g contact force was detected and then travelled a distance of 5 mm through the cracker at a speed of 3.0 mm/s.

Measurements were performed 24 hours after baking in six replicates per batch at ambient temperature (25±1 °C).

Colour measurements

The colour of the crackers top surface was measured 24 hours after baking using a Chroma meter Minolta CR-400 (Konica Minolta Co., Osaka, Japan). The results were expressed as *L** (lightness/darkness), *a** (redness/greenness), *b** (yellowness/blueness).

The influence of different type of pumpkin seed press cake flour incorporation on the total colour difference (ΔE) between control and the pumpkin flour containing crackers was measured according to the following the Eq. 3:

$$\Delta E = \sqrt{\Delta {L^*}^2 + \Delta {a^*}^2 + \Delta {b^*}^2} \qquad /3/$$

where Δ*L**, Δ*a** and Δ*b** indicate the difference in *L**, *a** and *b** values measured for crackers with virgin and cold press seed press cake flour, respectively.

Colour measurements were taken on 5 randomly selected crackers per sample batch.

Sensory evaluation

Sensory analysis of crackers was performed by semi-trained sensory panellists (28 female and 12 male, 27–50 years of age). All panellists were recruited from a staff working at the Institute of Food Technology in Novi Sad. Panellists evaluated liking of the crackers colour, taste, flavour, texture and overall liking on a 9-point structured hedonic scale where 1 indicates extreme disliking and 9 indicates extreme liking. Panellists worked in a sensory laboratory in individually sensory booths. They received two crackers per sample one at a time, in closed odourless plastic containers at ambient temperature (25±1 °C) labelled with three randomly chosen digit numbers and drinking water for palate cleansing. Before analysis all panellists received written information about the study and they signed informed consent to participate. The study was approved by the Ethics Committee of the Institute of Food Technology in Novi Sad, University of Novi Sad, Novi Sad, Serbia (Ref. No. 175/I/13-3).

Statistical analysis

All measurements were performed at least in triplicates if not stated differently. The means of replicates for analysed parameters were statistically processed by using the software package XLSTAT

2019.4.2. (*27*). Analysis of variance (ANOVA) and Fisher's least significant differences test ($p<0.05$) were used to determine significance of differences between sample means.

RESULTS AND DISCUSSION

Proximate analyses of crackers

Proximate composition of crackers is presented in Table 2. Changes in the chemical composition are in function of the gradual substitution of chickpea flour with pumpkin seed cake flours. Although tested samples showed significant differences in moisture content, the values were below 6 % which could be considered favourable for quality and stability of the produced cracker (*28*). The increase in the ratio of virgin (VF) or cold press (CF) pumpkin seed press cake flour influenced the higher content of proteins, fats and ash whereas total carbohydrates content decreased ($p<0.05$). The highest values of proteins and fats were observed for crackers with 35 % cold press (CF) pumpkin seed press cake flour. According to Regulation (EC) No. 1924/2006 (*29*), a claim that a food is "source of protein" may only be made where at least 12 % of the energy value of the food is provided by protein. According to this Regulation, the control cracker and crackers with 20 % of VF and CF can be labelled as protein source since 13.94 % (Control), 18.76 % (VF) and 18.91 % (CF) of their energy value is provided by protein. In the case of crackers with higher level of VF and CF, they may bear a claim "high protein" as their proteins provide 22.74 % (VF) and 23.98 % (CF) of the energy value. The values of total dietary fibres slightly varied between samples, and ranged from 9.17 % (20CFC) to 10.22 % (35VFC). Since all cracker samples contained more than 6 g of fibre per 100 g, they also can be labelled as "high in fibre" (*29*).

Table 2

Mineral content of crackers

Potassium was the most abundant macroelement in all cracker samples, followed by calcium and magnesium (Table 2). In comparison with gluten free crackers enriched with hemp flour and decaffeinated green tea leaves (*30*), crackers produced in this study had higher content of all tested minerals except Ca. Missbach *et al.* (*31*) found that potassium content was significantly lower in gluten free snacks than in their gluten counterparts (190.4±160 mg/100 g compared to 247.5±130 mg/100 g), while in this study potassium content was in range from 926 to 1007 mg/100 g. Substitution of chickpea flour with VF and CF significantly ($p<0.05$) altered the content of minerals. With an increase in the amount of pumpkin seed cake flours the content of almost all minerals progressively rose. The exception is Ca content where the highest content was recorded in the control sample. To declare product as a product with a significant amount of certain minerals, it must contain 15 % of Dietary Reference Values (DRV) in 100 g of product (*32*). Based on the report of European Food Safety Authority (2017) (*32*), all samples are significant source of Zn, Fe, Mg and K. Bearing in mind that gluten-free products have low amount of minerals, which can be harmful for patients suffering from celiac disease (*33*), the selection of used raw materials for crackers production is justified.

Fatty acid composition of crackers

The analysis of fatty acid profile has shown that the dominant fatty acids in all the samples were palmitic acid (C16:0), oleic acid (C18:1n9c) and linoleic acid (C18:2n6c) (Table 2). The content of linoleic acid was increased as a consequence of increasing the amount of pumpkin seed cake flours. In the case of alpha linolenic acid, the presence of CF in the cracker formulation affected its reduction. According to WHO/FAO in balanced died ratio of PUFA/SFA should been over 0.4. In all samples ratio were over 0.4, which indicates that choice of ingredients contributes to favourable fatty acid composition. Considering the MUFA intake, the Adult Treatment Panel III (2004) (*34*) recommended up to 20 % of energy intake as MUFA. Regarding the obtained results for fatty acid composition, the content of MUFA is in accordance with proposed recommendation.

Total phenolic content (TPC) and antioxidant activity

Polyphenols, secondary plant metabolites, could possess different biological activities such as ability to inhibit reactive oxygen and nitrogen species, oxidative enzymes, to activate antioxidant enzymes and could act as free radical scavengers (*35*). Values of TPC were in the range from 0.72 mg GAE/g for Control sample to 1.06 mg GAE/g for sample 35CFC (Table 3). The pumpkin oil cake incorporated crackers showed significant ($p<0.05$) increase in the TPC.

The antioxidant activity of the formulated crackers determined by ABTS assay revealed a similar trend to that found in the Folin-Ciocalteu assay (Table 3). The incorporation of pumpkin seed press cake flours resulted in higher values of antioxidant activity ranging from 11.5 mM TE/g to 17.0 mM TE/g. The higher values of antioxidant activity in the 35CFC might resulted from the initial presence of phenolic compounds in the cold pressed cake, while in the virgin pressed cake the amount of phenolic compound, which are known to be thermolabile components, can be reduced due to the thermal treatment of the seeds before oil extraction. In this respect, the pumpkin seed press cake flour inclusion in cracker formulation enhanced their antioxidant potential which could be beneficial for stability against oxidative damage during storage.

Table 3

Glycemic index of crackers

Compared to their gluten containing counterparts, gluten free baked products are often considered as products with high glycemic index. This is due to the prevalent of carbohydrate components of commonly used gluten free flours such as rice and maize flour. Moreover, there is a theoretical presumption that in the absence of gluten protein network that usually surrounds the starch granules, the starch granules become more susceptible to action of amylolytic enzymes which additionally contribute to the higher glycemic index (*36*,*37*). Considering the complex matrix characteristics of the bakery products, in addition to above, glycemic index are highly influenced by several factors such as type of starch (the amylose/amylopectin ratio), the physical entrapment of starch molecules within food, food formulations and processing, and by the present of other ingredients, such as sugars, fat, protein, dietary fiber and anti-nutrient content (*5,36,38,39*). Accordingly, it is of great importance to introduce raw materials with potential of reducing glycemic index in gluten free products manufacturing. The starch hydrolysis curves during the second intestinal phase of *in vitro* digestion of all produced crackers along with white bread are presented in Fig. 1. The results of hydrolysis index (HI), calculated from the starch hydrolysis curves, and the corresponding predicted glycemic index (pGI) are shown in Table 3. Glucose concentration increased during *in vitro* digestion for all samples, presenting a sharper growth during the first 30 min. As expected, the maximum glucose concentration was recorded in white bread which served as a benchmark while all created crackers had significantly lower glucose concentration where maximum occurred at 60 min and remained almost at the same level during the all examined digestion time. According to available literature data, the predicted glycemic index for gluten free cookies and crackers with addition of pulses flours ranged from low (<55) to intermediate (55–70) (*5,40*). The control cracker presented a glycemic index of 67.8±0.3 while substitution of chickpea flour by pumpkin seed press cake flours at both levels (20 and 35 %) significantly ($p<0.05$) reduced cracker glycemic index to approximately 60. The results obtained in this study showed that the selected combination of raw materials used in this study is feasible to obtain gluten-free crackers with a moderate glycemic index (*41*) where the use of flour from pumpkin seed cakes allows a further reduction in the predicted glycemic index.

Fig. 1

Physical properties of crackers

Eccentricity, spread factor and puffiness

Physical properties of crackers are summarized in Table 4. There were no significant differences between eccentricity and spread factor of samples. However, an increase in spread factor values is observed with substitution of chickpea flour with VF and CF. Compared to the Control sample, crackers substituted with VF and CF have higher mass values. Furthermore, addition of VF and CF affected the puffiness of samples. Significant dimensional changes are typical in the formulation of cookies with wheat flour and composite blends as well as in gluten free formulations for cookies based on rice or maize flours as a consequence of incorporation of high protein raw materials (*15,42*). Since the all tested samples had a high protein and fibre content it can be assumed that used raw materials caused a higher amount of water retention in the dough system, which limited the free water and thus did not affect significant changes in dimensional parameters. This is in accordance with the research of Mancebo *et al.* (*43*), where presence of flours with higher protein content in gluten free formulations had no significant influence on spread ratio of cookies. Although a certain increase in cookies diameter and spread ratio is desirable for better quality (*42*), the advantage of the uniform cookies dimensions could be reflected in the simplified control of the products packaging.

Table 4

Colour of crackers

Crackers containing pumpkin seed press cake flour, regardless of type, possessed unusual colour for this type of product, as can be observed from the Fig. S1. Regarding the lightness parameter *L**, a reduction with increasing VF and CF concentration can be observed (Table 4). Parameters *a** and *b** have also been reduced, indicating an increase in green colour and decrease in the yellowness of biscuits. The green colour of pumpkin seed press cake flours derives from protochlorophyll found in chlorenchyma, the dark green layer around cotyledons of pumpkin cultivar *Cucurbita pepo* (*44*). The results showed that VF containing crackers were more red and less yellow in comparison to CF containing crackers. This could be related to the higher pigment degradation with the seed thermal treatment before oil extraction and later crackers baking process. The total colour difference (ΔE) was highly influenced by the amount of pumpkin seed press cake flours.

Hardness and fracturability of crackers

Textural properties of crackers expressed as hardness (maximum peak force recorded from force/distance curve) and fracturability (peak distance which represents the distance the cracker will deform before breaking) are listed in Table 4. According to obtained results partial replacement of chickpea flour with CF did not influence cracker texture although there was significant difference in crackers composition in terms of protein and starch content (Table 2). The similar hardness and fracturability of Control and CFC crackers were probably influenced with the combined effect of gelatinized starch granules embedded in the protein matrix, where the former were more dominant in Control sample while the latter contributed to texture formation in CFC samples. On the contrary, incorporation of VF led to an increase in cracker hardness and decrease in its fracturability. Unlike CF, VF passed through thermal treatment during oil extraction which influenced protein denaturation to higher degree and consequently led to higher water retention in VFC cracker dough (*45*). Higher content of retained water in VFC formulations in comparison to CFC produced stiffer doughs and subsequently harder crackers (*46*). However, no significant difference in crackers hardness and fracturability was observed with the increase in both VF and CF replacement level.

Sensory analysis (overall liking, colour, taste, texture and flavour) of crackers

Results of sensory study showed that all sensory scores for crackers were in the range from like slightly to like very much (Fig. 2). Replacement of chickpea flour with VF slightly decreased while replacement with CF significantly increased overall liking of crackers, especially in terms of taste and flavour. Cracker 35CFC was superior in terms of all analysed sensory properties, followed by the 20CFC. Cracker 35VFC was the least acceptable. In their study, Kaur and Sharma (*47*) showed that cookies supplemented with raw pumpkin seed flour were more acceptable in comparison with the cookies supplemented with roasted pumpkin seed flour which is in agreement with the results presented in this study. Although colour differences between samples were clearly visible, the colour liking between samples was not significant ($p<0.05$), except between samples 35VFC and 35CFC ($p>0.05$). Liking scores were in the range from “slight liking” (5.80) for 35VFC to “like very much” (7.50) for 35CFC. Han *et al.* (1) studied the effect of various pulse flours and fractions (desi chickpea, green lentil, red lentil, pinto bean, navy bean, and yellow pea) on development of gluten-free pulse-based cracker snacks, and similar to our findings, they found no statistical difference in colour acceptability scores that were in the range from 6.0 (for crackers containing navy bean flour) to 6.7 (for crackers containing pea protein isolate). Moreover, they concluded that within the evaluated range of samples, the colour was not barrier to product acceptability.

Fig. 2

CONCLUSIONS

The present study revealed that the combination of chickpea and pumpkin seed press cake flours could be successfully utilised in gluten-free crackers production without using conventional gluten-free starch-rich ingredients. This selected raw materials had a multiple benefits which are reflected in increased nutritional quality of final product, including increased protein and dietary fibre content, improved mineral and fatty-acid profile, enhanced total phenolic content and antioxidant activity compared to the control sample. The selected combination of raw materials used in this study allows the production of gluten-free crackers with a moderate glycemic index. Moreover, it was shown that cold press pumpkin seed cake would perform better than virgin pumpkin seed cake in terms of product texture and overall acceptability. Further research should be focused on the crackers shelf life assessment but also on the safety aspects of this novel food product.

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CONFLICT OF INTEREST

The manuscript is original, and it strictly followed all ethical procedures. No part of the manuscript has been published before, nor is any part of it under consideration for publication in another journal. The authors declare that there is not any conflict of interest.

SUPPLEMENTARY MATERIAL

All supplementary materials are available at: www.ftb.com.hr.

AUTHORS' CONTRIBUTION

Jelena Tomić was in charge of the conceptualization, formal analysis, writing the original draft and investigation. Dubravka Škrobot was in charge of the conceptualization, formal analysis, writing the original draft. Ljiljana Popović was in charge of the investigation, writing the original draft. Tamara

Dapčević Hadnađev was in charge of the supervision and writing the original draft. Jelena Čakarević was in charge of investigation and writing the original draft. Nikola Maravić was in charge of the formal analysis and investigation. Miroslav Hadnađev was in charge of the resources, supervision and project administration.

ORCID ID

J. Tomić https://orcid.org/0000-0002-8674-6932

D. Škrobot https://orcid.org/0000-0003-3292-8738

Lj. Popović https://orcid.org/0000-0001-9264-285X

T. Dapčević-Hadnađev https://orcid.org/0000-0001-6222-2889

J. Čakarević https://orcid.org/0000-0002-8446-1722

N. Maravić https://orcid.org/0000-0001-9926-7802

M. Hadnađev https://orcid.org/0000-0002-0055-5642

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Table 1. Formulation of crackers

Sample/raw material	Chickpea flour *w*/%	Virgin pumpkin seed press cake flour (VF) *w*/%	Cold press pumpkin seed press cake flour (CF) *w*/%	Vegetable fat *w*/%	Salt *w*/%	Soy lecithin *w*/%	Baking powder *w*/%	Water *w*/%
Control	100	-	-	25	2.5	0.5	0.5	40
20VFC	80	20	-	25	2.5	0.5	0.5	40
35VFC	65	35	-	25	2.5	0.5	0.5	40
20CFC	80	-	20	25	2.5	0.5	0.5	40
35CFC	65	-	35	25	2.5	0.5	0.5	40

Table 2. Proximate composition, mineral content and fatty acid composition of crackers

Parameters	Control	20VFC	35VFC	20CFC	35CFC
Proximat composition w/%					
Moisture	(4.62±0.06)[d]	(5.12±0.04)[b]	(3.99±0.03)[e]	(5.61±0.06)[a]	(5.00±0.03)[c]
Carbohydrates	(42.2±0.2)[a]	(35.2±0.4)[b]	(28.8±0.0)[c]	(34.9±0.2)[b]	(26.0±0.4)[d]
Crude Fat	(24.3±0.1)[d]	(24.8±0.1)[c]	(26.1±0.1)[b]	(24.9±0.1)[c]	(26.8±0.1)[a]
Protein	(16.5±0.1)[d]	(22.2±0.1)[c]	(27.3±0.0)[b]	(22.3±0.1)[c]	(28.7±0.1)[a]
Total dietary fibre	(9.95±0.13)[a]	(9.61±0.10)[b]	(10.2±0.2)[a]	(9.17±0.07)[c]	(9.42±0.17)[bc]
Ash	(2.47±0.03)[e]	(3.06±0.01)[d]	(3.67±0.03)[b]	(3.14±0.03)[c]	(3.99±0.02)[a]
Energy/(kcal/kJ)	473/1976	472/1971	479/2000	471/1967	479/1999
*Mineral content w/(*mg/100g*)*					
Zn	(3.00±0.04)[d]	(4.76±0.08)[c]	(5.90±0.06)[b]	(4.91±0.05)[c]	(6.17±0.13)[a]
Fe	(4.02±0.09)[d]	(4.67±0.11)[b]	(5.51±0.06)[ab]	(5.21±0.13)[b]	(5.79±0.24)[a]
Mg	(103±0)[e]	(170±4)[d]	(221±6)[b]	(181±3)[c]	(265±6)[a]
K	(926±17)[c]	(932±1)[bc]	(952±9)[b]	(986±11)[a]	(1007±5)[a]
Na	(810±16)[c]	(904±2)[b]	(1034±3)[a]	(827±1)[c]	(832±15)[c]
Ca	(32.4±0.5)[a]	(20.5±1.0)[b]	(17.5±0.0)[c]	(20.4±0.5)[b]	(16.0±0.2)[c]
Fatty acids composition w/%					
Lauric acid (C12:0)	(0.18±0,02)[ab]	(0.18±0.01)[ab]	(0.17±0.02)[ab]	(0.16±0.01)[b]	(0.19±0.01)[a]
Myristic acid (C14:0)	(0.79±0.01)[a]	(0.77±0.01)[bc]	(0.75±0.01)[cd]	(0.74±0.01)[d]	(0.77±0.01)[b]
Palmitic acid (C16:0)	(36.8±0.1)[a]	(35.6±0.0)[c]	(34.9±0.0)[e]	(36.0±0.0)[b]	(35.5±0.0)[d]

Parameters	Control	20VFC	35VFC	20CFC	35CFC
Palmitoleic acid (C16:1)	(0.18±0.01)[a]	(0.17±0.02)[a]	(0.17±0.01)[a]	(0.16±0.01)[a]	(0.14±0.01)[b]
Stearic acid (C18:0)	(4.21±0.02)[c]	(4.55±0.03)[b]	(4.72±0.02)[a]	(4.56±0.01)[b]	(4.74±0.02)[a]
Oleic acid (C18:1n9c)	(38.3±0.1)[c]	(38.6±0.1)[ab]	(38.7±0.1)[a]	(38.5±0.1)[abc]	(38.4±0.1)[bc]
Linoleic acid (C18:2n6c)	(18.0±0.1)[d]	(18.6±0.1)[b]	(18.9±0.1)[a]	(18.5±0.0)[c]	(18.9±0.1)[a]
Arachidic acid (C20:0)	(0.44±0.01)[b]	(0.44±0.01)[b]	(0.52±0.02)[a]	(0.46±0.01)[b]	(0.46±0.01)[b]
α-Linolenic acid (C18:3n3)	(0.77±0.03)[a]	(0.74±0.01)[a]	(0.74±0.01)[a]	(0.68±0.02)[b]	(0.67±0.01)[b]
Eicosanoic acid (C20:1n9)	(0.26±0.01)[bc]	(0.33±0.01)[a]	(0.26±0.01)[b]	(0.24±0.02)[c]	(0.25±0.00)[bc]
Total SFA	42.5	41.6	41.1	41.9	41.7
Total MUFA	38.8	39.1	39.2	38.9	38.8
Total PUFA	18.8	19.4	19.7	19.1	19.5
PUFA/SFA	0.44	0.47	0.48	0.46	0.47

Control=chickpea crackers, 20VFC and 35VFC=crackers in which 20 % or 35 % of chickpea flour was replaced with seed press cake flour remaining after Virgin pumpkin oil extraction, 20CFC and 35CFC=cookies in which chickpea flour was replaced with seed press cake flour remaining after cold pressed oil extraction, SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids. Values are presented as mean (n=3) ± standard deviations. Means in the same row with different superscript are statistically different ($p \leq 0.05$) according to Fisher (LSD) test

Table 3. Total phenolic content, antioxidant activity, hydrolysis index and predicted glycemic index of crackers

Parameters	Control	20VFC	35VFC	20CFC	35CFC
Total antioxidant activity (*c*(Trolox)/*m*(sample))/(mM/g)	(7.96±0.35)[d]	(12.0±0.2)[b]	(11.9±0.2)[b]	(11.5±0.3)[b]	(17.0±0.4)[a]
Total phenolic compounds *w*(galic acid)/(mg/g)	(0.72±0.00)[d]	(0.84±0.00)[bc]	(0.82±0.03)[c]	(0.88±0.01)[b]	(1.06±0.00)[a]
Hydrolysis index	(51.2±0.1)[a]	(38.8±0.3)[d]	(36.8±0.1)[e]	(40.1±0.2)[b]	(39.5±0.4)[c]
Predicted glycemic index	(67.8±0.1)[a]	(61.0±0.6)[b]	(59.9±0.1)[c]	(61.7±0.5)[b]	(61.4±0.3)[b]

Control=chickpea crackers, 20VFC and 35VFC=crackers in which 20 % or 35 % of chickpea flour was replaced with seed press cake flour remaining after Virgin pumpkin oil extraction, 20CFC and 35CFC=cookies in which chickpea flour was replaced with seed press cake flour remaining after cold pressed oil extraction. Values are presented as mean (n=3) ± standard deviations. Means in the same row with different superscript are statistically different ($p \leq 0.05$) according to Fisher (LSD) test

Table 4. Physical properties of crackers

Parameters	Control	20VFC	35VFC	20CFC	35CFC
Mass/g	(4.85±0.05)^{b}	(4.89±0.06)^{ab}	(4.98±0.08)^{a}	(4.98±0.10)^{a}	(4.95±0.08)^{ab}
Eccentricity/%	(1.00±0.01)^{a}	(0.99±0.00)^{a}	(0.99±0.01)^{a}	(1.00±0.01)^{a}	(0.99±0.00)^{a}
Spread factor/%	(9.92±0.23)^{a}	(10.1±0.2)^{a}	(10.1±0.1)^{a}	(9.94±0.10)^{a}	(9.98±0.06)^{a}
Puffiness/%	(28.9±3.4)^{a}	(25.2±4.5)^{ab}	(23.4±3.4)^{ab}	(21.6±8.0)^{b}	(23.8±3.8)^{ab}
Colour properties					
*L**	(69.0±0.3)^{a}	(50.5±0.7)^{d}	(46.2±0.5)^{e}	(57.1±0.7)^{b}	(51.4±0.9)^{c}
*a**	(5.18±0.15)^{a}	(4.18±0.21)^{b}	(4.38±0.15)^{b}	(1.90±0.23)^{c}	(1.18±0.25)^{c}
*b**	(34.6±0.3)^{a}	(19.1±0.7)^{c}	(15.1±0.6)^{d}	(23.9±1.0)^{b}	(18.7±0.8)^{c}
ΔE		(24.2±1.0)^{b}	(30.0±0.8)^{a}	(16.4±1.2)^{c}	(23.9±1.2)^{b}
Textural properties					
Hardness/kg	(2.79±0.67)^{ab}	(3.30±0.51)^{ab}	(3.46±0.44)^{a}	(2.65±0.51)^{b}	(2.96±0.48)^{ab}
Fracturability/mm	(2.15±0.31)^{a}	(1.04±0.10)^{b}	(0.95±0.09)^{b}	(2.22±0.22)^{a}	(2.02±0.25)^{a}

Control=chickpea crackers, 20VFC and 35VFC=crackers in which 20 % or 35 % of chickpea flour was replaced with seed press cake flour remaining after Virgin pumpkin oil extraction, 20CFC and 35CFC=cookies in which chickpea flour was replaced with seed press cake flour remaining after cold pressed oil extraction, *L**=lightness/darkness, *a**=redness/greenness, *b**=yellowness/blueness, ΔE=total colour difference. Values are presented as mean (n=3) ± standard deviations. Means in the same row with different superscript are statistically different (p ≤ 0.05) according to Fisher (LSD) test

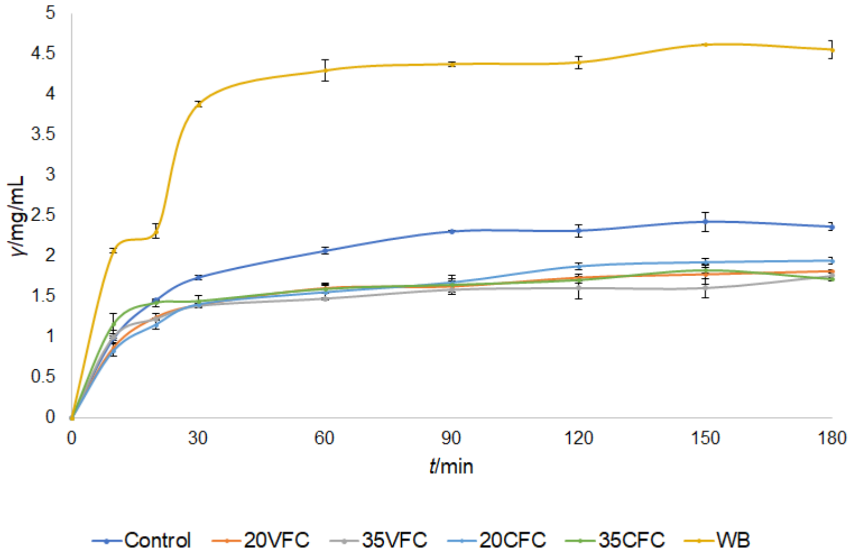

Fig. 1. Glucose concentration as a function of time during the second intestinal phase, relevant to examined crackers

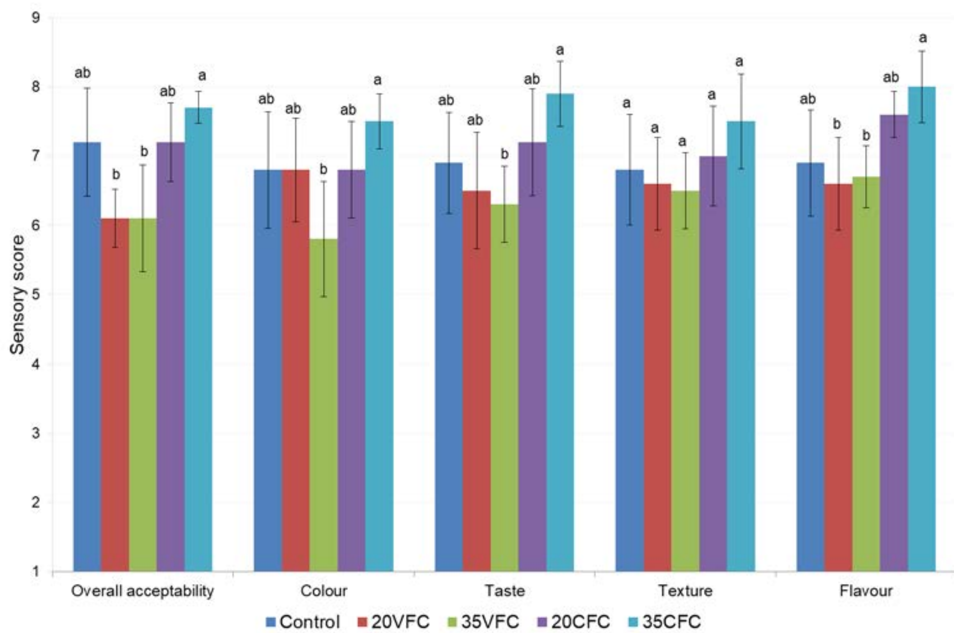

Fig. 2. Sensory evaluation scores for overall liking, liking of colour, taste, texture and flavour of gluten-free crackers based on chickpea (Control), chickpea and virgin pumpkin seed press cake

flour blend (20VFC and 35VFC) and chickpea and cold pressed pumpkin seed press cake flour blend (20CFC and 35CFC)

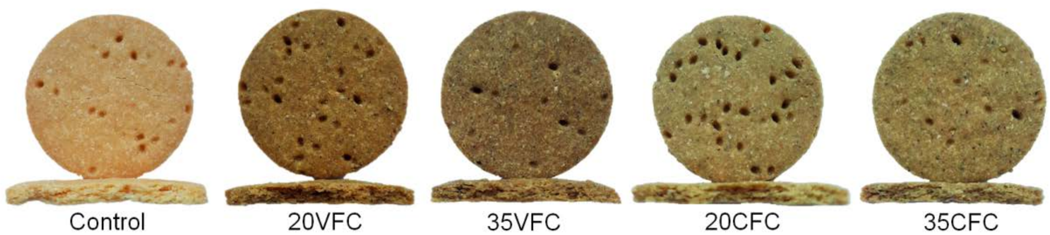

Fig. S1. Appearance of the gluten-free crackers based on chickpea (Control), chickpea and virgin pumpkin seed press cake flour blend (20VFC and 35VFC) and chickpea and cold pressed pumpkin seed press cake flour blend (20CFC and 35CFC)