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Effect of the pumpkin seed flour and pumpkin seed oil cake flour addition on quality of wheat bread

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Abstract: Pumpkin seeds and their oil cake are often treated as by-products, but their composition and high nutritive and biological value makes them a promising material for nutritional enrichment of bakery products. The purpose of this study was to characterise pumpkin seed flour (PSF) and pumpkin seed oil cake flour (PSOCF), and evaluate the effects of their addition on the chemical composition including the fatty acid composition, textural parameters, sensorial characteristics and antioxidant activity of the wheat bread. Six experimental bread samples with 5, 10 and 15% of PSF or PSOCF added to wheat basic formulation, and a control wheat sample, were produced. Results indicated that addition of PSF and PSOCF led to higher ash, cellulose, protein, fat, total carotene and unsaturated fats content, which means that the experimental bread samples had a higher nutritional value. PSF addition had more positive effect on the textural characteristics and antioxidant activity of bread, than PSOCF addition. All bread samples (evaluated by 5-point scale multiplied with the coefficient of importance) had very high sensory quality (89.88–96.59% of maximal possible quality). The results confirmed that PSF and PSOCF are suitable for use in food industry for nutritional enrichment of wheat bread and related products.

Keywords: wheat bakery products; seeds; pumpkin; chemical composition; product quality

Bakery products fortification with nutritionally valuable ingredients of plant origin is highly recommended (Eren and Akkaya 2024). Bread is consumed worldwide, because of its nutritive value, simplicity to use and low price, and in many countries, it is a complete meal rather than a complementary food and because of that it is ideal food for fortification. Lately, seeds and nuts have been recognised as plant additives of great importance, because of their bioactive components, with special reference to pumpkin seeds (Dotto and Chacha 2020). Today, pumpkin is among the most important agricultural crops in the world because it can grow

in different agroclimatic conditions and it has very wide and diverse use in agricultural, food, pharmaceutical and animal feed industry. Different species of pumpkin are all in the genus *Cucurbita* (Lestari and Meiyanto 2018), but not all *Cucurbita* species are used for food purposes. Common field pumpkin (*Cucurbita pepo* L.) is most commonly used for culinary use (bread, pie, desserts, biscuits, soups and beverages) and for decoration purposes (festivals, flower and vegetable shows). It is characterised by numerous varieties and forms, including pumpkin oil with two forms (with seed coat and without seed coat – so called 'golica'). Almost all of the

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parts of pumpkin can be used in food industry. Pumpkin seeds are used in production of oils with very high nutritive quality recognised and appreciated in food industry, pharmacy and traditional medicine. Pumpkin seeds, with their unique taste, are used as snack food (salted, roasted, toasted) or ground and used as a flour ingredient or food additives. Although pumpkin seeds were generally considered as agricultural or industrial waste, many studies shows that they can have very important role in disease prevention and health promotion (Dotto and Chacha 2020). They can be eaten every day as an excellent source of lipids, proteins, dietary fibres, minerals and phytonutrients (Jahan et al. 2023). They have high levels of tocopherols, carotenoids, saponins, pyrazine, phytosterols, triterpenoids, unsaturated fatty acids, phenolic components, flavonoids and minerals (Dotto and Chacha 2020). Because of the presence of these bioactive components, pumpkin seeds have several health benefits such as: reduction of the risk of cancer occurrence and infections, lowering blood cholesterol and glucose levels, lowering the depression and complications associated with oxidative stress, prevention of prostate and urinary bladder disorders, hepatoprotective properties (Dotto and Chacha 2020). Also, pumpkin seeds, because of their high content of beta-carotene, can help prevent skin diseases and support vision. During the production of pumpkin oil, through the extraction process from pumpkin seeds, oil cake remains as by-product with significant nutritional value. Pumpkin seed oil cake (PSOC) is mainly used as animal feed, because of its chemical composition rich in nutrients (moisture content 5.5–8.2%, crude protein content 45.6–52.3%, crude fat content 11.7–19.4%, crude cellulose content 2.2–4.8%, ash content 8.1–8.7%) (Bochkarev et al. 2016). Considering that pumpkin oil production is growing more and more, it would be effective to use PSOC not as a by-product but as an enriching ingredient for food production, viewed from the perspective of the sustainable side of food industry (Jeevitha and Bhuvana 2019). PSOC can be considered as a valuable raw material, rich in high quality proteins, oil and dietary fibres, which can lead to the production of nutritionally superior bread.

The aim of this study was to characterise pumpkin seed flour (PSF) and pumpkin seed oil cake flour (PSOCF), and evaluate the effects of their addition at different levels in the wheat flour (WF), on the chemical composition, nutritive and sensory quality of the WF bread. The purpose of this study was to deepen the knowledge related to the use of pumpkin and its by-products for the production of nutritionally enriched bakery products.

MATERIAL AND METHODS

Material

Pumpkin seeds and pumpkin seed oil cake flour, was kindly supplied by PZ Agrojapra [Bosnia and Hercegovina (B&H)]. Commercial cultivar of oil seed pumpkin (*Cucurbita pepo* subsp. var. *styriaca*), traditional type 'Gleisdorfer Ölkürbis' (Austrian origin), were used for oil production and obtaining by-products. Variety 'Gleisdorfer Ölkürbis' was chosen because it is grown locally and for its unique hull-less seeds, which make them very suitable for oil extraction (elimination of dehulling operation can significantly reduce the costs and complexity of oil production). Pumpkin cultivation was conducted at the location Crkvina, Šamac, B&H (45° 01' 07.2" N, 18° 24' 05.9" E), according to the established cultivation technology. The seeds were grounded in a laboratory mill Knifetec 1095 (Foss Tecator, Sweden) immediately before bread making. For the preparation of the bread samples, the following ingredients were used: wheat flour (WF, type 500) ('Mlinpek-Žitar' d.d., Bravnice b.b., B&H); pumpkin seeds flour (PSF) and pumpkin seed oil cake flour (PSOCF) (PZ 'Agrojapra', B&H); water from public water supply system; yeast 'Digo' (Lessafre Adriatic d.o.o., Hungary); table salt ('Solana' d.d., B&H); bakery improver 'Hit' with the following composition: soy flour, anti-clumping agent E170, emulsifier E472e and E471, dextrose, puffed wheat flour, agents for flour treatment E-300 and E341, enzymes ('IREX AROMA' d.o.o., Croatia). Commercial wheat flour, yeast, salt and improver were purchased from local markets in Banja Luka, B&H. The effects of 5, 10 and 15% PSF and PSOCF addition (related to WF weight) on the chemical composition, nutritive and sensory quality of the WF bread were evaluated. The PSF and PSOCF addition selection of 5, 10 and 15% were based on previous studies (Malkanathi et al. 2018, Wahyono et al. 2018, Jeevitha and Bhuvana 2019, Shevchenko et al. 2022). Bread samples are made according to the formulations given in Table 1.

Bread making

Bread samples were produced according to the traditional wheat bread making technology in bakery within the public institution 'Agricultural School' Banja Luka, through the following stages: the flour and other ingredients mixing in a spiral mixer IS 50 (MF Italy, Italy) for 4 min on low speed, 6 min on high speed, dough proofing for 10 min at room temperature, manual dough dividing (350 ± 2 g), manual rounding, round dough pieces proofing for 10 min at room temperature, final manual

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Table 1. The formulations of bread samples (% of ingredients on the flour basis)

| Bread samples | Ingredients (%) | | | | | | |
|---------------|-----------------|-----|-------|-------|-------|------|----------|
| | WF | PSF | PSOCF | water | yeast | salt | improver |
| Control | 100 | 0 | 0 | 55 | 3 | 2 | 0.5 |
| PSOCF 5% | 100 | 0 | 5 | 55.5 | 3 | 2 | 0.5 |
| PSOCF 10% | 100 | 0 | 10 | 57.5 | 3 | 2 | 0.5 |
| PSOCF 15% | 100 | 0 | 15 | 60 | 3 | 2 | 0.5 |
| PSF 5% | 100 | 5 | 0 | 57.5 | 3 | 2 | 0.5 |
| PSF 10% | 100 | 10 | 0 | 59.5 | 3 | 2 | 0.5 |
| PSF 15% | 100 | 15 | 0 | 60 | 3 | 2 | 0.5 |

WF – wheat flour; PSOCF – pumpkin seed oil cake flour; PSF – pumpkin seed flour; PSOCF 5% – bread sample with 5% added PSOCF; PSOCF 10% – bread sample with 10% added PSOCF; PSOCF 15% – bread sample with 15% added PSOCF; PSF 5% – bread sample with 5% added PSF; PSF 10% – bread sample with 10% added PSF; PSF 15% – bread sample with 15% added PSF

loaf shaping, final proofing for 45 ± 5 min in fermenting chamber (on 45°C and 80% humidity), baking in an electrical convection oven with steam production (Wiesheu GmbH, Germany) for 15 ± 2 min at $220 \pm 10^\circ\text{C}$, cooling on room temperature and then packaging in paper bags.

Determination of chemical properties of bread

Proximate chemical composition. The chemical composition of the flour and bread was determined using the international standardised method: moisture, ash, protein and fat content were determined according to the AOAC methods (2000). Moisture content was determined according to AOAC International Method 925.10; ash content according to AOAC 923.03; protein content according to AOAC 920.87 ($\text{N} \times 6.25$); and fat content according to AOAC 945.16. Cellulose content was determined by using the L. Bellucci method (Kaluderski and Filipović 1990). Available carbohydrates content (expressed in %) was determined by calculating the difference between the value of 100 and the sum of moisture, ash, lipids, proteins and cellulose levels. Total carotene content (TCC) was estimated following the method of Jahan et al. (2023), with results expressed as mg of TCC per 100 g of sample. All proximate analysis were performed in triplicate and results were showed as mean value \pm SD.

Fatty acid composition analysis. The fatty acid composition was determined using gas chromatography (GC) of fatty acid methyl esters (FAME), dissolving samples in hexane and by transesterification with saturated KOH/methanol solution (David et al. 2005). Instrument that has been used for fatty acid analysis was gas chromatograph Clarus 680 (Perkin Elmer, USA) with FID detector, column Elite-wax L 60 m, ID 0.32, DF 0.5, with standard Sigma Aldrich Supelco 37 components FAME MIX (Supelco, Sigma Aldrich, a brand of Merck KGaA, Germany).

Temperature regime: 60°C for 2 min, $10^\circ\text{C}\cdot\text{min}^{-1}$ up to 200°C , $5^\circ\text{C}\cdot\text{min}^{-1}$ up to 240°C , and 30 min at 240°C , injector and detector temperature 250°C , sample volume $1\ \mu\text{L}$, flow $1.5\ \text{mL}\cdot\text{min}^{-1}$, and nitrogen gas was carrier. Fatty acids composition was expressed as a percentage of individual fatty acids in the total amount of total identified fatty acids [$\text{g}\cdot(100\ \text{g})^{-1}$ of total fatty acids].

Antioxidant activity analysis

Ultrasound-assisted extraction with 70% ethanol (solid to liquid ratio 1 : 10) was used as a preparative method given by Vasileva et al. (2018), with optimised time and phase ratio. Prior to extraction bread samples were sliced (1.5 cm thick), dried (24 h, at 40°C) and ground in a laboratory mill. Ethanol extracts were obtained in the ultrasonic bath Witeg WUC-A-03H (Witeg Labortechnik GmbH, Germany) for 20 min at 45°C . Liquid extracts were filtered and used for further analysis. Determination of total phenolic content (TPC) in bread samples was done by Folin-Ciocalteu method with some modifications (Wolfe et al. 2003). Exactly 0.2 mL of bread extract was mixed with 1.5 mL of 7.5% of sodium hydrogen carbonate solution and 1.5 mL of 0.2M Folin-Ciocalteu reagent, and the mixture reacted for 30 min in dark and on room temperature. The absorbance was measured at 765 nm wavelength using the spectrophotometer Milton Roy, Spectronic 1201 UV-VIS (Milton Roy Company, USA) against the blank. The values of total phenolic content were expressed as gallic acid equivalents (mg GAE) per gram of sample ($\text{mg GAE}\cdot\text{g}^{-1}$). Antioxidant activity was determined by using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) method narrated by Liyana-Pathiranan and Shahidi (2005). Trolox was used as a standard for calibration curve, plotting DPPH scavenging activity (%)

versus Trolox concentration ($\text{mmol}\cdot\text{L}^{-1}$) and the following linear equation was obtained: $y = 1.7466x - 2.8742$. Antioxidant activity was also determined by using 2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt radical (ABTS) method given by Re et al. (1999). Standard Trolox was used to create a calibration curve ABTS scavenging activity (%) versus Trolox concentration ($\text{mmol}\cdot\text{L}^{-1}$) and the following linear equation was obtained: $y = 5.1479x - 5.5997$. The results of DPPH and ABTS analysis were expressed as mmol Trolox equivalents (TE) per gram of sample ($\text{mmol TE}\cdot\text{g}^{-1}$).

Determination of physical properties of bread

Bread texture. Profile analysis (TPA) was carried out 24 h after baking, using a texture analyser TA-Xt plus (Stable Microsystems, UK), Texture Expert software and plane cylindrical aluminium probe P/75. Bread pieces for TPA, with 35 mm in diameter, were cut out from 25 mm thick bread slices. Bread pieces were compressed with the double compression method, with the following settings: pre-test speed of $1.0\text{ mm}\cdot\text{sec}^{-1}$, test speed of $5\text{ mm}\cdot\text{sec}^{-1}$, post-test speed of $5\text{ mm}\cdot\text{sec}^{-1}$, 40% of strain, trigger force was 5 g, time between the two compressions was 5 s. The parameters recorded by TPA were: hardness, springiness, chewiness, cohesiveness and resilience.

Descriptive sensory analysis

The descriptive sensory analysis of bread quality was conducted 24 h after baking, by 17 trained panellists, students and teachers from the Faculty of Technology, University of Banja Luka (B&H). The analysed sensory attributes included: volume and shape, external appearance, appearance of cross-section, aroma and taste. The panellists used 5-point scoring system to assess the bread samples' quality level (points were in the range of 1 – unacceptable to 5 – expected). The scoring was based on national regulations for sensory assessment of bread quality and ISO standards for food sensory analysis. Sum of corrected values (value multiplied with the coefficient of importance – CI) is expressed in %

and called 'percentage of max level of quality' (shape and volume – CI 4, external appearance – CI 3, appearance of cross-section – CI 5, aroma of bread – CI 3, taste of bread – CI 5). Results of calculated percentage of max level of quality were classified as follows: < 40% as very bad; 40–49% as bad; 50–59% as poor; 60–69% as satisfactory; 70–79% as good; 80–89% as very good; 90–100% as excellent (Grujić 2015).

Statistical analysis

Data are expressed as mean \pm SD using three replicates ($n = 3$). Statistical analysis was performed using the software package IBM SPSS Statistics 26 (USA). One-way analysis of variance (ANOVA) and Tukey post-hoc test was used to evaluate and assess significant differences between samples, at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical and antioxidant properties. The chemical composition of ingredients, especially flour, is the main factor that can have an impact in the development of formulations for new bakery product.

As presented in Table 2, PSOCF contained 15.6 times more ash, 4.6 times more protein, 11.5 times more fat and 16.7 times more cellulose than WF. Findings regarding the chemical composition of WF are similar to results of Shevchenko et al. (2022). Results for chemical analysis of PSOCF were similar to the results of Bochkarev et al. (2016). Also, PSF contained 9.2 times more ash, 2.7 times more protein, 41.2 times more fat and 17.3 times more cellulose than WF (Table 2), which is similar to the ash and protein content reported in Shevchenko et al. (2022) study. Chemical composition of PSF was in accordance with the basic nutritional composition of pumpkin seed in study of Batool et al. (2022).

According to the results of the proximate composition of bread samples shown in Table 3, with increasing PSOCF and PSF proportions in bread formulations, a significant increase in the content of evaluated chemical

Table 2. Chemical composition of WF, PSOCF and PSF

| Flour samples | Content of chemical components (%) | | | | | |
|---------------|------------------------------------|-------------------|--------------------|--------------------|-------------------|-------|
| | moisture | ash | protein | fat | cellulose | ACH |
| WF | 12.07 ± 0.02^c | 0.51 ± 0.01^a | 11.94 ± 0.10^a | 1.08 ± 0.00^a | 0.18 ± 0.01^a | 74.22 |
| PSOCF | 8.10 ± 0.04^b | 7.96 ± 0.01^b | 54.81 ± 0.05^c | 12.45 ± 0.01^b | 3.00 ± 0.07^b | 13.68 |
| PSF | 6.34 ± 0.01^a | 4.69 ± 0.01^b | 32.78 ± 0.13^b | 44.50 ± 0.26^c | 3.11 ± 0.13^b | 8.58 |

^{a-c}different letters in the same column indicate significant difference ($P < 0.05$); values are expressed as mean \pm SD ($n = 3$), except value for ACH

ACH – available carbohydrates; PSF – pumpkin seed flour; PSOCF – pumpkin seed oil cake flour; WF – wheat flour

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Table 3. Chemical composition of bread samples

| Bread sample | Content of chemical components (%) | | | | | |
|--------------|------------------------------------|---------------------------|---------------------------|---------------------------|--------------------------|-------|
| | moisture | ash | protein | fat | cellulose | ACH |
| Control | 34.70 ± 0.09 ^e | 1.96 ± 0.01 ^a | 7.79 ± 0.11 ^a | 0.12 ± 0.01 ^a | 0.25 ± 0.02 ^a | 55.18 |
| PSOCF 5% | 31.21 ± 0.06 ^c | 2.26 ± 0.03 ^{bc} | 11.21 ± 0.07 ^c | 0.28 ± 0.01 ^{ab} | 0.26 ± 0.03 ^a | 54.79 |
| PSOCF 10% | 29.56 ± 0.62 ^b | 2.26 ± 0.00 ^{bc} | 12.77 ± 0.03 ^f | 0.43 ± 0.04 ^b | 0.27 ± 0.06 ^a | 54.71 |
| PSOCF 15% | 32.72 ± 0.07 ^d | 2.35 ± 0.05 ^c | 13.69 ± 0.15 ^g | 0.74 ± 0.05 ^c | 0.32 ± 0.04 ^a | 50.18 |
| PSF 5% | 27.88 ± 0.20 ^a | 2.12 ± 0.05 ^{ab} | 10.81 ± 0.09 ^b | 1.31 ± 0.06 ^d | 0.26 ± 0.02 ^a | 57.62 |
| PSF 10% | 30.71 ± 0.50 ^c | 2.13 ± 0.01 ^b | 11.60 ± 0.03 ^d | 2.82 ± 0.08 ^e | 0.31 ± 0.02 ^a | 52.43 |
| PSF 15% | 29.05 ± 0.27 ^b | 2.30 ± 0.09 ^c | 12.00 ± 0.00 ^e | 4.46 ± 0.10 ^f | 0.41 ± 0.01 ^b | 51.78 |

^{a–g}different letters in the same column indicate significant difference ($P < 0.05$); values are expressed as mean ± SD ($n = 3$), except value for ACH

ACH – available carbohydrates; PSF – pumpkin seed flour; PSOCF – pumpkin seed oil cake flour

components was observed, with the exception in carbohydrate and moisture content. The moisture content in the control sample was higher than in the PSOCF and PSF bread samples and that finding correlates with the observation of Kaur and Sharma (2018) who experimented with cakes supplemented with PSF. Contents of ash, protein, fat and cellulose increased with increasing addition of PSOCF and PSF from 5% to 15% in bread formulations. This can be attributed to the fact that PSOCF and PSF have much higher levels of ash, protein, fat and cellulose than wheat flour. However, significant statistical difference at $P < 0.05$ of all analysed chemical components was observed between control and enriched bread samples, with the exception of cellulose. Similar results of increased protein, ash, fat and cellulose content were reported in the studies of Kaur and Sharma (2018), Jeevitha and Bhuvana (2019) and Shevchenko et al. (2022). Results from Table 3 also indicated that there was a reduction in available carbohydrates levels with increasing PSOCF and PSF proportion in bread formulation. From the results it could be noticed that bread samples with addition of PSOCF and PSF can be considered as products with enhanced nutritional value.

Total carotene content (TCC) increased significantly ($P < 0.05$) with the increased PSOCF and PSF proportion in bread formulation, and TCC values from all enriched

bread samples was higher than control sample of bread (Table 4). This increase of TCC was particularly pronounced in samples with PSOCF addition. These findings were similar to the results of Kampuse et al. (2015) and Kaur and Sharma (2018), where increase in total carotenoids content or β -carotene content in bakery products correlated with the concentration of pumpkin product substitution.

Fats from the pumpkin seeds and pumpkin seed oil cake have low level of saturated fatty acids (SFAs), and high levels of linoleic acid (C18:2) and oleic acid (C18:1) content (Table 5). In PSF and PSOCF the predominant saturated fatty acid was palmitic acid (C16:0), while regarding the unsaturated fatty acids the analysed flours were rich in oleic (C18:1) and linoleic acid (C18:2) (Table 5). In case of both PSF and PSOCF monounsaturated (MUFA) group of FA was dominant over the polyunsaturated (PUFA) contents, compared to WF which had more prevalent PUFA than MUFA group of FA. Content of SFA and palmitic acid in PSF, was similar to results of Białek et al. (2016), but MUFA and PUFA concentrations were different. Analysis of the fatty acid composition in PSOCF and PSF showed that the unsaturated FA accounted for more than 80%, and that results were close to findings of Peng et al. (2021). FA composition was similar to finding of Lestari and Meiyanto (2018) results for PSOCF and PSF, and of Kalonov and Khaidar-Zade (2022) for WF.

Table 4. Total carotene content (TCC) of bread samples (mean ± SD; $n = 3$)

| | Bread samples | | | | | | |
|------------------------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | control | PSOCF 5% | PSOCF 10% | PSOCF 15% | PSF 5% | PSF 10% | PSF 15% |
| TCC (mg·100g ⁻¹) | 0.00 ± 0.00 ^{as} | 2.98 ± 0.37 ^c | 4.22 ± 0.37 ^d | 8.68 ± 0.46 ^e | 1.31 ± 0.26 ^b | 2.96 ± 0.05 ^c | 4.60 ± 0.06 ^d |

^{a–e}different letters in the same row indicate significant difference ($P < 0.05$); *not detected

PSF – pumpkin seed flour; PSOCF – pumpkin seed oil cake flour

Table 5. Fatty acid composition of flour samples (mean \pm SD; $n = 3$)

| Fatty acid [g·(100 g) ⁻¹ FA] | WF | PSOCF | PSF |
|---|-------------------------------|-------------------------------|-------------------------------|
| C16:0 | 16.51 \pm 0.06 ^c | 11.22 \pm 0.24 ^b | 10.55 \pm 0.02 ^a |
| C18:0 | 1.18 \pm 0.01 ^a | 4.98 \pm 0.04 ^c | 4.81 \pm 0.02 ^b |
| C18:1 | 13.80 \pm 0.05 ^a | 42.01 \pm 1.12 ^b | 43.16 \pm 0.10 ^b |
| C18:2 | 62.55 \pm 0.27 ^c | 36.72 \pm 0.05 ^b | 35.90 \pm 0.08 ^a |
| C18:3 | 3.54 \pm 0.04 ^c | 0.52 \pm 0.04 ^b | nd ^a |
| C24:0 | nd ^a | 0.71 \pm 0.58 ^a | 2.68 \pm 0.36 ^b |
| C22:6+C24:1 | nd ^a | 1.86 \pm 1.06 ^a | 1.41 \pm 0.04 ^a |
| Total SFA | 18.36 | 17.50 | 18.52 |
| Total MUFA | 15.28 | 43.07 | 43.96 |
| Total PUFA | 66.35 | 39.40 | 37.51 |

^{a-d}different letters in the same row indicate significant difference ($P < 0.05$); for control bread sample it was not possible to extract enough fat sample for further analysis

FA – fatty acid; SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; nd – not detected; PSF – pumpkin seed flour; PSOCF – pumpkin seed oil cake flour; WF – wheat flour

Increase of the added proportion of PSOCF and PSF in bread formulations caused a decrease of SFAs in bread samples, although in case of PSF breads that decreasing trend was irregular (Table 6). That finding is similar to the results in Białek et al. (2016) study. Regarding the fatty acid belonging to the MUFA group, content of the main MUFA acid – oleic acid – was increased in case of PSF bread samples with the increase of the proportion of PSF, but that was not observed in PSOCF breads, where the trend was irregular. It was the opposite situation with the PUFA group, where the increasing trend in content of the

main PUFA – linoleic acid – in PSOCF bread samples was observed with the increasing share of PSOCF in wheat bread samples, and small decreasing trend in case of PSF bread samples.

The unsaturated fatty acids have been widely studied due to their many health benefits such as protective effect against cardiovascular diseases, hypertension, arthritis, inflammation, autoimmune related disorders, cancer, and promotion of healthy growth, brain and nervous system development (Dotto and Chacha 2020).

Total phenolic content, ABTS and DPPH depended on the quantity of PSF added to bread samples (Table 7),

Table 6. Fatty acid composition of bread samples (mean \pm SD; $n = 3$)

| Fatty acid [g·(100 g) ⁻¹ FA] | Bread samples | | | | | |
|---|--------------------------------|--------------------------------|-------------------------------|--------------------------------|-------------------------------|-------------------------------|
| | PSOCF 5% | PSOCF 10% | PSOCF 15% | PSF 5% | PSF 10% | PSF 15% |
| C16:0 | 23.99 \pm 0.08 ^d | 16.90 \pm 0.86 ^c | 14.49 \pm 0.57 ^b | 13.37 \pm 0.09 ^{ab} | 12.76 \pm 0.07 ^a | 12.24 \pm 0.01 ^a |
| C18:0 | 7.12 \pm 0.02 ^b | 5.61 \pm 0.44 ^a | 5.34 \pm 0.31 ^a | 4.94 \pm 0.05 ^a | 5.01 \pm 0.06 ^a | 5.24 \pm 0.05 ^a |
| C18:1 | 36.47 \pm 0.11 ^{bc} | 31.86 \pm 1.69 ^a | 35.13 \pm 1.02 ^b | 35.08 \pm 0.39 ^b | 38.20 \pm 0.52 ^c | 39.33 \pm 0.63 ^c |
| C18:2 | 25.16 \pm 0.06 ^a | 39.16 \pm 1.08 ^{bc} | 40.60 \pm 0.23 ^c | 40.76 \pm 0.19 ^c | 39.45 \pm 0.09 ^c | 37.60 \pm 0.44 ^b |
| C18:3 | 0.88 \pm 0.63 ^a | 1.35 \pm 0.03 ^a | 1.33 \pm 0.02 ^a | 0.98 \pm 0.02 ^a | 0.72 \pm 0.02 ^a | 0.64 \pm 0.00 ^a |
| C24:0 | 0.33 \pm 0.03 ^a | 1.79 \pm 1.19 ^a | 0.39 \pm 0.05 ^a | 1.18 \pm 0.11 ^a | nd ^a | 1.32 \pm 1.08 ^a |
| C22:6+C24:1 | 1.36 \pm 0.03 ^b | 0.80 \pm 0.13 ^{ab} | 0.54 \pm 0.19 ^a | 1.06 \pm 0.02 ^{ab} | 2.30 \pm 0.45 ^c | 2.13 \pm 0.16 ^c |
| Total SFA | 33.11 | 25.34 | 21.02 | 20.25 | 18.39 | 19.39 |
| Total MUFA | 38.49 | 33.15 | 36.16 | 36.49 | 38.47 | 39.96 |
| Total PUFA | 28.39 | 41.50 | 42.81 | 43.25 | 43.14 | 40.64 |

^{a-d}different letters in the same row indicate significant difference ($P < 0.05$); for control bread sample it was not possible to extract enough fat sample for further analysis

FA – fatty acid; SFA – saturated fatty acid; MUFA – monounsaturated fatty acid; PUFA – polyunsaturated fatty acid; nd – not detected; PSF – pumpkin seed flour; PSOCF – pumpkin seed oil cake flour

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Table 7. Antioxidant activity of bread samples (mean \pm SD; $n = 3$)

| Bread samples | Total phenolic content (mg GAE·g ⁻¹) | DPPH (mmol TE·g ⁻¹) | ABTS (mmol TE·g ⁻¹) |
|---------------|---|------------------------------------|------------------------------------|
| Control | 0.98 \pm 0.00 ^a | 0.31 \pm 0.01 ^b | 1.26 \pm 0.01 ^a |
| PSOCF 5% | 1.06 \pm 0.11 ^a | 0.26 \pm 0.01 ^a | 1.26 \pm 0.01 ^a |
| PSOCF 10% | 0.93 \pm 0.09 ^a | 0.29 \pm 0.00 ^{ab} | 1.25 \pm 0.00 ^a |
| PSOCF 15% | 0.94 \pm 0.11 ^a | 0.28 \pm 0.02 ^{ab} | 1.25 \pm 0.01 ^a |
| PSF 5% | 1.20 \pm 0.03 ^b | 0.38 \pm 0.02 ^c | 1.31 \pm 0.00 ^b |
| PSF 10% | 1.20 \pm 0.00 ^b | 0.39 \pm 0.03 ^c | 1.41 \pm 0.01 ^c |
| PSF 15% | 1.27 \pm 0.01 ^b | 0.40 \pm 0.01 ^c | 1.43 \pm 0.01 ^c |

^{a-c}different letters in the same column indicate significant difference ($P < 0.05$)

ABTS – 2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid); DPPH – 2,2-diphenyl-1-picrylhydrazyl; GAE – gallic acid equivalents; TE – Trolox equivalents; PSF – pumpkin seed flour; PSOCF – pumpkin seed oil cake flour

and had statistically significant ($P < 0.05$) increase compared to control sample. Antioxidant activity had relatively small increasing trend in accordance with the increased level of added PSF. Statistically significant influence of added PSOCF on total phenolic content, DPPH and ABTS was not observed, in case of PSOCF bread samples and control bread ($P > 0.05$). Therefore, addition of PSOCF had no effect on antioxidant activity of bread samples. Addition of PSF had positive effect on antioxidant activity of bread samples, which could be explained with higher level of fat content in PSF than in PSOCF and with the fact that pumpkin seed oil has biologically active components rich in antioxidant properties (Nilova et al. 2017). Kaur and Sharma (2018) found that total antioxidant activity was higher in the cake supplemented with PSF in comparison to control sample, and Malkanthi et al. (2018) also found that antioxidant activity was increased in biscuits with addition of pumpkin seed powder compared to control sample. According

to Nyam et al. (2013), bread samples substituted with PSF showed a higher total phenol compound content and increase in DPPH value, compared to the control sample. Increased antioxidant properties in bakery products by almost double was also observed by Nilova et al. (2017) while investigating bakery products enriched with vegetable oils. Similar results were obtained in the study of Wahyono et al. (2018) which concluded that producing a bread with a plentiful antioxidant activity is possible by using the yellow pumpkin powder.

Textural properties and sensory evaluation. The results of the instrumental texture profile analysis (TPA) indicated that the hardness and chewiness of bread samples were reduced with the addition of PSOCF and PSF into bread formulas, compared to the control bread sample. However, this effect was not statistically significant ($P > 0.05$) for hardness of PSOCF bread samples (Table 8). Also, in PSF bread samples hardness and chewiness decreased with the increased level of added PSF.

Table 8. Textural properties of bread samples (mean \pm SD; $n = 3$)

| Bread samples | Textural properties | | | | |
|---------------|-------------------------------|-------------------------------|------------------------------|--------------------------------|------------------------------|
| | hardness (N) | springiness | cohesiveness | chewiness (N) | resilience |
| Control | 6.16 \pm 1.35 ^b | 0.95 \pm 0.00 ^b | 0.76 \pm 0.05 ^a | 4.42 \pm 0.78 ^c | 0.33 \pm 0.02 ^a |
| PSOCF 5% | 4.06 \pm 0.79 ^{ab} | 0.94 \pm 0.02 ^{ab} | 0.77 \pm 0.01 ^a | 2.94 \pm 0.56 ^{abc} | 0.35 \pm 0.00 ^a |
| PSOCF 10% | 5.90 \pm 1.05 ^b | 0.93 \pm 0.02 ^{ab} | 0.72 \pm 0.04 ^a | 3.91 \pm 0.43 ^{bc} | 0.31 \pm 0.04 ^a |
| PSOCF 15% | 3.87 \pm 0.75 ^{ab} | 0.91 \pm 0.01 ^a | 0.72 \pm 0.02 ^a | 2.52 \pm 0.44 ^{ab} | 0.29 \pm 0.02 ^a |
| PSF 5% | 4.34 \pm 0.16 ^{ab} | 0.96 \pm 0.01 ^b | 0.76 \pm 0.02 ^a | 3.16 \pm 0.06 ^{abc} | 0.32 \pm 0.01 ^a |
| PSF 10% | 3.67 \pm 0.08 ^{ab} | 0.96 \pm 0.01 ^b | 0.75 \pm 0.01 ^a | 2.64 \pm 0.12 ^{ab} | 0.31 \pm 0.01 ^a |
| PSF 15% | 2.81 \pm 0.17 ^a | 0.96 \pm 0.01 ^b | 0.72 \pm 0.01 ^a | 1.95 \pm 0.12 ^a | 0.29 \pm 0.01 ^a |

^{a-c}different letters in the same column indicate significant difference ($P < 0.05$)

PSF – pumpkin seed flour; PSOCF – pumpkin seed oil cake flour

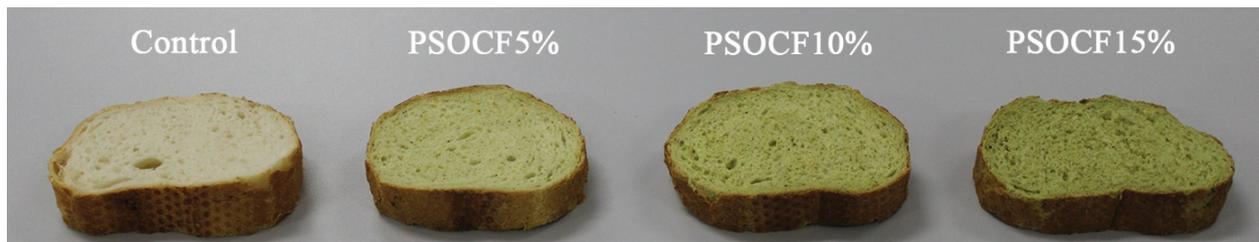


Figure 1. Comparison of slices for control bread and experimental PSOCF bread samples
PSOCF – pumpkin seed oil cake flour

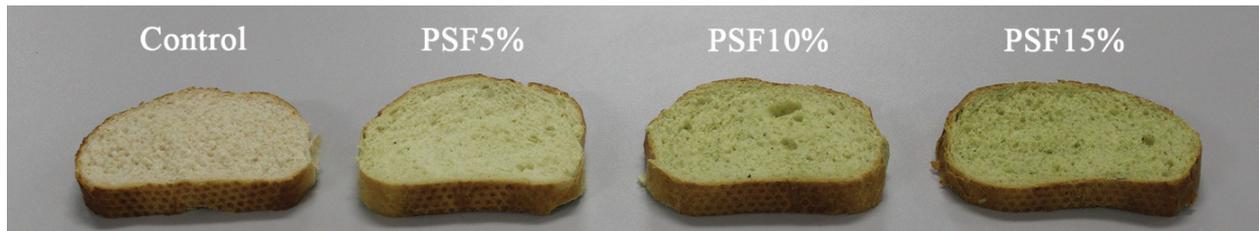


Figure 2. Comparison of slices for control bread and experimental PSF bread samples
PSF – pumpkin seed flour

Kampuse et al. (2015) and Nyam et al. (2013) came to a similar conclusion in their studies. Increased softness of the PSOCF and PSF bread samples could be the result of the chemical composition, especially the higher fat content, compared to the control bread. Enrichment of bread samples with PSOCF and PSF had no statistically significant effect on cohesiveness and resilience ($P > 0.05$). Results for springiness were very similar for all bread samples, although there was

statistically significant difference between analysed samples ($P < 0.05$), and they were all close to the value of 1 which would be ideal springiness of bread crumb.

Although consumer awareness about including the nutritionally enriched products in their diet is increasing, during the production of such products extra care must be taken to avoid compromising consumers' product acceptance. Sensory evaluation results (Figures 3 and 4) revealed that shape and volume, external

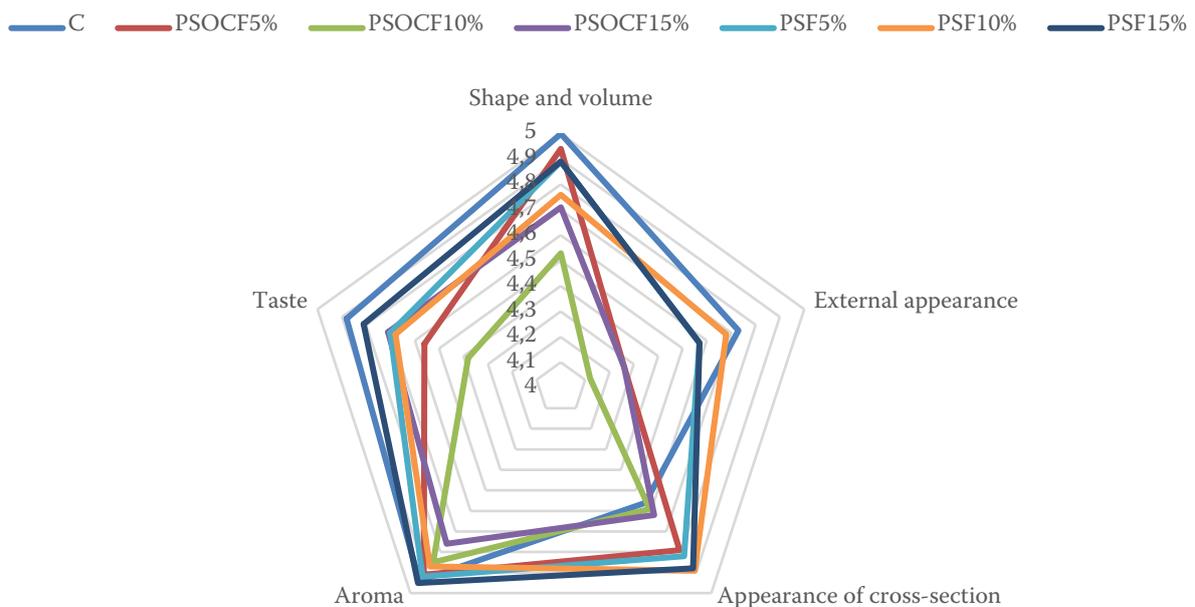


Figure 3. Radar plot of the sensory profile of bread samples with PSOCF and PSF addition
PSF – pumpkin seed flour; PSOCF – pumpkin seed oil cake flour

<https://doi.org/10.17221/190/2024-CJFS>

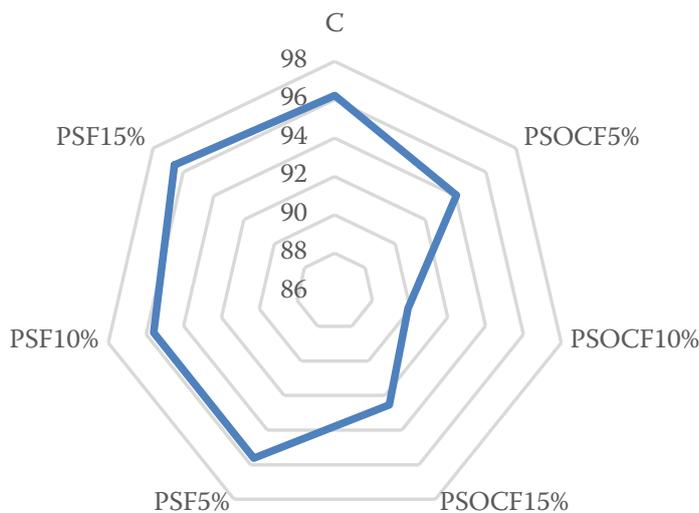


Figure 4. Radar plot of the percentage of max level of sensory quality for bread samples (80–89% is very good quality; 90–100% is excellent quality)

PSF – pumpkin seed flour; PSOCF – pumpkin seed oil cake flour

appearance and taste had been significantly different ($P < 0.05$) and they were similar or lower in comparison with the control bread. There was no significant difference between evaluated appearance of cross section and aroma of analysed samples ($P > 0.05$). PSF 15% bread and control bread had the highest overall quality score (4.82 of 5 possible points), while PSOCF 10% bread had the lowest overall quality score of 4.49 points. Analysis revealed that PSF 15% bread was evaluated as a sample with excellent quality and had the highest score, 96.59% of max level of sensory quality. All other bread samples also had excellent quality, with exception of PSOCF 10% bread which had very good quality but relatively the lowest score for percentage of max level of quality (89.88%). The bread samples with the addition of 5 and 15% PSOCF and 5, 10 and 15% PSF had excellent sensory quality (90–100%), and light olive-green colour shade was proportional to the amount of added pumpkin ingredients, which was in accordance with the results of Kampuse et al. (2015), Kaur and Sharma (2018), Jeevita and Bhuvana (2019).

CONCLUSION

In this work addition of PSOCF and PSF in bread preparation was investigated. This approach allows enrichment of bread products with pumpkin and its by-products with the possibility of reducing costs in waste treatment. The addition of PSOCF and PSF in the amount 5–15% to wheat flour in the bread production led to increase in ash, protein, fat, cellulose, total carotene and unsaturated fat levels. Based on these results, it could be concluded that bread samples with the addition of PSOCF and PSF

can be considered as products with increased nutritional quality. The addition of PSOCF did not affect antioxidant activity of bread samples, while addition of PSF had positive effect on antioxidant properties. Hardness and chewiness of bread samples were reduced with the addition of PSOCF, and even more with the addition of PSF. Sensory analysis of all bread samples revealed that they had very good or excellent quality of shape, volume, external appearance, cross-section appearance with light olive-green colour shade, aroma and taste of bread. However, the PSF bread samples were rated higher. All obtained results indicated that PSOCF and PSF have many positive effects on original characteristics of the wheat bread, without substantial changes and that they could be a viable alternative for the nutritive enrichment process of bakery products. The addition of PSF had slightly higher positive effect on the bread quality. This research was based on the development of new bakery products with a shelf life of 24 h, but further research could be focused on optimisation of formulations and technology for industrial production of bakery products enriched with pumpkin seed or pumpkin seed oil cake flour. Also, the sensory, nutritional and microbiological quality analysis of such and similar products with a longer shelf life are recommended.

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