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# Sensory Assessment and Physicochemical Properties of Wheat Bread Supplemented with Chia Seeds

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Quantitative descriptive analysis, consumer acceptability as well as physicochemical characteristics were employed to analyse the effect of ground chia seeds on the texture and quality of wheat bread. Chia seed oil changes and its impact on selected characteristics were determined. The share of chia seeds did not contribute to bread quality deterioration. The addition of 7.5% chia seeds significantly reduced consumer acceptance as well as contributed to the development of undesirable features in the sensory profile, including fatty, rancid, and off-flavour. The share of chia seeds in an amount not exceeding 5% did not deteriorate the overall quality of bread. The composition of fatty acids of bread with chia seeds, especially with their 5 and 7.5% addition, could be valuable from the nutritional point of view. The fatty acid profile of bread with 5 and 7.5% chia seeds was characterised by over 50% higher polyunsaturated fatty acids to saturated fatty acids ratio compared to wheat bread. The n-6/n-3 ratio in the fatty acid profile of wheat bread was 18.77, while in the fatty acid profile of chia seed bread it ranged from 1.42 to 0.67. Oil extracted from bread exhibited better quality features as compared to oil extracted from chia seeds and subjected to the oil oxidative stability test.

# **INTRODUCTION**

Chia (*Salvia hispanica* L.), a plant that belongs to the *Lamiaceae* family, has been known for many centuries. Due to nutritional and health effects, chia seeds become more and more popular in many countries, especially across the Europe. Nowadays, they have found various applications, such as functional food, animal feed, and cosmetics [Dinçoğlu & Yeşildemir, 2019]. Some studies have shown their consumption to yield beneficial effects on such health problems as insulin resistance, dyslipidemia, cardiovascular diseases, or inflammation [Vuksan *et al.*, 2017]. In recent years, consumers expect food with additional health benefits resulting from its consumption. They seek for a healthy lifestyle, which is also associated with the growing food awareness.

In 1997, the European Parliament approved chia seeds as a novel food, which resulted in its increased use in food production. Consumption of chia seeds is not typical in the European culture, but bread is still a staple food product in the diet of many people. The addition of chia seeds to popular food products, including bread, can be a perfect dietary supplement and bring a number of health benefits for consumers. Additives used for food supplementation can, on the one

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hand, improve the nutritional value of bread, but on the other hand, contribute to its diminished sensory acceptance.

Chia seeds, due to their chemical composition, have a high nutritional potential. They contain protein of a high biological value with such essential amino acids as isoleucine, leucine, lysine, and valine. For this reason, chia seeds can be a supplement of cereal proteins that are deficient in essential amino acids, especially lysine. In addition, the seeds contain fatty acids, and in particular are a valuable source of n-3 and n-6 fatty acids [Sandoval-Oliveros & Paredes-Lopez, 2013; Vázquez--Ovando *et al.*, 2010]. Due to the high content of fatty acids, including unsaturated ones, product with seeds of chia may be susceptible to rancid processes, which may lead to lowered consumer acceptance. Chia seeds are characterised by a high dietary fibre content, mainly the water soluble fraction [Vázquez-Ovando et al., 2010], and are an important source of natural antioxidants [Reves-Caudillo et al., 2008]. They can be a valuable supplement in conventional therapy for overweight and obesity in diabetics because of the glycaemic control and a high dietary fibre content [Vuksan et al., 2017]. Mohd Ali et al. [2012] concluded that chia oil can maintain a balanced serum lipid profile, while Citelli et al. [2016] observed changes in the lipid profile in the liver caused by chia oil supplementation. Chia inter alia has been used for the production of bread [Coelho & Salas-Mellado, 2015; Hrušková & Svec, 2015; Miranda-Ramos et al., 2020; Romankiewicz et al., 2017], pasta [Oliveira et al., 2015], and even ice cream



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[Campos et al., 2016]. Several studies of bread with chia seeds showed that they contributed to the technological quality and texture parameters, depending on additive type - flour or seeds, and the method of their preparation. For example, Coelho & Salas-Mellado [2015] observed a decrease in the specific volume and the total score values, and an increase in the firmness of bread crumbs with chia seeds. In turn, Romankiewicz et al. [2017] noticed that the addition of chia seeds decreased baking losses, but also bread volume and crumb hardness, whereas Hrušková & Švec [2015] determined higher elasticity of dough containing hydrated chia resulting in firmer crumb compared to the bread with dry chia flour. All listed authors concluded that the addition of chia seeds increased the nutritional value of the bread, for example bread with chia contained more dietary fibre and mineral components. Products with chia seeds were characterised by a rich fatty acid composition and a higher content of phenolic compounds. Miranda--Ramos et al. [2020] pointed out that phytates of chia seeds may affect the bioavailability of such microelements like Zn and Fe. They also emphasised that the glycaemic index was lower in bread with chia ingredients compared to wheat bread, which in combination with nutritional benefits could be clinically important for the prevention of metabolic diseases. In sensory evaluation, the breads with chia seeds met with consumer acceptance. Romankiewicz et al. [2017] concluded that the substitution of wheat flour with chia seeds up to 6% did not negatively affect the final product acceptance.

Due to the composition of chia seeds, it was hypothesised that although their addition can improve the nutritional value of the bread, it may, on the other hand, contribute to a decreased consumer acceptance resulting from oxidative changes of fats. However, there is a lack of data regarding the influence of chia seeds on the oxidative stability of fats contained in the products with their addition. In this study, ground chia seeds were added to the dough, which can contribute to the better utilization of nutrients but also could result in faster oxidation changes of fats during bread baking. It should be emphasised that most of the studies on the nutritional, technological, and sensory value of bakery and pastry products are carried out with seeds or grains, defatted or refined flour. Considering that the intestinal transit time in humans is too short to digest whole seeds and grains, it seems important to supplement bread with crushed seeds or grains. In the present study, wheat flour was partially replaced with ground chia seeds, thanks to which the bread was enriched with all the valuable nutrients contained in the whole grain, which offers both technological, nutritional, and sensorial novelty. For this reason, the aim of this study was to evaluate the effect of substituting some part of wheat flour with ground chia seeds on selected physicochemical and textural properties, on the technological and sensory quality, as well as on the acceptance of bread with 2.5, 5, or 7.5% of ground chia seeds.

# **MATERIALS AND METHODS**

# Materials

Formulation and bread making method by Mikulec *et al.* [2019] was applied. Some part of wheat flour (WF) type 650 (PZZ Stoislaw, Poland) was replaced with ground chia seeds (Natura Food, Intenson Europe sp. z o. o., Poland) in the fol-

lowing wheat/chia ratios (*w/w*): 97.5/2.5 (WCh2.5); 95/5 (WCh5) and 92.5/7.5 (WCh7.5). The chia seeds were ground using a laboratory mill (KM13, Bosch, Gerlingen, Germany). Standard wheat bread (WS) was also prepared. Forty loaves were prepared for each sample from two batches.

## Chemicals used in the experiment

The following chemicals were used for analyses: petroleum ether, chloroform, acetic acid glacial, potassium iodide, sodium thiosulfate, starch soluble phenolphthalein, sodium hydroxide, potassium hydroxide, sodium chloride, *n*-hexane, methyl orange, ethanol, sodium methylate (Avantor Performance Materials S.A., Gliwice, Poland), 12% boron trifluoride, Supelco 37 component FAME Mix and CLA isomers, MES hydrate, TRIZMA® base (Sigma-Aldrich Co., St. Louis, MO, USA), sulphuric acid, acetone (PPH Stanlab, Sp. z o.o., Lublin, Poland), Kjeltabs (Foss, Hillerød, Denmark), hydrochloric acid (PPUH Tarchem Sp. z o.o., Tarnowskie Góry, Poland), Tashiro indicator, boric acid, (Chempur, Piekary Śląskie, Poland), buffer solutions pH 4.0, 7.0, and 9.0 (Eurochem BGD. Sp. z o.o., Tarnów, Poland), and total dietary fibre assay kit (Megazyme, Bray, Ireland).

# Analysis of the proximate composition of wheat flour and chia seeds

The contents of water (AOAC 925.10), protein (AOAC 950.36), crude fat (AOAC 935.38), starch (AOAC 996.11), ash (AOAC 923.03), total, and soluble and insoluble dietary fibre (AOAC 991.43) were determined following the Association of Official Analytical Chemists (AOAC) methods [AOAC, 2006]. Analyses was performed in duplicate. Results were expressed as g/100 g dry matter (d.m.).

#### Analysis of the basic quality features of bread

Two hours after baking, some instrumental quality parameters of bread, such as bread volume [AACC, 2000], total baking loss [Majzoobi *et al.*, 2011], and crumb moisture (AOAC 925.10) [AOAC, 2006] were determined.

#### **Texture analysis**

Selected texture parameters of the bread crumb, such as hardness, cohesiveness, chewiness, gumminess, springiness, and resilience, were determined acording to the procedure described by Szcześniak [2002]. Cylindrical samples of crumb (r = 40 mm, h = 30 mm) were analysed with a TA.XT2.Plus texture analyzer (Stable Micro System, Surrey, UK) equipped with 5 kg load cell, using an aluminium, cylinder probe ( $\phi = 36$  mm), at the test speed of 2 mm/s, deformation of the entire sample height reaching 75%, and pause of 10 s. Measurements were done in ten replications.

# **Consumer acceptability**

Consumer acceptability tests using a 9-point hedonic scale [Stone & Sidel, 2004] were performed by a panel consisting of 75 untrained potential consumers (20–60 years old, 49 females and 26 males) who were habitual consumers of bread. Consumers were recruited from different institutes of the State Higher Vocational School in Nowy Sacz, Poland (staff and students).

## **Sensory profile**

The assessment was carried out in a sensory laboratory at the State University of Applied Sciences in Nowy Sącz, Poland, which meets the requirements of the Internatioanl Organization for Standarization (ISO) 8589 standard [ISO, 2010]. Descriptive sensory profiling of breads using a quantitative descriptive analysis (QDA), according to ISO 13299 standard [ISO, 2016] was carried out 4 h after baking by a panel of 8 assessors (in two independent repetitions) (n = 8, 7 women and 1 man, aged from 21 to 49), qualified as experts - according to ISO 8586 standard [ISO, 2014] and having appropriate methodological (theoretical and practical) preparation in the field of sensory methods and extensive experience in conducting assessments using quantitative descriptive analysis. Bread samples (35 g, a thickness of 12±2 mm) were placed on previously prepared and coded disposable paper trays. Pure water having a temperature of 20°C was used as a taste neutralizer between samples. Samples were presented to the assessors simultaneously.

The attributes of the analysed products were selected in accordance with the ISO 13299 procedure [ISO, 2016] and their definitions were established. Twenty qualitative attributes were selected and defined (Table 1). The 100-mm unstructured linear scale with marked boundary values from 0 to 10 conventional units (c.u.), where 0 meant no intensity of a given attribute, and 10 meant high intensity of a given attribute, was used. Results were calculated using Microsoft Excel 10.0 software (Microsoft, Redmond, WA, USA).

#### Analysis of oil extracted from bread and chia seeds

Chia seeds as well as bread supplemented with 7.5% chia seeds (WCh7.5) were analysed for oil fraction stability. The WCh7.5 sample was selected from among breads, due to the highest content of fat and possibly the greatest effect on the oxidative changes in fat. For this purpose, chia seeds/ bread samples were milled using a laboratory mill (KM13, Bosch Germany), and the oil was extracted with petroleum ether (1:3, w/v) for 3 h with continuous stirring. After decanting, fresh petroleum ether was added to the solid residue, and the extraction procedure was repeated twice. After collection, all liquid phases were centrifuged and the supernatant was removed from the oil fraction by evaporation using a vacuum evaporator (RV 10 C S99, IKA, Königswinter, Germany).

The oils obtained were subjected to the following analyses:

### Oil oxidative stability test

Chia oil extracted from chia seeds/bread was subjected to the oxidation stability test according to Pike [2001]. The analysis kit consisted of a purified compressed air cylinder with a flow regulator, and a thermostat. The air used for the measurement was drawn through the filter and the water vapour was removed by means of a molecular sieve. Oil sample was thermostated at 60°C under a constant air flow (20 L/h) and continuously aerated for 5 days. The samples were then analysed for peroxide and iodine values as well as free fatty acid content. TABLE 1. Sensory attributes of breads used in the quantitative descriptive analysis (QDA).

Attribute	Definition	Word anchors (0–10 c.u.)		
l	Appearance			
Beige colour of the crust	Visual perception of the bread crust colour	$light \rightarrow dark$		
Porosity	Visual perception of the bread crumb porosity/porous	poreless → porous		
	Odour			
Flour	Odour typical of flour	none $\rightarrow$ very intensive		
Grain-seed	Odour typical of cereal grains, dried seeds, bran	$none \rightarrow very$ intensive		
Alcoholic	Odour typical of the ethyl alcohol (ethanol)	$none \rightarrow very$ intensive		
Buttery	Odour typical of butter	none $\rightarrow$ very intensive		
Rancid	Odour typical of rancid oil	none $\rightarrow$ very intensive		
Fatty	Odour typical of fat	none → very intensive		
Off-odour	Smell of something other than "regular" smell of bread	none $\rightarrow$ very intensive		
	Flavour			
Sweet	Taste characteristic to sugar or substance containing or resembling sugar, as honey or saccharin (basic taste illustrated by sucrose diluted in water 1.5%)	none → very intensive		
Bitter	The taste experience when quinine or coffee is taken into the mouth (basic taste illustrated by caffeine diluted in water, 0.5%)	none $\rightarrow$ very intensive		
Grain-seed	Taste characteristic to cereal grains, dried seeds, bran	none $\rightarrow$ very intensive		
Acidulous	The intensity of the acidulous taste	none $\rightarrow$ very intensive		
Rancid	Taste typical of rancid oil	none → very intensive		
Fatty	Taste typical of fat	none → very intensive		
Off-flavour	Taste of something other than "regular" flavour of the bread	none → very intensive		
	Just of the intensity of the actuatious tasteintensiveTaste typical of rancid oilnone $\rightarrow$ very intensiveTaste typical of fatnone $\rightarrow$ very intensivevourTaste of something other than "regular" flavour of the breadTexture (mouth feel)The force required toThe force required to			
Hardness	The force required to bite bread samples	not hard $\rightarrow$ hard		
Chewiness	The energy required to chew a product until it is ready to be swallowed (the number of chews used to prepare the product to be swallowed)	not chewy → chewy		
Adhesiveness	The force required to remove bread crumbs	$low \rightarrow high$		
Moistness	Slightly or moderately wet	$dry \rightarrow moist$		

#### Peroxide value determination

The analysis was done according to the 3960 ISO standard [ISO, 2017]. The peroxide value was expressed in mmol of active oxygen per kilogram of the sample. As a result, the mean of two parallel determinations was assumed.

#### *Free fatty acid determination*

Free fatty acid (FFA) content was determined according to Del Flores-Álvarez *et al.* [2012] procedure. The results were expressed as lauric acid equivalents. The assay was carried out in duplicate for each sample. FFA content was calculated as follows (Eq. 1):

%FFA as lauric acid = =  $\frac{\text{mL NaOH} \times \text{NaOH molarity} \times 20 \text{ (equivalent weights)}}{\text{weight (g)}}$ 

*Iodine value determination* 

The test was made according to the European Committee for Standardization (CEN) DIN EN 14111 standard [CEN, 2003]. The iodine value was expressed as grams of  $I_2$  absorbed by 100 g of the sample. As a result, the mean of two parallel determinations was assumed.

#### **Determination of fatty acid profile**

The extraction of total lipids was performed in duplicate according to the Folch et al. [1957] method, while the derivatisation and determination of total fatty acid composition were performed according to the AOAC – approved method 991.39 [AOAC, 2006]. Briefly, to the fat extracted (10 mg), 0.5 mL of 0.5 N KOH (methanol solution) was added and heated to 85°C, then 1 mL of 12% BF, (methanol solution) was added, and the mixture was reheated at 85°C. After cooling to room temperature, 1 mL of hexane and 5 mL of saturated NaCl solution were added. About 1  $\mu$ L of the resulting sample was injected onto the column connected to the TRACE GC UL-TRA chromatograph (Thermo Electron Corporation, Milano, Italy) with a flame ionization detector (FID). Operating parameters were as follows: FID temperature 250°C; dispenser temperature 220°C; oven temperature 160°C (3 min) to 210°C (3°C/min) (210°C, 35 min). SUPELCOWAX10 column (30 m, 0.25 mm, 0.25  $\mu$ m) was used and helium was applied as a carrier gas at a flow rate of 1 mL/min. Split flow was 10 mL/min. Individual fatty acid methyl esters were identified by comparison to the standards of a mixture of Supelco 37 component FAME Mix, and of CLA isomers.

## **Nutritional properties**

Nutritional properties of lipids were evaluated using three indexes: the atherogenicity index (AI), the ratio of hypocholesterolemic to hypercholesterolemic fatty acids (HH), and the thrombogenicity index (TI) (Eq. 2–4) [Ulbricht & Southgate, 1991]:

$$AI = \frac{C12:0 + [4 \times C14:0] + C16:0}{\sum MUFA + \sum (n-6) + \sum (n-3)}$$
(2)

$$HH = \frac{C18:1n9 + C18:2 + C20:4 + C18:3 + C20:5 + C22:5 + C22:6}{C14:0 + C16:0}$$
(3)

$$TI = \frac{C14:0+C16:0+C18:0}{0.5 \cdot C18:1+0.5(MUFA-C18:1)+0.5 \times PUFA(n-6)+3} \times PUFA(n-3) + \frac{PUFA(n-3)}{PUFA(n-6)}$$
(4)

where: MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

## Statistical analysis

(1)

The analysis of variance (ANOVA) and Duncan posthoc test (p < 0.05) as well as principal component analysis (PCA) were performed using Statistica 13.3 (Stat Soft, Kraków, Poland).

## **RESULTS AND DISCUSSION**

# Analysis of the basic quality features of wheat flour and chia seeds

The wheat flour and chia seeds used for bread making differed significantly (p<0.05) in the content of proximate chemical components. In comparison with the wheat flour, chia seeds had approx. 1.5 times higher protein content, approx. 22 times higher crude fat content, approx. 10 times higher total dietary fibre content, and approx. 8 times higher mineral components content, which confirms their high nutritional value (Table 2). The chemical composition of chia seeds used in the present study is similar to that reported by other authors [Coelho & Salas-Mellado, 2015; Hrušková *et al.*, 2015]. Therefore, substituting a part of wheat flour with ground chia seeds had to contribute to the increase in the nutritional value of the bread.

## Analysis of the basic quality features of bread

The bread with chia seeds did not differ significantly in the total baking loss, which ranged from 14.25% for wheat bread to 14.75% for bread with 2.5% chia seeds (Table 3). No influence of chia seeds on bread volume was observed, which ranged from 883.25 mL for bread with 7.5% chia seeds to 897.67 mL for wheat bread. A decrease in crumb moisture was observed in the bread with chia seeds addition, on the day of baking (Table 3). Opposite results were obtained by Romankiewicz *et al.* [2017], who observed an increase in the baking loss after chia seeds addition and a reduction in bread volume, compared to wheat bread. However, Hrušková *et al.* [2015] obtained a larger volume of bread with chia flour, which was caused by the initial hydration of chia flour due to water absorption by chia polysaccharides. It is well known

TABLE 2. Chemical composition of chia seeds and wheat flour.

		Chia seeds	Wheat flour	
Moisture (g/100 g)		$6.87 \pm 0.06^{b}$	$13.73 \pm 0.06^{a}$	
Protein (g/100 g d.m.)		20.58±0.01 <sup>a</sup> 14.12±0.03		
Crude fat (g/100 g d.m.)		33.99±0.01ª	$1.56 \pm 0.01^{b}$	
	Soluble	$3.45 \pm 0.04^{a}$	$1.61 \pm 0.01^{b}$	
Dietary fibre (g/100 g d.m.)	Insoluble	$25.81 \pm 0.05^{a}$	$1.36 \pm 0.04^{\text{b}}$	
	Total	$29.25 \pm 0.01^{a}$	$2.97 \pm 0.04^{\text{b}}$	
Ash (g/100 g d.r	Ash (g/100 g d.m.) 4.9		$0.65 \pm 0.00^{\text{b}}$	
Starch (g/100 g	Starch (g/100 g d.m.) $0.00 \pm 0.00^{a}$ 73.1		$73.13 \pm 0.01^{b}$	

Values (mean  $\pm$  standard deviation) in the same row marked with different letters are significantly different at p<0.05; d.m. – dry matter.

that free water evaporates quicker during baking and storage, compared to the bound one. Despite the reduction of the gluten protein in bread, a larger loaf volume was obtained as a result of the addition of a gluten-free raw material, such as chia flour. Bread volume depends on the type and amount of protein used in dough formulation, as well as on its interaction with starch. Although chia seeds have a high protein content, it cannot form a continuous gluten-like network, thus impairing water entrapment. Additionally, proteins compete with starch for water, delaying starch swelling and gelatinization [Gnanavinthan *et al.*, 2010]. Therefore, in reference to our research and that of other authors, it can be concluded that the method of bread dough preparation, especially involving raw gluten-free and high-fibre content, can be very important for loaf volume, baking loss, or bread crumb moisture content.

## **Texture analysis**

The hardness of the bread crumb is one of the most important and often determined parameters in bread texture studies. It is the main determinant of bread quality and is closely related to the perception of bread as fresh to consumers. The highest hardness on the day of baking was determined in the breads with 2.5 and 5% share of chia seeds, reaching 3.10 and 3.25 N, respectively (Table 3). There was no significant difference between the hardness of the wheat bread and bread with the 7.5% chia seed content. An increase

in cohesiveness, gumminess, and chewiness of bread crumbs with chia seeds was observed, compared to wheat bread. A positive effect of 5 and 7.5% of chia seeds on the reduction of gumminess and chewiness of crumb was observed in comparison to other breads (Table 3). The changes in the texture of the bread crumb were mainly due to the decreased gluten content caused by the addition of chia seeds, an ingredient with a higher dietary fibere and fat content [Collar et al., 2007]. Bread dough is a complex system in which the interaction between the different ingredients may cause various effects. Depending on their amount, the additives used in bread making can change textural properties, for example fat restricts starch swelling during baking. During baking, anti-firming components interact with wheat flour starch molecules and decrease starch swelling. As a result, less surface area is exposed to gluten and weaker cross-linking with protein occurs; therefore, the firming rate is reduced [Scheuer et al., 2016]. For resilience, a significant increase (p<0.05) was observed in the bread with 7.5% share of chia seeds (Table 3). Coelho & Salas-Mellado [2015] obtained similar results; they observed an increase in the firmness of bread crumbs with chia on the baking day. Meanwhile, Romankiewicz et al. [2017] reported a 4 to 8% decrease in the crumb hardness, gumminess, and chewiness of bread with chia addition on the baking day, compared to the standard one. Comparing the texture profile assessed by the sensory panel to the results obtained from

TABLE 3. Characteristics of wheat bread and breads with addition of chia seeds.

Bread type	WS	WCh2.5	WCh5	WCh7.5
	Physico	chemical parameters		
Total baking loss (%)	14.25±0.55 <sup>a</sup>	$14.75 \pm 0.60^{a}$	14.28±0.60 <sup>a</sup>	$14.60 \pm 0.46^{a}$
Volume (mL)	$898 \pm 13^{a}$	$892 \pm 12^{a}$	$885 \pm 10^{a}$	$883\pm10^{a}$
Moisture of bread crumb (g/100 g)	45.70±0.01ª	45.28±0.09 <sup>b</sup>	45.17±0.13 <sup>b</sup>	45.30±0.08b
	Tex	ture parameters		
Hardness (N)	2.74±0.12 <sup>b</sup>	$3.10 \pm 0.13^{a}$	$3.25 \pm 0.12^{a}$	2.79±0.12 <sup>ab</sup>
Springiness (-)	$0.94{\pm}0.03^{ab}$	$0.89 \pm 0.01^{b}$	$0.89 \pm 0.04^{\text{b}}$	$0.96 \pm 0.02^{a}$
Cohesiveness (-)	$0.66 \pm 0.03^{d}$	$0.81 \pm 0.01^{b}$	$0.78 \pm 0.02^{\circ}$	$0.85 \pm 0.02^{a}$
Gumminess (N)	2.12±0.09°	$3.54 \pm 0.07^{a}$	2.49±0.16 <sup>b</sup>	2.52±0.09 <sup>b</sup>
Chewiness (N·mm)	1.96±0.09°	$3.34 \pm 0.13^{a}$	2.69±0.14 <sup>b</sup>	2.84±0.17 <sup>b</sup>
Resilience (-)	$0.40 \pm 0.01^{b}$	$0.40 \pm 0.02^{\text{b}}$	$0.41 \pm 0.01^{\text{b}}$	$0.51 \pm 0.01^{a}$
	Const	umer acceptability		
Taste	8.9±0.30ª	$8.8 \pm 0.47^{a}$	$8.3 \pm 0.48^{b}$	7.5±0.45°
Texture	$8.9 \pm 0.30^{a}$	$8.8 \pm 0.40^{a}$	8.2±0.31 <sup>b</sup>	7.6±0.32°
Aroma	$8.7 \pm 0.47^{a}$	$8.6 \pm 0.50^{a}$	$8.4 \pm 0.68^{b}$	7.6±0.65°
Crumb colour	$8.8 \pm 0.40^{\circ}$	$8.7 \pm 0.67^{a}$	8.1±0.53 <sup>b</sup>	$7.1 \pm 0.48^{\circ}$
Crust colour	$8.8 \pm 0.42^{a}$	$8.7 \pm 0.51^{a}$	$8.0 \pm 0.42^{b}$	7.2±0.67°
Overall acceptability	$8.9 \pm 0.30^{a}$	$8.8 \pm 0.52^{a}$	$8.2 \pm 0.45^{b}$	7.3±0.42°

WS – wheat bread, WCh2.5 – bread with 2.5% addition of chia seeds, WCh5 – bread with 5% addition of chia seeds, WCh7.5 – bread with 7.5% addition of chia seeds. Values (mean ± standard deviation) in the same row marked with different letters are significantly different at p<0.05.

#### **Consumer acceptability**

Consumer preference assessments provide very important and valuable information for food and drink producers, however they are not sufficient. Food producers should meet the expectations and needs of consumers, because without consumer acceptance they will not achieve the intended success. A new product should meet the certain level of quality required by the consumers because only a few percent of new products are fitted on the market.

In the consumer acceptance test, wheat bread and bread with 2.5% of chia seeds obtained similar scores (Table 3) and did not differ significantly ( $p \ge 0.05$ ). Other breads differed (p < 0.05) by means of the organoleptic evaluation compared to WS and WCh2.5, as well as differed among each other. The lowest scores were given to the bread with a 7.5% share of chia seeds. This bread obtained much lower scores, especially for the colour of crumb and crust and overall acceptability, which ranged from 7.1 to 7.3 (Table 3).

Other authors observed deterioration in the organoleptic characteristics, lower rating and consumer acceptance of bread with chia seeds [Coelho & Salas-Mellado, 2015; Hrušková *et al.*, 2015]. Hrušková *et al.* [2015] observed lower scores given for the appearance, texture, and overall acceptability to the bread with 8% addition of chia seeds. Deterioration of the organoleptic properties of bread enriched with such non-cereal additives like hemp [Mikulec *et al.*, 2019], cistus extract [Mikulec *et al.*, 2020], herbs [Adams *et al.*, 2011], and others can be explained by the content of essential oils that cause its specific taste and smell. The same explanation can be applied to the changes observed in the present study.

### **Descriptive sensory profiling**

The manufacturer must know not only how product is rated by consumers, but also why it has received a specific rating. Sensory profile analysis provides valuable answers to the "why" question. The method of sensory profiling (QDA) assumes that sensory characteristics are not homogeneous, but consist of a number of individual distinguishing attributes, a significant part of which can be identified and separately analysed.

In the sensory profile of the obtained breads, the flour and alcoholic odours were significantly less perceptible in the breads with 5% share of chia seeds, but significantly more grain-seed odour and flavour were detected in all breads with chia seeds (Table 4), compared to wheat bread. The share of chia seed contributed to the appearance of fatty flavour as well as rancid and off-flavour, mainly in WCh7.5 (Table 4). Regarding the textural properties, the assessors did not observe the difference between chia seed breads and wheat bread.

The PCA analysis allows finding dependencies in a data set, better data representation, classification, pattern searching, or dimension reduction. Values are not evenly distributed along all directions of the multidimensional coordinate sys-

TABLE 4. Intensity of the sensory attributes of breads.

Attribute	WS	WCh2.5	WCh5	WCh7.5
Crust colour	3.0±0.5°	3.2±0.5°	4.5±0.7 <sup>b</sup>	7.5±0.2ª
Porosity	$9.5 \pm 1.6^{a}$	$9.2\pm0.9^{a}$	$8.6 \pm 1.9^{a}$	$8.4 \pm 2.2^{a}$
Flour (odour)	$3.1 \pm 0.4^{a}$	$2.8 \pm 0.6^{a}$	$1.1 \pm 0.3^{b}$	$0.9 \pm 0.3^{b}$
Grain-seed (odour)	$0.6 \pm 0.5^{d}$	2.1±0.5°	3.9±0.5 <sup>b</sup>	$5.5 \pm 0.9^{a}$
Alcoholic (odour)	$3.6 \pm 1.0^{a}$	$2.3 \pm 0.8^{a}$	1.4±0.5 <sup>b</sup>	$1.3 \pm 0.3^{b}$
Buttery (odour)	$0.5\pm0.0^{a}$	$0.0 \pm 0.0^{\text{b}}$	$0.0 \pm 0.0^{\text{b}}$	$0.0\pm0.0^{\text{b}}$
Rancid (odour)	$0.0 \pm 0.0^{\text{b}}$	$0.0 \pm 0.0^{\text{b}}$	$0.0 \pm 0.0^{\text{b}}$	$1.5 \pm 0.5^{a}$
Fatty (odour)	$0.0 \pm 0.0^d$	$0.9 \pm 0.2^{\circ}$	1.5±0.5 <sup>b</sup>	$2.5 \pm 0.5^{a}$
Off-odour	$0.0 \pm 0.0^{\text{b}}$	$0.0 \pm 0.0^{\text{b}}$	$0.0 \pm 0.0^{\text{b}}$	$1.5 \pm 0.5^{a}$
Sweet	$0.0\pm0.0^{a}$	$0.0 \pm 0.0^{a}$	$0.0 \pm 0.0^{a}$	$0.0\pm0.0^{a}$
Bitter	$0.0\pm0.0^{a}$	$0.0 \pm 0.0^{a}$	$0.0 \pm 0.0^{a}$	$0.0\pm0.0^{a}$
Grain-seed (flavour)	$0.6 \pm 0.2^{d}$	3.5±0.5°	$5.3 \pm 1.1^{b}$	$6.6 \pm 1.9^{a}$
Acidulous	$0.0\pm0.0^{a}$	$0.0 \pm 0.0^{a}$	$0.0 \pm 0.0^{a}$	$0.0\pm0.0^{a}$
Rancid (flavour)	$0.0 \pm 0.0^{\text{b}}$	$0.0 \pm 0.0^{\text{b}}$	$1.6 \pm 0.5^{a}$	$2.5 \pm 1.5^{a}$
Fatty (flavour)	$0.0 \pm 0.0^d$	1.5±0.6°	2.50+0.9 <sup>b</sup>	$4.5 \pm 1.5^{a}$
Off-flavour	$0.0 \pm 0.0^{\text{b}}$	$0.0 \pm 0.0^{\text{b}}$	$0.0 \pm 0.0^{\text{b}}$	$2.1\pm0.9^{a}$
Hardness	$3.6 \pm 1.5^{a}$	$4.1 \pm 2.2^{a}$	$4.3 \pm 1.9^{a}$	$5.4 \pm 2.3^{a}$
Chewiness	$4.5 \pm 1.8^{a}$	$5.1 \pm 2.5^{a}$	$4.8 \pm 1.0^{a}$	$5.2 \pm 0.9^{a}$
Adhesiveness	$8.6 \pm 1.2^{a}$	$8.2 \pm 0.9^{a}$	$7.8 \pm 0.5^{a}$	$7.4 \pm 0.8^{a}$
Moistness	$5.6 \pm 0.5^{a}$	$6.1 \pm 0.7^{a}$	5.8±0.9 <sup>a</sup>	4.9±1.1ª

WS – wheat bread, WCh2.5 – bread with 2.5% addition of chia seeds, WCh5 – bread with 5% addition of chia seeds, WCh7.5 – bread with 7.5% addition of chia seeds. Values (mean  $\pm$  standard deviation) in the same row marked with different letters are significantly different at p<0.05.

tem, but show concentrations in certain subspaces. Finding these subspaces allows seeing patterns that are invisible or unnoticeable in the original data layout. The PCA transformation determines the correlation that occurs between multiple variables in a data set. If the data are correlated, then knowing only some of them is enough to determine the characteristics of the tested product [Jolliffe & Cadima, 2016].

The PCA of textural and sensory data of the analysed breads shows that principal component 1 and principal component 2 described 91.42% of the total variation. The PCA plots (Figure 1a and 1b) indicate that standard wheat bread (WS) was closely related to porosity, alcoholic odour, and adhesiveness. WCh2.5 bread was well correlated with moisture content, hardness, and gumminess (both parameters from instrumental texture analysis). WCh7.5 bread was related to such sensory attributes like crust colour, grain-seed, rancid and off-odour, rancid and off-flavour, and with such instrumental texture parameters like springiness and resilience. In turn, WCh5 bread showed a poor correlation with the attributes correlated with the first principal component but correlated with grain-seed, and off-odour, grain-seed, and fatty taste as well as with hardness from sensory assessment.

TABLE 5. Quality characteristics of chia oil during oxidative stability test.

Sample	Time (day)	Iodine value (g $I_2/100$ g)	Peroxide value (mmol O <sub>2</sub> /kg)	Free fatty acid content (mg NaOH/g)
Oil autroated from broad WCb7.5 (OED)	0	$86.12 \pm 0.39^{a}$	22.51±0.49 <sup>b</sup>	3.41±0.15°
Oil extracted from bread WCh7.5 (OFB)	5	$86.66 \pm 0.59^{a}$	$37.93 \pm 0.84^{a}$	$6.24 \pm 0.04^{b}$
	0	$85.70 \pm 0.79^{a}$	5.04±0.17°	$0.85 \pm 0.1^{d}$
Oil extracted from chia seed (OCS)	5	$88.73 \pm 2.99^{a}$	$37.74 \pm 0.22^{a}$	$8.79 \pm 0.03^{a}$

WCh7.5 – bread with 7.5 % addition of chia seeds. Values (mean  $\pm$  standard deviation) in the same columns marked with different letters are significantly different at p<0.05.

#### Analysis of bread and chia oil oxidative stability

During storage, fat may undergo some changes as a result of hydrolysis, oxidation, and polymerization processes, which lead to undesirable organoleptic and chemical changes, lowering the nutritional value as well as consumer acceptance. Such changes can also result in the formation of compounds harmful to health. Especially changes among the fat fraction result in undesired sensory deterioration. There are many factors influencing the oil stability, like fatty acid composition, presence of thermally-oxidised compounds, oxidative enzymes, and antioxidants [Manzocco *et al.*, 2016].

According to the Codex Alimentarius [Joint WHO/ FAO Codex Alimentarius Commission, 2017] requirements, in cold-pressed oils, the content of free fatty acids resulting from fat hydrolysis should be  $\leq$ 4.0 mg KOH/g and the peroxide value should be at <15 mmol O<sub>2</sub>/kg. Oil samples from chia seed (OCS) and from bread with 7.5% of chia seeds (OFB), extracted on the baking day, met the requirements specified in the Codex Alimentarius [Joint WHO/FAO Codex Alimentarius Commission, 2017] for the free fatty acid content (Table 5). After five days of storage, the FFA content of OFB increased to 6.24 mg KOH/g (almost double) and in OCS to 8.79 mg KOH/g (more than 10-fold). The peroxide value of OCS did not exceed the allowable level on the first day of the oxidation stability test. The peroxide value of OFB was higher compared to OCS, which may be due to the thermal processes that take place in bread during baking. Qualitative changes in OCS after five days of aerating at 60°C corresponded to the changes that took place in OFB. The iodine value, which is a measure of the degree of unsaturation of fats used to identify them in products, did not change during bread storage, compared to the value obtained on the bread baking day. For OCS, it changed slightly from 85.70 to 88.73 g  $I_2/100$  g (Table 5) and the increase observed was statistically insignificant ( $p \ge 0.05$ ). The obtained results indicate that in breads after 5 day of storage, the process of chia seeds fat oxidation was less advanced as compared to pure chia seeds oil extracted from raw material and subjected to the oil oxidative stability test. The high value of the iodine value indicates a high content of unsaturated fatty acids in the tested samples, which was confirmed by the fatty acid profile. Similar observations were made by Maire et al. [2013] who investigated baked products enriched with raw materials, being the source of unsaturated

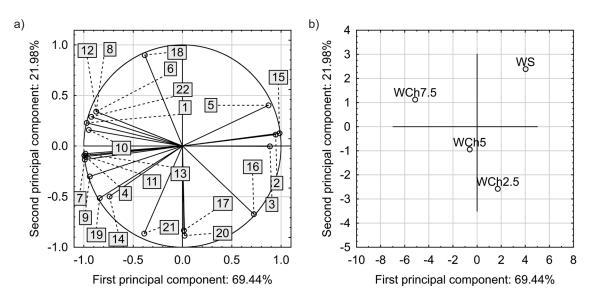


FIGURE 1. a) Projection of variables on the factor plane x: Crust colour (1), Porosity (2), Flour (3), Grain-seed (odour) (4), Alcoholic (odour) (5), Rancid (odour) (6), Fatty (odour) (7), Off-odour (8), Grain-seed (taste) (9), Rancid (taste) (10), Fatty (taste) (11), Off-flavour (taste) (12), Hardness (13), Chewiness (14), Adhesiveness (15), Moistness (16), Hardness txt (17), Springiness txt (18), Cohesiveness txt (19), Gumminess txt (20), Chewiness txt (21), Resilience txt (22); b) Projection of cases on the factor plane x: WS – wheat bread, WCh2.5 – bread with 2.5% of chia seeds, WCh5 – bread with 5% of chia seeds.

Fatty acids	Chia seeds	WS	WCh2.5	WCh5	WCh7.5
		Content (% of t	otal fatty acids)	-	
12:0	$0.004 \pm 0.001^{d}$	$0.028 \pm 0.003^{a}$	$0.011 \pm 0.000^{\text{b}}$	$0.007 \pm 0.000^{\circ}$	$0.007 \pm 0.000^{\circ}$
14:0	$0.027 \pm 0.004^{d}$	$0.233 \pm 0.009^{a}$	$0.101 \pm 0.002^{b}$	$0.076 \pm 0.000^{\circ}$	$0.071 \pm 0.005^{\circ}$
15:0	$0.013 \pm 0.001^{\circ}$	$0.212 \pm 0.004^{a}$	$0.089 \pm 0.000^{\text{b}}$	$0.078 \pm 0.001^{\circ}$	$0.052 \pm 0.000^{d}$
16:0	6.178±0.092°	$20.636 \pm 0.201^{a}$	$16.308 \pm 0.219^{b}$	14.625±0.541°	$13.341 \pm 0.476^{d}$
16:1 <i>n</i> -9	$0.046 \pm 0.007^{\circ}$	$0.560 \pm 0.0040^{a}$	$0.199 \pm 0.019^{\text{b}}$	$0.141 \pm 0.011^{\circ}$	$0.098 \pm 0.003^{d}$
16:1 <i>n</i> -7	$0.062 \pm 0.004^{\circ}$	$0.298 \pm 0.004^{a}$	$0.221 \pm 0.002^{b}$	$0.149 \pm 0.009^{\circ}$	$0.119 \pm 0.006^{d}$
17:0	$0.028 \pm 0.000^{\circ}$	$0.091 \pm 0.004^{b}$	$0.072 \pm 0.001^{\circ}$	$0.103 \pm 0.000^{a}$	$0.063 \pm 0.000^{d}$
17:1	$0.011 \pm 0.001^{d}$	$0.042 \pm 0.006^{a}$	$0.034 \pm 0.000^{b}$	$0.027 \pm 0.003^{\circ}$	$0.022 \pm 0.009^{\circ}$
18:0	2.178±0.095°	$0.801 \pm 0.021^{d}$	$2.161 \pm 0.012^{\circ}$	$3.166 \pm 0.065^{b}$	$3.376 \pm 0.035^{a}$
18:1 <i>n-</i> 9	4.699±0.057°	$9.380 \pm 0.047^{a}$	$7.405 \pm 0.057^{b}$	6.998±0.052°	6.513±0.133 <sup>d</sup>
18:1 <i>n</i> -7	$0.623 \pm 0.033^{d}$	$0.817 \pm 0.044^{a}$	$0.724 \pm 0.006^{\circ}$	$0.619 \pm 0.012^{d}$	$0.741 \pm 0.005^{b}$
18:2 <i>n</i> -6	18.658±0.062°	$63.155 \pm 0.181^{a}$	42.421±0.139 <sup>b</sup>	30.724±0.401°	$30.072 \pm 0.028^{d}$
18:3 <i>n</i> -3	$67.216 \pm 0.085^{a}$	$3.365 \pm 0.042^{\circ}$	$29.876 \pm 0.025^{d}$	42.813±0.136°	45.097±0.263 <sup>b</sup>
20:0	$0.172 \pm 0.004^{d}$	$0.078 \pm 0.013^{\circ}$	$0.180 \pm 0.001^{\circ}$	$0.318 \pm 0.009^{a}$	$0.277 \pm 0.003^{b}$
20:1	$0.088 \pm 0.000^{d}$	$0.308 \pm 0.008^{a}$	$0.200 \pm 0.008^{b}$	$0.160 \pm 0.009^{\circ}$	$0.155 \pm 0.005^{\circ}$
		Nutritional i	ndexes of fat		
Σ SFA	8.600	22.079	18.922	18.373	17.187
Σ MUFA	5.529	11.405	8.783	8.094	7.648
ΣPUFA	85.874	66.520	72.297	73.537	75.169
AI	0.06	0.28	0.21	0.18	0.17
TI	0.04	0.46	0.16	0.12	0.11
HH	14.6	3.64	4.86	5.48	6.09
PUFA/SFA	9.98	3.01	3.82	4.00	4.37
n-6/n-3	0.28	18.77	1.42	0.72	0.67

TABLE 6. Fatty acid profile and fat nutritional quality of chia seeds and supplemented breads.

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; AI – atherogenicity index; HH – ratio of hypocholesterolemic to hypercholesterolemic fatty acids, TI – thrombogenicity index; WS – wheat bread, WCh2.5 – bread with 2.5% addition of chia seeds, WCh5 – bread with 5% addition of chia seeds, WCh7.5 – bread with 7.5% addition of chia seeds. Values (mean ± standard deviation) in the same row marked with different letters are significantly different at p<0.05.

fats, and observed that lipid oxidation occurred at a very early stage (during dough preparation) and to a minor extent during the baking process.

# **Determination of fatty acid profile**

Chia seed oil can be an alternative for vegetarians and people who are allergic to fish products [Zettel & Hitzmann, 2018]. PUFA *n*-6 and *n*-3 are essential fatty acids that must be derived from the diet. The human body does not have a suitable enzyme for the endogenic synthesis of  $\alpha$ -linolenic and linoleic acid, due to the lack of desaturases introducing double bonds at the 3<sup>rd</sup> and 6<sup>th</sup> carbon atoms in the carbon chain. These acids are precursors for the synthesis of long--chain polyene fatty acids such as eicosapentaenoic acid and docosahexaenoic acid [Martynowicz *et al.*, 2019]. In the fatty acid profile of chia seeds as well as bread supplemented with chia seeds, predominant were the 18-carbon chain fatty acids, with their contents reaching up to 82.42% in WCh7.5 and 93.37 in chia seeds (Table 6).

 $\alpha$ -Linolenic acid (18:3n-3) had the greatest share in the fatty acid profile of chia seeds and the lowest one in the fatty acid profile of wheat bread (67.216 and 3.365%, respectively). Analyses showed a significant increase in the share of 18:3*n*-3 in the fatty acid profile of breads along with chia seed content increase from 29.876 in WCh2.5 to 45.097% in WCh7.5, and a significant decrease in the share of linoleic acid (18:2*n*-6) in the fatty acid profile of breads with increasing chia seed content. In the fatty acid profile of wheat bread, a much greater share of 18:2*n*-6 was observed compared to the profiles of other breads (Table 6). Osuna *et al.* [2016] and Melilli *et al.*  [2020] observed similar proportions in the fatty acid profile of wheat bread.

Chia oil is a valuable source of  $\alpha$ -linolenic acid and phytosterols. The nutritional value of bread with chia seeds was evaluated by such nutritional indexes as AI, HH, TI, PUFA/ SFA, and the n-6/n-3 ratio. These indexes evaluate the nutritional quality of foods based on their fatty acid compositions. AI and TI can be used as predictors or risk factors for cardiovascular diseases and should be kept at low levels in a healthy everyday diet [Ulbricht & Southgate, 1991]. The AI ranged from 0.17 for WCh7.5 to 0.28 for WS, meanwhile for chia seeds it reached 0.06. A decrease in the value of this index was observed along with an increase in chia seed content in bread. The TI ranged from 0.11 to 0.46 for WCh7.5 and wheat bread, respectively, and was 0.04 for chia seeds. The HH index indicates the effect of fatty acids on cholesterol metabolism and its high value is important from a nutritional point of view. The HH values ranged from 3.64 for WS to 6.09 for WCh7.5 (14.6 for chia seeds). An increase in the value of this index was observed with the increase in chia seed content in bread. Another increase was observed in the breads with chia seeds for the PUFA/SFA ratio. Its values ranged from 3.01 (WS) to 4.37 (WCh7.5), while for chia seeds it was 9.98 (Table 6). A low PUFA/SFA ratio in diets is considered a risk factor for increased blood cholesterol levels. In the present study, the PUFA/SFA ratio determined for the breads with chia seeds was by 0.8 to 1.4 times higher than that determined for wheat bread. Due to a high content of linolenic acid in chia seeds, their addition to bread formulations caused a significant decrease in the n-6/n-3 ratio which ranged from 1.42 (WCh2.5) to 0.67 (WCh7.5). While, for wheat bread, its value was as high as 18.77. Modern western diets provide excessive levels of n-6 but very low levels of n-3 fatty acids, leading to an undesired (from a nutritional point of view) n-6/n-3 ratio, characterised by 10- to 20-fold predominance of n-6 acids [Simopoulos, 2008]. Therefore, the ratio of 18:3n-3 to 18:2n-6 in chia seeds and hence bread supplemented with this seeds may have beneficial effects on the human health [Smith et al., 2011]. Other authors observed similar relationships regarding the improvement of the nutritional value of bread upon the addition of functional ingredients, like purslane flour [Melilli et al., 2020] or oilseeds, such as flax or soybeans [Osuna et al., 2016]. Therefore, consuming the bread with chia seeds would help increase the n-3 intake in the dietary habits of the population. Overall, the nutritional indexes used for quality evaluation indicated that breads with chia seeds, especially with their 5% and 7.5% content, could have beneficial health effects for consumers.

# CONCLUSION

The wheat grain and chia seeds used for bread making differed significantly in the content of proximate chemical components. The addition of chia seeds to dough formulations at the three levels tested did not deteriorate bread quality. The total baking loss and loaf volume of the breads with chia seed addition did not differ significantly from these of the wheat bread. The sensory and organoleptic quality of food products is considered to be the key factor driving consumers' purchase decisions. The chia seed content not exceeding 5% did not contribute to reducing the overall sensory quality of bread. The addition of 7.5% chia seeds significantly reduced consumer acceptance due to the appearance of fatty, rancid, and off-flavour. The chemical composition of chia seeds, especially the composition of fatty acids, is very valuable from the nutritional point of view. Enrichment and fortification of bakery products with chia seeds could be justified for nutritional reasons. The results do not confirm the negative impact of chia seeds on shortening the bread shelf life resulting from oxidative changes of fats.

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## **CONFLICT OF INTEREST**

Authors declare no conflict of interest.

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