

Evaluation of the quality, nutritional value and antioxidant activity of gluten-free biscuits made from corn-acorn flour or corn-hemp flour composites

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Abstract The aim of this study was to evaluate the effect of substituting 20–60% of corn flour with acorn or hemp flour on the quality, nutritional, pro-health value, and sensory properties of gluten-free biscuits. Partial replacement of corn flour with the mentioned above flours resulted in a significant reduction of biscuit volume and an increase of their hardness. The biscuits in the experiment were significantly darker than the control ones. Increasing the content of both studied flours resulted in a distinct trend of color variation from yellow toward red-purple. The protein content in biscuits with the addition of acorn flour did not differ significantly in comparison with the control biscuits, whereas biscuits with flour hemp contained 40–122% more protein in comparison with the control ones. The total dietary fiber content increased correspondingly with the addition of the investigated flours. The total polyphenols content (TPC) in biscuits with the acorn flour addition increased in the range of 308–801% in relation to the control, and the addition of hemp flour increased the TPC in the range of 41–143%. It was found that acorn flour

contributed to higher growth (an average of 367%) of the antioxidant activity of the biscuits in relation to the control samples than the hemp flour (an average of 114%). The control biscuits obtained the highest sensory score, similarly biscuits with 20 and 40% acorn flour addition. The most favorable replacement should be that not more than a 40% substitute of corn flour with acorn flour.

Keywords Gluten-free biscuits · Acorn flour · Hemp flour · Quality · Nutritional value · Polyphenols

Introduction

Biscuits are one of the most consumed baked goods worldwide [1]. They can be produced both from raw materials containing gluten and naturally gluten-free raw materials. However, the kind of raw materials used largely affects the properties of the final product as well as its nutritional value [2]. Gluten-free foods, produced for people suffering from various gluten-related disorders, often have less nutritional and pro-health value than their gluten counterparts, but more fats and therefore they have a higher caloric value [3, 4]. That is why a gluten-free diet may lead to deficiency in minerals, protein, fiber and other nutritional compounds [5]. It should be stressed that, however, these gluten-free products are less available on the market [4]. As the main ingredients of gluten-free bakery products are very often raw starch materials, in particular corn, rice and tuber starches, lately there has been an increase in interest in alternative gluten-free raw materials which would allow for the creation of gluten-free products with increased nutritional values and similar in quality to products based on raw materials containing gluten [5, 6]. Such ingredients

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which are not yet widely used could be flours from acorns and hemp.

The acorn seeds, genus *Quercus* family *Fagaceae*, have been used as food products for a long time [7]. In certain countries, they are still used in various forms—whole seeds in desserts, ground seeds in breads and cakes or as coffee ersatz, as well as in the form of an oil [8, 9]. Due to a significant number of species belonging to the *Fagaceae* family, the chemical composition of the acorn is very diverse. In general, it can be assumed that the acorn seeds contain approx. 3–8% protein with a high content of essential amino acids, 1–31% fat with a high content of unsaturated fatty acids, as well as significant amounts of antioxidants, the amount of which increases after heat treatment due to the degradation of tannins [7, 10–15].

The non-drug variety *Cannabis sativa* subsp. *sativa*, called hemp, contains small amounts of psychoactive Δ^9 -tetrahydrocannabinol THC which is allowed for legal cultivation in some countries [16]. Hemp seeds contain approx. 20–25% protein with a biological value similar to the hen egg white, 25–35% fat consisting essentially of polyunsaturated fatty acids (PUFAs), as well as considerable quantities of minerals and fiber [17–20]. Similarly to the acorn, hemp seeds have long been used (and to a minor degree they are still used now) for culinary purposes, e.g., as a snack or in the form of an oil [17].

Flours from acorn and hemp are a rich source of bioactive compounds from the polyphenols group [15, 21]. These compounds have an anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-viral, anti-thrombotic, anti-cancer and cardioprotective effects, as well as vasodilatory effects and neuroprotective features [22, 23].

Therefore the aim of this study was to assess the impact of the partial substitution of corn flour by acorn or hemp flour on the hydration properties of their mixtures, as well as on the quality, nutritional and pro-health value of gluten-free biscuits, and the sensory properties of these products.

Materials and methods

Materials

The basic biscuit formula consisted of 100 g of corn flour (the basic composition: protein 4.8 g 100 g⁻¹, fat 3.1 g 100 g⁻¹, dietary fiber 2.6 g 100 g⁻¹, carbohydrates 76.0 g 100 g⁻¹, ash 0.6 g 100 g⁻¹), 50.3 g sucrose, 26.8 g margarine, 17.9 g milk, 7.3 g fresh hen eggs, 4.5 g potato starch, 0.3 g salt and 3.6 g of gluten-free baking powder. All ingredients came from local supermarkets or retail shops. In the biscuits with the added test flours, the appropriate amount of corn flour (20, 40 or 60%, i.e., 20, 40 or 60 g) was replaced with the debittered acorn flour (Dary

Natury, Poland) which has the basic composition: protein 5.7 g 100 g⁻¹, fat 5.2 g 100 g⁻¹, fiber 18.1 g 100 g⁻¹, carbohydrates 80.9 g 100 g⁻¹, ash 1.2 g 100 g⁻¹, or hemp flour (Radgeb, Poland) with the basic composition: protein 19.7 g 100 g⁻¹, fat 7.8 g 100 g⁻¹, fiber 51.8 g 100 g⁻¹, carbohydrates 60.9 g 100 g⁻¹, ash 6.6 g 100 g⁻¹. The basic composition of the studied flours was determined in the laboratory (see section “[Chemical analyses of flours and biscuits](#)”). Samples (flour blends or biscuits) were marked with the following symbols: C—control (corn flour or the biscuits containing only corn flour); A—acorn flour; H—hemp flour; 20, 40, 60—the percentage of studied flours in each blend to study the hydration properties and the finished baked biscuits.

Methods

Hydration properties of flour mixtures and their oil absorption capacity

The corn flour and its blends with acorn or hemp flour were investigated in the same proportions as the baking biscuits. Swelling volume SV (i.e., the volume occupied by a known weight of sample) was determined by the Mancebo et al.'s [2] procedure. In brief: 100 mL of distilled water was added to 5 g of flour and after mixing it was left for 24 h, and then the volume of the precipitation was measured. Water holding capacity WHC (i.e., the amount of water bound by the sample not subjected to the action of any external force) was determined by water separation on a sintered glass filter, from the sample used to denote swelling volume [2]. Water absorption, WA (i.e., the maximum amount of water that can be absorbed and retained by sample after its centrifugation at low speed), was carried out according to the AACC standard nr 56-30.01 [24]. Oil absorption capacity, OAC (i.e., the amount of oil that can be absorbed and retained by centrifuged sample), was determined according to the Raghavendra et al. [25] procedure. In brief, 10 mL of oil was added to 0.5 g of the sample and after mixing by glass rod the blend was left for 24 h, and then it was centrifuged for 30 min at 3000×g. All the analyses were performed in triplicate.

Biscuits preparation

All ingredients listed in the section “[Materials](#)” were mixed for 5 min in a mixer Major Classic KM636 (Kenwood, UK), at the second speed stage. Then, the biscuit dough was rolled out to a thickness of 5 mm and cut into a diameter of 5 cm. The biscuits were baked for 10 min at a temperature of 190 °C of the lower heating element and 220 °C of the upper heating element (electric oven MIWE Condo type CO 2 0608, MIWE GmbH, Germany). The biscuits

were baked in two separately prepared batches of dough, and each batch yielded at least 30 biscuits.

Chemical analyses of flours and biscuits

The basic chemical composition of the flours and biscuits was determined by the following methods: protein content ($N \times 6.25$) by Kjeldahl method, using the Büchi B324 extraction system (Büchi Labortechnik AG, Switzerland) (AOAC 950.36), fat content by Soxhlet method using Büchi B811 (Büchi Labortechnik AG, Switzerland) (method 935.38), ash content by carbonization (method 923.03), and soluble, insoluble and total dietary fibre by the enzymatic–gravimetric method (991.43) [26]. Total carbohydrates amount was calculated by subtracting from 100% the sum of protein, fat, ash and water. The available carbohydrates content was calculated by subtracting total dietary fiber from total carbohydrates. The energy value of biscuits was calculated by the use of Atwater coefficients (protein and available carbohydrates 4 kcal g⁻¹, fat 9 kcal g⁻¹) [27]. Analyses were performed in duplicate.

The polyphenol compounds content and antioxidant activity in biscuits were analyzed by extracting 0.6 g of analyzed material in 10 mL of 80% ethanol under ambient conditions, for 2 h in a type WB22 water bath (Memmert, Germany) that was equipped with a shaking device. Then, the extract was separated in a type MPW-350 centrifuge (MPW Med. Instruments, Poland) at 1050×g for 15 min and stored in a refrigerator. The following chemical analyses concerning the content of polyphenolic compounds and antioxidant activity were performed: total content of polyphenols by spectrophotometric method, according to Singleton et al. [28]; content of flavonoids by spectrophotometric method, according to El Hariri et al. [29]; content of phenolic acids, flavonols and anthocyanins by spectrophotometric method, according to Mazza et al. [30]; with the modification of Oomah et al. [31]; content of tannins by spectrophotometric method, according to Broadhurst and Jones [32]. Additionally, antioxidant activity was analyzed by a method with free ABTS radical [33], and expressed as Trolox Equivalent Antioxidant Capacity (TEAC—mM Tx kg⁻¹ d.m. of sample). The experiment was repeated three times.

Physical properties of flours and biscuits

The volume of biscuits was determined with the laser volume meter Volscan profiler 600 (Stable Micro Systems, England). The hardness of biscuits was determined using the texture analyzer TA-XT2plus (Stable Micro Systems, England), and the results were calculated by the use of the Texture Exponent (Stable Micro Systems, Surrey, England). The SMSP/6 probe, test speed 2 mm s⁻¹, distance

10 mm, and trigger force 0.03 N were applied. The diameters of biscuits were measured using a caliper (in two perpendicular directions, the results are the average) and thickness of six biscuits (three biscuits, each prepared from separate batches of dough). Spread factor SF was calculated by dividing width of the biscuits by their thickness.

The color assessment of flours and biscuits was performed by instrumental method using the CIE $L^*a^*b^*$ [34] system. Measurements were done using the reflection method with the use of Konica Minolta CM-3500d (Konica Minolta, Inc., Japan). The visual angle was 10°. The diaphragm with a diameter of 30 mm and Petri dish with a diameter of 55 mm were used. The following parameters were measured: L^* —brightness of the color ($L^*=0$ black, $L^*=100$ white), a^* —share of the green color ($a^*<0$) or red ($a^*>0$), b^* —share of the blue ($b^*<0$) or yellow ($b^*>0$), C^* (chroma)—saturation of color, h (hue)—shade of color. Each sample was measured in six replicates (three biscuits with each separately prepared batches of dough). Color differences (ΔE^*) between samples were calculated according to:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}. \quad (1)$$

Sensory evaluation

The sensory evaluation of biscuits was conducted by 15 people from the age of 21–49. A 7-point hedonic scale (from “extremely dislike” to “extremely like”) was used. Color, smell, taste, consistency and appearance were evaluated.

Statistical analysis

To determine the significance of differences between biscuits, one-way analysis of variance (ANOVA) and the least significant difference (LSD) at significance level of 0.05, using Fisher post hoc test, were calculated. The calculations were performed with statistical software package Statistica 10.0 (StatSoft Inc., USA).

Results and discussion

Hydration properties of flour mixtures

The partial substitution of the corn flour (control sample) for the above-mentioned flours contributed to a significant increase of swelling volume. SV increased with the increasing amount of acorn or hemp flour in the blend, in the range 20–57%, but higher SV value was observed in mixtures containing hemp flour (Table 1). Similarly, the

Table 1 Hydration properties of flour composites and their oil absorption capacity

Sample	SV (mL g ⁻¹)	WHC (g g ⁻¹)	WA (g g ⁻¹)	OAC (g g ⁻¹)
Control	2.30 ^a ±0.10	2.11 ^a ±0.05	1.06 ^a ±0.04	0.57 ^a ±0.08
A20	2.77 ^b ±0.15	2.12 ^a ±0.04	1.10 ^{ab} ±0.04	0.62 ^{ab} ±0.05
A40	2.94 ^{bc} ±0.12	2.18 ^a ±0.11	1.17 ^{bc} ±0.09	0.66 ^{ab} ±0.05
A60	3.14 ^{cd} ±0.21	2.25 ^a ±0.10	1.26 ^{cd} ±0.06	0.69 ^b ±0.06
H20	3.17 ^{cd} ±0.12	2.24 ^a ±0.11	1.32 ^{de} ±0.07	0.63 ^{ab} ±0.07
H40	3.37 ^{de} ±0.27	2.63 ^b ±0.18	1.38 ^e ±0.08	0.71 ^b ±0.04
H60	3.62 ^e ±0.22	3.13 ^c ±0.21	1.42 ^e ±0.04	0.85 ^c ±0.05

Mean value ± standard deviation

Values signed with the same letters in particular columns do not differ significantly at 0.05 level of confidence

partial replacement of corn flour for both flours increased the value of water absorption in comparison with control sample by 10–39%, except for A20. Slight differences were found in the water holding capacity, because despite the increase of this parameter in all samples compared with control, only in the samples H40 and H60 this increase was statistically significant (by 25 and 48%, respectively). Furthermore, partial substitution of the corn flour for the above-mentioned flours resulted in an oil absorption capacity increase, in the range 9–21% in the case of flour with acorn and 11–49% in the case of flour with hemp. The hydration properties of flour are most significantly affected by dietary fiber, protein and starch content [2, 25, 35]. The protein binds approx. twice as much liquid compared to its weight, native starch approx. 33%, but with the increasing amount of damaged granules the water absorption significantly increases. Dietary fiber, depending on its botanical origin and composition, binds approx. 3–10 fold more water in relation to its weight [2, 25, 36]. The protein content in the acorn flour was at a higher level than in corn flour, while in hemp flour was approx. fourfold higher. In turn, dietary fiber content varied widely, the lowest in corn

flour, approx. sevenfold higher in acorn flour and approx. 20-fold higher in hemp flour. This had a direct impact on the significant differences in hydration properties of the tested blends (Table 1).

Chemical composition of biscuits

The protein content of biscuits with acorn flour did not differ significantly in comparison with the control ones, and biscuits with the hemp flour addition contain about 40–122% more protein than the control (Table 2). Švec et al. [37] proved that a 20% hemp flour (wholemeal dehulled and hulled, industrial or laboratory obtained) substitution for wheat flour (containing 12.5% of protein) increased the protein content in the range of 16–27%. Corn flour used in these studies contained 4.8% of protein (however, Akubor and Onimawo [38] reported 9.2% in their study); therefore, the increase in the amount of this component in the biscuit due to the hemp flour addition was greater.

In terms of fat content, only biscuits enriched with 40 and 60% of hemp flour differed from the control biscuits and contained 7 and 12% more fat, respectively. Inconsiderable differentiation of fat in biscuits resulted from the fact that the base formulation contained approx. 27% fat (in relation to the flour), and the amount of this component brought by the two examined flours was so small that did not significantly impact the overall fat content.

The mineral content of all types of biscuits differed due to the large difference of the ash content in the test flours. The hemp flour, which contained the most ash, increased the minerals content more than 16-fold in biscuits with a 60% blend, and the smallest increase, about 88%, was recorded in biscuits with 20% acorn flour addition. The ash content was significantly higher in biscuits with the incorporation of hemp flour than in wheat flour biscuits with an inulin or bee pollen addition: 0.4–0.5% [39, 40], wheat taro composite biscuits 0.92–1.32% [41], oat cookies containing

Table 2 Chemical composition and energy value of gluten-free biscuits

Sample	Protein (g kg ⁻¹)	Fat (g kg ⁻¹)	Ash (g kg ⁻¹)	TDF (g kg ⁻¹)	SDF (g kg ⁻¹)	IDF (g kg ⁻¹)	TC (g kg ⁻¹)	AC (g kg ⁻¹)	Energy (kcal kg ⁻¹)
Control	35.6 ^a ±2.1	120.4 ^a ±5.9	8.7 ^a ±1.7	22.0 ^a ±1.0	5.5 ^a ±0.8	16.5 ^a ±0.3	780.6 ^e ±9.1	758.6 ^g ±8.3	4259 ^g ±19
A20	35.8 ^a ±1.2	122.7 ^{ab} ±2.6	16.3 ^b ±2.6	40.7 ^b ±1.7	9.0 ^b ±0.6	31.7 ^b ±1.9	753.1 ^d ±3.3	712.3 ^f ±4.6	4103 ^f ±41
A40	36.2 ^a ±1.6	124.8 ^{ab} ±2.1	24.4 ^c ±1.7	58.3 ^c ±4.1	12.4 ^c ±0.8	45.8 ^c ±3.5	744.2 ^d ±1.2	686.0 ^e ±4.7	4011 ^e ±33
A60	36.5 ^a ±1.0	126.6 ^{ab} ±5.6	32.8 ^d ±2.2	76.8 ^d ±3.7	16.2 ^c ±0.8	60.7 ^d ±3.1	744.6 ^d ±6.1	667.8 ^d ±3.0	3965 ^d ±44
H20	50.0 ^b ±3.1	125.6 ^{ab} ±4.0	56.0 ^e ±4.2	75.0 ^d ±3.4	8.4 ^b ±0.5	66.7 ^d ±3.0	706.8 ^c ±2.7	631.8 ^c ±5.9	3857 ^c ±39
H40	64.2 ^c ±4.2	129.3 ^{bc} ±5.2	102.1 ^f ±6.1	127.2 ^e ±6.1	11.2 ^c ±0.9	116.1 ^e ±5.2	646.4 ^b ±15.8	519.2 ^b ±11.5	3505 ^b ±20
H60	79.2 ^d ±3.8	134.9 ^c ±4.1	149.2 ^g ±9.1	179.9 ^f ±7.9	14.0 ^d ±1.0	166.0 ^f ±8.9	577.7 ^a ±10.6	397.7 ^a ±4.3	3122 ^a ±22

Mean value ± standard deviation

Values signed with the same letters in particular columns do not differ significantly at 0.05 level of confidence

oat bran 1.59–2.58% [42], wheat flour substituted with 25% defatted corn germ flour: 2.9% [43], wheat biscuits supplemented with 10–50% partially de-oiled maize germ cake: 1.09–4.39% [44], corn cookies: 4.3% [45] and similar to the wheat–sesame peels biscuits: 8.45–12.87% [46].

Similarly, partial replacement of corn flour with hemp or acorn flours contributed to a substantial and significant increase in dietary fiber content. The total dietary fiber (TDF) content increased as the amount of examined flours increased; however, the hemp flour had a more pronounced effect than acorn flour. The same trend was found in the case of insoluble dietary fiber (IDF), as well as the soluble dietary fraction (SDF). The recommended Adequate Intake of dietary fiber for adults is 25–38 g day⁻¹ (14 g 1000 kcal⁻¹ day⁻¹), and the ratio of the insoluble to the soluble fraction should be 3:1 [47, 48]. Meanwhile, actual fiber intake is much lower and in the US is approx. 15 g day⁻¹ [49], therefore an increase of its content in the sweets and snack products is beneficial from a nutritional point of view. The ratio of IDF to SDF in the control biscuits was compatible with the recommended daily allowance, and in the biscuits with the addition of acorn flour the rate was close to optimal (3.5–3.7:1). In the biscuits enriched with hemp flour, the content of insoluble dietary fiber was much higher, which resulted in IDF:TDF ratio in the range of 8–12:1.

The carbohydrate content, both total and available, in the investigated biscuits enriched with hemp and acorn flour was lower than that in control ones, and the energy value decreased correspondingly. The most calorific were the control set biscuits, and the least calorific were the biscuits with 60% hemp flour.

Total polyphenols content (TPC) in flours with acorn and hemp were determined at the level of 16.09 or 4.11 mg catechin g⁻¹ and tannins 8.79 or 0.71 mg catechin g⁻¹, respectively. The contents of other phenolic compounds in the acorn and hemp flours were: phenolic

acids (mg ferulic acid g⁻¹)—0.878, 1.555; flavonoids (mg rutin g⁻¹)—3.012, 1.170; flavonols (mg quercetin g⁻¹)—0.548, 1.580; anthocyanins (mg cyanidin-3-glucoside g⁻¹)—0.161, 0.407, respectively. The content of polyphenols and tannins in acorn flour according to Rakić et al. [15] is 11.76–14.93 mg catechin g⁻¹ and 7.76–10.9 mg catechin g⁻¹, and the content of polyphenols in hemp flour—7.72 mg catechin g⁻¹ [21]. It can be said that the results presented in this paper concerning the above-mentioned phenolic compounds are consistent with the literature, and some differences most likely result from different extraction methods, various methods of determinations, as well as the volatility of varied, climatic and soil conditions.

Taking into account the large quantities of bioactive compounds from the polyphenols group in the analyzed flours, their use in gluten-free biscuits production seems to be fully justified, because such additions should enhance these products with regard to health promoting compounds.

The total polyphenols content (TPC) in biscuits with 20–60% acorn flour addition increased in the range of 308–801% in relation to the control, and the addition of hemp flour increased the TPC in the range of 41–143%. A similar tendency was observed in the flavonoids content; their increase in acorn flour biscuits was on average 488%, and in hemp flour biscuits at 90% relative to control (Table 3). The content of TPC and flavonoids increased with increasing the amount of acorn and hemp flours in biscuits; however, the more favorable additive in terms of a greater amount of introduced phenolic compounds was flour from acorn. The 60% addition of flour from acorn was the most preferred addition to biscuits because it guaranteed the greatest amount of polyphenols and flavonoids in this product (Table 3). It may be noted that the acorn flour was richer in flavonoids and TPC than hemp flour and, hence, a far bigger amount of these compounds in biscuits with acorn flour than hemp flour.

Table 3 Polyphenols content and antioxidant activity of gluten-free biscuits with acorn and hemp flour addition

Sample	TPC Total phenolic content (mg catechin g ⁻¹)	Content of flavo- noids (mg rutin g ⁻¹)	Content of phenolic acid (mg ferulic acid g ⁻¹)	Content of flavonols (mg quercetin g ⁻¹)	Content of anthocyanins (mg cyanidin-3-glu- coside g ⁻¹)	Content of tannins (mg catechin g ⁻¹)	ABTS TEAC (mmol Tx kg ⁻¹)
Control	0.87 ^a ±0.02	0.356 ^a ±0.070	0.235 ^a ±0.029	0.180 ^a ±0.012	0.384 ^b ±0.066	0.097 ^c ±0.000	15.10 ^a ±1.36
A20	3.55 ^e ±0.05	1.956 ^d ±0.022	0.971 ^b ±0.035	1.009 ^d ±0.032	2.474 ^d ±0.134	1.043 ^d ±0.023	67.93 ^e ±1.01
A40	5.99 ^f ±0.01	1.988 ^d ±0.081	1.356 ^c ±0.058	1.563 ^e ±0.02	3.0854 ^e ±0.066	1.161 ^e ±0.011	69.89 ^f ±0.00
A60	7.84 ^g ±0.16	2.364 ^e ±0.076	1.461 ^c ±0.070	1.714 ^f ±0.089	3.875 ^e ±0.050	1.375 ^f ±0.005	75.53 ^g ±0.85
H20	1.23 ^b ±0.02	0.576 ^b ±0.039	1.312 ^c ±0.039	0.183 ^a ±0.008	0.228 ^a ±0.022	0.032 ^a ±0.000	22.10 ^b ±2.34
H40	1.65 ^c ±0.04	0.632 ^{bc} ±0.089	1.388 ^c ±0.031	0.237 ^b ±0.028	0.368 ^b ±0.000	0.057 ^b ±0.003	32.58 ^c ±1.19
H60	2.12 ^d ±0.02	0.846 ^c ±0.046	1.480 ^c ±0.110	0.377 ^c ±0.072	0.672 ^c ±0.143	0.058 ^b ±0.006	43.76 ^d ±0.97

Mean value ± standard deviation

Values signed with the same letters in particular columns do not differ significantly at 0.05 level of confidence

In the case of other bioactive compounds from the polyphenols group (phenolic acids, flavonols, anthocyanins), it was observed that although their amount was lower in acorn flour compared to hemp flour, the biscuits fortified with acorn flour were characterized by a higher content of these polyphenols than the biscuits with hemp flour (Table 3). It can be probably explained by the fact that the acorn flour contains a high amount of tannins, which under high temperature during baking biscuits turn into low-molecular weight phenolic compounds [15], i.e., phenolic acids, flavonols, anthocyanins and undetermined in this work other phenolic compounds. Therefore, a larger amount of these compounds were observed in biscuits with the addition of acorn flour than in the biscuit with hemp flour. Both studied flours contributed to the enrichment of the gluten-free biscuits in the phenolic acids, flavonols and anthocyanins relative to the control sample. It was also observed that despite the decomposition of tannins during baking biscuits with acorn flour, there was a greater amount present in the final product. The effect of tannins degradation was high anthocyanin content in the aforementioned biscuits. This is particularly important because such compounds are phytochemicals capable of scavenging free radicals and thus reducing the risk of chronic diseases (such as cardiovascular disease, cancer) and age-related neuronal degeneration [50].

It was found that acorn flour contributed to higher growth (an average of 367%) in the antioxidant activity of biscuits in relation to the control samples than the hemp flour (an average of 114%) (Table 3). This is most likely related to the large amounts of polyphenols and tannins in biscuits with the addition of acorn flour and in flour itself, because tannins have 15–30-times greater activity than other polyphenols (TEAC for acorn flour: 76 mM Tx kg⁻¹, and for hemp flour: 53 mM Tx kg⁻¹). The coefficient correlation between the TPC and the antioxidant activity was $r=0.92$, and between tannins content and the antioxidant activity was $r=0.93$. In the research of Chauhan et al. [51], the antioxidant activity of gluten-free cookies made with addition of raw and germinated amaranth flour was 15.10 or 21.43 g 100 g⁻¹, depending on the kind of additive. In turn, in the research of Sharma et al. [52], the content of total polyphenols in gluten-free cookies made with addition of foxtail millet, Barnyard millet or Kodo millet flour increased in a range of 30%–200% (23.5–58.4 mg 100 g⁻¹), and antioxidant activity increased on average by about 40%, compared with the control. Krystyjan et al. [39] examined the biscuits, in which 20–50% of the fat was replaced with inulin, and found that the total polyphenols content was in the range of 0.875–0.723 mg catechin g⁻¹ d.m., and TEAC 13.8–15.6 mM Tx kg⁻¹ d.m. In studies of the baked goods with hemp, TPC was at the

level 5.21 mg gallic acid g⁻¹ [53]. TPC in biscuits with mango peel powder ranged from 1.8 to 4.5 gallic acid g⁻¹ [54]. According to Pasqualone et al. [55], biscuits enriched with grape mare extract contained 0.629 mg gallic acid g⁻¹ of TPC, 0.481 mg catechin g⁻¹ of flavonoids, 0.140 mg cyanidin-3-glucoside g⁻¹ of anthocyanins, and antioxidant activity was 790 mM Tx kg⁻¹. The acorn and hemp flours used in this study are a rich source of bioactive compounds from the polyphenols group. They enrich biscuits in phenolic compounds (phenolic acids, flavonoids, flavonols, anthocyanins and tannins), despite the possible loss of these compounds during baking [56].

Physical properties of biscuits

The partial substitution of the corn flour with the studied flours contributed to a significant decrease of the biscuit volume (in the range –8 to –17% relative to control), although in most cases there was no significant difference between biscuits containing hemp flour or acorn flour at different levels (Table 4). Molavi et al. [57] also observed the decrease of the volume of wheat biscuits with the increased addition of acorn flour. The spread factor varied in the range from –3% (H20) to 14% (A60), compared with the control.

The analyzed flours contributed to an increase of the hardness of biscuits, although in relation to the control only the biscuits A60 (hardness increase of 87%) as well as H40 and H60 (an increase of 59 and 81%, respectively) differed significantly. The hardness of the biscuits depends on the protein, fat, carbohydrates and starch content, as well as their interactions [58]. Mancebo et al. [2] noted the decrease of biscuit hardness with increasing amounts of protein, while Singh and Mohamed [59] did not observe a significant effect of gluten or soybean protein supplementation on this parameter. In turn, Arun

Table 4 The effect of studied flours on physical properties of gluten-free biscuits

Sample	V (mL)	SF	Hardness (N)
Control	24 ^c ± 0	5.44 ^b ± 0.10	29.23 ^a ± 1.84
A20	21 ^{ab} ± 1	5.49 ^{bc} ± 0.09	30.04 ^a ± 2.29
A40	20 ^a ± 1	6.17 ^d ± 0.21	34.37 ^a ± 2.09
A60	20 ^a ± 1	6.18 ^d ± 0.25	54.81 ^c ± 1.82
H20	22 ^b ± 1	5.27 ^a ± 0.13	28.44 ^a ± 4.90
H40	20 ^a ± 1	5.57 ^{bc} ± 0.17	46.34 ^b ± 2.20
H60	20 ^a ± 1	5.61 ^c ± 0.16	53.03 ^c ± 0.64

Mean value ± standard deviation

Values signed with the same letters in particular columns do not differ significantly at 0.05 level of confidence

et al. [58] observed lower hardness of biscuits containing plantain peel fiber. However, in the case of strongly water-binding ingredients a relevant parameter affecting the hardness is the quantity of liquid in the formulation. According to Sharma and Gujral [60], strongly hydrophilic ingredients absorb significant quantities of water, as the constant content in the formulation leads to harder dough. The consequence of harder dough can be a volume reduction and hardness increase of the biscuit, which was observed in this study which was conducted with a constant amount of liquid in the formulation. The substitution of wheat flour with acorn flour causing an increase in the hardness of biscuits was also confirmed by Molavi et al. [57]. The authors explained it by higher amylose content in acorn starch, which according to Correia et al. [61] varies from approx. 40 to more than 80%, depending on the species and method of flour obtaining. The retrogradation of amylose is faster than the branched amylopectin; therefore, in bakery products it is responsible for the initial hardness [62].

The color of the product is one of the important parameters contributing to choices made by consumers [63]. All biscuits containing both tested flours were significantly darker than the control, while the hemp flour influenced this parameter to a greater extent (Table 5, Online Resource 1). Biscuits containing acorn flour were more noticeably red than the control ones and biscuits containing hemp flour had lower share of redness. However, all biscuits with the studied flours had less yellow color than the biscuits containing only corn flour (control biscuits). On the other hand, it can be concluded that the hemp flour reduced the share of yellow color to a greater extent than acorn flour. The dependencies described result mainly from the properties of the flours which are the main ingredient of

the biscuits. The brightest flour was the corn flour and the darkest was the hemp flour (Table 5). The highest value of the parameter b^* was found in the corn flour and the lowest in the hemp flour, whereas the share of the red color determined in the flours did not have such univocal effect on the color of biscuits as the difference of the a^* parameter between corn and hemp flours was statistically irrelevant. Parameter h (hue angle) describes the color wheel in such a way that $0/360^\circ$ value corresponds to the red-purple color, 90° for yellow, 180° for green and 270° for blue [64]. In the studied biscuits, the gradations of color from yellow towards red-purple in correlation with increasing the content of both studied flours were observed. Acorn flour had a greater impact on the change of color than flour from hemp. These observations are confirmed in the parameters of the color of the flours as the measure of red color was the highest in acorn flour. In turn, chroma (C^*) informs about the color intensity (the higher the value, the more intense and vivid the color is perceived). Biscuits with studied flours had a lower value of this parameter because they were darker. The yellow color characteristic of corn flour is perceived as more vivid than the brown one. Based on the data provided in Table 5, it may be observed however that the corn biscuits had a higher value of C^* than the corn flour itself. It may be explained with the impact of other ingredients, e.g., the fat which makes the color seem more vivid compared with the color of the dry flour. The perception of the color of the two samples can be inferred from color difference ΔE^*_{ab} . Color difference can be interpreted as follows: $0 < \Delta E^* < 1$ differences in color are unrecognizable by standard observer, $1 < \Delta E^* < 2$ only an experienced observer can recognize differences, $2 < \Delta E^* < 3.5$ inexperienced observer can spot the differences, $3.5 < \Delta E^* < 5$ any observer can easily see the differences, $\Delta E^* > 5$ an observer

Table 5 Color parameters of flours and gluten-free biscuits

Sample	L^*	a^*	b^*	C^*	h
Flour					
Corn	$83.99^C \pm 0.23$	$3.36^A \pm 0.11$	$26.60^C \pm 0.81$	$26.81^C \pm 0.80$	$82.80^C \pm 0.24$
Acorn	$59.91^B \pm 0.28$	$5.57^B \pm 0.10$	$23.28^B \pm 0.15$	$23.89^B \pm 0.15$	$77.01^A \pm 0.24$
Hemp	$49.29^A \pm 0.22$	$3.52^A \pm 0.03$	$20.16^A \pm 0.31$	$20.24^A \pm 0.15$	$80.10^B \pm 0.06$
Biscuits					
Control	$71.32^c \pm 1.63$	$7.00^c \pm 1.12$	$32.73^f \pm 1.05$	$33.47^f \pm 1.25$	$77.96^e \pm 1.53$
A20	$55.04^d \pm 0.80$	$8.23^d \pm 0.22$	$26.81^e \pm 0.30$	$28.05^e \pm 0.34$	$72.94^c \pm 0.32$
A40	$48.98^c \pm 0.30$	$8.64^d \pm 0.13$	$24.95^d \pm 0.38$	$26.41^d \pm 0.40$	$70.89^b \pm 0.15$
A60	$43.14^b \pm 0.50$	$8.85^d \pm 0.24$	$22.09^c \pm 0.50$	$23.79^c \pm 0.56$	$68.17^a \pm 0.09$
H20	$50.19^c \pm 0.44$	$5.80^b \pm 0.02$	$25.95^e \pm 0.53$	$26.59^d \pm 0.51$	$77.40^e \pm 0.28$
H40	$42.02^b \pm 0.64$	$5.27^{ab} \pm 0.01$	$19.47^b \pm 0.23$	$20.17^b \pm 0.22$	$74.86^d \pm 0.15$
H60	$36.85^a \pm 0.44$	$4.72^a \pm 0.16$	$14.75^a \pm 0.03$	$15.49^a \pm 0.07$	$72.26^c \pm 0.53$

Mean value $\pm$ standard deviation

Values signed with the same letters in particular columns and groups (flours or biscuits) do not differ significantly at 0.05 level of confidence

sees two different colors [65]. Comparing the biscuits with the same share of studied flours (A20/H20, A40/H40 and A60/H60), it can be concluded that the differences were so significant that the color of the biscuits could be seen as two different colors ($\Delta E^* = 5.49, 9.48, 10.51$, respectively). The color of biscuits depends on various factors, e.g., composition or Maillard reaction products formed during baking that affect the brown surface [40]. But it seems that in the presented study the investigated flours had a decisive influence due to their intensive brown color. According to Hrušková and Švec [66] color of hemp flour depends on the method of its production. Laboratory-made wholemeal flour had a grayish shade, and the flour produced on an industrial scale, as by-product of oil extraction had a similar color to cocoa (middle-brown), just like flour used in this study.

Sensory evaluation

As shown in Fig. 1, the control biscuits obtained the highest scores. Their color was rated 6.9 points, while a slightly lower color rating was given to biscuits with acorn flour in the range 5–6.3 points. In contrast, the color of biscuits enriched with hemp flour was rated much lower: 2.7–4.3 points depending on the content of the flour. Lower differences were observed in the case of smell. The control biscuits obtained the highest scores (7.0 points), whereas the biscuits with acorn flour received scores in the range 6.4–5.0 points, and with hemp flour 6.4–4.1 points. Taste of control biscuits was assessed at 6.6 points; the biscuits with acorn flour received 6.3–4.9 points and with hemp flour 6.1–2.4 points. A similar trend was observed with regard to consistency and appearance, i.e., biscuits with hemp flour were rated lower than the biscuits containing acorn flour.

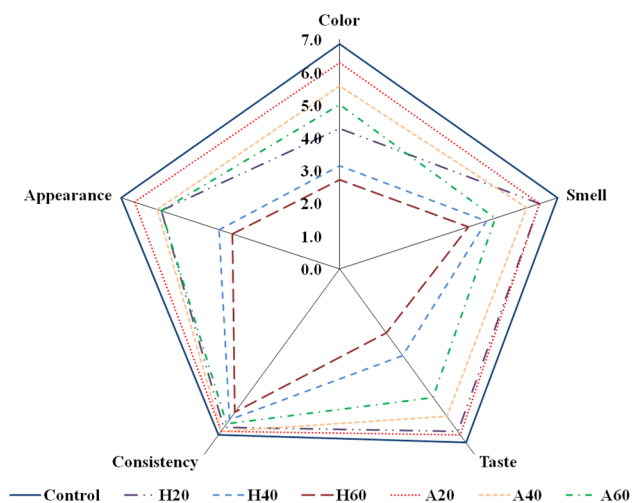


Fig. 1 Results of sensory evaluation of gluten-free biscuits

In the case of biscuits from hemp flour, the lower scores were due to a greenish color and bitter taste resulting from thicker granulation. The lower scores of texture, color, flavor and overall acceptance of wheat biscuits with the addition of acorn flour were also observed by Molavi et al. [57].

Conclusions

The studied flours, which were used as partial substitution (20–60%) of corn flour, contributed to a significant increase of the hydration properties of mixtures and the oil absorption capacity. This is important, because increasing water content in the dough and final products increases the profitability of production while reducing the energy value of biscuits. In terms of physical characteristics of biscuits, studied flours had a measurable influence on their color. Both investigated flours rated higher in nutritional value of gluten-free biscuits than those made with corn flour (hemp flour was to this respect more advantageous) as well as in bioactive compounds (here, acorn flour was more advantageous); however biscuits with hemp flour got less points in sensory evaluation. A decrease in energy value of the studied biscuits in the range of –4 to –27% relative to the control ones was observed.

Therefore, the most advantageous option to be considered is the replacement of 40% corn flour with acorn flour. This increased the nutritional and pro-health value of biscuits, with almost unchanged sensory attributes.

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Compliance with ethical standards

Conflict of interest None.

Ethics requirements The sensory evaluation involving human participants was carried out in accordance with the binding national and institutional provisions and all participants expressed a voluntary consent to participate in the testing.

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**Evaluation of the quality, nutritional value and antioxidant activity of
gluten-free biscuits made from corn-acorn flour or corn-hemp flour
composites**

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Online Resource 1

Fig. S1 Image of studied gluten-free biscuits: Control (100% corn flour), A – acorn flour; H – hemp flour; 20, 40, 60 – the percentage of acorn or hemp flour in the mixtures

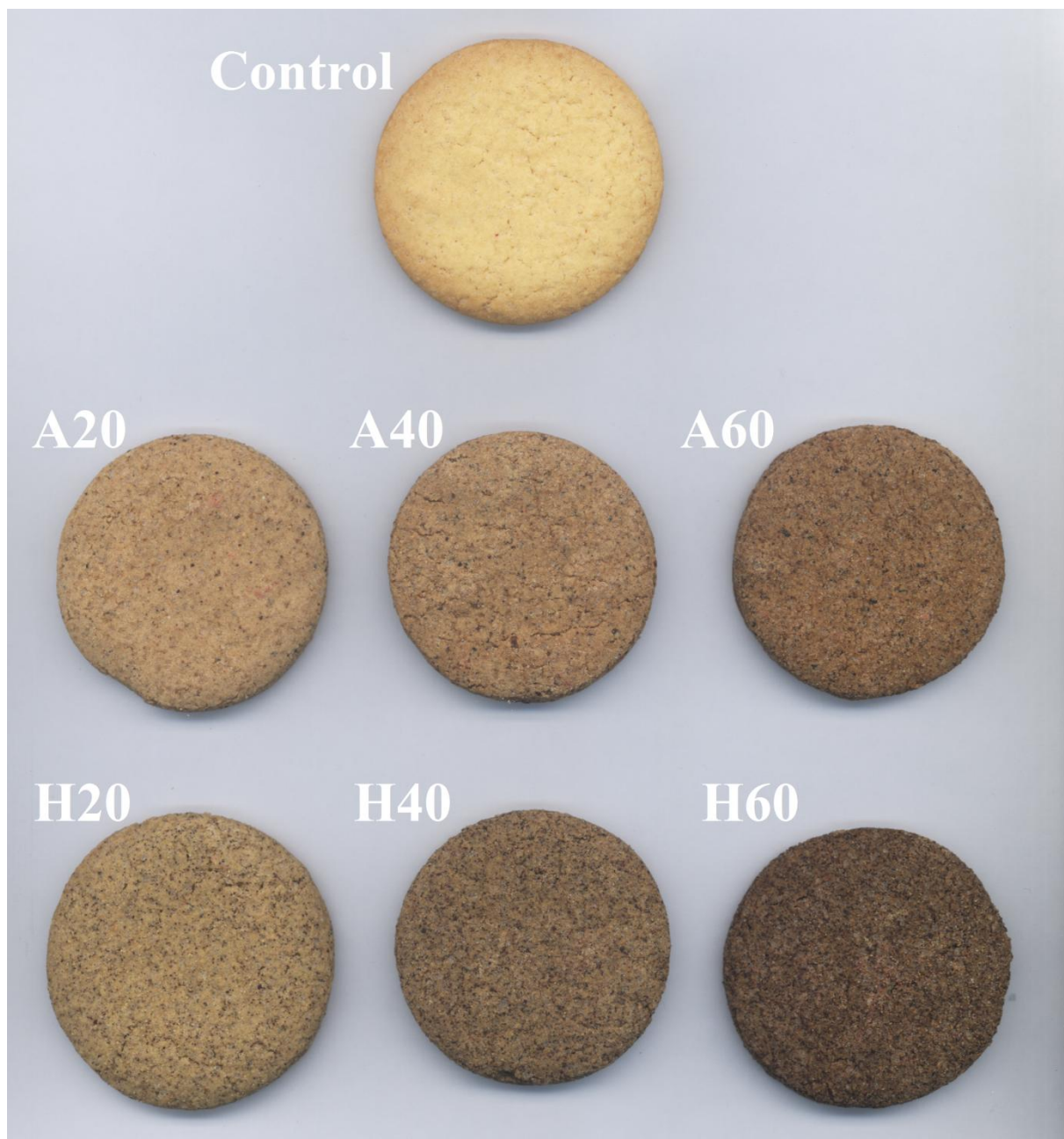


Fig. S1 Image of studied gluten-free biscuits: Control (100% corn flour), A – acorn flour; H – hemp flour; 20, 40, 60 – the percentage of acorn or hemp flour in the mixtures