EJFA Emirates Journal of Food and Agriculture

Emirates Journal of Food and Agriculture 36: 1–11
doi: 10.3897/ejfa.2024.127265

RESEARCH PAPER

Assessment of the quality of bread made from ancient wheat flour with the addition of amaranth, flax and hemp seeds

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Academic editor: Angelo Maria Giuffrè ♦ **Received** 10 May 2024 ♦ **Accepted** 20 June 2024 ♦ **Published** 27 August 2024

Abstract

Consumers are increasingly seeking bakery products with better nutritional value and health benefits. The aim of the research was to assess the quality of bread made from ancient wheat flours (spelt, einkorn, emmer) with the addition of amaranth (*Amaranthus cruentus* L.), hemp (*Cannabis sativa* L.) and flax (*Linum usitatissimum* L.) seeds. The study evaluated bread made using only equal proportions of ancient wheat flours (Group I), and three variants of bread with the addition of amaranth, flax, and hemp seeds with the following proportions: Group II (10%, 5%, 5%, respectively), Group III (10%, 10%, 0%, respectively), Group IV (10%, 0%, 10%, respectively). The assessment of bread quality included: nutritional value (total protein, fat, fatty, amino acids, acid profile, ash, fiber, minerals), caloric value, physical characteristics (baking loss, color, shear force) and evaluation of organoleptic characteristics. It was demonstrated that bread enriched with amaranth, flax, and hemp seeds exhibited increased nutritional value (higher protein content with a favorable amino acid profile, higher fat content, including polyunsaturated fatty acids, and fiber), acceptable sensory attributes, but darker crust color and poorer crumb texture compared to bread without these additives. Among the examined groups, the highest protein and fat content with a favorable composition of fatty acids were observed in bread from Group IV. Group III bread had the highest hardness and fiber content and fiber content. On the other hand, bread from Group II received the best evaluation in terms of taste, aroma, as well as crust thickness.

Keywords

Physical features, Sensory features, Nutritional value, Relic wheat, Amaranth, Flax, Hemp

Introduction

In food processing, there is a search for innovative ideas for food products or the improvement of traditional recipes. The nutritional value of bakery products is mainly determined by the content of nutrients: protein, fat, mineral components or vitamins (Krochmal-Marczak et al. 2020; Tareh et al. 2022). Enriching bakery products with natural bioactive compounds and reducing the amount of adverse ingredients commonly used in food processing are important directions in food production (Chikpah et al. 2023; Różyło et al. 2023). However, new bakery products must be tested and gain consumer approval before being marketed (Wendin et al. 2020; Dabija et al. 2023). Ensuring food safety for the growing human population, especially considering climate change, remains a persistently relevant issue. As a result, many countries indicate the need to implement changes in the agri-food industry and popularize new dietary habits among contemporary populations (Budzulak et al. 2022; Longin et al. 2023).

Enriching bakery products seems to be a very cheap and easy way for improving food quality. A compromise between nutritional value and sensorial quality is generally achieved via the enrichment of wheat flour by 10%–30% with other cereals and/or pseudo-cereals and up to 5% of functional components, eg. dietary fiber (Dziki et al. 2014). In particular, ancient cereals are gaining interest since several studies suggested that they present a healthier nutritional profile than modern wheats cultivars (Dinu et al. 2018).

At present, flours from common wheat or durum wheat are widely used in baking. However, there has been a recent surge of interest in so-called ancient wheats (also known as hulled wheats) due to their good chemical composition and the fractional composition of the gluten protein complex; these ancient species include einkorn, emmer, and spelt (Goriewa-Duba et al. 2018; Brouns et al. 2022). Many authors (Arzani and Ashraf 2017; Şerban et al. 2021; Golea et al. 2023) point to the high nutritional values of food products made from spelt, emmer and einkorn. Hidalgo et al. (2008), examining the content of individual nutrients in wheat grains, found that regardless of environmental changes, all varieties of emmer and spelt exhibit higher protein content (on average by 59%), fat (by 50%), tocotrienols (by 88%), total tocopherols (by 46%), lutein (by 483%), as well as monounsaturated fatty acids (by 53%), with lower levels of saturated fatty acids (by 21%) and polyunsaturated fatty acids (by 8%) in relation to traditional wheat varieties. These authors compared the carotenoid content in spelt flour and traditional bread flour and found that the carotenoid content in einkorn (from 8.1 to 9.8 mg kg^{-1}) was 8 times higher than in wheat flour. Moreover, this flour showed slower degradation rate during storage. Rachoń et al. (2015, 2020) and Keçeli et al. (2021) obtained a high content of protein, ash, fat, P, K, Mg, Ca, Cu, Zn, Fe, and Mn in einkorn wheat grain. Çığ et al. (2021) reported that einkorn wheat grain contained large quantities of potassium and trace elements, including iron, manganese and zinc. Abdel Aal et al. (1995) proved that einkorn grain was of the highest quality as it contained a substantial amount of protein, phosphorus, and potassium. Spelt grain is rich in protein, and some varieties contain a significant amount of starch and fat. Biel et al. (2021) and Fan et al. (2008) showed that potassium, phosphorus, magnesium, and calcium had the highest concentrations among the analyzed macronutrients in wheat grain. Therefore, the above-mentioned authors advocated for increased consumption of whole grain bread or bread made from ancient wheat varieties.

There are also reports indicating better food tolerance for products made from spelt, einkorn, and emmer by individuals allergic to gluten (Nakamura et al. 2005). Kaur et al. (2022) and Bradauskiene et al. (2023) indicated that due to food intolerances, such as celiac disease, there is an increasing demand for gluten-free food. Saeed et al. (2022) conclude that gluten-free diet is the only solution for individuals diagnosed with celiac disease. However, gluten-free products available on the market are lacking in macronutrients and phytochemicals.

Sharma et al. (2020) have concluded that approximately 8.4% of the entire human population is affected by issues related to wheat consumption, while the rest of the consumers should not eliminate wheat from their diet.

Previous research has shown that the nutritional value of food products, such as bakery products, can be modified by adding various unconventional ingredients to flour (Tareh et al. 2022; Chikpah et al. 2023; Różyło et al. 2023) or by using flour from ancient wheats as a substitute for common or hard wheat flour (Kulathunga et al. 2023; Roumia et al. 2023). The dissemination of new baking recipes has already allowed to make bread with improved nutritional, physicochemical, and sensory qualities. Therefore, more knowledge about ancient wheat species is needed to improve bakery products.

Due to their chemical composition, including bioactive components, amaranth, flax or hemp seeds can be used as additives to bread, thereby enhancing the quality of bakery products (Bădărău et al. 2018; Pecyna et al. 2023). The main advantage of amaranth seeds is their high content of protein rich in amino acids (all essential amino acids, including lysine, tryptophan, and sulfur amino acids). Amaranth seeds also have a favorable lipid composition, containing a valuable component, squalene, known for its beneficial antioxidant properties. The seeds contain many vitamins, minerals, and bioactive compounds such as tocopherols, phytosterols, and polyphenols. They also have a high content of fiber and minerals, including iron and calcium (Coțovanu et al. 2023).

Flax seeds have a broad application in the food industry and are considered one of the best sources of nutrition (Augustyńska-Prejsnar et al. 2022). Flax seeds contain approximately 40% fat, of which 60% are omega-3 fatty acids. Additionally, they have a favorable amino acid profile with high content of lysine, leucine and phenylalanine (Cócaro et al. 2020). Fiber accounts for 28% dry matter, with around 25% being soluble fractions known for properties that reduce cholesterol levels. Mineral compounds constitute approximately 3 to 8%. Phytoestrogens are present in flax seeds in the form of sterols, known for their antioxidant properties (Cócaro et al. 2020; Gao et al. 2022).

Hemp seeds are a rich source of protein, fat (30%), fiber, Ca, P, Fe, Zn, and Mg. Characteristic components of hemp seeds include edestin, choline, phytic acid, trigonelline, lecithin, tocopherols and vitamin K. Terpene hydrocarbons, mainly β-caryophyllene and α-humulene (sesquiterpenes), as well as monoterpene and myrcene, are responsible for the taste and aroma of hemp (Gambuś et al. 2020). Hemp protein is known for its high digestibility and favorable amino acid profile, allowing for effective utilization by the human body, serving as a source of bioactive peptides (Farinon et al. 2020; Kotecka-Majchrzak et al. 2021).

Bakery products, especially bread, are among the main foods in the human diet. Enriching the composition of bread with seeds, e.g. flax, hemp or amaranth, makes it possible to obtain a product with increased nutritional value, desired by consumers. Enriching bread with ingredients such as amino acids, unsaturated fatty acids, fiber and microelements is particularly important in the modern diet. Cooper (2015) showed that the gluten-free food market worth almost $ 1.6 bn, there is every reason for renewed interest in ancient species.

The aim of the study was to demonstrate that amaranth, flax, and hemp seeds are suitable additives for baking bread made from a blend of flours from ancient wheat varieties (spelt, einkorn and emmer). The research hypothesis posited that adding amaranth, flax, and hemp seeds to flour from ancient wheat varieties would enhance the nutritional value and sensory characteristics of the bread.

Materials and methods

Bread ingredients

The flour from ancient wheat varieties (spelt, einkorn, emmer) was purchased from an organic store in Rzeszów, Poland. According to the manufacturer's declaration:

- Light spelt flour contained per 100 g: 1.6 g of fat, 74 g of carbohydrates, 10.6 g of protein, 3.9 g of fiber.
- Whole grain einkorn flour contained per 100 g: 1.8 g of fat, 62 g of carbohydrates, 10 g of protein, 12 g of fiber.
- Whole grain emmer flour contained per 100 g: 2.6 g of fat, 59 g of carbohydrates, 13 g of protein, 9.8 g of fiber.

Amaranth (*Amaranthus cruentus* L.), flax (*Linum usitatissimum* L.) and hulled hemp (*Cannabis sativa* L.) seeds were used as additives. They were purchased from "Bio Planet," and were certified as an organic cultivation product. Amaranth seeds contained: total ash 3.44%, protein 15.40%, lysine 0.76%, methionine 0.29%, cysteine 0.29%, tryptophan 0.21%; fat 6.72%, and 48.50/10.20% n-6/n-3 fatty acids ratio. Flax seeds contained: total ash 3.69%, protein 22.5%, lysine 0.86%, methionine 0.29%, cysteine 0.29%, tryptophan 0.36%, fat 35.94%, and 23.00/42.50% n-6/n-3 fatty acids ratio. Hemp seeds contained: total ash 5.18%, protein 32.5%, lysine 1.10%, methionine 0.29%, cysteine 0.29%, tryptophan 0.36%; fat 53.1%, and 56.00/18.00% n-6/n-3 fatty acids ratio. The other ingredients were: uniodized natural salt "Kłodawska," cane sugar from "Bio-ekowital," and baking yeast from "Biorela," purchased at the health food store "Spices without chemicals".

Technology of enriched bread production

Based on previous baking, the best variants of flours and additives were selected for the experiment. In each of the two experimental series, four variants of rolls were produced from a mixture of ancient wheat flours (spelt, einkorn, emmer) using a fixed recipe composition (Table 1). Preparation of dough and baking of wheat bread, control bread (without seeds) and with added seeds was carried out according to the direct method using yeast according to (46). Group I comprised standard rolls made from ancient wheat flours (in equal proportions), serving as the control. Groups II, III, and IV were experimental groups, in which a mixture of amaranth, flax, and hemp seeds was added in various proportions (Table 1). Dry amaranth and flax seeds were milled, resulting in particle size fractions ranging from 0.02 to 1.50 mm (Φ). Hemp seeds were used whole, without any processing or milling. The dough production process was the same for each of the examined groups. A starter was prepared from yeast, sugar, water and flour, which was subsequently combined with the remaining ingredients. All ingredients were introduced into a mechanical mixer with a stainless steel stirrer (Titanium, Havand, UK) and kneaded for 5 minutes to form the dough. The dough was fermented for 60 minutes at 23 °C. Afterwards, the dough was divided into 100-g portions and shaped into small round rolls, and left to rise for 30 minutes. The baking process was conducted in a convection-steam oven with an integrated temperature probe (AEG BSK792320M, Berlin, Germany) at a temperature of 180 °C for 30 minutes. After removing from the oven and allowing them to cool completely, the rolls were weighed on a scale to the nearest 0.01 g (Ohaus V1193, Parsippany, NJ, USA).

Table 1. Recipe composition of enriched bread (%).

Ingredients	Variants of product			
	Group I	Group II	Group III	Group IV
Spelt flour	20.00	13.33	13.33	13.33
Einkorn flour	20.00	13.33	13.33	13.33
Emmer flour	20.00	13.33	13.33	13.33
Water	35.00	35.00	35.00	35.00
Amaranth seeds	-	10.00	10.00	10.00
Flax seeds	-	5.00	10.00	-
Hemp seeds	-	5.00	-	10.00
Yeast	3.50	3.50	3.50	3.50
Salt	1.00	1.00	1.00	1.00
Sugar	0.50	0.50	0.50	0.50

Quality parameters

The cross-section surface and the crust surface were subject to instrumental color evaluation using a colorimeter (CR-400; Minolta, Osaka, Japan) equipped with an 8-mm diameter measuring head. The colour assessment of the cross-sectional surface of products was determined, based on the reflection method, using a Chrome Meter colorimeter (Konica Minolta Osaka, Japan), fitted with a CR 400 head (ø = 11 mm). The colorimeter was calibrated with a Konica Minolta calibration plate (observer 20, illuminant D65). The reading of the measurement results was achieved in a CIE LAB colorimetric system, with L* (lightness), a* (redness) and b* (yellowness).

The temperature of the products during all physical measurements was equal to the ambient temperature, maintained at 20 ± 1 °C. Measurements were taken at 10 different representative points on the crumb and crust surfaces. Brittleness was measured based on the cutting force (Fmax), using a Zwick/Roell machine BT1-FR1.OTH.D14 (from Zwick CmbH & Co.KG. Ulm, Germany), applying a wide-width Warner-Bratzler (V-blade) with a head speed of 100 mm·min-1 and a 0.2 N pre-cut force (min. 3 replicates per sample). The results of the shear force measurement were compiled using the Test Xpert II software. Total baking loss - is the difference between the mass of the formed dough piece and the mass of cooled bread, expressed as a percentage of the mass of dough formed for baking.

The determination of the protein content bread rolls was determined using the Kjeldahl method. The calculated amount of nitrogen determined in the tested samples was convert-ed into protein using a conversion factor of 6.25.

The distillation-titration method was used (sample mineralization, steam distillation of ammonia, which is bound in boric acid solution, titration with hydrochloric acid solution). The analyses were performed using a Kjeldatherm apparatus (Gerhardt, Königswinter, Germany), with controlled temperature regulation. Automatic distillation was carried out using a Vapodest Carousel (Gerhardt, Königswinter, Germany).

The determination of the free fat was conducted by the Soxhlet extraction-gravimetric method using a Soxtherm extractor apparatus (Gerhardt, Königswinter, Germany), with an electric dryer in a controlled temperature range of 105 ± 2 °C Additionally, the ash content was determined by burning the sample at 525 ± 25 °C in a muffle furnace (Nobetherm P330, Lilienthal, Germany).

The dietary fiber content of the rolls was determined using the enzymatic-gravimetric method. The setup consisted of a Kjeldatherm mineralization block (Gerhardt, Königswinter, Germany), a Vapodest Carousel automatic distillation unit (Gerhardt, Germany), and a vacuum filtration set (Foss Analytical A/S, Denmark). The obtained values were used to calculate the dietary fiber content in the roll sample. Assimilable carbohydrates were calculated based on the results of fat, protein, water, ash and dietary fiber determination. The sodium content was determined using the ICP-OES method (inductively coupled plasma excitation emission spectroscopy) after high-pressure microwave mineralization with nitric acid. The salt content was calculated according to Regulation (EU) No 1169/2011 of the European Parliament and of the Council (salt = sodium × 2.5).

The amino acid composition was determined by gas chromatography using a Clarus 580 gas chromatograph (Perkin Elmer) with an autosampler, equipped with a flame ionization detector (GC-FID) (EC Regulation 152/2009, III F (Tryptophan III G).

Fatty acid analysis was performed using DGF C-VI 11a:2016 mod +DGF C VI 10a: 2016 mod (Agilent Technologies 7890A GC System with FID Detector and a CP-Sil 88 Column, Agilent, Santa Clara, USA) The principle of the method was based on the separation of fatty acids (identification of fatty acids after retention time) by gas chromatography with flame-ionisation de-tection. Samples of fatty acid methyl esters were prepared by trans-esterification with boron trifluoride BF3 according to PN-EN ISO 12966-2:2017-05.

Sample measurement was performed in accordance with PN-EN ISO 12966-4:2015-07 (GC-FID). Methyl esters of fatty acids were determined by capillary gas chromatography technique.

To determine the individual elements, bread samples were mineralized in HNO_3: $HClO_4$: H_2SO_4 at a ratio of 20:5:1, in an open system in a Tecator heating block (FOSS, Denmark). The content of calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), and trace elements in the samples was determined using flame atomic absorption spectroscopy (FAAS) with a Hitachi Z-2000 instrument (Tokyo, Japan). To determine the phosphorus content, a Shimadzu UV-VIS spectrophotometer (Kyoto, Japan) and the vanadium-molybdenum method were employed.

Sensory evaluation

The evaluation of the sensory properties of the samples was performed using the scaling method. Sensory analysis panel consisted of 10 people with confirmed sen-sory sensitivity and with at least 4 years of experience, which included 8 women and 2 men, aged 26–55 assessment, no smoking. The study used special assessment cards to describe the sensory properties of the samples. In order to properly evaluate the samples, the was cut into slices measuring 2 cm × 2 cm × 4 cm. All samples to be assessed were placed in covered vessels, marked with numerical codes. The samples were randomly assessed. Each panelist assessed a sample in three replications. The external appearance of the rolls was evaluated directly after removal from the oven. Other parameters, such as crust and crumb characteristics, taste and aroma were determined in the cooled bread. In order to ensure proper assessment, bread samples were sliced, coded, and presented to the evaluators in white containers. Sample sets for individual evaluators were presented in a specific order, which was changed during the second evaluation session to avoid any potential influence of the previous sample evaluation on the subsequent one. Between each sample evaluation, the tasters took a break (30 seconds) and rinsed their mouths with mineral water. Each characteristic was assigned a numerical score of organoleptic evaluation. Each taster evaluated the sample in three replicates. The evaluation was conducted in an odor-free room at 20 ± 2 °C. The evaluation of the bread was carried out in accordance with the PN-A-74108:1996 standard.

Statistical analyses

The research results were subjected to statistical analysis using the Statistica 13.3 software. The arithmetic mean and SEM (standard error of measurement) were calculated. A one-way analysis of variance was applied. The significance of differences between the means was tested using Tukey test at a significance level, α = 0.05. The results related to the effect of seed additives on the sensory properties of the rolls were verified using Kruskal-Wallis nonparametric tests.

Results and discussion

Cereals in their natural form or whole grain, they are a rich source of nutrients. However, when refined by the removal of the bran and germ, the remaining endosperm is mostly carbohydrate (Cooper 2015). Therefore, further studies are urgently required, including medical on the genotypes of ancient and modern wheat species (Shewry 2018).

The addition of amaranth, flax, and hemp seeds significantly reduced the losses in the baked bread. However, flaxseed reduced losses during bread baking the least. This was due to the higher content of fiber and lignans, which retained water and prevented its evaporation during heat processing. Park and Morita (2004) reported that the loaf volumes of bread baked with 20%, 10% and 5% of amaranth flour substitution were 83%, 89% and 94%, respectively, of the control, wheat only.

The color of a food product is an important visual feature that influences its selection and purchase. In addition to the primary ingredients, additives and their proportions in the recipe have a significant impact on the color of bakery products (Adamczyk et al. 2021). The present study showed a significant ($p < 0.05$) effect of the additives used on the color of the cross-section of bakery products. In all groups, seed-enriched products were characterized by a darker color and a higher degree of color saturation towards yellow range compared to the control group (Table 2). The color changes in the tested groups were mainly due to the addition of hemp seeds containing chlorophyll and as a result of increased fiber and fat content of flax seeds. In addition, the results were consistent with the findings of Adamczyk et al. (2021) conducted on bread enriched with Chia seeds. Wiedemair et al. (2022) reported that the addition of hemp raw materials often negatively affected the technological parameters of baked goods: increased bread hardness, decreased elasticity and even darker color compared to the reference bread.

Texture evaluation is important for the acceptability of the product by consumers. Changes in texture parameters depend on the type and quantity of additives used (Adamczyk et al. 2021; Wiedemair et al. 2022). Hardness (N) is the most commonly determined texture parameter of bread crumb, as it describes the maximum force during the first bite (Armero and Collar 1997). The current study demonstrated that the rolls made from ancient wheat flours with the addition of amaranth, flax and hemp seeds had higher shear force (hardness) compared to the control group. In the tested groups, the rolls from Group III showed the highest hardness (with 10% amaranth seeds and 10% flax seeds) (Table 2). The addition of seeds to bread weakens the gluten matrix, leading to a looser cellular structure of the crumb, which affects the volume and texture of the resulting crumb. One of the components of flax seeds is mucilage, which increases the hardness of bread. The decrease in the volume of bread could also be caused by enriching it with fiber-rich additives. However, the presence of fiber in the dough negatively affects the formation and properties of gluten, which reduces the ability to retain gases, thereby affecting the volume and texture of the bread. Różyło and Laskowski (2008) reported that the addition of amaranth flour or flakes at 5% led to a decrease in the softness of bread crumb. Mikulec et al. (2019) showed that hemp flour content significantly inhibited changes in bread crumb hardness by reducing the bread stalling index from 1.12 (wheat bread) to 0.05 (50% of the additive). Rainero et al. (2022) showed that breadstick fortification with red grape pomace has a positive effect on nutritional, technological without affecting sensory acceptability.

Proteins are an essential dietary component, and their consumption determines the proper functioning of the body. They are used to rebuild tissues and are a component of blood, lymph, enzymes and some hormones. They are part of antibodies, help maintain the proper pH of body fluids, and participate in the transport of certain vitamins and minerals (Wu 2016). Protein deficiency in the diet leads to the inhibition of growth and development of the body, hinders tissue repair, and reduces disease resistance. Synthesis of new proteins occurs using both body proteins and those supplied with food (Tavarini et al. 2019). In the present research, the addition of amaranth, flax, and hemp seeds had a beneficial effect ($p \leq 0.05$) on increasing the protein content in all experimental groups compared to the control (Table 3); they also improved the nutritional value of the bread (Table 4).

Among the study groups, breads with a higher proportion of hemp (Groups IV and II) were characterized by higher protein content and more favorable amino acid composition. The increase in the proportion of aspartic acid, lysine, leucine, methionine, serine, tyrosine, and arginine in Group IV (with 10% addition of amaranth and hemp) can be considered beneficial for improving bread

Table 2. The influence of the addition of amaranth, flax and hemp seeds on the physical characteristics of bread.

Parameter	Group I	Group II	Group III	Group IV
Baking loss (%)	9.97b ± 4.24	6.71a ± 3.20	7.48a ± 2.44	6.42a ± 3.35
Colour cross-section: L* - lightness	69.75c ± 2.35	67.10b ± 2.43	65.19a ± 2.00	68.09b ± 3.49
a*- redness	3.99b ± 0.22	3.05ab ± 0.20	2.58a ± 0.51	2.64a ± 0.13
b* - yellowness	15.03a ± 0.68	17.18b ± 0.73	16.96b ± 1.12	17.20b ± 0.58
Texture parameters: Warner–Bratzler shear force (N)	6.58a ± 1.15	8.16b ± 0.27	10.30c ± 0.66	9.10b ± 0.56

Explanations: Group I - control, group II - amaranth 10%, flax 5%, hemp 5%, group III - amaranth 10%, flax 10%, group IV - amaranth 10%, hemp 10%; a, b, … - values in rows with different letters differ significantly, $p < 0.05$.

Table 3. The influence of the addition of amaranth, flax and hemp seeds on the basic chemical composition of bread.

Parameter	Group I	Group II	Group III	Group IV
Dry matter %	75.39 ± 0.77a	75.84 ± 0.08a	76.27 ± 071a	81.37 ± 0.86b
Protein (N × 6.25) %	13.24 ± 0.24a	14.99 ± 0.08c	14.27 ± 0.04b	16.10 ± 0.20d
Fat %	3.57 ± 0.06a	9.46 ± 0.09c	7.08 ± 0.04b	11.33 ± 0.27d
Total ash %	2.52 ± 0.05a	3.24 ± 0.09b	2.70 ± 0.10a	2.66 ± 0.15a
Fiber %	3.95 ± 0.07a	6.11 ± 0.09b	7.93 ± 0.36c	3.94 ± 0.11a
Bioavailable carbohydrates %	52.38 ± 0.71c	42.48 ± 1.16a	43.79 ± 0.87a	47.15 ± 0.71b
Sodium mg/kg	6100.3 ± 50.0c	5453.3.7 ± 68.0b	5351.3 ± 49.2b	3739.0 ± 15.40a
Salt from the calculations %	1.52 ± 0.01c	1.36 ± 0.02b	1.33 ± 0.02b	0.98 ± 0.04a
Energy value kJ/100	1274.67 ± 13.2a	1367.33 ± 1.53c	1317.33 ± 8.08b	1508.67 ± 18.23d

Explanations: Group I - control, group II - amaranth 10%, flax 5%, hemp 5%, group III - amaranth 10%, flax 10%, group IV - amaranth 10%, hemp 10%; a, b, … - values in rows with different letters differ significantly, $p < 0.05$.

Table 4. The influence of the addition of amaranth, flax and hemp seeds on the amino acid composition of protein (%).

Parameter	Group I	Group II	Group III	Group IV
Lysine	0.36 ± 0.01a	0.45 ± 0.03b	0.47 ± 0.03b	0.51 ± 0.06c
Methionine, expressed as methionine sulfone	0.21 ± 0.003a	0.29 ± 0.001c	0.23 ± 0.002b	0.29 ± 0.004c
Cysteine, expressed as cysteic acid	0.30 ± 0.004a	0.32 ± 0.002b	0.31 ± 0.010ab	0.35 ± 0.012c
Threonine	0.40 ± 0.15a	0.58 ± 0.015d	0.45 ± 0.015b	0.49 ± 0.004c
Aspartic acid	0.65 ± 0.005a	0.92 ± 0.059b	0.87 ± 0.020b	1.10 ± 0.021c
Glutamic acid	4.59 ± 0.025c	4.09 ± 0.085a	3.98 ± 0.030a	4.24 ± 0.020b
Serine	0.64 ± 0.010a	0.73 ± 0.015c	0.69 ± 0.016b	0.80 ± 0.005d
Proline	1.50 ± 0.025b	1.21 ± 0.010a	1.21 ± 0.020a	1.26 ± 0.025a
Isoleucine	0.51 ± 0.006a	0.54 ± 0.008b	0.54 ± 0.006b	0.59 ± 0.009c
Alanine	0.47 ± 0.009a	0.58 ± 0.010c	0.53 ± 0.020b	0.60 ± 0.016c
Valine	0.60 ± 0.002a	0.71 ± 0.046b	0.64 ± 0.011ab	0.71 ± 0.007b
Glycine	0.52 ± 0.009a	0.72 ± 0.015c	0.68 ± 0.007b	0.72 ± 0.009c
Leucine	0.98 ± 0.015ab	1.01 ± 0.020bc	0.95 ± 0.012a	1.03 ± 0.015c
Arginine	0.59 ± 0.005a	1.12 ± 0.026c	0.87 ± 0.020b	1.19 ± 0.035c
Phenylalanine	0.68 ± 0.007a	0.75 ± 0.009b	0.69 ± 0.003a	0.76 ± 0.004b
Histidine	0.31 ± 0.015a	0.44 ± 0.042b	0.32 ± 0.015a	0.39 ± 0.020b
Tyrosine	0.39 ± 0.010a	0.49 ± 0.025bc	0.44 ± 0.015ab	0.51 ± 0.002c

Explanations: Group I - control, group II - amaranth 10%, flax 5%, hemp 5%, group III - amaranth 10%, flax 10%, group IV - amaranth 10%, hemp 10%; a, b, … - values in rows with different letters differ significantly, $p < 0.05$.

quality. Krupa-Kozak et al. (2022) demonstrated favorable results after adding flaxseed cake as a byproduct after oil extraction from the seeds. Other authors have confirmed that flax protein is a source of valuable amino acids such as arginine, aspartic acid, glutamic acid (Chung et al. 2005), as well as cysteine and methionine (Tavarini et al. 2019).

The current study showed a significant effect of the addition of amaranth, flax and hemp seeds on the ash and carbohydrate content in the analyzed product. The bread enriched with a mixture of all seeds (10% amaranth, 5% flax, 5% hemp seeds) from Group II was characterized by a higher ash content. Carbohydrate content, on the other hand, was lowest in the bread from Groups II and III. Dietary fiber, although not a nutrient, has a significant impact on human health, including prolonging satiety, promoting the development of beneficial gut microbiota, supporting proper intestinal function, affecting cholesterol levels in the blood, and reducing blood glucose levels (Barber et al. 2020). In this study, it was shown that bread containing 10% amaranth seeds and 10% flax seeds had the highest fiber content. A significant increase in the fiber content was also observed in Group II (with 10% amaranth, 5% flax, and 5% hemp seeds) (Table 3).

Amaranth seeds, depending on the species, contain between 7.6% and 19.6% dietary fiber (Danz and Lupton 1992), which consists mainly of compounds such as lignin, cellulose, hemicellulose, uronic acids and pentosans. Other authors (Stamatie et al. 2022) have concluded that the addition of hemp increases the fiber and protein content in bread. Makowska et al. (2023) have shown that wheat bread with the addition of fermented flaxseed cake can be used as a product for people with diabetes due to its high fiber and protein content, as well as reduced starch content.

The primary source of salt in the diet is cereal products, especially bread. Therefore, reducing the level of salt in bread has important implications for human health. The present research showed a decrease in the salt content of products after flax and hemp seeds were added to bread. The largest decrease was observed in the experimental Group IV. International health agencies recommend salt intake at a level of about 5–6 g per day (Markus et al. 2012).

The energy contained in food depends on the type of food consumed, and it is released during metabolic processes occurring in the body. Among the dietary nutrients, fats provide the most energy (Krupa-Kozak et al.

2022). This study demonstrated that the energy value of enriched bread was higher in all groups compared to the control bread. The higher energy value of Group IV bread may have been due to the higher fat-rich hemp seed content. Alkurd et al. (2020) showed that the addition of other constituents to white flour wheat may have a positive effect on increasing the energy of the bread. On the other hand, Krupa-Kozak et al. (2020) obtained a bakery product with a lower energy value after adding flaxseed cake to gluten-free bread.

Enriching bakery products with amaranth, flax, and hemp seeds resulted in an increased fat content in the experimental groups (II, III, IV) compared to the control group (I). The highest increase was observed in Group IV with 10% amaranth seed and 10% hemp seed addition (Table 3). The fat in enriched products exhibited a more favorable profile of fatty acids compared to the fat in non-enriched bread. Control bread (group I) had the lowest content of omega 7 fatty acids. However, bread from group IV had the lowest content of omega 9 acids (Table 5). The proportion of unsaturated fatty acids in all experimental groups was significantly higher than in the control bread. This indicated a more favorable health profile of enriched bread. Particular attention should be given to bakery products with 10% amaranth seed and 10% hemp seed supplementation. Among polyunsaturated fatty acids, omega-3 fatty acids play a significant role. These acids in the human body lower the level of undesirable triglycerides in the blood, exhibit anticoagulant and anti-inflammatory effects, and support brain function (Farinon et al. 2020). In the present study, the content of beneficial omega-3 fatty acids was several times higher ($p < 0.05$) compared to the control product (Group I). The obtained results can be mainly attributed to the high content of alpha-linolenic acid in flax seeds. According to Farinon et al. (2020), hemp seed oil is characterized by a very favorable fatty acid profile with low saturated fatty acid content and high content of unsaturated fatty acids. Gao et al. (2022) have demonstrated that flaxseed flour is rich in unsaturated fatty acids, amino acids, and mineral components, making it a good addition to cereal flour. Strzelczyk et al. (2023) have reported that hemp nuts contain about 30–35% oil with 80–90% of essential fatty acids. Additionally, hemp nuts contain amino acids, vitamins and minerals.

In the conducted experiments, the mineral content of bread was influenced by the addition of amaranth, flax, and hemp seeds (Figs 1, 2). Pojić et al. (2015) found that bread with the addition of hemp flour was characterized by higher nutritional value as it contained more protein, as well as macro- and micronutrients, es-

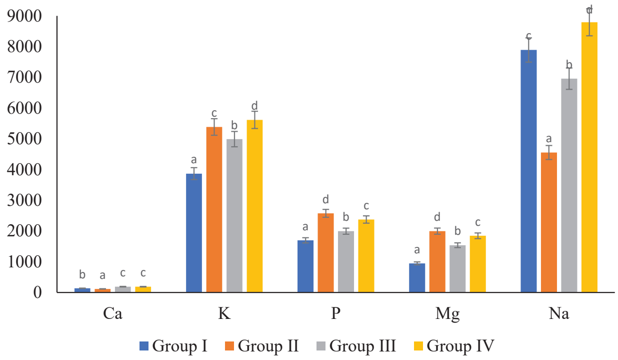

Figure 1. The influence of the addition of amaranth, flax and hemp seeds on the content of macroelements in bread mg/kg dry matter. Group I - control, group II - amaranth 10%, flax 5%, hemp 5%, group III - amaranth 10%, flax 10%, group IV - amaranth 10%, hemp 10%; a,b,c,d - values in rows with different letters differ significantly, $p < 0.05$.

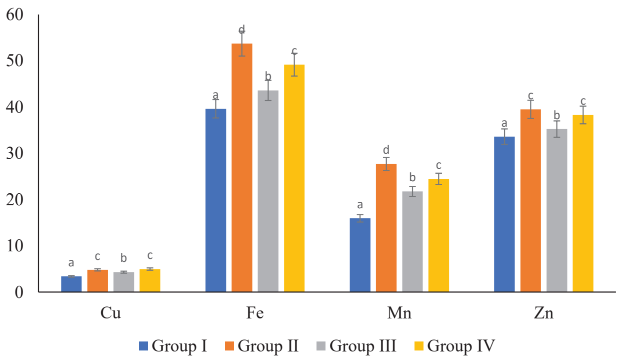

Figure 2. The influence of the addition of amaranth, flax and hemp seeds on the content of microelements in bread mg/kg dry matter. Group I - control, group II - amaranth 10%, flax 5%, hemp 5%, group III - amaranth 10%, flax 10%, group IV - amaranth 10%, hemp 10%; a,b,c,d - values in rows with different letters differ significantly, $p < 0.05$.

Table 5. The influence of the addition of amaranth, flax and hemp seeds on the fatty acid profile (g/100g).

Parameter	Group I	Group II	Group III	Group IV
Saturated fatty acids	1.72 ± 0.04c	1.48 ± 0.03b	1.02 ± 0.07a	1.35 ± 0.08b
Unsaturated fatty acids	1.87 ± 0.02a	7.91 ± 0.05c	6.07 ± 0.02b	9.91 ± 0.06d
Polyunsaturated fatty acids	0.83 ± 0.03a	5.57 ± 0.05c	3.77 ± 10b	8.14 ± 0.02d
Monounsaturated fatty acids	1.06 ± 0.03a	2.34 ± 0.02c	2.27 ± 0.02c	1.76 ± 0.01b
Omega 9 fatty acids	0.81 ± 0.02b	2.03 ± 0.03c	1.98 ± 0.02c	0.11 ± 0.02a
Omega 7 fatty acids	0.19 ± 0.02a	0.29 ± 0.02b	0.29 ± 0.01b	1.72 ± 0.03c
Omega 6 fatty acids	0.75 ± 0.03a	3.74 ± 0.04c	1.94 ± 0.03b	6.33 ± 0.08d
Omega 3 fatty acids	0.12 ± 0.02a	1.78 ± 0.02b	1.73 ± 0.02b	1.75 ± 0.02b

Explanations: Group I - control, group II - amaranth 10%, flax 5%, hemp 5%, group III - amaranth 10%, flax 10%, group IV - amaranth 10%, hemp 10%; a, b, … - values in rows with different letters differ significantly, $p < 0.05$.

pecially iron. On the other hand, Martinez-Lopez et al. (2020) pointed to the positive impact of adding amaranth seeds, which caused an increase in protein content, β-carotene, vitamins, mineral components, and dietary fiber in bread. However, these seeds also contain various antinutritional components, such as phytic acid and saponins, which bind to micro- and macronutrients, making these substances unavailable for our body. This study showed that in all experimental groups, the addition of amaranth, flax and hemp seeds caused an increase in the analyzed macro- and micronutrients, except for calcium and sodium (a decrease in Group II) (Figs 1, 2).

However, this increase varied depending on the proportion of seeds in the sample. The addition of 10% amaranth, 5% flax and 5% hemp seeds (Group II) resulted in a significant increase in potassium, phosphorus, magnesium, copper, iron, manganese and zinc, along with a decrease in calcium and sodium compared to Group III, where 10% amaranth and 10% flax seeds were used. In Group IV, the addition of amaranth seeds (10%) and hemp seeds (10%) to bread resulted in a significant increase in the potassium and sodium content of the bread, compared to the other study groups (Figs 1, 2).

Significant differences were observed between the evaluated products in the conducted sensory evaluation of bread made from ancient wheat flour enriched with amaranth, flax, and hemp seeds. Products from Group II (10% amaranth, 5% flax and 5% hemp seeds) and Group III (10% amaranth and 10% flax seeds) obtained higher ratings in terms of color and skin thickness. On the other hand, products from Group I (control) exhibited significantly better crumb characteristics compared to the enriched bread (Table 6). Products in Group II had a better taste and aroma compared to the other experimental groups. The sensory evaluation of products from Groups I and II allowed the evaluated rolls to be classified as bread of the second quality class, while products from Groups III and IV were classified as third-quality class (Table 6). Stamatie et al. (2022) demonstrated that the addition of hemp press cake increased the fiber and protein content in bread; but it cannot be too high, as it contributes to lower scores in sensory evaluation (Mikulec et al. 2019; Pecyna et al. 2023), bread elasticity and hardness, as well as crust color (Wiedemair et al. 2022). However, Ropciuc et al. (2022) reported that hemp oil used in small quantities (5–10%) had a positive impact on baked goods, while in larger doses, it weakened the dough and negatively affected its elasticity. Mikulec et al. (2019) demonstrated that the inclusion of hemp flour at 30% and 50% resulted in a lower sensory evaluation of the bread. Primarily, it affected the color of the crumb, increasing its browning index. The latter authors suggested that the share of hemp flour in industrial production should not exceed 30%. Scientists published unequivocal assessments of the effect of the sole addition of amaranth seeds on the sensory characteristics of bread. Malik et al. (2023) have pointed out that the slightly bitter and astringent taste of amaranth seeds limits their use in bakery products. Yeşil and Levent (2022) proved that bread with added amaranth seeds obtained high scores in terms of symmetry, texture and appearance, but was less appreciated by consumers in terms of aroma and taste. Gao et al. (2022) evaluated flax bread additives (seeds or flour) and produced bread with acceptable color, texture, flavor and better antioxidant activity. However, the percentage addition cannot be too high, as it darkens the color of the crumb and deteriorates the sensory properties of the bread. The results presented by other authors (Mikulec et al. 2019; Stamatie et al. 2022; Ropciuc et al. 2022; Wiedemair et al. 2022; Pecyna et al. 2023) were partially consistent with the present study. It should be stated that the food industry is undergoing a constant evolution process to meet the dynamic requirements of a growing population, increasingly concerned about health-related diseases (Carpentieri et al. 2022).

Enriching bread with amaranth seeds and 5% of flax and hemp additives (Group II) resulted in more favorable crust characteristics (color and thickness) and crumb compared to their 10% inclusion (Groups III and IV). Products from Groups III and IV with an increased proportion of flax (10%) and hemp seeds (10%) obtained a lower third class of bread quality in the sensory evaluation compared to Group II (5% of flax and hemp seeds each) (Table 6).

Table 6. The influence of the addition of amaranth, flax and hemp for the organoleptic evaluation of bread.

Parameter	Group I	Group II	Group III	Group IV
External appearance	3.85 ± 0.58ab	3.80 ± 0.48a	4.45 ± 0.50bc	4.60 ± 0.57c
The color of the bread crust	2.72 ± 0.78a	4.65 ± 0.41b	4.56 ± 0.49b	2.40 ± 0.46a
Bread crust thickness	3.75 ± 1.38b	4.85 ± 0.34c	4.00 ± 0.47bc	2.65 ± 0.48a
Other features of bread crust	2.60 ± 0.46a	3.15 ± 0.47b	3.95 ± 0.28c	4.75 ± 0.42d
Elasticity of bread crumb	4.83 ± 0.28d	3.75 ± 0.42c	3.0 ± 0.41b	2.12 ± 0.20a
Porosity of bread crumb	4.70 ± 0.48c	3.43 ± 0.56b	3.00 ± 0.67b	2.25 ± 0.54a
Other features of bread crumb	4.70 ± 0.42c	3.65 ± 0.34b	3.05 ± 0.28a	3.15 ± 0.24a
Taste and smell	4.00 ± 0.23b	4.35 ± 0.34b	3.65 ± 0.32a	3.60 ± 0.46a
Total points	31.15	31.63	29.66	25.52
Bread quality level	2	2	3	3

Explanations: Group I - control, group II - amaranth 10%, flax 5%, hemp 5%, group III - amaranth 10%, flax 10%, group IV - amaranth 10%, hemp 10%; a, b, … - values in rows with different letters differ significantly, $p < 0.05$.

Conclusions

The results have shown that the addition of amaranth, hemp and flax seeds as an additive to ancient flour bread is a good technological alternative and allows to obtain bread with increased nutritional value and acceptable sensory and physical characteristics. In all experimental groups (II, III, and IV), the addition of amaranth, flax, and hemp seeds to ancient wheat flours resulted in increased protein content, mineral components, fiber, fat, unsaturated and polyunsaturated fatty acids, as well as improved amino acid composition, compared to the control group (I), resulting in breads with increased health-promoting value. Furthermore, bread enriched with a mixture of amaranth, flax, and hemp seeds had lower baking losses, a darker color, and a higher degree of yellow color saturation compared to non-enriched bread. However, the shear force of seed-enriched bread was rated lower compared to bread from the control. In the analyzed groups, the highest protein and fat content with the most favorable proportion of fatty acids was obtained in bread from Group IV (10% amaranth seeds and 10% hemp seeds). On the other hand, bread enriched with a mixture of all seeds (10% amaranth, 5% flax, 5% hemp seeds) from Group II had higher ash content, as well as a more favorable taste and aroma. Bread from Group 3 with 10% flax and 10% amaranth seeds showed the highest hardness.

One of the most promising areas of development of functional food involves modifying food by using various substitutes and health-promoting additives. The results of the study allowed to obtain bakery products with high nutritional value and health-promoting qualities that could be intended for a broad range of consumers. However, further research is required to improve the recipe for baking bread from ancient wheat flours enriched with other additives.

Author contributions

Wacław Jarecki contributed to wrote the manuscript, data analysis and practical experimental work, Renata Tobiasz-Salach wrote the manuscript, practical experimental work and interpretation of the data, and Anna Augustyńska-Prejsnar participated in the conception and design of the experiment and practical experimental work.

Research funding

This research was funded by the program of the Minister of Science and Higher Education of Poland entitled "Regional Initiative of Excellence", 2019–2023, project number 026/RID/2018/19.

Conflict of interests

The authors declare that there is no conflict of interest.

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