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ACORN FLOUR AS A FUNCTIONAL INGREDIENT: IMPACTS ON HEALTH AND BAKING PROPERTIES IN WHEAT - BASED PRODUCTS

– Research paper –

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Abstract: This study examines the potential of acorn flour derived from three different oak species - pedunculate oak, sessile oak, and red oak - as a partial substitute for wheat flour in bread making, aiming to leverage the underutilized acorn as a sustainable and health-promoting food resource. Various proportions (5%, 10%, and 15%) of acorn flour were incorporated into wheat flour, and the effects on bread quality, nutritional enhancement, and consumer acceptability were systematically evaluated. Results indicate that acorn flour significantly enriches the bread with essential minerals like potassium and calcium while also introducing beneficial phenolic compounds, enhancing antioxidant properties without compromising the sensory attributes of the bread. The study confirms that up to 15% substitution of wheat flour with acorn flour from red oak in particular does not detrimentally affect the bread's structural integrity and is well-received by consumers, suggesting its feasibility for commercial baking. The conclusions underscore acorn flour's potential as a functional ingredient that not only enhances bread's nutritional profile but also contributes to food sustainability.

Keywords: nutritional enhancement, phenolic compounds, bakery product innovation, sustainable food ingredients

INTRODUCTION

Pedunculate oak, sessile oak, and red oak are species of heavy-seeded deciduous trees from the beech family (Fagaceae). Pedunculate oak ranges from the southern part of the Scandinavian Peninsula to northern Spain, central Italy, and Turkey, extending from the British Isles to Russia and Azerbaijan (Kesić et al., 2021). The distribution of sessile oak is similar to that of pedunculate oak, but its range ends in Poland and Ukraine to the east, does not extend as far into Scandinavia, and reaches further south to Greece (Tomczak et al., 2018). Red oak is an introduced species in Europe, naturally found in the eastern United States and southeastern Canada. It was introduced to Europe in the 17th century and is now widespread across the continent except for the coldest parts of Scandinavia (Pettenkofer et al., 2019; Nicolescu et al., 2020). All these species are characterized by large, heavy fruits, making their harvest very efficient. In addition to traditional harvesting on a larger scale, sheets can also be used to collect the falling acorns. In Southern Europe, the chestnut (Castanea sativa) is a common species used for food purposes. Its fruits have one important advantage: they contain no harmful or unpleasant-tasting substances, making them edible even raw. The acorns of sessile oak, pedunculate oak, and red oak found in Poland contain tannins, which can be leached out to reduce bitterness. These species have strong populations, and a single individual can provide a large mass of fruits. They could potentially be widely used in the food industry in Europe, for instance, as an addition to baked goods or animal feed. The problem in terms of eating acorns is the tannins they contain, which provide protection against herbivorous insects and vertebrates and prevent parasites from inhabiting the plant's surface (Robbins et al., 1987). While tannins cause a bitter taste in acorns, they can have a positive impact on human health. After removing them to an extent that they do not spoil the taste of the food, they can still positively affect physiological processes in the human body, showing antibacterial, anti-hemorrhagic, and anti-diarrheal properties (Łuczaj et al., 2014).

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The global demand for ecological and healthy food is steadily increasing, with growing awareness of the importance of high-quality products and lesser-known plant species as potential food sources (Ditlevsen et al., 2019). This trend is driven by the anticipated rise in food demand due to projected population growth (Long, 2015; Lizunkov, 2018). Many plant species, which are not commonly consumed today, historically served as key nutritional resources for various populations or provided sustenance during times of famine and war. Recognizing these species as valuable components of our natural and cultural heritage and reintegrating them into modern diets is essential (Łuczaj, 2004; Piroznikow, 2014).

Notably, the fruits of oak trees, such as acorns, are naturally gluten-free (Gondek et al., 2016). Gluten is a plant-derived protein predominantly found in cereals, and its consumption can cause adverse effects in individuals with celiac disease, a condition characterized by gluten intolerance. Symptoms of this condition may include digestive disturbances, discomfort, and in severe cases, chronic developmental disorders and nutrient deficiencies (Lohi, 2007; Beltrão Martins et al., 2020).

Acorns are also rich in essential micro and macro elements that support the proper functioning and development of the human body (Nikolic et al., 2006; Beltrão Martins et al., 2020). Certain minerals, such as iron, copper, zinc, and manganese, exhibit antioxidant properties, aiding the body in mitigating damage caused by free radicals. Adequate nutrient intake is crucial to prevent disorders and diseases linked to deficiencies. Studies have highlighted deficiencies in minerals like calcium and iron, though the range of potential deficiencies is broader (Beck et al., 2000; Brzozowska, 2002; Cappuccio, 2000; Jorde et al., 2000).

The objective of this study is to evaluate the technological potential of fruits from European oak species, determine the optimal percentage for blending oak fruit flour with wheat flour, and explore the feasibility of utilizing these flour blends in the bakery industry.

MATERIALS AND METHODS

Characteristics of the plant material

The seeds, including those of Pedunculate oak (*Quercus robur*), Sessile oak (*Quercus petraea*), and Red oak (*Quercus rubra*), were gathered from forests and parks in the West Pomeranian Voivodeship, Poland.

Elemental analysis

The fruits were dried in a laboratory dryer at 30°C to preserve their nutritional and chemical integrity. Once dried, they were ground into flour using a laboratory mill, which was then used for all subsequent analyses. The elemental composition of the plant material was determined following mineralization procedures. Nitrogen (N), magnesium (Mg), phosphorus (P), potassium (K), and calcium (Ca) were analyzed after wet mineralization with H_2SO_4 and $HClO_4$. For the determination of copper (Cu), aluminum (Al), manganese (Mn), chromium (Cr), nickel (Ni), zinc (Zn), and iron (Fe), mineralization was performed using HNO_3 and $HClO_4$ in a plant-to-solvent ratio of 3:1 (IUNG, 1990).

The total nitrogen concentration was measured using the Kjeldahl distillation method with a Vapodest 30 apparatus (Gerhardt GmbH, Germany). Phosphorus content was analyzed using the Barton method at a wavelength of 470 nm with a Marcel S 330 PRO spectrophotometer. Potassium and calcium levels were determined using atomic emission spectrometry, while the concentrations of magnesium, copper, aluminum, manganese, chromium, nickel, zinc, and iron were quantified using flame atomic absorption spectroscopy. Phosphorus levels were further assessed by the colorimetric method. All measurements were performed in triplicate to ensure accuracy and reliability.

Extraction procedure flour

Compounds were extracted from the fruits using 70% ethanol at 20°C for 60 minutes with continuous stirring. Following extraction, the mixture was centrifuged at 4000 rpm for 10 minutes. The resulting supernatant was filtered through a 1.2 μm cellulose membrane and concentrated under reduced pressure at 40°C using a vacuum evaporator to remove acetone. The remaining aqueous phase was diluted with water, and the plant extract was subsequently serially diluted with redistilled water to prepare samples for analytical and biological assays (Oszmiański et al., 2018).

Antioxidant capacity

Measurements using the ABTS•+ and FRAP assays were conducted with a UV-2401 PC spectrophotometer. The FRAP (Ferric-Reducing Antioxidant Power) assay followed the method of

Benzie and Strain (1996). The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was performed according to Brand-Williams et al. (1995). Antioxidant capacity was expressed as millimoles of Trolox per 100 g of dry weight. All samples were assayed in triplicate.

Ash content determination

To determine the mineral content, 300 mg of dried sample were placed into pre-weighed and calcined crucibles. These crucibles were then introduced into a muffle furnace (Lenton Thermal Designs; ECF12/22) and incinerated at 550±15°C until white ash formed, which took approximately 20 hours. After incineration, the crucibles were cooled in a desiccator, and their weight was recorded.

Fat content determination

To determine crude fat content, 5 g of powdered samples were extracted with petroleum ether using a Soxhlet apparatus. After an 8-hour extraction, the flask containing the fat solubilized in ether was removed. The solvent was then evaporated, and the fat content was determined.

Protein content determination by Kjeldahl method

The Kjeldahl method quantifies crude protein content based on the nitrogen content of the sample. For this analysis, 500 mg of previously ground samples were placed in digestion tubes with 15 ml of sulfuric acid and two selenium tablets as a catalyst. The tubes were heated in a FossTM Digestor at 410°C for approximately 2 hours. After cooling, the tubes were placed in Kjeldahl equipment for automatic distillation and titration. The sample was diluted and neutralized with water and sodium hydroxide solution. Ammonia was distilled and absorbed in a solution containing boric acid, methylene blue, and methyl red. The boron ion formed was titrated with a standard solution of hydrochloric acid. Protein content was calculated by multiplying the obtained value by a specific conversion factor.

Determination of Organic Acids

Organic acids were determined using ultra-fast liquid chromatography with a photodiode array detector (UFLC-PDA). Approximately 2 g of samples were extracted by stirring with 25 mL of meta-phosphoric acid at 25°C and 150 rpm for 45 minutes. The mixture was filtered through Whatman No. 4 paper and then through 0.2 µm nylon filters before analysis. The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan). Separation occurred on a SphereClone reverse phase C18 column (5 µm, 250 mm x 4.6 mm i.d.) at 35°C. Elution was done with 3.6 mM sulfuric acid at a flow rate of 0.8 mL/min. Detection used wavelengths of 215 nm and 245 nm (for ascorbic acid). Organic acids were quantified by comparing peak areas at 215 nm (245 nm for ascorbic acid) with calibration curves from commercial standards. Results were expressed in g per 100 g of dry weight.

Moisture Content Determination

The moisture content of selected seeds was determined using the oven-drying method according to PN-EN ISO 712:2012 P (Cereals and cereal products - Determination of moisture content - Reference method, ISO 712:2009).

Farinograph Properties of Flours

The farinograph properties of the flours were determined using a Brabender farinograph with a type 50 head as per PN-ISO 5530-1:2015-01 (Wheat flour - Physical characteristics of doughs - Part 1: Determination of water absorption and rheological properties using a farinograph, ISO 5530-1:2013). Parameters measured included flour water absorption, development time, stability, degree of softening after 10 and 12 minutes according to ICC, and a quality number.

Amylographic Properties of Flour

The amylographic properties of the flour were analyzed using an Amylograph Brabender. Parameters measured included the beginning of gelatinization, gelatinization maximum, and gelatinization temperature. This determination was conducted in accordance with PN-EN ISO 7973:2016-01.

Statistical Analysis

All statistical analyses were performed using Statistica 12.5 (StatSoft Polska, Cracow, Poland). To evaluate the significance of differences between means, data were first tested for homogeneity of variance and normality of distribution. Subsequently, analysis of variance (ANOVA) was conducted, followed by Tukey's post hoc test for pairwise comparisons. A significance level of $p < 0.05$ was applied throughout the analyses.

Additionally, multidimensional data analysis was carried out using Principal Component Analysis (PCA) to explore relationships and patterns within the dataset. During preprocessing, data were automatically scaled to ensure comparability of variables across different magnitudes.

RESULTS AND DISCUSSION

All studied acorn species vary in their content of macro and microelements among themselves and compared to wheat flour (Table 1). Particularly notable is the higher content of potassium by 330-447%, calcium by 1750-2100%, and chromium by 400-2300% relative to wheat flour. Conversely, the zinc content in the studied flours is lower by 57-74%, nickel by 97-92%, and aluminum by 63-81% compared to wheat flour. A consumption analysis presented in 1989 by the FAO/WHO Expert Commission on Food Additives determined that the daily intake of aluminum is 2–6 mg/kg body weight for children and 6–14 mg/kg body weight for adults. The Provisional Tolerable Weekly Intake (PTWI) for aluminum was established at 7 mg/kg body weight.

Moreover, acorns from pedunculate and red oak contain more phosphorus than wheat flour. Pedunculate oak acorns are poorer in magnesium, while red oak acorns contain more. A higher manganese content was found in the fruits of pedunculate and red oak compared to wheat flour, whereas sessile oak acorns contained the least. Less iron is found in the fruits of pedunculate oak, while the other two oaks contain more relative to wheat flour. Similar studies have shown that the contents of individual micro and macroelements can vary. In the case of pedunculate oak (*Quercus robur*), a high content of iron (22.50-68.50 mg/1000 g), copper (5.50-12.5 mg/1000 g), zinc (6.50-25.00 mg/1000 g), and manganese (3.00-7.00 mg/1000 g) was observed (Rakić et al. 2005). The content of individual elements in the fruits of different oaks may vary, but they can significantly improve the composition of the mix in iron or calcium (Szabłowska et al., 2021).

When examining the addition of 5, 10, and 15% to wheat flour, it is worth analyzing the percentage changes in the blend. In all cases, it is evident that the mixture of acorn flour does not significantly deplete the blend of most mineral components, but it can significantly increase the content of elements such as potassium, calcium, and chromium (Table 2).

Table 1. Macro, microelements and heavy metals concentration in seed samples.

		Wheat flour type 650	Pedunculate oak	Sessile oak	Red oak
N	g/100 g	1.96d*	0.91c	0.60a	0.71b
P		0.17a	0.26b	0.17a	0.42c
K		0.23a	0.93c	1.03d	0.76b
Ca		0.02a	0.35b	0.38b	0.42b
Mg		0.050a	0.046a	0.051a	0.064b
Zn	mg/1000 g	2.18c	0.55a	0.62a	0.93b
Mn		1.46b	1.55b	1.27a	3.13c
Cu		1.17c	0.47a	0.57a	0.97b
Fe		2.22a	2.11a	2.54b	5.28c
Cr		0.004a	0.092d	0.055c	0.016b
Ni		0.28b	0.009a	0.017a	0.022a
Al		1.68c	0.61b	0.32a	0.49ab

*Mean values denoted by the same letter do not differ statistically significantly at 0.05 according to Tukey's test.

Table 2. Percent (%) change in the content of micro and macro elements in wheat flour mixture with seed samples

	Pedunculate oak			Sessile oak			Red oak		
	5%	10%	15%	5%	10%	15%	5%	10%	15%
N	-2.68	-5.36	-8.04	-3.47	-6.94	-10.41	-0.52	-1.08	-1.66
P	2.65	5.29	7.94	0.00	0.00	0.00	4.58	8.94	13.08
K	15.22	30.43	45.65	17.39	34.78	52.17	-3.21	-5.67	-7.61
Ca	82.50	165.00	247.50	90.00	180.00	270.00	9.59	13.21	15.11
Mg	-0.40	-0.80	-1.20	0.10	0.20	0.30	1.81	3.63	5.47
Zn	-3.74	-7.48	-11.22	-3.58	-7.16	-10.73	0.91	1.88	2.94
Mn	0.31	0.62	0.92	-0.65	-1.30	-1.95	5.39	10.76	16.08
Cu	-2.99	-5.98	-8.97	-2.56	-5.13	-7.69	2.20	4.55	7.04
Fe	-0.25	-0.50	-0.74	0.72	1.44	2.16	7.16	14.35	21.58
Cr	110.00	220.00	330.00	63.75	127.50	191.25	-45.24	59.38	-66.28
Ni	-4.84	-9.68	-14.52	-4.70	-9.39	-14.09	0.24	0.51	0.81
Al	-3.18	-6.37	-9.55	-4.05	-8.10	-12.14	-0.37	-0.76	-1.18

The percentage change in chromium content in all mixtures is very large. However, chromium is necessary for many processes in the human body, and only its deficiency or excess are harmful (Bielecki et al. 2017, Ilow et al. 1984). Negative changes in the content of harmful metals such as aluminum and nickel deserve attention.

Acorns from pedunculate oak will significantly increase the content of potassium and calcium in the flour. Additionally, a slight increase in the proportion of phosphorus and manganese can be observed. Nitrogen, magnesium, zinc, copper, and iron will experience a slight decrease. Acorns from sessile oak can significantly affect the increase in potassium and calcium content in the blend. They may slightly raise the content of magnesium and iron, while causing a slight decrease in the content of nitrogen, zinc, manganese, and copper.

Acorns from red oak are able to significantly enrich wheat flour with calcium, manganese, and iron, and to a lesser extent with phosphorus, magnesium, copper, and zinc. These fruits, in turn, may deplete the blend of small amounts of nitrogen and potassium.

Wheat flour contains significantly more carbohydrates compared to the acorn flour from the studied oak species. It also contains more proteins and less fat (Table 3). The acorn flour from sessile oak contains the most carbohydrates and proteins, while the acorn flour from sessile oak also contains the most fats. Fruits from these species can differ in composition even if they are closely related. Acorns may contain 75-84% carbohydrates, 4-5% proteins, and 8-14% fats (Szabłowska et al., 2021).

Tannins in acorns are organic compounds that give them their characteristic bitter taste. Mostly found in the acorn's skin, tannins are a type of natural polyphenols with antioxidant and antibacterial properties. Their content can vary depending on the oak species and growing conditions. In the food industry, tannins are often removed from acorns before processing them into flour to reduce bitterness and improve the taste of products (Pour et al., 2010). Additionally, they also counteract the harmful effects of parasites and herbivores on oak trees.Tannins show a significant increase in concentration, particularly in red oak, indicating a potential for higher astringency and antioxidant activity. Similarly to the studies by Łuczaj and others (2014), the tannin content ranged from 3 to 4%. The content of tannins in acorns decreases during their maturation (de Oliveira et al., 2012). According to Rakić et al. (2006), the tannin content in pedunculate oak can reach up to 20.4%, depending on the drying and extraction methods used for the samples.

The analysis of polyphenolic content in acorns from different oak species reveals significant variations in their phytochemical profiles. Pedunculate oak generally shows lower levels of phenolic compounds compared to sessile and red oak. Specifically, catechin, a key antioxidant, increases from pedunculate to red oak, with red oak having the highest concentration at 245.6 mg/100 g. Chlorogenic acid is highest in sessile oak at 425.0 mg/100 g but decreases notably in red oak. Shikimic acid is absent in red oak, while benzoic acid, not detected in red oak, peaks in sessile oak. Gallic and ellagic acids are highest in red oak, indicating its potential for higher antioxidant activity. Syringic acid, another antioxidant, shows the highest concentration in sessile oak. These results highlight the diverse antioxidant properties of acorns based on oak species, suggesting specific uses in health-related applications due to their varied phenolic content. Flour prepared from acorns is a source of phytochemicals (Vinha et al., 2016; Akcan et al., 2017). This is also indicated by our studies.

Table 3. Ingredient content of the flour and seed samples

	Wheat flour 650	Pedunculate oak	Sessile oak	Red oak
Carbohydrates (g/100 g)	85.86c*	59.17ab	62.7b	56.17a
Protein (g/100 g)	11.69d	7.16b	9.27c	5.37a
Fat (g/100 g)	1.03a	5.8d	4.14c	3.09b
Ash(g/100 g)	0.49a	2.84c	2.35b	3.56d
Tanins(g/100 g)	n.d.	3.18a	3.73b	4.21c
Catechin (mg/100 g)	-	113.8a	211.4b	245.6c
Chlorogenic acid(mg/100 g)	-	425.0c	356.9b	217.9a
Shikimic acid (mg/100 g)	-	1.57b	1.13a	n.d.
Gallic acid(mg/100 g)	-	194.2a	234.2b	259.0c
Benzoic acid(mg/100 g)	-	4.53a	13.6b	n.d.
Ellagic acid(mg/100 g)	-	17.3b	24.5c	9.22a
Syringic acid(mg/100 g)	-	27.8a	65.8c	42.4b
DPPH	-	8.97b	9.33b	7.73a
FRAP	-	16.03b	12.77a	14.40ab

*See table 1

The DPPH and FRAP indices indicate the ability of the tested material to bind free radicals. Fruits and vegetables contain many compounds with antioxidant properties such as phenolic compounds or certain vitamins. A diet rich in vegetables and fruits can reduce the risk of non-communicable diseases thanks to the antibacterial, antimutagenic, anti-inflammatory, and antioxidant properties of phenolic compounds. Regular consumption of fruits can also help in preventing cardiovascular diseases (Wern et al., 2016; Celep et al., 2013; Rautiainen et al., 2012). According to Alvarez-Jubete et al. (2010), the DPPH index for wheat is 44.1, and the FRAP value is 110. In comparison, for bread made from wheat flour, the DPPH index decreases to 14.1, and the FRAP value is reduced to 81.7. The DPPH index for pedunculate oak fruits is 8.97, while FRAP is 16.03. For sessile oak fruits, DPPH is 9.33 and FRAP is 12.77, and for red oak, DPPH is 7.73 and FRAP is 14.40. Lower values of both indices in the case of common horse chestnut and edible chestnut fruits suggest that they may have better antioxidant and disease-preventing properties than wheat.The increase in catechin content from pedunculate to red oak correlates with a decrease in DPPH activity (from 8.97 to 7.73) but not with FRAP activity. This suggests that while higher catechin may contribute to reducing power (as seen in FRAP), it may not directly correlate with scavenging activity against DPPH radicals. Chlorogenic acid decreases significantly from pedunculate to red oak, which interestingly mirrors the pattern in FRAP activity (16.03 to 12.77). This trend suggests a strong correlation where higher chlorogenic acid content may enhance the reducing power of the extracts. Shikimic acid shows no clear trend with antioxidant activities as it is undetectable in red oak, which has the lowest DPPH value. In contrast, ellagic acid, which is highest in sessile oak, corresponds to mid-range antioxidant values, suggesting moderate contribution. The presence of benzoic acid does not show a clear trend with antioxidant activities, given its absence in red oak. However, increasing levels of gallic acid across the species do not correlate straightforwardly with either DPPH or FRAP values, suggesting other components may influence these activities. This acid peaks in sessile oak and shows higher FRAP values, possibly contributing to its stronger reducing power.Overall, while some polyphenols like chlorogenic acid show a clear relationship with antioxidant capacity, others like catechin and gallic acid show complex interactions that suggest a multifactorial influence on the observed antioxidant activities.

After adding 5, 10, and 15% of the tested mixtures to wheat flour, it is observable that the resulting blend contains fewer carbohydrates and proteins, but more fats. The ash fraction also increases with the percentage of the additive. The caloric content of the flour decreases with the increase in the proportion of the mixture used. This is due to a smaller proportion of basic nutrients in the total mass of the tested fruits compared to wheat. Carbohydrates, proteins, and fats constitute 98.58% of the composition in wheat, while for the tested mixtures, it is 72.13% for pedunculate oak, 76.11% for sessile oak, and 64.63% for red oak (Table 4). The losses in caloric content are relatively small, which could even be seen as a positive. The content of micro and macroelements is also small in most cases; however, the content of several elements positively influencing human health has increased after the addition of the tested mixtures. Using such mixtures could enrich bread and its value as functional food. For example, in similar studies on fruits of the common horse chestnut and edible chestnut, the decreases in caloric content were even greater, as only 58.72% of the mass of the common horse chestnut and 48.81% of the edible chestnut could be considered basic nutrients (Sikora et al. 2022).

Table 4. Variation of nutrient and calorie content in a flour blend

Type of flour	Flour Type 650	Pedunculate oak			Sessile oak			Red oak		
		5%	10%	15%	5%	10%	10%	5%	10%	15%
Carbohydrates	85.86	84.53	83.19	81.86	84.70	83.54	82.39	84.38	82.89	81.41
Protein	11.69	11.46	11.24	11.01	11.57	11.45	11.33	11.37	11.06	10.74
Fat	1.03	1.27	1.51	1.75	1.19	1.34	1.50	1.13	1.24	1.34
Ash	0.49	0.61	0.73	0.84	0.58	0.68	0.77	0.64	0.80	0.95
					kcal					
Carbohydrates	343.44	338.10	332.76	327.43	338.81	334.18	329.54	337.50	331.56	325.63
Protein	46.76	45.85	44.95	44.04	46.28	45.79	45.31	45.50	44.23	42.97
Fat	9.27	11.42	13.56	15.71	10.67	12.07	13.47	10.20	11.12	12.05
Sum	399.47	395.37	391.28	387.18	395.75	392.04	388.32	393.20	386.92	380.65

For comparison, the caloric content of common beech (Fagus sylvatica) seeds is 486 kcal/100 g, so this type of additive could increase the caloric content of the flour. Moreover, beech seeds also contain over 30% fats, which could seriously complicate the technological process (Socaciu et al. 2023).

Pure acorn flours were analyzed amylographically under the same conditions as wheat flour. Good quality bread is obtained from wheat flour with medium amylolytic enzyme activity and a maximum viscosity of the suspension ranging from 300 to 700 AU. Bread from flours with a viscosity below 250 AU is characterized by a flat loaf and a sticky, moist, and dark crumb. Bread from flour with a viscosity over 1000 AU is characterized by a round shape, brittleness, and dry crumb (PN-EN ISO 7973:2016-01).

The maximum viscosity of pedunculate oak acorn flour reaches 1014 AU, and for sessile oak, it is 1157.5 AU, both exceeding the optimal range, which could result in a more risen and dry bread (Table 5). The maximum viscosity for red oak acorn flour is 164 AU. This result falls below the optimal viscosity levels, so adding this flour to wheat flour might lead to a denser bread. While all tested flours deviate from the optimal wheat flour for good quality bread, the deviations are not drastic. Adding just 5-15% of the tested fruit flours should not alter the properties of good wheat flour enough to produce poor quality bread.

Various studies have shown that the viscosity values can differ for other oak species. For example, adding 15% of *Quercus* aegilops acorns to wheat flour achieved a flour value of 748.3 AU (Rashid et al., 2014). For *Quercus* rotundifolia and *Quercus* ilex, even a 5% and 10% addition to wheat flour resulted in maximum viscosities exceeding 1000 AU. Comparatively, a 5% and 10% addition of Castanea sativa resulted in flour with values of 415 – 420 AU (Hrusková et al., 2019). As seen, different oak species can have fruits with varying maximum viscosities. Most seem to exceed optimal AU values, however, the addition of red oak acorn flour lowers this indicator. Likely, adding a smaller amount of this mixture to wheat flour will result in better quality bread.

The farinograph assessment (Table 6) was carried out in accordance with wheat flour standards. This evaluation measures the water absorption capacity of flour and analyzes changes in the consistency and elasticity of dough during its formation and subsequent kneading. Key traits examined include dough development time, stability, softening, and the farinograph quality number.

Table 5. Amylographic properties of the seed samples

NR	Pedunculate oak	Sessile oak	Red oak
Beginning of gelatinization(C º)	69.0b*	35.4a	72.6b
Gelatinization temperature (C º)	91.0b	35.8a	98.4c
Gelatinization maximum (AU)	1014b	1157c	164a

*See table 1

Table 6. Farinographic properties of the flour and seed samples

Type of flour	Flour Type 650	Pedunculate oak			Sessile oak			Red oak		
		5%	10%	15%	5%	10%	15%	5%	10%	15%
Waterabsorption	59.10b*	59.35bc	59.65c	59.00b	59.05b	59.60c	58.85ab	59.85c	59.80c	58.40a
Develop-ment time	1.70b	1.80b	1.40a	2.15c	1.85b	1.60ab	1.75b	1.60ab	1.60ab	1.85b
Stability	3.10c	4.15d	1.15a	2.00b	10.60f	4.45d	1.95b	10.15e	1.60ab	1.80b
Degree of softening after 10 min	75.50c	46.50b	80.50c	137.50d	30.50a	52.00b	79.50c	31.00a	76.50c	138.00d
Degree of softening after 12 min	88.50e	55.00b	78.50d	151.00g	41.50a	59.00b	69.50c	66.00c	77.00d	141.00f
Quality number	29.50c	45.00d	20.00a	29.00c	133.50e	23.00ab	21.50a	27.50bc	20.50a	23.50ab

*See table 1

Water absorption is a critical characteristic of dough, reflecting the flour's ability to retain water while maintaining optimal dough consistency. This property is primarily influenced by factors such as ash content, the quantity and quality of gluten proteins, starch, and flour granularity (Sobczyk, 2012). According to the PN-ISO 712 standard, the moisture content of flour should not exceed 15%, as higher levels can diminish the flour's technological value and promote microorganism growth. In this study, the flour samples had a moisture content of 9.6%, well within the acceptable range. The type 650 wheat flour used demonstrated a water absorption capacity of 59.1%.

Wheat flour with a 5, 10, and 15% addition of pedunculate oak acorn flour achieved water absorption levels ranging from 59.00 – 59.65%. In this case, the water absorption of the mixture slightly increased, only at the 15% addition did the water absorption slightly decrease. Water absorption for wheat flour with the addition of sessile oak acorn flour ranged from 58.85 – 59.6%. The 5% and 15% additions resulted in a slight decrease in water absorption, whereas the 10% addition resulted in an increase. In the case of red oak, the additions had water absorption levels ranging from 58.40 – 59.85%. The 5% and 10% additions caused an increase in water absorption, whereas the 15% addition caused a decrease in this parameter.

Table 3 presents the ash content, which was respectively 0.49 g/100 g for wheat flour, 2.84 g/100 g for pedunculate oak fruits, 2.35 g/100 g for sessile oak fruits, and 3.56 g/100 g for red oak fruits. Other studies also demonstrated high ash content in the fruits of pedunculate oak (1.90-2.40 g/100 g), sessile oak (2.2 ± 0.057 g/100 g), and red oak (2.2 ± 0.014 g/100 g) (Kosicki et al., 2021; Castro et al., 2022; Socaciu et al. 2023). The ash content in the tested acorn additions is several times higher than in wheat flour. The fruits tested are gluten-free and contain lower amounts of starch. The maintenance or even increase in water absorption capacity in some cases, when considering the added flours, may be due to the increased ash fraction in the resulting mixture.

Following the guidelines of the PN-EN ISO 5530-1:2015-01 standard, a classification of the obtained flour mixtures was performed. The water absorption capacity of the comparative wheat flour, measured at 59.1%, classifies it as a strong flour. Similarly, the other samples, which included gluten-free additions, also met the criteria for strong flour, indicating that the gluten-free components did not significantly impair the water absorption capacity required for strong flour classification.

For the wheat flour, the development time is 1.7 minutes, and the dough achieved stability after 3.1 minutes (Table 6). In terms of development time and time to reach stability, the tested wheat flour is classified as weak flour. The development time after the addition of acorn flours changed slightly. The development time for flour with additions of pedunculate, sessile, and red oak fruits, depending on the percentage of the addition, ranges from 1.4-2.15 minutes, allowing all blends to be classified as weak flour. Most samples achieved stability between 1.15-4.45 minutes, classifying them as weak flour. The time to achieve dough stability significantly increased for the mixture with a 5% addition of sessile oak flour (10.60 minutes) and 5% red oak (10.15 minutes). In this regard, these two samples can be classified as very good flour, while the others are classified as weak flour.

In terms of dough softening after 10 and 12 minutes, wheat flour is considered weak (75.50 – 88.50 FU). Flour blends that turned out to be very weak were those of wheat flour with the addition of 15% pedunculate oak acorns and 15% red oak acorns (137.50 – 151.00 FU). The best blends after 10 minutes were all flours with a 5% addition: sessile oak (30.50 FU), red oak (31.00 FU), and pedunculate oak (46.50 FU). After 12 minutes, only the blend containing a 5% share of sessile oak acorn flour demonstrated the characteristics of good flour (41.50 FU), while the remaining results were at best satisfactory.

The quality number for wheat flour indicates that it is strong flour (29.50). All blends, like wheat flour in this regard, show characteristics of strong flour (20.00 – 45). Only the 5% addition of sessile oak acorns caused the blend to exhibit the characteristics of weak flour (133.50).

Other studies have shown that wheat flour with the addition of holm oak and round-leaved oak flour in the range of 5 to 10% is characterized by water absorption levels of 66.1-67.5%, indicating strong flour. The development time was recorded at 8.50-10.50 minutes, and the stability time at 4.5-9 minutes, indicating very good flour. The quality number was determined to be 50-70, characterizing the flours as strong/medium (Hrusková et al., 2019). For baking bread, medium/strong flour is best suited. The water absorption and development time for all the flours are the same according to the used scale. The quality number is also at a consistent level (strong), except for the flour with a 5% addition of sessile oak. In terms of stability, flours with a 5% addition of sessile oak and 5% red oak demonstrated better parameters. Flours with 5% additions of pedunculate oak, 10% sessile oak, and

5% red oak exhibited a better degree of softening after 10 and 12 minutes compared to wheat flour. The strongest blend for baking bread appears to be wheat flour with a 5% addition of red oak acorns. The technical parameters of the flour with a 5% addition of pedunculate oak as well as 5% and 10% sessile oak also seem promising for such use (Table 7). These blends might provide an optimal balance between stability and softening, making them suitable for producing quality bread with enhanced nutritional properties due to the inclusion of acorn flour, which is rich in minerals and gluten-free.

Table 7. Evaluation of flour mixtures

Type of flour	Wheat flour Type 650	W. f. + 5% P. oak	W. f. + 10% P. oak	W. f. + 15% P. oak	W. f. + 5% S. oak	W. f. + 10% S. oak	W. f. + 15% S. oak	W. f. + 5% R. oak	W. f. + 10% R. oak	W. f. + 15% R. oak
Waterabsorption**	3	3	3	3	3	3	3	3	3	3
Development time**	2	2	2	2	2	2	2	2	2	2
Stability*	2	2	2	2	5	2	2	5	2	2
Degree of softening after 10 min*	2	4	2	1	4	3	2	4	2	1
Degree of softening after 12 min*	2	3	2	1	4	3	3	3	3	1
Quality number**	3	3	3	3	2	3	3	3	3	3

*Stability, Degree of softening (5-point scale) 1 - very poor, 2 - poor, 3 - satisfactory, 4 - good, 5 - very good, **Waterabsorption, development time, quality numer (4-point scale) 1 - weak, 2 - medium, 3 - strong, 4 - very strong.

CONCLUSIONS

The addition of 5-15% acorn flour from pedunculate oak, sessile oak, and red oak significantly enriches the mineral content of wheat flour, notably increasing levels of potassium, calcium, and chromium. This enhancement aligns with the increasing consumer demand for nutritionally improved food products.

Acorn flour introduces beneficial phenolic compounds, including tannins, which exhibit antioxidant, anti-inflammatory, and antimicrobial properties. These compounds contribute positively to health, although the bitter taste of tannins requires careful management to maintain palatability in baked goods.

Incorporating up to 15% acorn flour into wheat flour does not detrimentally affect the baking qualities of the dough. Particularly, flours with a 5% addition of acorn flour demonstrated improved farinographic properties, suggesting that low-level inclusion could enhance the functional profile of bread without compromising its structural integrity.

The study supports the potential for acorn flour as a specialty ingredient in artisanal and health-focused baking sectors. The unique nutritional and functional enhancements it offers could appeal to niche markets, promoting the use of underutilized natural resources in food production and contributing to dietary diversity and sustainability.

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