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EFFECTS OF DEFATTED WALNUT MEAL AS A POTENTIAL INGREDIENT IN BREAD: PHYSICOCHEMICAL, RHEOLOGICAL, FUNCTIONAL AND SENSORY PROPERTIES

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Abstract. Potential functional ingredients, resulting after partial oil extraction by cold pressing, present a promising direction with perspective for the nutritional improvement of traditional bakery products and may enhance their health-promoting properties. The current study sought to determine the effect of wheat flour substitution with walnut (*Juglans regia L.*) meal by 0, 2, 4, and 6% of the total amount. The influence of partially defatted walnut meal (BWF) incorporation on dough physicochemical, and rheological properties, as well as the final sensory properties of the bread was investigated. Mixolab test results indicated an increase in dough development time and stability, a reduction of hydration capacity, free water content, enzymatic activity as well as a reduction of the shelf life of the enriched bread. Physical and sensory analyses showed that substitution of wheat flour with partially defatted walnut meal below 6% (on a flour basis) in bread manufacturing recipes yielded products with an enhanced porosity (72.2 to 74.5%), titratable acidity (1.4 to 2.3 degrees), freshness (2.0 to 7.8%) and polyphenol content (0.09 to 2.20 µg GAE/100 g product). All samples show specific positive characteristics, well defined, without defects, with a total acceptability of 4.54 - 4.88. The samples with 2% and 4% BWF show better quality indicators, therefore they can be recommended to consumers as products with high nutritional value.

Keywords: *flour, walnut meal, dough, Mixolab, baking quality, bread.*

Abstract. Ingredientele funcționale potențiale, rezultate după extracția parțială a uleiului prin presare la rece, prezintă o direcție promițătoare cu perspectivă pentru îmbunătățirea nutrițională a produselor de panificație tradiționale și le pot îmbunătăți proprietățile de promovare a sănătății. Studiul actual a urmărit să determine efectul înlocuirii făinii de grâu cu șrot de nucă *(Juglans regia L.),* cu 0, 2, 4 și 6% din cantitatea totală. A fost investigată influența încorporării șrotului de nucă parțial degresat (BWF) asupra proprietăților fizico-chimice și reologice al aluatului, precum și asupra proprietăților senzoriale finale ale pâinii. Rezultatele testului Mixolab au indicat o creștere a timpului de dezvoltare și a stabilității aluatului, o reducere a capacității de hidratare, a conținutului de apă liberă, a activității enzimatice precum și o reducere a termenului de valabilitate al pâinii îmbogățite. Analizele fizice și senzoriale au arătat că înlocuirea făinii de grâu cu șrot de nucă parțial degresat sub

6% (pe bază de făină) în rețetele de fabricare a pâinii a dat produse cu o porozitate sporită (72.2 până la 74.5%), aciditate titrabilă (1.4 până la 2.3 grade), prospețime (2.0 până la 7.8%) și conținut de polifenoli (0.09 până la 2.20 μg GAE/100 g produs). Toate probele prezintă caracteristici pozitive specifice, bine definite, fără defecte, cu o acceptabilitate totală de 4.54 - 4.88. Probele cu 2% și 4% BWF prezintă indicatori de calitate mai buni, prin urmare pot fi recomandate consumatorilor ca produse cu valoare nutritivă ridicată.

Cuvinte cheie: *făină, șrot de nucă, aluat, Mixolab, calitatea coacerii, pâine.*

1. Introduction

The transformation of industrial by-products into high-quality food ingredients is a trend to combat the problem of food safety. Millions of tons of agricultural by-products are produced each year along agricultural and food processing procedures. It is necessary to take further steps to explore the internal potential of agri-food waste to stand out as a food ingredient to partially or completely replace the composition of other food products [1].

Currently, there is a multitude of plant sources of perspective in the view of researchers for which classification was made by genesis, processing, product, and production stage, and information on their effect in the industry was also systematized [2]. Some agricultural waste contains the most valuable nutrients, which combine a range of vitamins and minerals, and other important components: dietary fiber, phospholipids, and biologically active substances of natural origin with protective or stimulating action on physiological systems and body functions [2]. Potential ingredients obtained from industrial by-products can be a remedy for nutritional improvement of traditional bakery products, intensification of the technological process, fortification of flour [3], creating of new products with treatment effect and prophylaxis [4-7].

Nowadays, research is being done to obtain functional bakery products with the addition of protein preparations from defatted, deodorizing soybean flour [8], cotton [9], sunflower flour [10-11], pinus [12] rapeseed, black sesame, hazelnut [13, 14], which are also beneficial supplements that increase the quality bakery products [2]. For example, defatted, deodorizing soybean flour has very wide use: in the USA, bakery products and baby food are manufactured (2-6% is added to ordinary bread and 10-15% to special varieties); in France, Germany, the Netherlands - is added to diet bread [8].

For the preparation of bakery products (buns, baguettes, croissants, muffins) it is interesting to use flour enriched with „grits", partially defatted, from which can be obtained products with high nutritional value, intended for both healthy people and people with health problems (overweight, sports, vegetarians, etc.) [13, 15-18].

Partially defatted walnut meal is a secondary product that remains after nuts pressing [19, 20] and oil extraction. Extremely rich in proteins, essential amino acids, minerals [21], vitamins, and polyphenols with antioxidant activity [22], these by-product has attracted the attention of scientists [19, 23-27]. Characteristics of partially defatted walnut meal indicate that it could have applications as a food additive with nutritional benefits in the manufacture of food for consumption [1]. Due to its high protein content and partial solubility, partially defatted walnut meal *(Juglans regia L.)* has satisfactory functional properties and can be a good source of protein and a functional ingredient in heterogeneous food systems (emulsions, foams, and suspensions) [28, 29]. In addition, the partially defatted walnut meal is used in the confectionery industry in the production of fillings, cakes, candies, etc. [30-38].

The experimental results obtained by Blessing showed that the substitution of wheat flour with a walnut meal (in proportions of 0-50%) significantly increases the bread's content of proteins and fats (respectively 12.17% - 25.70% and 2.40% - 37.57%) and decreases the level of carbohydrates (63% - 19.4%).

As the degree of substitution of wheat flour with walnut meal increases, the volumetric density and hydration capacity decrease insignificantly, but the assimilation capacity of the composite flour increases [39]. Another study conducted by Almoraie demonstrates the effect of improving bread by adding walnut meal that was prepared by soaking, shelling, oven drying, and sifting. As a result, substitution with 30% walnut flour provided the best bread quality [40]. The presence of walnut meal, peanut meal, and membrane between the kernel of the nut, also significantly improves the organoleptic qualities of bakery and confectionery products [10, 14, 40, 41].

In this study, the purpose of the experiments was to establish compositions using a functional ingredient - partially defatted walnut meal to obtain products with high nutritional value. The effects of adding 2 to 6% BWF (on a flour basis) to bread formulation were compared. Emphasis was placed on its effect on the physicochemical, and rheological properties, as well as the properties of the final products. Rheological parameters were estimated using an empirical method, the Mixolab instrument/device that measures both protein (gluten matrix) and starch characteristics and can also provide information on dough development time, protein breakdown, starch gelatinization, enzyme activity, and gel strength.

By applying these methods, those skilled in the art can obtain key information regarding the performance characteristics of wheat flour when measuring the rheological properties of a dough sample. The initial configuration of the Chopin system offers 16 profiles for the following products: bread, biscuits, pizza, pasta, baklava, etc. If the flour does not meet the parameters of a configured profile, Mixolab suggests changes [42].

2. Methods and materials

2.1. Research materials

The main ingredients for the bakery products were first-grade wheat flour „Beatrice-Com" [43] and walnuts *(Juglans regia L.)* [44]. After walnut cold pressing in laboratory conditions, partially defatted walnut meal was obtained.

The manufacturing recipes include other ingredients as well: compressed yeast *(Saccharomyces Cerevisiae)*, salt [45], refined sunflower vegetable oil [46], water [47] and ascorbic acid (E300) [48].

2.2. Determination of the quality and technological properties of wheat flour and walnut meal

To determine the quality of wheat flour, sensory analysis methods and physicochemical methods were used according to international standard methods [49], namely: total ash content [50], moisture [51], titratable acidity [52], quantity [53] and quality of wet gluten [54].

In addition, it was rated the quality of the partially defatted walnut meal, following the characteristics: sensory analysis [55], physicochemical: moisture [51], crude fat [56], total ash content [50], peroxide value [57] and acidity [58].

To assess the physicochemical indicators of the dough, titratable acidity and moisture were determined before and after fermentation [59].

2.3. Evaluation of rheological properties

The dough rheological characteristics were tested by means of the Chopin Mixolab using the standard „Chopin+” protocol; the running parameters of the device are depicted in Table 1. The Mixolab analyzes the quality of the proteins during the dough's kneading (formation time, hydration capacity, stability, elasticity, softening), heating behavior when protein denaturation, starch gelatinization (gelatinization and gelatinization temperature), enzymatic activity (proteolytic), the behavior of the starch when cooling (jellification and relegation).

A typical curve recorded by Mixolab (Figure 1) illuminates the following parameters: C1 - maximum torque during mixing, Nm; C2 - measures the protein weakening based on the mechanical work and temperature, Nm; C3 - expresses the starch gelatinization, Nm; C4 - indicates the stability of the formed starch gel, Nm; C5 - measures the starch retrogradation during the cooling stage, Nm; *α* - represents the slope of the curve between the end of the period of 30 °C and C2 (gives an indication about the rate of the proteins thermal weakening); *ß* - represents the slope of the curve between C2 and C3 (gives indications about the gelatinization rate); *γ* - represents the slope of the curve between C3 and C4 (gives indications about the rate of enzymatic hydrolysis) [42, 60, 61].

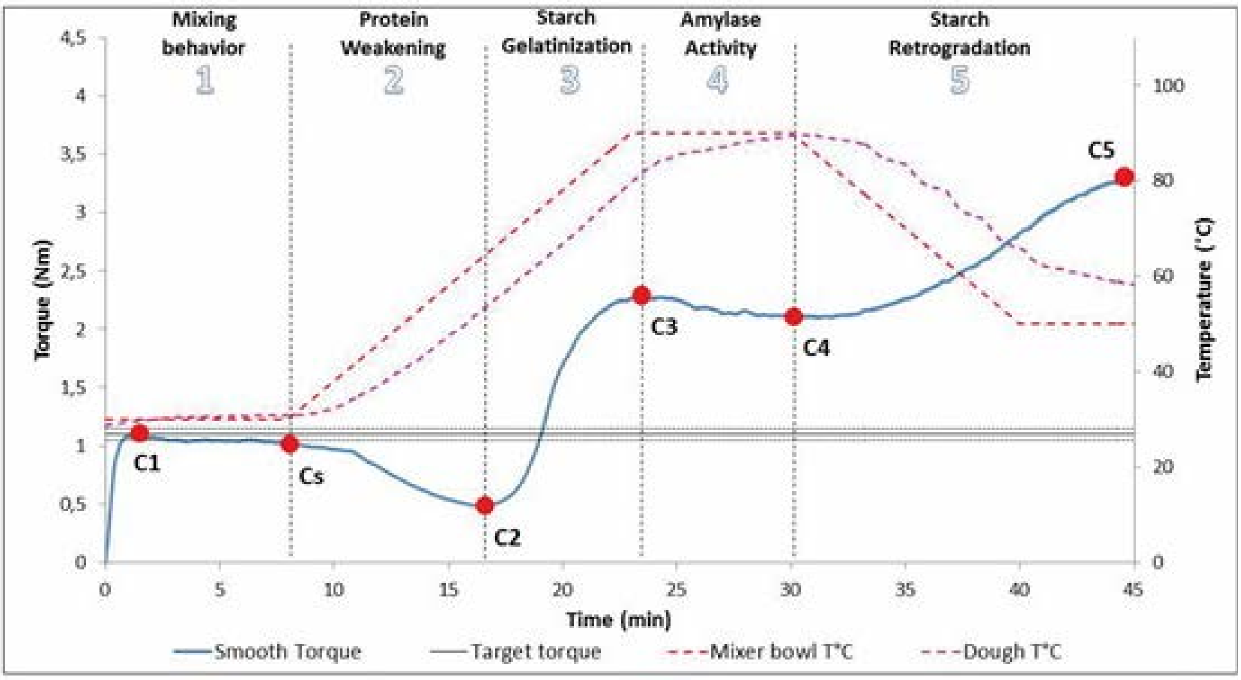


Figure 1. Typical curve recorded by Mixolab [60].

Table 1

Settings of the Mixolab [61]

Parameter	Value
Mixing rate, rpm	80
Dough weight, g	75
Tank temperature, °C	30
Temperature of the first plateau, °C	30
Duration of the first plateau, min.	8
Temperature of the second plateau, °C	90
First temperature gradient, °C/min.	4

Continuation Table 1

Duration of the second plateau, min.	7
Second temperature gradient, °C/min.	4
Temperature of the third plateau, °C	50
Duration of the third plateau, min.	5
Total analysis time, min.	45

The determinations were made in the laboratory of the Department of Food Technology, the Technical University of Moldova, Republic of Moldova and in research laboratory of the University „Dunarea de Jos", Galati, Romania.

2.4. Preparation of bread

Dough preparation is a crucial stage in the technological process of bread making. The dough was prepared using first-grade wheat flour, with or without the addition of walnut meal. The recipes for the different variants are as follows: a control sample without any addition (CS), and samples with 2, 4 and 6% partially defatted walnut meal (2% BWF, 4% BWF and 6% BWF). The control sample was made solely with first-grade wheat flour. Salt and baker's compressed yeast were added in doses of 1.5% and 3%, respectively, based on the weight of the flour mixture. The amount of water was calculated beforehand and adjusted based on the hydration capacity values determined using the Mixolab. After mixing the ingredients with a laboratory mixer (Mesko-AGD, Poland), the dough left to ferment for 60 min at a temperature of 31 ± 1 °C, with a puncture made after 30 minutes. The dough was then divided into pieces weighing 0.400 ± 0.010 kg and placed into molds. The final dough fermentation lasted 30 minutes. The bread was baked in an electric modular oven (Forni Mini-Rotor, Italy) at 240 ± 10 °C for 22 ± 2 min [8]. The obtained samples were analyzed 12 hours after baking.

2.5. Determination of the quality of the experimental samples

To determine the quality of the experimental samples, the following standardized physicochemical methods were used [62, 63]: porosity, freshness, the specific volume of bread, acidity titratable, polyphenol content [64], the water activity (Rotronic Hygro Palm) [65] and microbiological characteristics [66].

Sensory evaluation was performed using 9 panelists trained comprising of graduate students and staff members of the Department of Food Technology, UTM. Samples were randomly assigned to each panelist. The panelists were asked to evaluate each loaf for appearance, color, consistency, taste, smell and total score on organoleptic. A 5-point scoring scale was used [67]. The individual score was given in accordance with the table, which represents the magnitude of the deviation from the sensory requirements provided by the technical documents in force (Table 2) [68].

Table 2

The scoring scale that represents the magnitude of the deviation from the sensory requirements provided by the technical documents in force

Scoring	Description of the evaluation step
5	No deviation from sensory quality requirements
4	Minimal non-conformities from sensory quality requirements
3	Visible non-conformities from sensory quality requirements
2	Obvious/considerable non-conformities from sensory quality requirements

Continuation Table 2

1	Very obvious/considerable non-conformities from the sensory quality requirements
0	The product cannot be analyzed from the sensory point of view

The score awarded by the evaluators was recorded in the sensory analysis sheet.

2.5.1. Methodology for determining the peroxide value

The peroxide value is a physicochemical index that characterizes the degree of freshness of oils. But not only oils but also other products that contain fat can be affected by the rancidity process. In the present research study on the utilization of a secondary product of walnuts - defatted walnut meal *(Juglans regia L.)* in bakery products, we concluded that the quality of the proposed product may be affected by the oxidation of fats in defatted walnut meal, which is about 40%. To receive truthful results, methods of analysis approved on the territory of other countries were studied. Thus, to determine the peroxide value in food products containing flour, the methodology [69] was applied. The method consists in extracting the fat from 15 ± 0.1 g of the sample to be analyzed, with 100 ml of chloroform and treating a volume of 10 ± 0.1 mL of the chloroform extract in the medium of 15 ± 0.1 mL acetic acid with 1 ± 0.1 mL potassium iodide solution with a concentration of 50-55%. The released iodine is titrated with a solution of sodium thiosulfate, 0.01 n solution, in the presence of 1 mL of starch solution with a concentration of 0.5%.

2.5.2. Determination of total polyphenols content (TPC)

The evaluation of the total polyphenol content in the experimental samples was carried out according to the Folin-Ciocalteu method [70], with some non-essential modifications. The amount of sample, 0.2 ± 0.01 g, was dissolved in an amount of 15 ± 0.1 mL of methanol: deionized water (6:4, v/v) and sonicated (ISOLAB Laborgeräte GmbH) at 28 ± 2 °C, for 30 min and at maximum power (frequency 20 kHz, and power 130 W). Then the extracts were centrifuged (10 min, 2500 rpm) and the supernatants were collected. An aliquot (0.1 mL) of the aqueous methanolic phase was diluted with water to a total volume of 6.5 mL, to which 0.5 mL of Folin-Ciocalteu reagent was then added. After 8 min, 3 mL of sodium carbonate solution (10%) was added to the mixture. The reaction mixture was incubated at 40 ± 2 °C in a water bath for 30 min The spectra of the preparation extracts were recorded at spectrophotometer DR 5000 (HACH-Lange GmbH, USA-Germany) in the range of 200...1000 Nm. Results were expressed in μg gallic acid equivalents (GAE)/ 100 g product. The experiments were performed in 3 replications.

2.6. Statistical analysis

Statistical analysis of the experimental data was performed [71] through variance analysis (one-way ANOVA), by comparison at a significance level of 95% ($p \leq 0.05$). All tests were performed in triplicate and all results have been presented as average ± standard deviation. All calculations were made using IBM SPSS Statistics 23 and Microsoft Excel 2010.

3. Results and discussion

3.1. Evaluation of the raw material

3.1.1. Evaluation of wheat flour

Wheat flour was characterized from the point of view of the most important specific parameters that determine the baking quality.

The flour used in the study met the sensory quality requirements [43]. Following the physicochemical determinations, it was established that the registered parameters for the used flours fall within the permissible limits. The moisture of first-grade wheat flour was 14.4 ± 0.2%, the titratable acidity 2.2 ± 0.1 degrees and the ash content - 0.750 ± 0.001%, the content of wet gluten is 24.4 ± 0.2%, wet gluten quality at appliance IDC-1 60 ± 5 c.u., which demonstrates a high quality of gluten.

3.1.2. Evaluation of walnut meal

The partially defatted walnut meal *(Juglans regia L.)* was obtained under laboratory conditions, treated by mixing with ascorbic acid (1% by weight of flour mixture used) [72] and stored under vacuum conditions to prevent the fat oxidation [73], thus favoring the preservation of their organoleptic characteristics - color, taste and aroma.

Partially defatted walnut meal presents a fine-grained powder, light brown in color, with the taste and smell characteristic of nuts. The physicochemical characteristics indicate particles' size of 220 ± 10 µm [28], moisture content 5.8 ± 0.2%, crude fat 40.0 ± 0.1%, the ash content 4.57 ± 0.03%, peroxide value 9.2 ± 0.1 mmol/kg oil, acidity value 3.85 ± 0.10 mg KOH/ g oil.

According to USDA (2013), dry matter, crude fat and ash content of walnut meal are 95.3, 49.42 and 2.99, respectively [74]. The peroxide and acidity index of edible walnut oil should not exceed 10 mmol/kg oil and 4.0 mg KOH/g oil respectively [30].

3.2. Evaluation of the semi-finished product

Characterization of dough is an essential task to ensure good technological quality.

The inclusion in the technological recipe of different raw materials usually affects the properties of the dough and the quality of the bread. The properties of the dough depend directly on the state of the protein-proteinase complex, which is characterized by the quantity and quality of gluten [75].

Walnuts contain up to 11-26% protein (essential amino acids, especially lysine) [30]. The composition of the addition contributed to the intensification of microbiological processes, as a result of the vital activity of bacteria [22].

Lactic, acetic and propionic acid, accumulated in the fermentation process, have antagonistic activity against sporulating bacteria and molds, which would increase the microbiological purity of bakery products, given the high microbiological contamination of walnut meal [75]. In this case, it increases the gas formation capacity and the enzymatic, amylolytic activity, and the biochemical and microbiological processes are activated which will possibly reduce the fermentation time of the dough. The maximum value of titratable acidity was observed after fermentation of the sample 6% BWF, reaching the value of 3.3 degrees (Table 3).

Table 3

Physicochemical indices of quality wheat flour dough with the addition of secondary product of walnuts - partially defatted walnut meal

Characteristics / The product	Titratable Acidity, degrees		Moisture content, %	
	(before fermentation)	(after fermentation)	(before fermentation)	(after fermentation)
CS	1.9 ± 0.2	2.9 ± 0.2	40.0 ± 0.1	36.0 ± 0.2
2% BWF	2.0 ± 0.2	3.0 ± 0.1	38.8 ± 0.2	34.0 ± 0.2

Continuation Table 3

4% BWF	2.3 ± 0.1	3.2 ± 0.1	33.7 ± 0.1	33.0 ± 0.2
6% BWF	2.8 ± 0.1	3.3 ± 0.1	32.6 ± 0.2	32.0 ± 0.2

Note. Results are expressed as mean ± standard deviation. CS - control sample; BWF - sample with addition of partially defatted walnut meal.

It was shown that the added amounts of partially defatted walnut meal influence greatly moisture during fermentation dough fermentation. In the control sample, CS, the moisture values are the highest (40.0 ± 0.1% before and 36.0 ± 0.2 after fermentation) as a consequence of the significant content of carbohydrates in wheat flour [40]. According to our research, the dough moisture with the addition of 2% BWF before fermentation was reduced to 38.8 ± 0.2% and after fermentation to 34.0 ± 0.2%. When introducing a maximum amount of 6% BWF, the moisture value before fermentation was reduced to 32.6 ± 0.2% and at the end of fermentation to 32.0 ± 0.2%. Partially defatted walnut flour added to wheat flour tended to bind moisture, thus reducing the moisture content of the composition. A decrease in moisture was also observed due to the lack of gluten and starch in wheat flour, which have the property of binding water in the dough [39].

Similar patterns were observed by Burbano, Tsyganova, Gomez and Aghamirzaei et al. [4, 16, 17, 75], who argued that the protein and fiber in walnut meal could bind water in the dough, causing moisture to decrease as addition concentration increases. As the percentage level of walnut meal increases (20-50%), the results obtained by Almoraie [40] indicated increased moisture values. The author Tsugkieva claims that the addition of 1% membrane the kernel of the nut does not influence the quality parameters of the semi-finished product [41].

3.3. Testing the rheological character of the semi-finished products with the addition of partially defatted walnut meal

The rheological characteristics are a determining factor for obtaining quality products. In the work, it was followed the influence of the elaborated technology on the properties of the dough with the addition of partially defatted walnut meal using the Mixolab Chopin. The Mixolab is a complex device that measures the rheological properties of the dough. Briefly, this device cumulates the farinographic, extensographic and amyloographic analysis in one [76-78].

One of the features that are evaluated in this device is the hydration capacity. The hydration capacity of flour depends on the quantity and quality of gluten (increases as the quantity is greater and the quality is better); the degree of flour extraction (in flours with increased cellulose content it is higher); the fineness of the flour (increases with finer ground flours, whose starch particles absorb more water). For widely consumed wheat flour, the hydration capacity is 54-64% [25, 79].

The high-water absorption capacity of the dough accounts for consistency, which is one of the attractive characteristics of bread making. In this study the, BWF was used to replace an amount of wheat flour. In the first stage, for the control sample were obtained higher values of the hydration capacity, of 60%.

With the increase in the amount of partially defatted walnut meal in the composition of the recipe, a decrease in the hydration capacity values was observed: for the 2% BWF sample - 58.4%, the 4% BWF sample - 57.0% and respectively the 6% BWF samples – 56.3% (Table 4).

This can be explained by the presence of compounds with hydrophobic properties, as well as the fatty acids contained in the partially defatted walnut meal. Although BWF is high in fiber [30], it contains a great quantity of oil (40%) and is considered an oily additive that obviously reduces water absorption in the dough. Thus, the high hydration rate of the fiber and protein in the walnut meal requires the inclusion of a pre-hydration step before it is added to the dough.

Table 4

Influence of the addition of BWF on dough hydration capacity

Parameters	CS	2% BWF	4% BWF	6% BWF
Hydration capacity, %	60.0 ± 0.4	58.4 ± 0.3	57.0 ± 0.2	56.3 ± 0.5

Note. Results are expressed as mean ± standard deviation. CS - control sample; BWF - sample with addition of defatted walnut meal.

The values of the increase of the dough moisture in the case of the previous determinations are argued by the calculation of the recipes, but the values obtained for this device (Mixolab) prove the opposite. A similar pattern was observed by Aghamirzaei et al. [75] when the effects of the incorporation of grape seed flour on the rheological properties of the dough were studied. Water absorption decreased significantly by increasing the additive content from 0 to 25%.

The decrease in hydration capacity values is explained by the content in the walnut meal of non-polar hydrophobic amino acids (alanine, phenylalanine, proline, valine, methionine), which repel water [30].

The partially defatted walnut meal does not have the property of binding water because it does not contain gluten and starch. The authors argue that this could be due to increased concentrations of chemical compounds with hydrophobic properties, such as fatty acids. Although the additive has fiber-rich fractions, still its oil content is high and it is considered an oily additive that obviously reduced the water-holding capacity of the flour through reduced water absorption [4, 8, 39, 75].

In addition, other research, by Pycia et al. [80] was characterized by low water absorption capacity, and this parameter decreased as the share of walnut flour in the system increased (from 5 to 15 %). The average value of this parameter was 47.9%.

Burbano et al. [4] claimed that the addition of walnut flour (WF) greatly affected the microstructural characteristics of the dough, leading to differential molecular mobility and rheological behavior. Likewise, the addition of WF improved the consistency of the dough, making it much easier to mold.

In contrast, other studies [40] showed that the addition of walnut flour in dough progressively ($p \leqslant 0.05$) significantly affected water absorption capacity, from 60% in the control to 65% in wheat flour supplemented with 50% flour of walnut. This increase, the author mentions, is due to the protein solubility and dough composition following the addition of nuts whose protein is characterized by high solubility and water absorption capacity. Furthermore, the addition of walnuts could lead to a structural change in the dough that may allow more water to be absorbed due to hydrogen bonding.

Parameters of the rheological evaluation of the dough with the addition of partially defatted walnut meal are presented in Table 5.

Significant development time values were observed for the flour mixture with the addition of 4% BWF, with values of 0.98 min. Following the addition of more than 6% BWF, an obvious decrease in C1 development time to 0.63 min was observed.

From the presented results, higher stability with a value of 1.73 min was observed in the flour mixture with the addition of 4% BWF, thus showing a positive influence of partially defatted walnut meal on this dough characteristic. The value of the dough development time also increased, from 0.92 to 0.98 min, compared to the control sample.

In the second stage with the heating of the dough (53-59 °C) and the mechanical action of the kneading paddles increases and the minimum consistency C2 (with the increase of the amount of partially defatted walnut meal has reduced the denaturation of proteins, therefore the free water content from the dough).

Table 5

The variation of the rheological characteristics of the dough in the Chopin Mixolab, for the samples with added partially defatted walnut meal walnut meal

Stages		CS	2% BWF	4% BWF	6% BWF
C1	Development time, min.	0.92 ± 0.02	0.95 ± 0.03	0.98 ± 0.02	0.63 ± 0.08
	Standard consistency, Nm	1.08 ± 0.12	1.13 ± 0.12	1.12 ± 0.12	1.13 ± 0.12
	Dough temperature, °C	29.90 ± 1.10	31.30 ± 1.30	31.10 ± 1.10	31.40 ± 1.40
	Standard consistency amplitude, Nm	0.09 ± 0.00	0.08 ± 0.01	0.07 ± 0.02	0.10 ± 0.00
	Stability, min.	1.47 ± 0.03	1.38 ± 0.02	1.73 ± 0.07	1.70 ± 0.10
C2	Development time, min.	17.12 ± 0.15	17.5 ± 0.16	17.62 ± 0.24	18.02 ± 0.37
	Standard consistency, Nm	0.35 ± 0.08	0.37 ± 0.07	0.36 ± 0.02	0.37 ± 0.07
	Dough temperature, °C	53.80 ± 1.20	58.9 ± 1.10	57.40 ± 0.90	58.60 ± 1.40
C3	Development time, min.	23.95 ± 0.20	23.5 ± 0.30	23.73 ± 0.27	23.73 ± 0.07
	Standard consistency, Nm	1.70 ± 0.02	1.69 ± 0.02	1.64 ± 0.02	1.61 ± 0.02
	Dough temperature, °C	77.50 ± 1.50	79.80 ± 0.20	78.80 ± 1.20	78.80 ± 1.20
C4	Development time, min.	33.58 ± 0.56	33.87 ± 0.34	33.60 ± 0.65	32.67 ± 0.30
	Standard consistency, Nm	1.28 ± 0.02	1.22 ± 0.02	1.21 ± 0.02	1.16 ± 0.02
	Dough temperature, °C	84.10 ± 0.90	83.80 ± 1.20	84.00 ± 1.00	85.30 ± 0.30
C5	Development time, min.	45.00 ± 0.00	45.00 ± 0.00	45.00 ± 0.00	45.00 ± 0.00
	Standard consistency, Nm	1.84 ± 0.02	1.85 ± 0.02	1.77 ± 0.02	1.74 ± 0.02
	Dough temperature, °C	58.30 ± 0.70	57.30 ± 1.70	58.40 ± 0.60	57.50 ± 1.50

Note: Results are expressed as mean ± standard deviation. CS - control sample; BWF - sample with addition defatted walnut meal.

Research by Almoraie et al**.** [40] showed that increasing the level of walnut addition significantly reduced dough development time. This value is an important indicator of the hydration capacity of the flour particles and therefore of the presence of a hydrophobic substance. Based on published reports, dough stability parameters decreased while the mixing tolerance index increased with increasing amounts of addition in the mixes. This hypothesis is also supported by other authors [61, 80, 81]. The reduction of hydration capacity could be due to the fact that the addition of nut constituents could disrupt the wheat gluten-starch network, competing with wheat flour proteins for water, and then decreasing its stability.

In the second stage with the heating of the dough (53-59 °C) and the mechanical action of the kneading paddles increases and the minimum consistency C2 (with the increase of the amount of partially defatted walnut meal has reduced the coagulation of proteins, therefore the water content free from the dough).

In third stage, the control sample showed the highest values of the maximum gelatinization C3 with a maximum value of 23.95 min, and the consistency of 1.7 Nm. Due to the increase in temperature (78-80 °C), the proteins denature and release water which becomes available for starch. Starch granules absorb water and as a result, increase the consistency of the dough.

In the 4th stage, with the increase of the amount of partially defatted walnut meal, the C4 consistency decreases, also the enzymatic activity (proteolytic, amylolytic) and the most stable gel formed is presented by the sample with 6% of partially defatted walnut meal with 1.16 Nm (by 9.4% compared to the control sample).

In the 5th stage, we have a more efficient downgrading of the starch in the sample with 2% of partially defatted walnut meal, with 0.54% compared to the control sample, which gives us a longer shelf life compared to the other samples. The addition of 6% BWF reduces the shelf life of the products by 5.4% compared to the control sample.

Our results agreed with other reports that indicated that the addition of ground walnut increased the gelatinization temperature values and led to a very noticeable reduction in the viscosity of such systems [82].

Conversely, other studies have shown that increases in peak viscosity and gelatinization temperature were observed in composite wheat flours [40]. The difference could be attributed to the variation in added materials. Some additives are free of sugars or starches, while others contain materials that may contain sugars and starches that increase viscosity and gelatinization temperature.

The authors Pycia ed Juszczak (2021, 2022) [80, 82] claim that the retrogradation enthalpy and degree of retrogradation increase significantly compared to the control and reduce the storage modulus values that characterize the tested doughs.

Based on the results obtained at Mixolab, was built the graph regarding the rheological evaluation of the dough with the addition of BWF (Figure 2).

It is possible to compare the obtained curves with the associated profiles. The profiler is a translation into six qualitative index measurements, shown in the graphical test result (Figure 3).

Based on the baking test results, Mixolab C2, C3 and C4 characteristics were positively correlated with porosity, while C5 was significantly correlated with bread freshness. The correlation coefficients recorded were in the range of 0.64-0.80 ($p < 0.001$) and - 0.88 ($p < 0.001$), respectively.

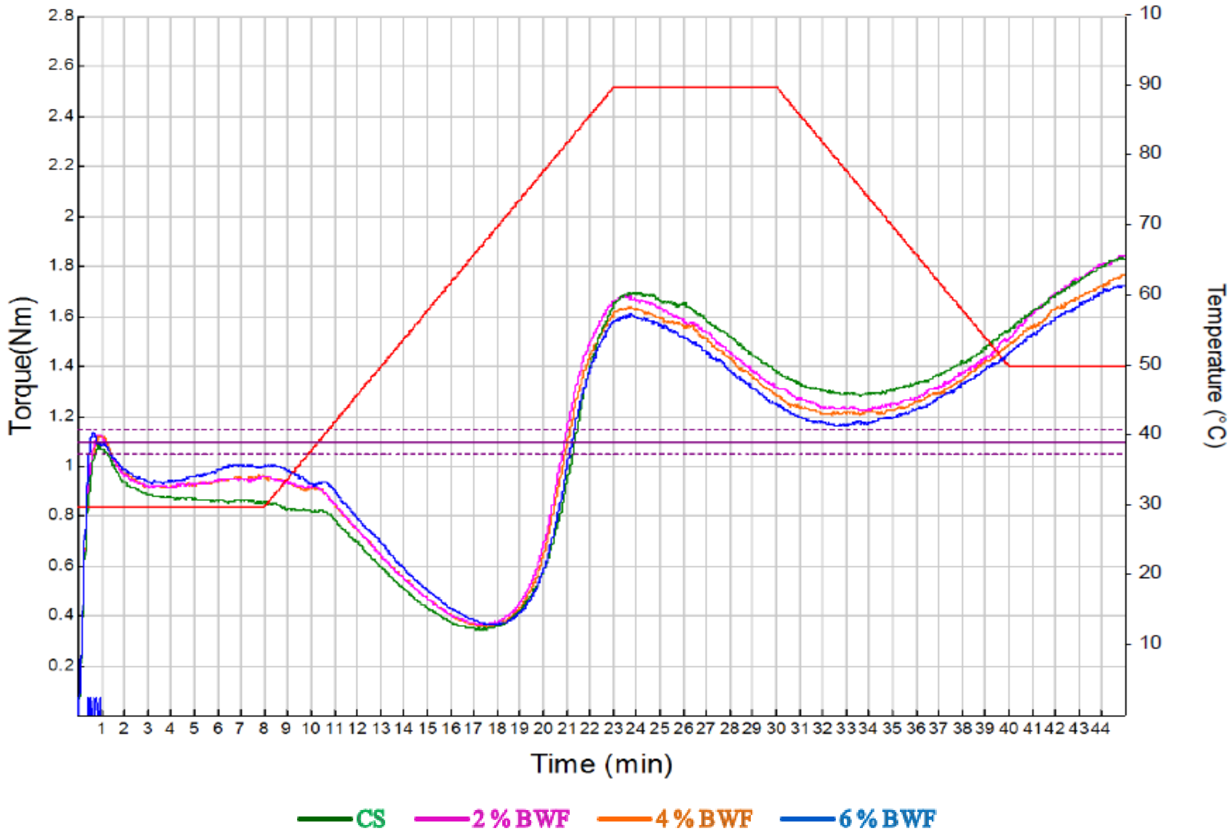


Figure 2. Rheological evaluation of the dough with the addition of BWF, in Mixolab (Done by author).

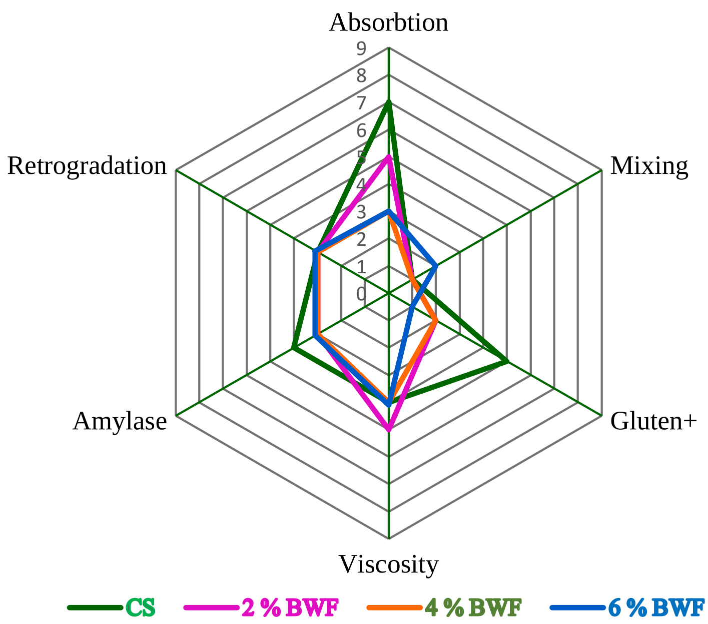


Figure 3. Graphic profile of overlapping experimental samples:
CS - control sample; BWF - sample with addition defatted walnut meal.

3.4. Testing the nutritional and functional characteristics of bread with the addition of walnut meal

The physicochemical quality indicators of the products obtained with the addition of partially defatted walnut meal and control are presented in Table 6.

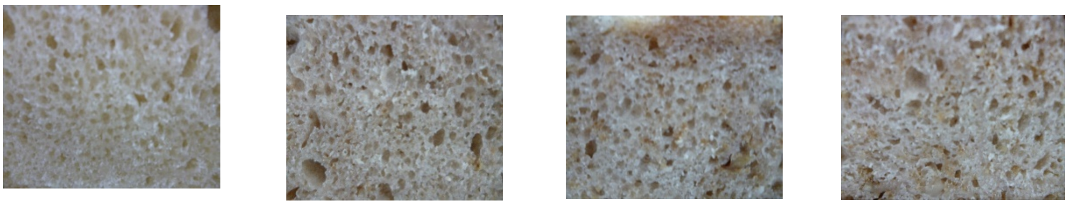
Table 6

Influence of the addition of BWF on wheat bread quality

Parameters	CS	2% BWF	4% BWF	6% BWF
Porosity, %	72.2 ± 1.4	72.8 ± 1.3	73.1 ± 1.2	74.5 ± 1.5
Specific volume of bread, cm^3/100 g	271 ± 12	268 ± 15	263 ± 10	261 ± 11
Acidity titratable, degrees	1.4 ± 0.2	1.8 ± 0.1	2.2 ± 0.1	2.3 ± 0.2
Freshness, %	2.0 ± 0.1	4.0 ± 0.1	6.0 ± 0.1	7.8 ± 0.1
Water activity, c.u.	0.227 ± 0.001	0.276 ± 0.003	0.301 ± 0.006	0.317 ± 0.003
Total polyphenol content, µg GAE/100 g product	0.09 ± 0.02	1.02 ± 0.05	1.44 ± 0.07	2.20 ± 0.08

Note. Results are expressed as mean ± standard deviation. CS - control sample; BWF - sample with addition partially defatted walnut meal.

The analysis of the results presented in Table 6 showed that the addition of partially defatted walnut meal influenced the bread quality parameters [68]. The porosity of bread increased linearly with the added concentration of partially defatted walnut meal, showing values of 72.8%, 73.1% and 74.5% in samples with 2%, 4% and 6% BWF, respectively, compared to control. This phenomenon is explained by the stimulation of the fermentation activity of baker's yeast by the components of the partially defatted walnut meal (Figure 4). Therefore, the porosity of products with the addition of walnut flour increased insignificantly, with a maximum of 3.09% compared to the control**.**



CS 2% BWF 4% BWF 6% BWF

Figure 4. Influence of partially defatted walnut meal addition on the porosity of experimental samples from wheat flour: CS - control sample; BWF - sample with addition partially defatted walnut meal.

In general, porosity is characterized by the uniformity, size and thickness of the pore walls. In this study the control sample is characterized by uniform, medium-sized pores with thin walls. With the increase of the amount of addition the pores have larger sizes and thicker wallswhich thus influencing the porosity results.

Increased values for porosity were also obtained by Chochkov [7] ed Sandulachi [83] for bread in the presence of defatted walnut meal. Pycia et al. (2020) state that the reduced and uneven porosity of the product enriched with walnut meal, over 20%, is probably due to the high amounts of fat that hinder the activity of the yeast and the retention of gas bubbles, and the reduced amount of gluten in the dough as a result of the replacement of wheat flour with walnut meal. Consequently, the support of the three-dimensional gluten network it's weaker [10].

On the contrary, the specific volume of bread was reduced by supplementing wheat flour with partially defatted walnut meal, where the specific volume was highest in the control (271 ± 12 cm3/100g) and lowest in the sample containing 6% BWF (261 ± 11 cm3/100g). The authors claim [61, 81] that these changes are due to the fact that the addition contributed to reducing the amount of gluten, but had a tonic effect on it. The amount of partially defatted walnut meal walnut meal increased the gas-forming capacity, but less the gas-holding capacity in the dough. Chochkov et al. [7] claim that the optimal addition of walnut meal to maintain the correct bread volume is 5-10%. Almoraie et al. [40] estimated that supplementing bread with walnut meal at a level higher than 20% has a very negative effect on its weight and volume. Following the interaction of the components in the recipe, the gluten network responsible for retaining the fermentation gases produced by the yeasts is weakened [17]. This is consistent with the results obtained in this study.

The values of titratable acidity and freshness of baked samples are important indicators in directing the evolution of physicochemical transformations in bread during the storage period. In this work, we observed that these parameters have changed depending on the amount of addition, manifesting an increasing trend. The bread samples with partially defatted walnut meal supplementation showed an increase in titratable acidity value within the range of 1.8 ± 0.1 to 2.3 ± 0.2 degrees compared to wheat flour (1.4 ± 0.2). This could be associated with the high percentage of fatty acids in the added supplement, which contributes to the intensification of biochemical and microbiological processes in the dough [40].

The freshness is due to the behavior of the starch during cooling (crystallization of amylopectin). The higher the index, the shorter the shelf life of bakery products [80]. According to our research, the freshness of the core in the sample with the addition of 2% BWF increased to 4.0% compared to 2.0% for control sample. Hydrophobic amino acids (non-polar) in the partially defatted walnut meal have the property of repelling water during the baking stage, significantly influencing the freshness of the bread core. Freshness increased to 6.0% and 7.8% in samples with 4% and 6% BWF. This can be explained by the fact that with a greater addition of BWF, the amount of free water will be greater [18, 40] and thus we will have greater decreases in baking losses.

Water activity is frequently used as a critical control point in HACCP programs aimed at controlling microbial stability or physicochemical and enzymatic degradation in products [65]. The tested samples showed increasing a_w values, from 0.227 c.u. (CS) at 0.317 c.u. (6% BWF).

According to the sources [65] it is considered that the values do not exceed the range determined for food quality and safety. Following the microbiological evaluation, the baked bread samples with the addition of partially defatted walnut meal showed no signs of mold contamination, having a color from golden yellow to dark yellow. Microbiological stability of work samples has been comparatively higher than those of control [83]**.** In the samples, 4% and 6% BWF were found to be a common microbiota, with the presence of *Streptococcus lactobacillus*, *Streptococcus lactis* and *Bacillus subtilis*.

According to the studies [6, 22, 84, 85], it can be stated unequivocally that walnuts (Juglans regia L.) are leaders in the content of phenolic substances compared to other nuts. Their value is 1.5-2.5 g gallic acid equivalents (GAE)/100 g fresh matter. In this study, the presence of bioactive compounds in partially defatted walnut meal increased the total content of phenolic compounds in bread. The highest results were recorded in the sample with 6% BWF, of 2.20 ± 0.02 µg GAE / 100 g product, compared to the control sample 0.09 ± 0.02 µg GAE / 100 g product.

The results of the sensory analysis of the wheat bread samples with the added partially defatted walnut meal are presented in Table 7.

Table 7

Sensory profiles of bread samples prepared with the addition of BWF

Sensory characteristics	CS	2% BWF	4% BWF	6% BWF
Exterior appearance	4.40 ± 0.03	5.00 ± 0.00	4.80 ± 0.00	5.00 ± 0.00
Color	4.40 ± 0.01	5.00 ± 0.00	4.80 ± 0.00	5.00 ± 0.00
Consistency	4.80 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00
Taste	4.00 ± 0.02	4.40 ± 0.03	4.60 ± 0.04	4,80 ± 0.00
Smell	5.00 ± 0.00	4.60 ± 0.02	4.80 ± 0.00	4.80 ± 0.00
The total score on organoleptic	4.54 ± 0.06	4.71 ± 0.05	4.78 ± 0.04	4.88 ± 0.00

Note. Results are expressed as mean ± standard deviation. CS - control sample; BWF - sample with addition defatted walnut meal.

The table 7 shows the sensory attributes of the composite bread of different percentages of supplementation of defatted walnut meal added to wheat flour. The prepared bread was evaluated by the evaluators regarding the external appearance and color of the crust. The control sample, CS, has a specific color of first-grade wheat flour, and at the samples with addition of BWF an increase in crust color intensity was observed with a higher level of walnut meal. Probele CS, 2%, and 6% BWF au obținut un scor de 5.0. The 2% and 6% BWF samples have a more pleasant and more homogeneous shade, with a score of 5.0. Three analysts indicated skin surface color differences in the 4% BWF sample (4.80) expressed by irregular shape and uneven surface color. But these differences were described as moderately perceptible. The color change is explained by the content of polyphenols *(TCP)* existing in the partially defatted walnut meal which, when homogenized with other components in the recipe and after heat treatment, lead to a change in the color of the finished product. On the other hand, the inclusion of 2, 4, 6% BWF significantly increased the core consistency compared to the control, from 4.80 to 5.00.

Following the sensory analysis of the samples taken in the research, a pleasant smell was noticed, specific to freshly baked bread, with a slightly sweet taste. With the evaluation for the smell of the bread, the quality score ranged from 4.00 to 4.80. The results clearly show that bread made from 100% wheat flour had the highest score (5.00), followed by bread with 4% and 6% BWF (4.80), while bread with 2% BWF had the lowest value (4.60). A reason for this may be caused by the bitter taste of some inherent compounds of BWF, especially at high temperatures, as reported by Santos et al. [86].

The overall evaluation also shows that the degree of supplementation influences the overall approval of the bread samples. Bread made with 6% walnut flour had the highest score with 4.88 compared to the control (4.54) and other walnut flour supplements.

Similar patterns were observed by [13, 16-18, 83] in their tries of enhancing the nutritive and biological value of bread.

4. Conclusions

In this study, the effects of the incorporation of partially defatted walnut meal on the dough physicochemical and rheological properties, as well as properties of the final products were investigated. Increasing the incorporation of BWF from 2 to 6% (on a flour basis) led to increased final dough moisture after fermentation (32.0 to 36.0%). The composition of the partially defatted walnut meal addition contributed to the intensification of microbiological processes, as a result of the vital activity of bacteria. The changes in the trend of the Mixolab curve depended on the doses of the walnut meal. The results demonstrate that increasing the amount of partially defatted walnut meal reduces the hydration capacity, the free water content of the dough, the enzymatic activity (amylolytic and proteolytic) as well as the shelf life of the bakery products. Significant correlations were observed between gluten quantity and C1, C3, C4, and C5 and also between gluten quality and C3 and C4. Stability is also correlated with wet gluten quantity and quality. Moreover, the parameters C2, C3 and C4 were positively correlated with porosity based on the results of baking tests, while C5 was correlated with bread freshness.

The addition of partially defatted *(Juglans regia L.)* walnut meal increased the bread final titratable acidity (1.4 to 2.3%) and freshness (2.0 to 7.8 %). The fiber content of the partially defatted walnut meal also hinders the leavening ability of the bread, as a result, the specific volume of the bread was progressively reduced, while the porosity of the bread increased (72.2 to 74.5%). The inclusion of bioactive compounds from partially defatted walnut meal in bread increased the overall amount of phenolic compounds, from 0.09 to 2.20 μg GAE/100 g product. Following the sensory analysis, all samples have specific positive characteristics, very well defined, without defects with a total acceptability of 4.54 - 4.88. The samples with 2% and 4% partially defatted walnut meal can be recommended to consumers as products with high nutritional value.

Conflicts of Interest: The author declares no conflict of interest.

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