Scientific Papers. Series B, Horticulture. Vol. LXX, No. 1, 2026
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON THE DEVELOPMENT OF A LOW-CARB BREAD WITH ANTIOXIDANT POTENTIAL, FORTIFIED WITH *SPIRULINA*, INTENDED FOR THE PREVENTION AND DIETOTHERAPY OF DIABETES MELLITUS AND OBESITY

Luminița CATANĂ[1], Monica CATANĂ[1], Anda-Grațiela BURNETE[1], Monica-Alexandra DÂRĂ[1], Denisa-Eglantina DUȚĂ[1], Florica CONSTANTINESCU[1], Adrian ASĂNICĂ[2]

[1]National Research & Development Institute for Food Bioresources, IBA Bucharest, 6 DinuVintila Street, District 2, 021102, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: mcatana1965@gmail.com

Abstract

The adoption of an unhealthy lifestyle (a diet high in sugars and fats, physical inactivity), genetic predisposition, chronic stress, and environmental factors are major causes of diabetes mellitus and obesity. The adoption of a healthy diet, including products adapted to the metabolic disorders associated with these chronic conditions, is of considerable interest. This paper presents research on the development of a low-carbohydrate bread fortified with Spirulina powder (fortification levels of 1-4%), with high nutritional value and antioxidant potential, intended for individuals with diabetes and obesity. This bread exhibits superior sensory qualities and has a low content of total carbohydrates (35.60%) and available carbohydrates (25.65%). It is distinguished by its protein content (14.15%), total dietary fiber (9.95%), mineral content (K = 282.45 mg/100 g; Mg = 83.21 mg/100 g; Ca = 63.42 mg/100 g; Fe = 3.27 mg/100 g), total polyphenols (262.83 mg GAE/100 g) and antioxidant capacity (295.48mg mg Trolox equivalents/100 g). Due to its complex biochemical composition and low content of total and available carbohydrates, this type of bread is suitable for the diet of individuals with diabetes and obesity.

Key words*: bread, low carb, Spirulina, diabetics, obese individuals.*

INTRODUCTION

Diabetes management, including the prevention and remission of type 2 diabetes, is based on effective evidence-based studies. Dietary recommendations and nutrition therapy are essential for improving both life expectancy and quality of life (Mette et al., 2023; Uusitupa et al., 2019). Among the key factors involved in the development of diabetes is the increase in postprandial glucose levels following the consumption of foods with a high glycemic index, such as wheat flour bread (Miller et al., 2024).

Bread is a staple food in countries around the world and is therefore important in global nutrition. The metabolic effect of bread depends on several qualitative aspects, such as the type of grains, carbohydrate and other nutrient content, and preparation methods, etc. Bread quality is determined by its content of dietary fiber, whole grains, seeds, and, respectively, added sugar in its composition (Ludwig et al., 2018; Pagliai et al., 2021). High consumption of refined grains, which are low in dietary fiber and have a high glycemic index, is associated with an increased risk of type 2 diabetes and other chronic diseases. Therefore, improvements in bread composition could influence population health. Thus, international research has highlighted the beneficial effect of reformulated breads rich in dietary fiber, whole grains, and/or functional ingredients on fasting blood glucose concentrations in adults, mainly among those with type 2 diabetes (Schadow et al., 2023).

The consumption of functional bakery products based on whole-grain flours, enriched with seeds and cereals, can improve health and reduce postprandial blood glucose levels as well as the risk of metabolic syndrome (Lange et al., 2024).

Burnete et al. (2020) developed a bread for individuals with diabetes using nutritionally valuable ingredients rich in bioactive compounds (polyphenols, carotenoids, etc.). The product is characterized by a low content of available carbohydrates (38.56-39.49%) and stands out due to its protein content (8.85-9.12%), total dietary fiber (3.15-3.70%), and total polyphenols (100.85-115.12 mg GAE/100 g), while also exhibiting antioxidant capacity (117.45-125.15 mg TE/100 g).

Chiranthika et al. (2024) conducted experiments to develop a functional bread intended for individuals with diabetes, fortified with flours derived from the rhizomes of *Lasia spinosa* and *Nelumbo nucifera* (fortification levels of 20, 40, 60, and 80%). Chemical analysis showed that the fortified breads had higher dietary fiber, resistant starch, and ash contents than the control breads, while protein, fat, and starch contents were lower. The authors also reported that bread fortified with 20% rhizome flour achieved the highest sensory score compared with breads produced with higher fortification levels. Moreover, it exhibited higher levels of dietary fiber, resistant starch, antidiabetic activity, and a lower starch digestibility rate than the control bread.

Jiménez et al. (2025) developed a low-glycemic-index white bread fortified with dietary fiber from *Oxalis tuberosa* (fortification levels of 3%, 6%, and 10%) as a functional ingredient providing nutraceutical benefits.

This paper presents research on the development of a low-carbohydrate bread fortified with *Spirulina* powder (fortification levels of 1-4%), with high nutritional value and antioxidant potential, intended for individuals with diabetes and obesity.

MATERIALS AND METHODS

Materials

"White bread" (Control sample) was obtained from white wheat flour, "Bucovina" still water, natural sourdough, and sea salt.

To obtain the product "Low Carb Bread Fortified with *Spirulina* Powder", ingredients were selected to ensure a reduction in glycemic index and an increase in fiber content. For this purpose, in addition to *Spirulina* powder, the following were used: natural sourdough fortified with *Spirulina*, whole wheat flour, *Aronia pomace* powder (20 g/kg whole wheat flour), a seed mix (85 g seeds/kg whole wheat flour; sunflower seeds 35%, flax seeds 20%, white sesame 15%, black sesame 15%, poppy seeds 5%, chia seeds 5%, millet 5%), chicory inulin. Natural sourdough (C) was obtained by fermentation at room temperature for 12 days of a mixture consisting of white wheat flour type 650, whole wheat flour, rye flour, and "Bucovina" still water. Then, 5%, of the amount of wheat flour used to feed the natural sourdough (C) was substituted with *Spirulina* powder. The fermentation of the culture obtained from the natural sourdough, white wheat flour, whole wheat flour, *Spirulina powder* and "Bucovina" still water was carried out under refrigeration conditions (3-8°C), for 30 days (Catană et al., 2024).

The use of wholegrain flour, sourdough, and oilseeds in the bread formulation leads to a reduction in carbohydrate content and glycemic index and, consequently, to a decrease in blood glucose levels approximately one hour after consumption (Lange et al., 2024).

Bread making

For the development of the "Low carb bread fortified with *Spirulina* powder", four experimental variants were created (Figures 2-3), corresponding to the levels of fortification with *Spirulina* powder: 1%, 2%, 3%, and 4%. In parallel, a control variant was used ("White bread"- C) (Figure 1).



Figure 1. "White bread" (Control sample)



Figure 2. "Low carb bread fortified with *Spirulina* powder" (V1, V2)



Figure 3. "Low carb bread fortified with *Spirulina* powder" (V3, V4)

For the development of "White bread" and "Low carb bread fortified with *Spirulina* powder", a biphasic method was applied.

Methods

Statistical Analysis

"Low carb bread fortified with *Spirulina* powder" (V1, V2, V3, V4) and "White bread" (Control sample) were analyzed in triplicate, and the results are presented as the arithmetic mean and standard deviation.

Sensory analysis

The sensory analysis of "Low carb bread fortified with *Spirulina* powder" (V1, V2, V3, V4) and "White bread" (Control sample) was conducted by evaluating the sensory characteristics (appearance, color, taste, and smell) and by "Comparison method with unitary score scales" method (Burnete et al., 2020).

Evaluation of the sensory attributes of the products by a panel of 10 tasters through comparison with scoring scales ranging from 0 to 5 points, followed by the calculation of the average score. Calculation of weighted average scores, summation of these to obtain the total average score, and determination of the product's sensory quality based on the total average score by comparison with a scale from 0 to 20 points.

Table 1. Correlation of total average score with product rating

Total average score	Product rating
18.1.....20	Very good
15.1...18	Good
11.1.....15	Satisfactory
7.1....11	Unsatisfactory
0...7	Failure

Instrumental analysis of color (L*, a* and b*) was performed with CM-5 colorimeter (Konica Minolta, Japan), using SpectraMagic NX software.

Instrumental texture analysis was performed with an Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA) equipped with Bluehill 3.13 software.

Slices of bread with a thickness of 25 mm were compressed using a compression piston with a diameter of 12 mm. The analysis method included a compression cycle to a depth of 4 mm, with a compression speed of 1 mm/s. Three measurements were performed for each sample. The testing was carried out at room temperature, and the test parameters were:

- compression speed: 100 mm/min;
- load cell: 50 N.

Using the Bluehill 3.13 software, the following texture parameters were calculated: firmness, elasticity, and cohesiveness.

Physico-chemical analysis

Nutritional composition of the bread samples was determined by the AOAC method: 979.09 (protein content), 963.15 (fat content), 923.03 (ash content) and 985.29 (total dietary fiber). Moisture of the bread samples was determined by a Halogen Moisture Analyzer HE53 (Mettler Toledo). Total carbohydrates were calculated using the formula: 100 (%) -moisture (%) - protein (%) - fat (%) - total dietary fiber (%). The energy value was calculated using the conversion factors of 9 kcal/g for fat, 4 kcal/g for carbohydrates and protein, and 2 kcal/g for fiber, in accordance with the provisions of Commission Regulation No. 1169/2011 (European Commission, 2011).

Determination of iron and zinc was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, model NexION300Q, PerkinElmer) after dry digestion of the samples. Determination of sodium, potassium, calcium, and magnesium was carried out by High-Resolution Continuum Source Atomic Absorption Spectrometry (Analytik Jena, model contrAA 700 - High-Resolution Continuum Source Atomic Absorption Spectrometer), flame technique, after dry digestion of the samples.

For the determination of minerals, two different analytical techniques were used in order to accurately quantify the mineral content of the studied elements (the zinc and iron content of the analyzed samples is significantly lower than that of potassium, calcium, and magnesium).

ICP-MS operating conditions are presented in Table 2, while HR-CS AAS operating conditions are presented in Table 3.

Table 2: ICP-MS operating conditions

Parameter	Value
Radio Frequency Generator	40 MHz
Radio Frequency Power	1000 W
Nebulizer Gas Flow	0.89 L/min
Auxiliary Gas Flow	1.20 L/min
Plasma Gas Flow	16 L/min
Sample Flow	1.0 mL/min
Replicate Read Time	15 s

Table 3. HR-CS AAS operating conditions

Element	Wavelength (nm)	Gas Flow (L/h)	Burner Height (mm)	Flame Type
Na	588.9953	90	6	Aer-acetilenă
K	766.4908	80	8	Aer-acetilenă
Ca	422.6728	80	6	Aer-acetilenă
Mg	285.2125	70	6	Aer-acetilenă

Bioactive compounds content

The determination of the total polyphenol content was carried out using the Folin-Ciocalteau spectrophotometric method, according to Horszwald and Andlauer (2011), with some modifications (extraction medium, extraction mode and time, and volume of extract used in analysis) using a UV-VIS Jasco V 550 spectrophotometer at a wavelength of λ = 755 nm. For quantification, a calibration curve of gallic acid in the concentration range of 0-0.20 mg/mL was used, and the results were expressed as mg of Gallic Acid Equivalents (GAE) per g of product.The content of total carotenoids was determined using a spectrophotometric method (Chinnadurai et al., 2013).

Antioxidant capacity

Antioxidant capacity was assessed using the DPPH (1,1-diphenyl-2-picryl hydrazyl) method, according to Horszwald and Andlauer (2011), with some modifications (extraction medium, extraction mode and time, and volume of extract used in analysis), using a UV-VIS Jasco V 550 spectrophotometer at a wavelength of λ = 517 nm. For quantification, a calibration curve of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) in the concentration range of 0-0.4375 mmol/L was used, and the results were expressed as µmol Trolox equivalents per g of bread sample.

Microbiological analysis

The microbiological analysis of bread samples was carried out using the following methods: SR ISO 21527-1:2009 (Yeasts and molds), SR EN ISO 21528-1:2017 method (*Enterobacteriaceae*), ISO 21807:2004 (Water activity). Water activity was determined using Aquaspector AQS 31 equipment.

Determination of shelf life (Real-time shelf-life study)

To determine the shelf life of the product "Low carb bread fortified with *Spirulina* powder" (V1–V4), a real-time stability study was conducted. Test batches of the product (for each experimental variant) were produced and stored at room temperature in sealed polypropylene bags. The low-carb bread samples fortified with *Spirulina* powder were analyzed daily for 7 days, as follows:

- Sensory analysis (appearance, taste, smell, aroma);
- Instrumental texture analysis (firmness, elasticity, cohesiveness);
- Moisture analysis;
- Microbiological analysis (yeasts and molds, *Enterobacteriaceae*, water activity).

Failure thresholds (end of shelf life) were established for each analytical method (Table 4), and the shelf life was considered to have ended when any of the analyzed parameters (sensory, microbiological, texture, moisture) exceeded the established limits. A safety margin was also applied: if the product remained within the acceptable limits for all parameters during the 7-day period, its shelf life was defined as 4 days.

Table 4. Failure thresholds (end of shelf life)

Parameter	Failure thresholds (end of life)
Sensory analysis (appearance, taste, smell, aroma)	Sensory analysis (appearance, taste, smell, aroma) does not comply with the provisions of the technical specification of the product
Instrumental analysis of texture Firmness (N) Elasticity Cohesiveness	 > 25 N < 0.95 < 0.40
Moisture	> 45.4 %
Microbiological analysis Yeasts and molds *Enterobacteriaceae* Water activity	 > 10 CFU/g > 10 > 0.98

RESULTS AND DISCUSSIONS

Sensory analysis

The sensory analysis of the product "Low Carb Bread Fortified with *Spirulina* Powder" showed that the addition of *Spirulina* powder (fortification levels of 1-4%) does not negatively affect its taste or smell/aroma. Thus, breads

fortified with this microalga, at the specified concentrations, exhibit a pleasant taste and aroma characteristic of a well-baked bakery product with added *Spirulina* powder. The bread fortified with *Spirulina* powder has a pleasant, characteristic aroma resulting from the synergism between the bread's aroma and the specific aroma of this microalga.

At the same time, the product "Low carb bread fortified with *Spirulina* powder" has an elastic, dense crumb with uniform pores, brown in color with a slight purplish hue, free of lumps, in which seeds (sunflower, white and black sesame, flax, millet, and chia) are visibly distinguishable.

Additionally, the products "Control bread" and "Low-carb bread with antioxidant potential, fortified with *Spirulina*" were also evaluated from a sensory perspective by a panel of 10 assessors, using the "comparison method with unit scoring scales".

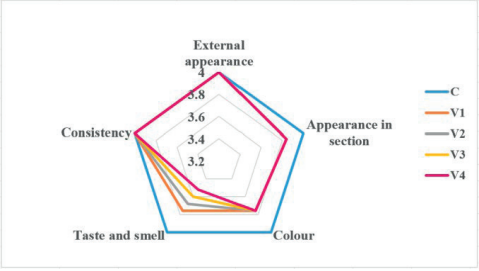

Figure 4. Sensory evaluation of the product "Low carb bread fortified with *Spirulina* powder" and of the "White bread" (C)

Following the sensory evaluation carried out using the "comparison method with unit scoring scales", the products received the rating "very good", achieving the following scores (Figure 4):

- "White bread" (control sample): 20 points;
- V1: 19.36 points;
- V2: 19.28 points;
- V3: 19.20 points;
- V4: 19.12 points.

The addition of ingredients required to reduce the carbohydrate content and glycemic index, as well as to increase antioxidant capacity, leads to a darker color of the product "Low carb bread fortified with *Spirulina* powder" compared to the "White bread" (C). This is reflected in a 1.30-1.37-fold decrease in lightness (L*) (Figure 5).

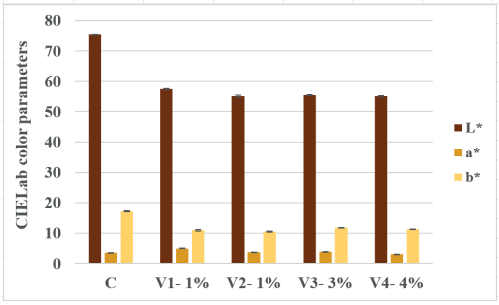

Figure 5. Color parameters of the product "Low carb bread fortified with *Spirulina* powder" (V1, V2, V3, V4) and of the "White bread" (C)

Instrumental texture analysis revealed an increase in firmness in the case of the product "Low carb bread fortified with *Spirulina* powder" compared to "White Bread" (control sample).

The increase in firmness of the low-carb bread is due to the ingredients used to reduce the glycemic index and enhance antioxidant capacity (whole wheat flour, seed mix, *Aronia* pomace powder, etc.).

Thus, 7 days after the date of manufacture, firmness recorded the following values:

- ✓ "White bread" (C)- Firmness = 7.09 N;
- ✓ "Low carb bread fortified with *Spirulina* powder" (V1)- Firmness =12.89 N;
- ✓ Low carb bread fortified with *Spirulina* powder" (V2)- Firmness =13.29 N;
- ✓ Low carb bread fortified with *Spirulina* powder" (V3)- Firmness =23.39 N;
- ✓ Low carb bread fortified with *Spirulina* powder" (V4)- Firmness =13.47 N.

Figures 6-10 present: the compression curves for the "White bread" (control sample) and the "Low carb bread fortified with *Spirulina* powder" (V1-V4).

The firmness of the product "Low Carb Bread Fortified with *Spirulina* Powder" (V1, V2, V3), measured 7 days after manufacture, is comparable to that reported by Burnete et al. (2020) for "Hypoglycemic Bread with Antioxidant Potential", also measured 7 days after manufacture (Firmness = 13.67 N).

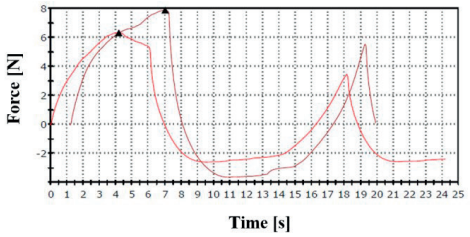

Figure 6. Compression curves for the product "White bread" (control sample)

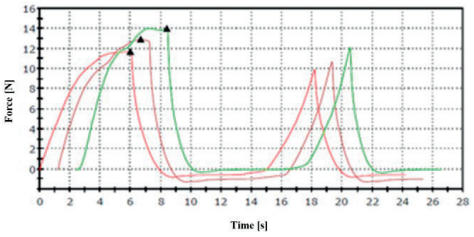

Figure 7. Compression curves for the product "Low carb bread fortified with *Spirulina* powder" (V1)

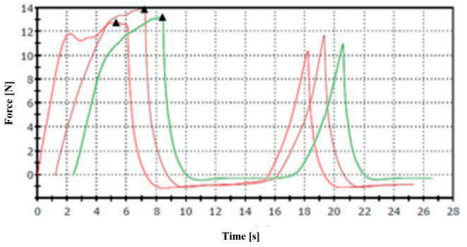

Figure 8. Compression curves for the product "Low carb bread fortified with *Spirulina* powder" (V2)

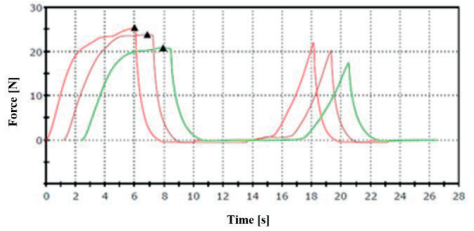

Figure 9. Compression curves for the product "Low carb bread fortified with *Spirulina* powder" (V3)

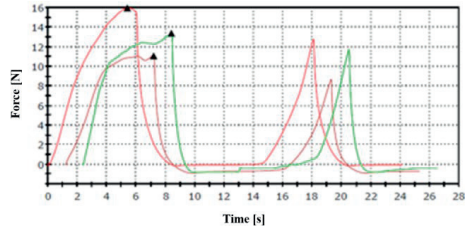

Figure 10. Compression curves for the product "Low carb bread fortified with *Spirulina* powder" (V4)

In Table 5 are presented texture parameters (firmness, elasticity and cohesiveness) of the products "Low carb bread fortified with *Spirulina* powder" and "White bread" (C).

Table 5. The textural properties of the products "Low carb bread fortified with *Spirulina* powder" and "White bread" (C)

Bread	Period (days)	Firmness (N)	Cohesiveness	Elasticity
"White bread"	7	7.09±0.42	0.45±0.04	1.18±0.03
V1	7	12.89±1.05	0.44±0.03	1.01±0.02
V2	7	13.29±0.28	0.43±0.03	1.03±0.03
V3	7	23.39±1.87	0.44±0.02	1.04±0.03
V4	7	13.47±1.86	0.45±0.02	1.02±0.02

Firmness is crucial for bread freshness and is often influenced by dietary fiber, which disrupt the gluten network, hinder gas retention, reduce bread volume, and increase crumb firmness (Djordjević et al., 2021; Ni et al., 2020). Despite high firmness, bread must also exhibit high cohesiveness to prevent crumbling and maintain freshness (Tōth et al., 2022).

Thanks to carefully selected ingredients that ensure proper moisture and high cohesiveness, the product "Low Carb Bread Fortified with *Spirulina* Powder" retains its freshness even 7 days after production. An important contribution to maintaining the freshness of this low-carb bread comes from the natural sourdough fortified with *Spirulina* powder used in its formulation, as well as the application of two-phase technology in its preparation.

Physico-chemical analysis

The physico-chemical composition of the products "Low Carb Bread Fortified with *Spirulina* Powder" and "White Bread" (C) is presented in Table 6.

Table 6. Physico-chemical composition of the products "Low carb bread fortified with *Spirulina* powder" and "White bread" (C)

Component	C	V1	V2	V3	V4
Moisture (%)	41.47±1.04	44.98±1.12	44.06±1.10	43.36±1.08	43.25±1.08
Acidity (degrees)	3.10±0.03	5.6±0.06	5.73±0.06	5.80±0.06	5.86±0.06
Ash (%)	0.47±0.007	1.60±0.02	1.73±0.03	1.86±0.03	1.95±0.03
Protein (%)	7.67±0.12	13.02±0.20	13.39±0.20	13.80±0.21	14.15±0.21
Fat (%)	0.76±0.01	4.77±0.06	4.89±0.06	4.98±0.06	5.05±0.07
Carbohydrates (%)	49.63±0.55	35.63±0.39	35.93±0.40	36.00±0.40	35.60±0.39
Available carbohydrates (%)	49.12±0.54	26.41±0.29	26.37±0.29	26.23±0.29	25.65±0.28
Total dietary fibers (%)	0.51±0.009	9.22±0.17	9.56±0.17	9.77±0.18	9.95±0.18
Salt (NaCl) (%)	0.72±0.005	0.70±0.005	0.69±0.005	0.68±0.005	0.67±0.005
Energy value (kcal/100g)	235	219	222	224	225
Energy value (kJ/100g)	998	921	933	943	943

The physico-chemical analysis revealed that the product "Low carb bread fortified with *Spirulina* powder" has a reduced carbohydrate content, specifically available carbohydrates, and stands

out for its protein, ash, and total dietary fiber contents compared to "White Bread" (C).

According to the results obtained, the moisture of the product "Low carb bread fortified with *Spirulina* powder" ranged between 43.25% and 44.98%, which is comparable to the values reported by Mahmoud et al. (2024) for breads fortified with the microalgae *Chlorella vulgaris*, *Phaeodactylum tricornutum*, and *Tetraselmis chuii* (4% fortification level), where moisture ranged from 41.47% to 42.93%.

Additionally, this bread has higher moisture and lower carbohydrate content compared to the low-glycemic white bread fortified with *Oxalis tuberosa* dietary fiber reported by Jiménez et al. (2025) (Moisture = 32.41%; Carbohydrates = 68.02% d.w.).

Furthermore, the carbohydrate content of "Low carb bread fortified with *Spirulina* powder" is comparable to that reported by Pereira et al. (2024) for multigrain (spelt and lupin) bread fortified with 1.9% (w/w of flour) *Chlorella vulgaris* (Carbohydrates = 36.23%).

The acidity of the product "Low carb bread fortified with *Spirulina* powder" is approximately 1.8 times higher than that of "White Bread" (C), due to the ingredients used in its formulation (whole wheat flour, *Spirulina* powder, *Aronia* pomace powder, etc.). It is comparable to that reported by Hoxha et al. (2023) for bread fortified with pumpkin flour at a 25% fortification level (Acidity = 5.5 degrees).

The ash content of the product "Low carb bread fortified with *Spirulina* powder" (V3 and V4) is comparable to that reported by Sya'niy et al. (2026) for white bread fortified with *Moringa oleifera* green leaf stalk powder (Ash = 1.90%) and to that reported by Hoxha et al. (2023) for bread fortified with pumpkin flour at a 5% fortification level (Ash = 1.82%).

The protein content of "Low carb bread fortified with *Spirulina* powder" is 1.3-1.4 times higher than that reported by Zahorec et al. (2024) functional bread fortified with 15% carob pulp powder and 10% dry sourdough (Protein = 10.02%), while the available carbohydrate content is 1.8-1.9 times lower compared with the values reported by the same authors (Available carbohydrates = 48.76%).

Additionally, the protein content of the low-carb bread fortified with *Spirulina* powder is higher than that reported by Görünmek et al. (2025) for breads fortified with the microalgae *Chlorella vulgaris* and *Micractinium simplicissimum* (fortification levels 1% and 3%; Protein = 9-12.3%).

It is noteworthy that "Low carb bread fortified with *Spirulina* powder" qualifies as a source of protein, as, according to Regulation (EC) No 1924/2006 of the European Parliament and of the Council, at least 12% of its energy value must come from proteins; in this case, proteins contribute 23.78-25.15% of the energy value.

The fat content of the product "Low carb bread fortified with *Spirulina* powder" is lower than that reported by Jiménez et al. (2025) for low-glycemic white bread fortified with *Oxalis tuberosa* dietary fiber at a 10% fortification level (Fat = 6.36% d.w.), but higher than that reported by Pereira et al. (2024) for multigrain (spelt and lupin) bread fortified with 1.9% (w/w of flour) *Chlorella vulgaris* (Fat = 2.55%).

The low-carb bread presented in this experimental study has a high total dietary fiber content (9.22-9.95%), comparable to that reported by Lange et al. (2024) for multiseed bread (Total dietary fibers = 9.3%), and 1.42-1.53 times higher than that reported by Zahorec et al. (2024) for functional bread fortified with 15% carob pulp powder and 10% dry sourdough (Total dietary fibers = 6.48%). Furthermore, the product "Low carb bread fortified with *Spirulina* powder" is rich in fiber, in accordance with Regulation (EC) No. 1924/2006 of the European Parliament and of the Council, having a fiber content greater than 6 g/100 g.

Dietary fiber, especially viscous fiber, can contribute to reducing the glycemic response after consuming carbohydrate-rich foods. Maintaining target blood glucose levels and controlling postprandial glycemia are essential for the prevention and management of diabetes (Giuntini et al., 2022). Additionally, Lu et al. (2023) reported that supplementation with viscous soluble dietary fibers offers potential benefits for glycemic and lipid control in patients with type 2 diabetes. The recommended supplementary dose is 8.3-10.2 g/day, and the recommended duration of supplementation is over 6 weeks.

The nutritional value of the product "Low Carb Bread Fortified with *Spirulina* Powder" is further enhanced by its mineral content (K, Ca, Mg, Zn, and Fe). The mineral element content of this product is presented in Figures 11 and 12.

According to the results obtained, the mineral content of the product increases proportionally with the level of fortification with *Spirulina* powder.

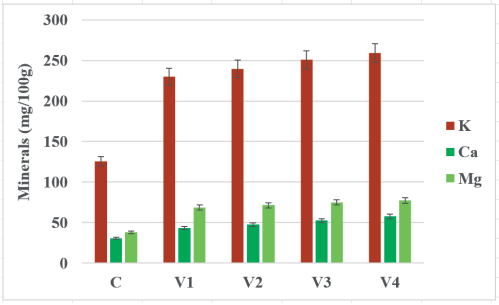

Figure 11. Mineral content (K, Ca, and Mg) of the product "Low Carb bread fortified with *Spirulina* powder" and of the "White bread" (C)

The potassium content of the product "Low Carb Bread Fortified with *Spirulina* Powder" ranged between 230.08 and 259.50 mg/100 g, with the highest value recorded in experimental variant V4 (fortification level with *Spirulina* powder: 4%). The potassium content of this functional bread is comparable to that reported by Zahorec et al. (2024) for functional bread fortified with 15% carob pulp powder and 10% dry sourdough (K = 247.44 mg/100 g), but 1.39-1.73 times higher than that reported by Mahmoud et al. (2024) for breads fortified with the microalgae *Chlorella vulgaris*, *Phaeodactylum tricornutum*, and *Tetraselmis chuii* at a 4% fortification level (K = 132.73-186.62 mg/100 g).

The calcium content of the product "Low Carb bread fortified with *Spirulina* powder" ranged from 43.23 to 57.50 mg/100 g, comparable to that reported by Hoxha et al. (2023) for bread fortified with pumpkin flour at 5% and 10% fortification levels (Ca = 41.61 mg/100 g and 50.51 mg/100 g, respectively), but lower than that reported by Mumtaz et al. (2025) for toasted and wholemeal breads enriched with buckwheat husk at fortification levels of 1.5-4.5% (Ca = 67.1-87.7 mg/100 g).

The magnesium content of the low-carb bread fortified with *Spirulina* powder is 1.8-2 times higher than that of "White Bread" (C) and 3-4.8 times higher than that reported by Mahmoud et al. (2024) for breads fortified with the microalgae *Chlorella vulgaris*, *Phaeodactylum tricornutum*, and *Tetraselmis chuii* at a 4% fortification level (Mg = 14.05-25.10 mg/100 g).

The iron (Fe) content of the product "Low carb bread fortified with *Spirulina* powder" ranged between 3.36 and 3.75 mg/100 g, which is higher than that reported by Mumtaz et al. (2025) for toasted and wholemeal breads enriched with buckwheat husk at 1.5-4.5% fortification levels (Fe = 0.97-1.95 mg/100 g).

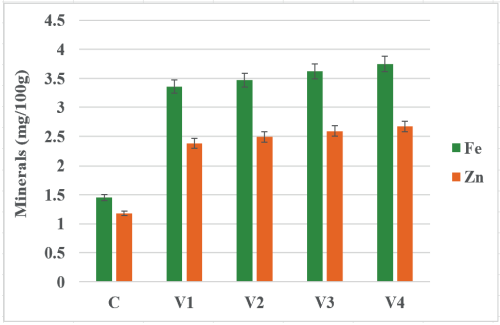

Figure 12. Mineral content (Fe and Zn) of the product "Low carb bread fortified with *Spirulina* powder" and of the "White bread" (C)

The zinc content of the product "Low carb bread fortified with *Spirulina* powder" is approximately 1.4 times lower than its iron content. However, the zinc content of this product is 1.7-1.9 times higher than that reported by Zahorec et al. (2024) for functional bread fortified with 15% carob pulp powder and 10% dry sourdough (Zn = 1.39 mg/100 g).

Bread, as a staple food consumed worldwide, offers significant potential for nutrient fortification due to its widespread popularity and simple production. Among staple foods, bread has been identified as a major contributor to macronutrients, minerals, and vitamins (Falsafi et al., 2025). In this context, fortifying bread with *Spirulina* is of particular interest for increasing its content of proteins, minerals, fiber, and other bioactive compounds.

Bioactive compounds content

Due to some of the ingredients used (whole wheat flour, *Spirulina* powder, *Aronia* pomace powder, sourdough, etc.), the product "Low carb bread fortified with *Spirulina* powder" has a high content of bioactive compounds (total polyphenols, total carotenoids) compared to "White bread" (C). Thus, the polyphenol content

of this bread variety ranged in the range of 244.76-262.83 mg GAE/100 g (Figure 13).

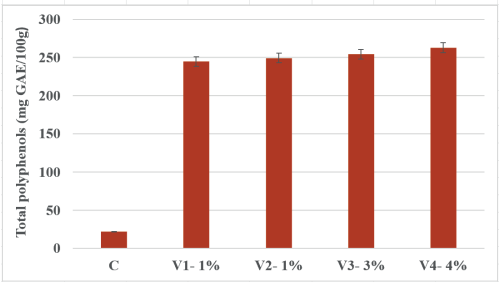

Figure 13. Total polyphenols of the product "Low carb bread fortified with *Spirulina* powder" and of the "White bread" (C)

The total polyphenol content of the product "Low carb bread fortified with *Spirulina* powder" is 1.3-1.4 times higher than that reported by Pandey et al. (2024) for bread enriched with black carrot (*Daucus carota* L.) powder at a 10% fortification level (total polyphenols = 185.78 mg GAE/100 g), and 2.4-2.6 times higher than that reported by Hernández-López et al. (2023) for bread fortified with 2.5% *Spirulina* (total polyphenols = 100 mg GAE/100 g).

Fortification with microalgae to increase polyphenol content is one of the main trends in functional foods aimed at preventing cardiovascular diseases and diabetes (Koli et al., 2022). Numerous studies support the antioxidant, anti-inflammatory, antithrombotic, anti-allergenic, antidiabetic, anticancer, and overall protective properties of polyphenols (Ahmed et al., 2025; Potì et al., 2019). In recent years, the complex symbiotic relationship between dietary polyphenols and the human gut microbiota has become a central focus of scientific investigation due to its profound implications for human health and disease (Jyoti and Dey, 2025). Considering these aspects, the consumption of low-carb bread with a high polyphenol content is of real interest.

The total carotenoid content of the product "Low carb bread fortified with *Spirulina* powder" ranged from 1.09 to 4.37 mg/100 g, with the highest value recorded at the 4% *Spirulina* fortification level (Figure 14).

The total carotenoid content of "Low carb bread fortified with *Spirulina* powder" is higher compared with that reported by Catană et al. (2025) for "Low carb Mini-Baguette Fortified with Spirulina Powder" (total carotenoids = 1.03-3.92 mg/100 g).

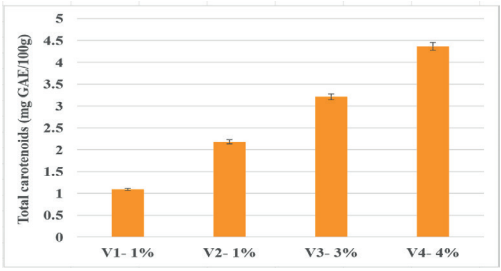

Figure 14. Total carotenoids of the product "Low carb bread fortified with *Spirulina* powder"

Carotenoids are tetraterpenoid pigments that are an essential part of the human diet and have a significant impact on human health. They possess antioxidant, cytoprotective, photoprotective, and other bioactive properties, enabling them to play a protective role against various diseases, including cancer, diabetes, cardiovascular diseases, obesity, and neurodegenerative disorders (Bufka et al., 2024).

Antioxidant capacity

Due to its content of antioxidants (phenolic compounds and carotenoids), the product "Low carb bread fortified with *Spirulina* powder" exhibits notable antioxidant capacity (Figure 15).

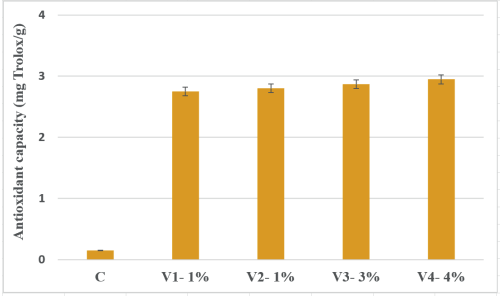

Figure 15. Antioxidant capacity of the product "Low carb bread fortified with *Spirulina* powder" and of the "White bread" (C)

The antioxidant capacity of this bread ranged between 2.75 and 2.95 mg Trolox/100 g, with the highest value recorded at the 4% *Spirulina* fortification level. This antioxidant capacity is 4.07-4.37 times higher than that reported by Calderón de la Barca et al. (2022) for bread made from a flour mix (68.7% wheat, 22.7%

amaranth, 8.6% orange sweet potato), where the antioxidant capacity was 0.675 mg Trolox/g.
Corroborating the results of sensory analyses, physicochemical properties, bioactive compound content, and antioxidant capacity, the experimental variant V4 of the product "Low carb bread fortified with *Spirulina* powder" was selected as the optimal formulation.

Microbiological analysis

The microbiological indicators of the "Low carb bread fortified with *Spirulina* powder" and of the "White Bread" (C) are presented in Table 7. The results of the microbiological analysis confirmed that both products comply with the requirements of the current legislation.

Table 7. Microbiological analysis of the product "Low carb bread fortified with *Spirulina* powder" and of the "White bread" (C)

Microbiological indicators	"White bread" (C)	"Low carb bread fortified with *Spirulina* powder"			
		V1	V2	V3	V4
Yeasts and molds (CFU/g)	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10
Water activity	0.935	0.957	0.949	0.943	0.941

The water activity of the products ranged from 0.935 to 0.957. Based on sensory analysis (appearance, taste, smell, and aroma), instrumental texture analysis (firmness, elasticity, cohesiveness), moisture analysis, and microbiological analysis (yeasts and molds, *Enterobacteriaceae*, water activity), it was found that the product "Low carb bread fortified with *Spirulina* powder" (V1-V4) remained compliant even after 7 days from the date of manufacture.
According to the established protocol for shelf-life evaluation of the product, and based on the results of microbiological analyses, sensory evaluation, moisture content, and instrumental texture measurements, the shelf life of the product "Low carb bread fortified with Spirulina powder" (V1-V4) was set at 4 days.

CONCLUSIONS

The product "Low-carb bread fortified with *Spirulina* powder" has superior sensory qualities and a complex biochemical composition, making it suitable for the metabolic requirements of diabetics and obese individuals. Thus, this fortified bread has a low carbohydrate content (35.60-36.00%) and available carbohydrates (25.65-26.41%) and stands out with its protein content (13.02-14.51%), total dietary fiber (9.22-9.95%), and minerals (K: 230.08-259.50 mg/100 g; Ca: 43.23-57.50 mg/100 g; Mg: 68.74-77.43 mg/100 g; Fe: 3.36-3.75 mg/100 g; Zn: 2.38-2.67 mg/100 g).
Additionally, "Low-carb bread fortified with *Spirulina* powder" is notable for its content of bioactive compounds: total polyphenols (244.76-262.83 mg/100 g) and total carotenoids (1.09-4.37 mg/100 g). Due to its antioxidant content, this product exhibits antioxidant capacity (2.75-2.95 mg Trolox/g).
The shelf life of "Low-carb bread fortified with *Spirulina* powder" was established at 4 days.

ACKNOWLEDGEMENTS

This work was carried out through the Core Program within the National Research Development and Innovation Plan 2022-2027, carried out with the support of the National Research Authority, project no. PN 23 01 01 01.

REFERENCES

Ahmed, Z.S.O., Khan, E., Elias, N., Elshebiny, A., Dou, Q. (2025). Updated Review on Natural Polyphenols: Molecular Mechanisms, Biological Effects, and Clinical Applications for Cancer Management. *Biomolecules*, *15*, 629.

AOAC International. (2016). Official Methods of Analysis of AOAC International, 20th ed.; *Association of Official Analytical Chemists*: Gaithersburg, MD, USA.

Bufka, J., Vãnková, L., Sýkora, J., Křížková, V. (2024). Exploring carotenoids: Metabolism, antioxidants, and impacts on human health. *Journal of Functional Foods, 118*,106284.

Burnete, A.G., Catană, L., Catană, M., Lazăr, A. M., Teodorescu, R.I., Asănicã, A. C., Belc, N. (2020). Use of vegetable functional ingredients to achieve hypoglycemic bread with antioxidant potential for diabetics. *Scientific Papers. Series B, Horticulture, Vol. LXIV,* No. 2, p. 367-374.

Calderón de la Barca, A.M., Mercado-Gómez, L.E., Heredia-Sandoval, N.G., Luna-Alcocer, V., Porras Loaiza, P.M.A., González-Ríos, H., Islas-Rubio, A.R. (2022). Highly Nutritional Bread with Partial Replacement of Wheat by Amaranth and Orange Sweet Potato. *Foods*, *11*, 1473.

Catană L., Catană M., Constantinescu F., Burnete A.G., Stamatie G., Asanică A.C. (2024). Research on the realization and testing of the fermentation capacity of natural sourdough fortified with *Spirulina* powder. *Scientific Papers. Series B, Horticulture, vol. LXVIII,* No. 2, p. 764-772.

Catană L., Catană M., Burnete, A.G., Dâră, M.A., Constantinescu, F., Criveanu, G., Asănică A.C. (2025). Research on the development of a low-carb

mini-baguette fortified with *Spirulina* powder, intended for diabetics and obese individuals. *Scientific Papers. Series B, Horticulture, Vol. LXIX*, No. 1, 715-724.

Chiranthika, N.N.G., Chandrasekara, A., and Gunathilake, K. D. P. P. (2024). Physicochemical, *in vitro* antidiabetic and sensory characteristics of leavened.

Functional bread made with *Lasia spinosa* and *Nelumbo nucifera* rhizome flours composited with wheat flour. *Food Production, Processing and Nutrition, 6:*33.

Chinnadurai, S., Karthik, G., Chermapandi, P., Hemalatha, A., Anantharaman (2013). Estimation of major pigment content in seaweeds collected from the Pondicherry coast. *The Experiment, Vol. 9*(1), 522-525.

Djordjević, M., Djordjević, M., Šoronja-Simović, D., Nicolić, I., & Šereš, Z. (2021). Delving into the role of dietary fiber in gluten-free bread formulations: Integrating fundamental rheological, technological, sensory, and nutritional aspects. *Polysaccharides*, *3*, 59–82.

European Commission. Regulation (EC) No. 1169/2011 of 25/10/2011, on the Provision of Food Information to Consumers. *Off. J. Eur. Union* 2011, L304, 18–63.

European Commission. Regulation (EC) No. 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. *Off. J. Eur. Union* 2011, L404, 9–25.

Görünmek, M., Kibar, A.A., Cakmak, Z.E., and Çakmak, T. (2025). Nutritional and functional assessment of fortified bread with psychrotolerant and mesophilic microalgae. *Algal Research*, *86*, p.103900.

Giuntini, E.B., Sardá, F.A.H., de Menezes, E.W. (2022). The Effects of Soluble Dietary Fibers on Glycemic Response: An Overview and Futures Perspectives. *Foods*, *11*(23):3934.

Hernández-López, I., Alamprese, C., Cappa, C., Prieto-Santiago, V., Abadias, M., Aguiló-Aguayo, I. (2023). Effect of *Spirulina* in bread formulated with wheat flours of Different Alveograph Strength. *Foods, 12*, 3724.

Horszwald, A., & Andlauer, W. (2011). Characterisation of bioactive compounds in berry juices by traditional photometric and modern microplate methods. *Journal of Berry Research*, *1,* 189–199.

Hoxha, I., Besiana Hoxha, B., Xhabiri, G., Shala, N., Dreshaj, A., Namik Durmishi, N. (2023). The Effect of the addition of pumpkin flour on the rheological, nutritional, quality, and sensory properties of bread. *Ecological Engineering & Environmental Technology, 24*(7), 178–185.

ISO 21807:2004. Microbiology of food and animal feeding stuffs — Determination of water activity.

Jyoti, and Dey, P. (2025). Mechanisms and implications of the gut microbial modulation of intestinal metabolic processes. *Npj Metabolic Health Dis.*, *3* (1), 1–19.

Koli, D.K., Rudra, S.G., Bhowmik, A., Pabbi, S. (2022). Nutritional, functional, textural, and sensory evaluation of *Spirulina* enriched green pasta: A potential dietary and health supplement. *Foods, 11*, 979.

Lange, E., Pałkowska-Goździk, E., Kęszycka, P. (2024). The Influence of various types of functional bread on postprandial glycemia in healthy adults. *Appl. Sci.*, *14*, 11900.

Lu, K., Yu, T., Cao, X., Xia, H., Wang, S., Sun, G., Chen, L. and Liao, W. (2023) Effect of viscous soluble dietAry fiber on glucose and lipid metabolism in patients with type 2 diabetes mellitus: a systematic review and meta-analysis on randomized clinical trials. *Front. Nutr. 10*:1253312.

Ludwig, D.S., Hu, F.B., Tappy, L., Brand-Miller, J. (2018). Dietary carbohydrates: role of quality and quantity in chronic disease. *BMJ* 361, k2340.

Mette, A.M., Chaitong, C., Kjeld, H., Cyril, W. C. K, Hana, K., et al. (2023). Evidencebased European recommendations for the dietary management of diabetes. *Diabetologia, 66*:965–85.

Miller, V., Jenkins, D.A., Dehghan, M., Srichaikul, K., Rangarajan, S., Mente, A, et al. (2024). AssociAtions of the glycaemic index and the glycaemic load with risk of type 2 diabetes in 127 594 people from 20 countries (PURE): a prospective cohort study. *Lancet Diabetes Endocrinol.,* 12:330–8.

Ni, Q., Ranawana, V., Hayes, H. E., Hayward, N. J., Stead, D., & Raikos, V. (2020). Addition of broad hull to wheat flour for the development of high-fiber bread: Effects on physical and nutritional properties. *Foods, 9*, 1192.

Mahmoud, N., Ferreira, J., Raymundo, A., Nunes, M.C. (2024). Enhancing the Protein, Mineral Content, and Bioactivity of Wheat Bread through the Utilisation of Microalgal Biomass: A Comparative Study of *Chlorella vulgaris*, *Phaeodactylum tricornutum*, and *Tetraselmis chuii*. *Applied Sciences*, *14*, 2483.

Mumtaz,W., Klepacka, J., Czarnowska-Kujawska, M. (2025). Modification of Mineral Content in Bread with the Addition of Buckwheat Husk. *Applied Sciences, 15*, 4455.

Pagliai, G., Dinu, M., Madarena, M.P., Bonaccio, M., Iacoviello, L., Sofi, F. (2021). Consumption of ultra-processed foods and health status: a systematic review and meta-analysis. *Br J Nutr*, *125*, 308–318.

Potì, F., Santi, D., Spaggiari, G., Zimetti, F., Zanotti, I. (2019). Polyphenol health effects on cardiovascular and neurodegenerative disorders: A review and meta-analysis. *Int. J. Mol. Sci.*, *20*, 351.

Pereira, T., Costa, S., Barroso, S., Teixeira, P., Mendes, S., Gil, M.M. (2024). Development and optimization of high-protein and low-saturated fat bread formulations enriched with lupin and microalgae. *LWT- Food Science and Technology, 191,* 115612.

Schadow, A.M., Revheim, I., Spielau, U., Dierkes, J., Lukas Schwingshack, L., Frank, J., Hodgson, J.M., Moreira-Rosário, A., Seal, C.J., Buyken, A.E., Rosendahl-Riise, H. (2023). The effect of regular consumption of reformulated breads on glycemic control: A systematic review and meta-analysis of Randomized Clinical Trials. *Advances in Nutrition*, *14*(1), 30-43.

Sya'niy, A.L., Estiasih, T., Nur, M., Ahmadi, K., Martati, E., Wulan, S.N., Fidyasari, A. (2026). Antihyperglycemic potential of white bread substituted with *Moringa oleifera* leaf powder from

different leaf stalk colors in *in vitro* and *in vivo* models. *Food Chemistry Advances, 10,* 101227.

Tóth, M., Kaszab, T., & Meretei, A. (2022). Texture profile analysis and sensory evaluation of commercially available gluten-free bread samples. *European Food Research and Technology, 248*, 1447–1455.

Zahorec, J., Šoronja-Simović, D.; Petrović, J., Šereš, Z.; Pavlić, B., Božović, D., Perović, L., Martić, N., Bulut, S., Kocić-Tanackov, S. (2024). Development of Functional Bread: Exploring the Nutritional, Bioactive and Microbial Potential of Carob (*Ceratonia siliqua* L.) Pulp Powder. *Processes, 12*, 2882