

## RESEARCH ON THE REALIZATION AND TESTING OF THE FERMENTATION CAPACITY OF NATURAL SOURDOUGH FORTIFIED WITH *SPIRULINA* POWDER

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### Abstract

*Bread is one of the most important product of cereal origin. It was, from ancient times, a worldwide basic food, and undoubtedly of great value to both human nutrition and the economy. Natural sourdough is a leavening agent that has many benefits, both in terms of bread quality (pleasant taste and aroma, high bioavailability of minerals, etc.) and consumer health. This paper presents the results of the research undertaken for the realization and testing of the fermentation capacity of natural sourdough fortified with Spirulina powder. For the fortification of natural sourdough with Spirulina powder, two levels of fortification were used (3% and 5%). Natural sourdough fortified with Spirulina (fortification level 5%) has the highest protein content (7.65%), total ash (0.74%), total polyphenols (124.95 mg GAE/100g), vitamin C (3.40 mg/100 g), mineral elements and the highest antioxidant capacity. The fermentation capacity of this sourdough was tested in the preparation of white bread, with very good results in terms of sensory and physico-chemical qualities.*

**Key words:** sourdough, Spirulina, fortification, fermentation, bread

### INTRODUCTION

Bread is one of the most consumed fermented foods in the world, being a basic element in the people diet in many countries (Grața et al., 2021). One of the oldest ways of leavening bread is sourdough fermentation, transforming cereal flour into attractive, tastier and more digestible products (Grața et al., 2021; Fraberger et al., 2023).

Sourdough is a key element in traditional and artisan bread making (Hernández-Figueroa et al., 2024). Sourdough bread is obtained by spontaneous fermentation or inoculation of a mixed microbial culture, called sourdough starter. Sourdough starter is a mixture of water and flour fermented by yeasts and bacteria (usually lactic acid bacteria) which is traditionally used to produce bread, panettone, pancakes, pizza, cakes, cookies, buns and waffles (Albagli et al., 2021).

Sourdough fermentation is a technique that can use several types of flour, such as wheat, rye or other grains, and water. The oldest process of

sourdough preparation is spontaneous fermentation and acidification due to the local microbiota, in a complex process of interaction, mainly between lactic acid bacteria and yeasts (Arora et al., 2021; De Vuyst and Neysens, 2005; Gänzle, 2014). Using sourdough to ferment the dough ensures a higher quality bread with health benefits. During fermentation, through the chemical reactions that take place, metabolites and flavor compounds result, which give the bread a superior sensory quality. At the same time, longer fermentation allows adjusting the gluten level, delaying starch digestibility and increasing the bioavailability of vitamins and minerals in the human body (Akamine et al., 2023).

During the fermentation of sourdough dough, various compounds such as organic acids, peptide compounds and exopolysaccharides are formed, which ensure the increase of the shelf life of the bread and the reduction of the growth rate of molds on its surface, preserving the quality characteristics of the product

(Chavan et al., 2011; Park et al., 2006; Corsetti et al., 2012; Luz et al., 2019). International studies have shown that aqueous extracts from fermented sourdoughs inhibit the growth of the main molds that can affect the quality of bread, and can be considered natural antimicrobials for bread and bakery products (Luz et al., 2019; Ryan et al., 2011; Samapundo et al., 2017; Hernández-Figueroa et al., 2023). Fermentation of sourdough dough results in bread with a lower glycemic index compared to traditional bread (Maioli et al., 2008; Fekri et al., 2018). Also, the fermentation of the dough with sourdoughs lead to other advantages of the final product: the increase in the content of soluble fibers (Coda et al., 2014; Coda et al., 2017), the increase in the content of soluble phenolic compounds and the antioxidant capacity (Gobeti et al., 2019; Wang et al., 2019); phytate content decrease (Coda et al., 2015). Chavan et al. (2011) and Zou et al (2016) mention the fact that the use of sourdough as a leavening agent of the dough increases the bioavailability of minerals, the production of peptides with antioxidant activity and the increase of the shelf life of the bread. This paper presents the results of the research undertaken for the realization and testing of the fermentation capacity of natural sourdough fortified with *Spirulina* powder.

## MATERIALS AND METHODS

### Materials

To obtain the natural sourdough fortified with *Spirulina* powder the following materials were used: *Spirulina*, white wheat flour, whole wheat flour, rye flour and "Bucovina" still water.

### Natural sourdough fortified with *Spirulina* powder-making

Natural sourdough fortified with *Spirulina* powder was obtained and tested at the Human Nutrition Laboratory in IBA Bucharest. Firstly, experiments followed the achievement of control natural sourdough (C) by fermenting a mixture of white wheat flour type 650, whole wheat flour, rye flour and "Bucovina" still water. The experiments were carried out at room temperature, over 12 days. The control natural sourdough (C) was kept under

refrigeration conditions (4-8°C) and fed in a ratio of 1:3:3 = natural sourdough: "Bucovina" still water: mix of white wheat flour and whole wheat flour, once every three days.

Secondly, experiments followed the achievement of natural sourdough, fortified with *Spirulina* powder (fortification levels were 3% and 5%, respectively). Thus, 3% and 5%, respectively, of the amount of wheat flour used to feed the control natural sourdough (C) was substituted with *Spirulina* powder. The fermentation of the culture obtained from the control natural sourdough (C), white wheat flour, whole wheat flour, *Spirulina* powder and "Bucovina" still water was carried out under refrigeration conditions (3-8°C), for 30 days. The culture was fed in a ratio of 1:3:3 = natural sourdough fortified with *Spirulina* powder: "Bucovina" still water: mix of white wheat flour whole wheat flour and *Spirulina* powder, once every three days, for a period of 30 days.

In Figure 1 are presented "Control Natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" (V1 - fortification level 3%; V2 - fortification level 5%).

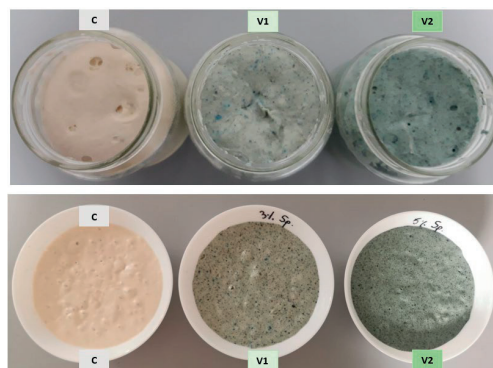


Figure 1. "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" (V1 - fortification level 3%; V2 - fortification level 5%)

### Testing the fermentation capacity of natural sourdough

The fermentation capacity of "Natural sourdough fortified with *Spirulina* powder" compared to that of "Control natural sourdough" (C) was tested by making the "White bread" product, using the biphasic process. For this purpose, the following

technological operations were carried out: preparation of preferment, dough kneading, dough fermentation, dough division and intermediate shaping, final shaping, final leavening, baking, cooling, packaging, marking.



Figure 2. "White bread" (general aspect and section), prepared with "Control natural sourdough" (C)

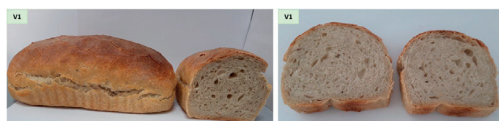


Figure 3. "White bread" (general aspect and section), prepared with "Natural sourdough fortified with *Spirulina* powder" (V1 - fortification level 3%)



Figure 4. "White bread" (general aspect and section), prepared with "Natural sourdough fortified with *Spirulina* powder" (V2- fortification level 5%)

## Methods

### Statistical Analysis

The samples were analyzed in triplicate, the results are presented as arithmetic mean and standard deviation.

### Sensory analysis

Sensory analysis (appearance, taste, smell) was performed using the descriptive method.

The determination of instrumental color parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) was carried out using CM-5 colorimeter (Konica Minolta, Japan) with SpectraMagic NX software.

The determination of the texture parameters (firmness, elasticity) was carried out using the Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA) and Bluehill 3.13 software.

### Physico-chemical analysis

Physico-chemical analysis was performed using the following methods: AOAC 979.09 (protein content), AOAC 923.03 (ash content), AOAC 985.29 (total dietary fiber), AOAC 963.15 (fat content), ACC 44-15A (moisture content).

Physical indicators (volume, porosity, elasticity) and acidity of the bread made with natural sourdough were determined with SR 91:2007 "Bread and fresh pastry products. Methods of analysis". Total carbohydrate content was analytically determined using the following formula: Total carbohydrate (%) = 100 – moisture (%) – ash (%) – protein (%) – fat (%).

Energy analyses (expressed in kcal/100 g and kJ/100 g) was carried out according to the provisions of Commission Regulation no. 1169/2011 (European Commission, 2011).

### Bioactive compounds content

Total polyphenol content was performed by extracting the sample in a methanol:water = 1:1 mixture, applying the Folin-Ciocalteu spectrophotometric method, using UV-VIS Jasco V 550 spectrophotometer (Horszwald and Andlauer, 2011). Determination of the absorbance of the extracts was performed at wavelength  $\lambda = 755$  nm and a gallic acid calibration curve was used, in the concentration range of 0-0.20 mg/mL.  $\beta$ -carotene content was determined using a chromatographic method (Catană et al., 2020). Vitamin C content was determined using a chromatographic method (Asănică et al., 2019).

### Antioxidant capacity

The antioxidant capacity was determined by applying the DPPH spectrophotometric method Horszwald and Andlauer (2011). The method is based on measuring the ability of antioxidants to scavenge stable radicals. The free radical DPPH (1,1-diphenyl-2-picryl hydrazyl) is reduced to the corresponding hydrazine, when reacting with hydrogen donors, and this stability is visible by the discoloring test, which evaluates the decrease in absorbance at 517 nm produced by the addition of the antioxidant to the solution of DPPH in methanol. UV-VIS Jasco V 550 spectrophotometer and calibration curve of Trolox (0-0.4375 mmol/L) were used.

## Microbiological analysis

The microbiological parameters were determined by using the following methods: SR ISO 21527-1:2009 (Yeasts and molds), SR EN ISO 21528-1:2017 (*Enterobacteriaceae*), SR ISO 15214/2001 (Lactic acid bacteria).

## RESULTS AND DISCUSSIONS

### Sensory analysis

Following the sensory analysis, it was found that the "Control Natural Sourdough" (C) is like a fermented acid dough, with an aerated appearance, white-yellowish and has a pleasant taste and smell, specific to wild yeasts and lactic bacteria. At the same time, the sensory analysis of "Natural sourdough fortified with *Spirulina* powder" revealed that has the form of a fermented acid dough, with an aerated appearance, gray-green to light green colour and a pleasant, specific taste and smell.

Following the sensory analysis of the breads made with "Control natural sourdough" (C), respectively with "Natural sourdough fortified with *Spirulina* powder" it was found that they are well leavened, have an elastic, dense core and present a pleasant taste and smell/aroma, characteristic of well-baked bread. It is noteworthy that the addition of *Spirulina* to natural sourdough did not affect the taste and smell/aroma of bread made with this natural leavening agent.

Following the instrumental analysis of the color (Figure 5), it was found that the addition of powder *Spirulina* to the composition of the natural leaven caused a slight darkening of the color of the core of the breads made with this natural fermentation agent, a fact that is reflected in the decrease of the luminance value  $L^*$ . Thus, the bread made with "Control natural sourdough" (C), had the luminance  $L^* = 75.37$ , and the bread made with "Natural sourdough fortified with *Spirulina* powder" (fortification level 5%) had the luminance  $L^* = 74.44$ .

At the same time, the addition of powder *Spirulina* in the composition of natural sourdough caused a decrease in the values recorded by the parameters  $a^*$  (red-green color coordinate) and  $b^*$  (yellow-blue color coordinate).

The luminance  $L^*$  of the breads made with "Control natural sourdough" (C), respectively

with "Natural sourdough fortified with *Spirulina* powder" is lower compared to that reported by Illueca et al. (2023) in the case of bread made with sourdough ( $L^* = 86.30$ ).

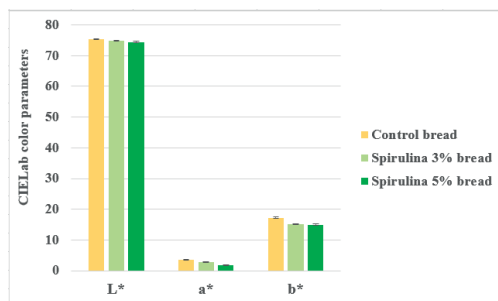


Figure 5. Color parameters of the breads prepared with "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" (V1 - fortification level 3%; V2 - fortification level 5%)

The textural properties of the breads made with "Control natural sourdough" (C), respectively with "Natural sourdough fortified with *Spirulina* powder" packed in a polypropylene bag, for 7 days from the date of manufacture, are presented in Table 1.

Table 1. The textural properties of breads made with "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder"

Bread	Period (days)	Firmness (N)	Elasticity
Control bread	1	5.40±0.16	1.00±0.01
	2	5.83±0.14	1.00±0.01
	3	6.59±0.16	1.15±0.01
	6	6.89±0.19	1.17±0.03
	7	7.09±0.21	1.18±0.03
<i>Spirulina</i> 3% bread	1	5.29±0.08	0.99±0.01
	2	5.42±0.09	0.99±0.01
	3	5.50±0.08	1.07±0.06
	6	5.56±0.09	1.08±0.05
	7	5.67±0.09	1.10±0.07
<i>Spirulina</i> 5% bread	1	5.37±0.05	0.98±0.00
	2	5.51±0.06	1.00±0.00
	3	5.67±0.06	1.03±0.01
	6	5.78±0.07	1.06±0.03
	7	5.84±0.09	1.09±0.03

According to the results, the addition of *Spirulina* powder in the composition of natural sourdough causes a decrease in the firmness of bread prepared with this natural fermentation agent, compared to Control bread. Thus, after 7 days from the date of manufacture, these bread samples had a firmness of 5.67 N, respectively, 5.84 N, compared to Control bread which had a firmness of 7.09 N. The firmness correlates



sensorially with the softness of the core: a high firmness represents a denser, harder core. The elasticity of the core was not influenced by the fortification with *Spirulina* powder of the natural sourdough.

### Physico-chemical analysis

The physico-chemical composition of "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" is presented in Table 2.

Table 2. The physico-chemical indicators of the "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder"

Physico-chemical indicators	„Control natural sourdough“ (C)	„Natural sourdough fortified with <i>Spirulina</i> powder“	
		V1- <i>Spirulina</i> 3%	V2- <i>Spirulina</i> 5%
Moisture (%)	49.34±1.23	48.76±1.22	48.88±1.22
Acidity (degrees)	11.0±0.17	13.0±0.20	14.6±0.22
Ash (%)	0.36±0.006	0.52±0.009	0.69±0.01
Protein (%)	5.95±0.09	6.74±0.10	7.28±0.11

Fortification with *Spirulina* of natural sourdough increases acidity, protein and ash content. Thus, "Natural sourdough fortified with *Spirulina* powder" has 1.44-1.92 times higher ash content, 1.13-1.22 times higher protein content and 1.18-1.33 times higher acidity, compared to "Control natural sourdough" (C). The "Natural sourdough fortified with *Spirulina* powder" has a protein content higher than that reported by Burnete et al. (2019), in the case of sourdough enriched in phenolic compounds and inulin (Protein = 5.83%). *Spirulina* is an important source of proteins, minerals, vitamins, antioxidants and polyunsaturated fatty acids Janda-Milczarek et al. (2023).

The physico-chemical indicators of the breads made with "Control natural sourdough" (C), respectively, with "Natural sourdough fortified with *Spirulina* powder" are presented in Table 3.

The physico-chemical analysis revealed that the white bread prepared with "Natural sourdough fortified with *Spirulina* powder" (V2-fortification level 5%) recorded the highest values of the physico-chemical indicators: volume (297 cm<sup>3</sup>/100 g), porosity (77.85%), ash (0.60%), protein (8.85%). Also, this bread recorded the lowest value for the carbohydrate content (47.88%), respectively, available carbohydrates (47.28%). The volume of white

bread prepared with "Natural sourdough fortified with *Spirulina* powder" is higher than that reported by Burnete et al. (2020) in the case of white bread prepared with natural sourdough (Volume = 272 cm<sup>3</sup>/100 g), respectively that reported by Plessas et al. (2023) in the case of breads prepared with sourdough with the addition of freeze-dried *L. plantarum* (1-3%) (Volume = 245-252 cm<sup>3</sup>/100 g).

Table 3. The physico-chemical indicators of breads made with "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder"

Physico-chemical indicators	Control bread	<i>Spirulina</i> 3% bread	<i>Spirulina</i> 5% bread
Nominal mass (%)	0.421±0.002	0.423±0.002	0.425±0.002
Volume (cm <sup>3</sup> /100g)	275±1.38	289±1.44	297±1.49
Porosity (%)	71.30±0.29	73.30±0.29	77.85±0.31
Elasticity (%)	95±0.10	97±0.10	97±0.10
Moisture (%)	41.47±1.04	41.62±1.04	41.85±1.05
Acidity (degrees)	3.10±0.05	3.30±0.05	3.50±0.05
Ash (%)	0.47±0.007	0.52±0.008	0.60±0.009
Protein (%)	7.67±0.12	8.23±0.12	8.85±0.13
Fat (%)	0.76±0.01	0.80±0.01	0.82±0.01
Carbohydrates (%)	49.63±0.55	48.83±0.54	47.88±0.53
Available carbohydrates (%)	49.12±0.54	48.27±0.53	47.28±0.52
Total fiber (%)	0.51±0.008	0.56±0.009	0.60±0.01
Energy value (kcal/100g)	235	234	233
Energy value (kJ/100g)	998	995	989

The protein content of white bread prepared with "Natural sourdough fortified with *Spirulina* powder" is comparable to that reported by Lazo-Vélez et al. (2021) in the case of breads prepared with selenized chickpea sourdoughs (Protein = 8.89-8.98%). Also, this bread has a carbohydrate content, respectively, available carbohydrates, lower than that reported by Burnete et al. (2020) in the case of white bread prepared with natural sourdough (Carbohydrates = 49.79%, Available carbohydrates = 49.21%) and compared to that reported by Lazo-Vélez et al. (2021) in the case of breads prepared with selenized chickpea sourdoughs (Carbohydrates = 55.09-55.88%).

### Bioactive compounds content

The content in bioactive compounds of "Natural sourdough control" (C) and "Natural sourdough fortified with *Spirulina* powder" is presented in Table 4. According to the results, "Natural sourdough fortified with *Spirulina* powder" (V2-fortification level 5%) recorded the highest values of total polyphenols (120.60 mg GAE/100 g), vitamin C (3.40 mg/100 g) and β-carotene (8.54 mg/100 g).

The total polyphenols content of the breads made with "Control natural sourdough" (C),

respectively with “Natural sourdough fortified with *Spirulina* powder” is presented in Table 5.

Table 4. Bioactive compounds content of the “Control natural sourdough” (C) and “Natural sourdough fortified with *Spirulina* powder”

Bioactive compounds	„Control natural sourdough” (C)	„Natural sourdough fortified with <i>Spirulina</i> powder”	
		V1- <i>Spirulina</i> 3%	V2- <i>Spirulina</i> 5%
Total polyphenols (mg/100g)	55.23±1.38	109.21±2.73	120.60±3.01
Vitamin C (mg/100g)	Nd*	2.12±0.06	3.40±0.10
β-carotene (mg/100g)	Nd*	5.15±0.12	8.54±0.20

\*Undetectable

Table 5. Total polyphenols content of the breads made with “Control natural sourdough” (C) and “Natural sourdough fortified with *Spirulina* powder”

Bioactive compound	Control bread	<i>Spirulina</i> 3% bread	<i>Spirulina</i> 5% bread
Total polyphenols (mg/100g)	21.75±0.54	35.44±0.88	46.67±1.16

White bread prepared with "Natural sourdough fortified with *Spirulina* powder" (V2 - fortification level 5%) recorded the highest values of total polyphenols content (46.67 mg GAE/100 g). The total polyphenol content of this bread is comparable to that reported by Plessas et al. (2023) in the case of bread with sourdough with the addition of freeze-dried *L. plantarum* (1%) (Total polyphenols content = 49 mg GAE/100 g), but lower compared to that reported by the same author in the case of bread prepared with sourdough with freeze-dried *L. plantarum* (1-3%) and freeze-dried pomegranate juice (6%) (Total polyphenols content = 54.4-90.1 mg GAE/100 g).

International epidemiological studies (Dini and Grumetto, 2022) suggested that the introduction in to the diet and the long-term consumption of foods containing polyphenols protect against cancer, osteoporosis, cardiovascular diseases, diabetes and neurodegenerative diseases. Bread has been a basic element in the human diet for thousands of years, being one of the most consumed foods in the world (Papadimitriou et al., 2019) and obtaining some types of bread, enriched in polyphenols, using "Natural sourdough fortified with *Spirulina* powder", is of real interest.

### Antioxidant capacity

Due to its antioxidant content (polyphenols, vitamin C, β-carotene), "Natural sourdough fortified with *Spirulina* powder" has

antioxidant capacity. The highest value of the antioxidant capacity was recorded in the case of "Natural sourdough fortified with *Spirulina* powder", V2 - fortification level 5% (Figure 6).

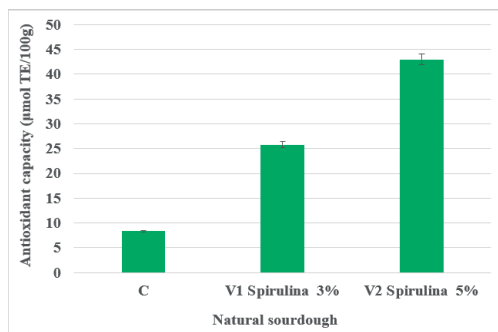


Figure 6. Antioxidant capacity of the “Control natural sourdough” (C) and “Natural sourdough fortified with *Spirulina* powder”

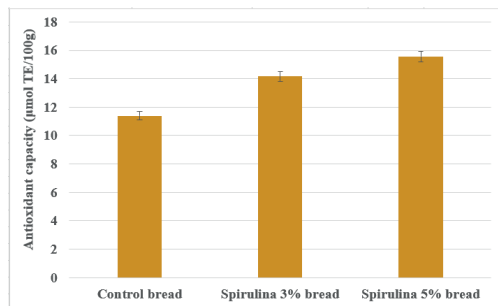


Figure 7. Antioxidant capacity of the breads prepared with "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" (V1 - fortification level 3%; V2 - fortification level 5%)

Antioxidant capacity of the breads prepared with “Natural sourdough fortified with *Spirulina* powder” (Figure 7) is comparable with that reported by Burnete et al. (2019), for the white bread prepared with natural sourdough enriched in phenolic compounds and inulin (Antioxidant capacity = 13.68-14.8 μmol TE/100 g).

### Microbiological analysis

The microbiological indicators of the “Control natural sourdough” (C) and “Natural sourdough fortified with *Spirulina* powder” are presented in Table 6.

The concentrations of yeasts, respectively lactic bacteria of "Natural sourdough fortified with *Spirulina* powder" are comparable to those

reported by Burnete et al. (2019), in the case of natural sourdough enriched in phenolic compounds and inulin: Yeasts =  $2.8 \times 10^6$  CFU/g; Lactic acid bacteria =  $1.7 \times 10^8$  CFU/g.

Table 6. The microbiological indicators of the "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder"

Microbiological indicators	„Control natural sourdough" (C)	„Natural sourdough fortified with <i>Spirulina</i> powder"	
		V1- <i>Spirulina</i> 3%	V2- <i>Spirulina</i> 5%
Yeast (CFU/g)	$1.5 \times 10^5$	$5 \times 10^5$	$3 \times 10^6$
Lactic acid bacteria (CFU/g)	$7.5 \times 10^6$	$8 \times 10^7$	$2.2 \times 10^8$
<i>Enterobacteriaceae</i> (CFU/g)	< 10	< 10	< 10

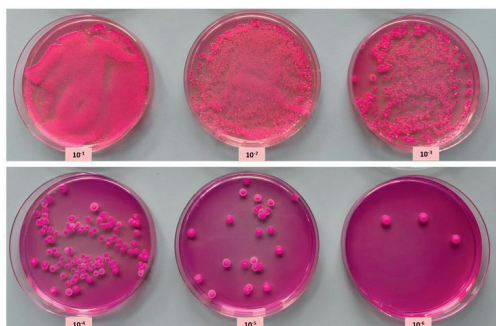


Figure 8. Yeasts on DRBC medium for the "Natural sourdough fortified with *Spirulina* powder" (V2 -fortification level 5%)

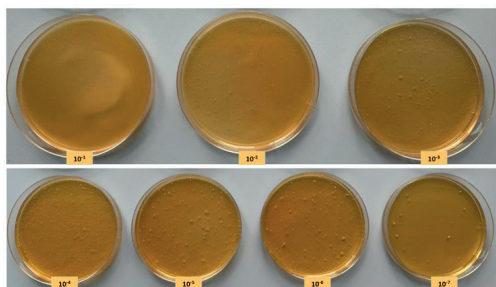


Figure 9. Lactic acid bacteria for the "Natural sourdough fortified with *Spirulina* powder" (V2 - fortification level 5%)

Based on microbiological analysis, in the case of "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder", yeasts of the genus *Saccharomyces cerevisiae* and *Zygosaccharomyces* spp. and lactic acid bacteria belonging to the genera *Lactobacillus* were identified.

Following the microbiological analysis of bread samples prepared with "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" (V1 -

fortification level 3%; V2 - fortification level 5%) packed in polypropylene bags, it was found that they fall within the provisions of the legislation in force even 9 days after the date of manufacture (Table 7).

Table 7. The microbiological indicators of breads with "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder"

Product	Yeast and molds (CFU/g)			<i>Enterobacteriaceae</i> (CFU/g)		
	2 days	5 days	9 days	2 days	5 days	9 days
Control bread	< 10	< 10	< 10	< 10	< 10	< 10
<i>Spirulina</i> 3% bread	< 10	< 10	< 10	< 10	< 10	< 10
<i>Spirulina</i> 5% bread	< 10	< 10	< 10	< 10	< 10	< 10

The microbiological stability of the bread samples is due to the acidity conferred by the use in their composition of "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder". Also, it should be mentioned that in the case of breads prepared with "Natural sourdough fortified with *Spirulina* powder", their microbiological stability is due to the antimicrobial activity of *Spirulina* powder. Recent international research has demonstrated the antioxidant and antimicrobial activity of extracts obtained from *Spirulina* (Abdel-Moneim et al., 2022).

## CONCLUSIONS

"Natural sourdough fortified with *Spirulina* powder" (fortification level 3% and 5% *Spirulina*) stands out for the content in protein (6.74-7.28%), ash (0.52-0.69%), polyphenols (109.21-120.60 mg GAE/100 g), vitamin C (2.12-3.40 mg/100 g) and  $\beta$ -carotene (5.15-8.54 mg/100 g) and has antioxidant capacity (25.81-42.95  $\mu$ mol TE/100 g). Also, "Natural sourdough fortified with *Spirulina* powder" is a natural dough fermentation agent, notable for its content in yeast ( $5 \times 10^5$ - $3 \times 10^6$ ) and lactic acid bacteria ( $8 \times 10^7$ - $2.2 \times 10^8$ ).

The fermentation capacity of "Natural sourdough fortified with *Spirulina* powder" was tested in the preparation of white bread, applying the biphasic process, obtaining products with superior sensory qualities and corresponding physico-chemical indicators. Thus, white bread prepared with "Natural sourdough fortified with *Spirulina* powder" (fortification level with 5%) has an elastic,

dense core, pleasant taste and smell/aroma, has the largest volume (297 cm<sup>3</sup>/100g), the highest protein content (8.85%), ash (0.60%), total polyphenols (46.67 mg GAE/100 g) and the highest antioxidant capacity (15.56 μmol TE/100 g).

"Natural sourdough fortified with *Spirulina* powder" can be considered a natural preservation agent, since the bread prepared with this fermentation agent, from a microbiological point of view, falls within the provisions of the legislation in force, and can be consumed up to 9 days after the date of manufacture.

## REFERENCES

- Abdel-Moneim, A.M.E., El-Saadony, M.T., Shehata, A.M., Saad, A.M., Aldhumri, S.A., Ouda, S.M., Mesalam, N.M. (2021). Antioxidant and antimicrobial activities of *Spirulina platensis* extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. *Saudi Journal of Biological Sciences*, 29, p. 1197–1209.
- Akamine, I.T., Mansoldo, F.R.P., Vermelho, A.B. (2023). Probiotics in the Sourdough Bread Fermentation: Current Status. *Fermentation*, 9, 90.
- Albagli, G., Schwartz, I.d.M., Amaral, P.F.F., Ferreira, T.F., Finotelli, P.V. (2021). How dried sourdough starter can enable and spread the use of sourdough bread. *LWT - Food Science and Technology*, 149, 111888.
- Arora, K., Ameer, H., Polo, A., Di Cagno, R., Rizzello, C.G., Gobbetti, M. (2021). Thirty Years of Knowledge on Sourdough Fermentation: A Systematic Review. *Trends Food Sci. Technol.*, 108, p. 71–83.
- Arte, E., Rizzello, C.G., Verni, M., Nordlund, E., Katina, K., Coda, R. (2015). Impact of Enzymatic and Microbial Bioprocessing on Protein Modification and Nutritional Properties of Wheat Bran. *J. Agric. Food Chem.*, 63, p. 8685–8693.
- Asănică, A.C., Catană, L., Catană M., Burnete, A.G., Lazăr, M.A., Belc, N., Martínez Sanmartín, A. Internal Validation of the Methods for Determination of Water-Soluble Vitamins from Frozen Fruits by HPLC-HRMS. *Rom Biotechnol Lett.*, 24(6), p. 1000-1007.
- Burnete A. G., Lazăr A. M., Catană M., Catană L., Stamatie G., Teodorescu R.I., Belc N., Vlăduț V. (2019). Research regarding the achievement of sourdough enriched in phenolic compounds and inulin, using flour from Jerusalem artichoke tubers (*Helianthus Tuberosus*). *Volume International Symposium ISB – INMA TEH, Agricultural and Mechanical Engineering*, p. 822-829.
- Burnete A.G., Catană L., Catană M., Lazăr A.M., Teodorescu R.I., Asănică A.C., Belc N. (2020). Use of vegetable functional ingredients to achieve hypoglycemic bread with antioxidant potential, for diabetics. *Scientific Papers Series B. Horticulture*, Volume LXIV, No. 2, p. 367-374.
- Catană L., Catană M., Iorga E., Asănică A.C., Lazăr M.A., Lazăr A.G., Belc N., Pîrvu G. (2020). Internal Validation of Rapid and Performant Method for Carotenoids Determination in Tomato Waste Powder by HPLC. *Revista de Chimie*, 71(1), p. 342-349.
- Chavan, R.S., Chavan, S.R. (2011). Sourdough Technology-A Traditional Way for Wholesome Foods: A Review. *Compr. Rev. Food Sci. Food Saf.*, 10, p. 169–182.
- Coda, R., Di Cagno, R., Gobbetti, M., Rizzello, C.G. (2014). Sourdough lactic acid bacteria: Exploration of non-wheat cereal-based fermentation. *Food Microbiol.*, 37, p. 51–58.
- Coda, R., Varis, J., Verni, M., Rizzello, C.G., Katina, K. (2017). Improvement of the protein quality of wheat bread through faba bean sourdough addition. *LWT—Food Sci. Technol.*, 82, p. 296–302.
- Corsetti, A., Settanni, L. (2007). Lactobacilli in Sourdough Fermentation. *Food Res. Int.*, 40, p. 539–558.
- De Vuyst, L. and Neysens, P. (2005). The Sourdough Microflora: Biodiversity and Metabolic Interactions. *Trends Food Sci. Technol.*, 16, p. 43–56.
- Dini, I., Grumetto, L. (2022). Recent Advances in Natural Polyphenol Research. *Molecules*, 27, 8777.
- Fekri, A., Torbati, M., Khosrowshahi, A.Y., Shamloo, H.B., Azadmard-Damirchi, S. (2020). Functional effects of phytate-degrading, probiotic lactic acid bacteria and yeast strains isolated from Iranian traditional sourdough on the technological and nutritional properties of whole wheat bread. *Food Chem.*, 306, 125620.
- Fraberger, V., Ozulku, G., Petrova, P., Nada, K., Petrov, K., Johann, D.K., Rocha, J.M.F. (2023). Sourdough as a Source of Technological, Antimicrobial, and Probiotic Microorganisms. In *Sourdough Innovations*; CRC Press: Boca Raton, FL, USA, p. 265–310.
- Garofalo, C., Zannini, E., Aquilanti, L., Silvestri, G., Fierro, O., Picariello, G.; Clementi, F. (2012). Selection of Sourdough Lactobacilli with Antifungal Activity for Use as Biopreservatives in Bakery Products. *J. Agric. Food Chem.*, 60, p. 7719–7728.

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- Gänzle, M.G. (2014). Enzymatic and Bacterial Conversions during Sourdough Fermentation. *Food Microbiol.*, 37, p. 2–10.
- Gobbetti, M., De Angelis, M., Di Cagno, R., Calasso, M., Archetti, G., Rizzello, C. (2019). Novel insights on the functional/nutritional features of the sourdough fermentation. *Int. J. Food Microbiol.*, 302, p. 103–113.
- Graça, C., Lima, A., Raymundo, A., Sousa, I. (2021). Sourdough Fermentation as a Tool to Improve the Nutritional and Health-Promoting Properties of Its Derived-Products. *Fermentation*, 7, 246.
- Hernández-Figueroa, R.H., Mani-López, E., López-Malo, A. (2022). Antifungal Capacity of Poolish-Type Sourdough Supplemented with *Lactiplantibacillus Plantarum* and its Aqueous Extracts in Vitro and Bread. *Antibiotics*, 11, 1813.
- Hernández-Figueroa, R.H.; Mani-López, E.; López-Malo, A. (2023). Antifungal Activity of Wheat-Flour Sourdough (Type II) from Two Different *Lactobacillus* in Vitro and Bread. *Appl. Food Res.*, 3, 100319.
- Hernandez-Figueroa, R.H., Mani-Lopez, E., Palou, E., Lopez-Malo, A. (2024). Sourdoughs as Natural Enhancers of Bread Quality and Shelf Life: A Review. *Fermentation*, 10, 7.
- 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.
- Illueca, F., Moreno, A., Calpe, J., Nazareth, T.d.M., Dopazo, V., Meca, G., Quiles, J.M., Luz, C. (2023). Bread Biopreservation through the Addition of Lactic Acid Bacteria in Sourdough. *Foods*, 12, 864.
- Janda-Milczarek, K., Szymczykowska, K., Jakubczyk, K., Kupnicka, P., Skonieczna-Żydecka, K., Pilarczyk, B., Tomza-Marciniak, A., Ligenza, A., Stachowska, E., Dalewski, B. (2023). Spirulina Supplements as a Source of Mineral Nutrients in the Daily Diet. *Appl. Sci.* 13, 1011.
- Jiang, X., Liu, X., Xu, H., Sun, Y.; Zhang, Y., Wang, Y. (2021). Improvement of the nutritional, antioxidant and bioavailability properties of corn gluten-wheat bran mixture fermented with lactic acid bacteria and acid protease. *LWT - Food Sci. Technol.*, 144, 111161.
- Lazo-Vélez, M.A., Garzon, R., Daniela Guardado-Félix, D., Serna-Saldivar, S.O., Rosell, C.M. (2021). Selenized chickpea sourdoughs for the enrichment of breads. *LWT - Food Science and Technology*, 150 112082.
- Luz, C., D'Opazo, V., Mañes, J., Meca, G. (2019). Antifungal Activity and Shelf Life Extension of Loaf Bread Produced with Sourdough Fermented by *Lactobacillus* Strains. *J. Food Process. Preserv.*, 43, e14126.
- Maioli, M., Pes, G.M., Sanna, M., Cherchi, S., Dettori, M., Manca, E., Farris, G.A. (2008). Sourdough-leavened bread improves postprandial glucose and insulin plasma levels in subjects with impaired glucose tolerance. *Acta Diabetol.*, 45, p. 91–96.
- Park, Y.-H., Jung, L.-H., Jeon, E.-R. (2006). Quality Characteristics of Bread Using Sour Dough. *Prev. Nutr. Food Sci.*, 11, p. 323–327.
- Plessas, S., Mantzourani, I., Alexopoulos, A., Alexandri, M., Kopsahelis, N., Adamopoulou, V., Bekatorou, A. (2023). Nutritional Improvements of Sourdough Breads Made with Freeze-Dried Functional Adjuncts Based on Probiotic *Lactiplantibacillus plantarum* subsp. *plantarum* and Pomegranate Juice. *Antioxidants* 12, 1113.
- Ryan, L.A.M., Zannini, E., Dal Bello, F., Pawlowska, A., Koehler, P.; Arendt, E.K. (2011). *Lactobacillus Amylovorus* DSM 19280 as a Novel Food-Grade Antifungal Agent for Bakery Products. *Int. J. Food Microbiol.*, 146, p. 276–283.
- Samapundo, S., Devlieghere, F., Vroman, A., Eeckhout, M. (2017). Antifungal Activity of Fermentates and Their Potential to Replace Propionate in Bread. *LWT - Food Sci. Technol.*, 76, p.101–107.
- Wang, Y., Compaoré-Séréme, D., Sawadogo-Lingani, H., Coda, C., Katina, K., Maina, N. (2019). Influence of dextran synthesized in situ on the rheological, technological, and nutritional properties of whole-grain pearl millet bread. *Food Chem.*, 285, p. 221–230.
- Zou, T.B., He, T.P., Li, H.B., Tang, H.W., Xia, E.Q. (2016). The Structure-Activity Relationship of the Antioxidant Peptides from Natural Proteins. *Molecules*, 21, 72.