

Article

Influence of Technological Parameters on Sourdough Starter Obtained from Different Flours

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Abstract: One of the oldest biotechnological processes used in bread manufacture is sourdough production which relies on wild yeast and lactobacillus cultures naturally present in flour. The aim of this paper was to evaluate the influence of selected flours of different cereal grains (ancient wheat, corn, and rye), different dough variations, and temperature of fermentation on the quality of spontaneous sourdough. Two values of fermentation temperatures were tested (25 °C and 35 °C), and for each temperature analyzed, three backslopping steps were carried out to obtain mature doughs according to the traditional type I sourdough scheme. In total, 14 different sourdoughs were produced, and microbiology, pH, and total titration acidity for 96 h were determined. Optimal pH values for the samples determined that the optimal fermentation period was 48 h. The acidification rate of the dough was faster at 35 °C than at 25 °C. This fact became evident via the pH values obtained in the first 24 h. However, from this point, the pH values were lower in the samples kept at 25 °C, showing that a cooler fermentation temperature allows the acidification activity of the microorganisms to be prolonged for a longer time. In the study carried out, the ideal fermentation time for the population of LAB and yeasts is 72 h at a temperature of 25 °C, and the most productive sourdoughs were the dough with 100% Einkorn wheat flour and the dough obtained from the 1:1 combination of flour rye and corn flours.

Keywords: different flours; sourdough starter; spontaneous fermentation; technological factors; LAB; yeast



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1. Introduction

The food industry is in continuous transition and is constantly evolving from its traditional status based on raw materials to adopting an innovative system and a market-oriented position. This transformation is accompanied by increasing demand for high value-added products that meet consumer demands—for taste, convenience, health, food safety and well-being. In many cases, innovations in biotechnology research succeed in meeting these requirements.

Bakery products provide over 50% of humanity's food sources, and therefore, worldwide, a growing number of specialists and institutions undertake studies and carry out extensive research in order to finalize in the industry the processes and technologies that ensure obtaining quality products and varied assortments, adapted to local specifics and consumer taste.

To improve the quality of bakery products in terms of texture, shelf life, and flavor, an important step is dough fermentation, largely attributed to the metabolic interaction of microorganisms [1]. Lactic bacteria represent, along with yeasts, the predominant microflora. Most of the microorganisms isolated from doughs are represented by the *Lactobacillus* genus, and among the yeast species, *Candida* and *Saccharomyces* are the most common [2]. In the study by Dan Xu [3], mixed starter cultures of yeast and lactobacilli were used to evaluate their ability to improve bread quality and enhance its flavor. The

survival of fermentation microorganisms depends on the competitiveness against certain microbial strains present in the conditions of the dough ecosystem. Their survival and growth are also determined by the presence of suitable substrates. However, a thorough understanding of the behavior of microorganisms and the impact of certain substrates in the ingredients used can lead to obtaining new, stable doughs and implicitly to innovative bread recipes with the addition of sourdough [4]. It was shown that dough that was fermented with both lactic bacteria and yeast had an extended shelf life, and its sensory properties were also improved due to the presence of organic acids, amino acids, and a group of B vitamins produced by the lactic starter culture [5]. The results showed that the synergistic activity between lactic acid bacteria (*Lactobacillus bulgaricus*) and baker's yeast (*Saccharomyces cerevisiae*) improved the sensory properties of bread and also extended its shelf life.

Studies and specialized literature have highlighted the importance of biotechnological applications of lactic bacteria in grain-based products, creating new opportunities to improve the functional and nutritional quality and texture of flour products using sourdoughs. Bread obtained by the addition of sourdough obtained from a combination of lactobacilli and yeast had a more complex profile of volatile substances. In the study by Noriko K. [6], different effects of lactic acid bacteria and yeast on sourdough bread were examined, and the development of a new sourdough bread made with a yeast isolated from fruit and lactic acid bacteria, namely *Lactobacillus paracasei* NFRI 7415 isolated from a traditional Japanese fermented fish (funa-sushi). An increased content of organic acids and free amino acids was found in this dough, which favored the flavor of the final product. Also, in 2021, a study presented new hypotheses for the successful management of sourdough and proposed different directions for research and their application [7].

Although taste and convenience are important factors for consumers, most products that meet these consumer demands are line extensions or packaging innovations that involve limited biotechnological research. In any case, there is a continuous search for new products and processes that offer ingredients with new functionalities and cost-effective processing. Food fermentations offer these advantages, and there is continuous improvement in the microbial bioconversions that are at the heart of the production of functional metabolites. Microbial fermentation with the help of lactic acid bacteria strains and yeasts is of real interest to the bakery industry as a result of their significant antifungal activity and the ability to contribute to the extension of the shelf life. New dimensions have been introduced to improve fermentations by applying post-genomic approaches to almost all microorganisms involved in major food fermentations or used to produce different value-added compounds.

Lactic acid bacteria (LAB), with a tradition in industrial food fermentation, are used as starters for the fermentation of raw materials of plant and animal origin. Using lactic acid bacteria in food fermentation has an important role in the quality of food products. Fermented food products with lactic acid bacteria are less perishable, and their nutritional value may be enhanced, but one of the most important aspects is the safety of these products, which may be improved due to the inhibition of pathogenic bacteria and spoilage microorganisms by the low pH and the presence of organic acids and antimicrobial compounds. Bio-preservation is one of the many attributes of lactic acid bacteria under the scope of food safety.

The microbial ecosystem in sourdough obtained from different flours may present distinct lactic acid bacteria communities that can contribute to many variations of sourdough flavors and antimicrobial compounds. In this study, different flours (wheat, corn, and rye), variations, and technological parameters were used in order to obtain sourdough with different microbial ecosystems and characteristics. Therefore, the aim of this study is to evaluate the influence of different cereal grains (ancient wheat, corn, and rye), different dough variations, and temperature of fermentation on the quality of the spontaneous sourdough in order to obtain active food ingredients with a role in optimizing baking technology, improving nutritional quality and extending the shelf life of food products.

2. Materials and Methods

2.1. Raw Materials

The raw materials used in experiments were procured from Romanian manufacturers. Corn flour from SC Paradisul Verde SRL, Brasov, Romania, obtained from 100% corn ecologic crop produced in Romania, Einkorn wheat flour (*Triticum monococum*), whole wheat flour, rye flour from Biofarmland Manufactura SRL, Arad, Romania, 100% organic flours produced in Romania, packed in 5 kg paper bags.

The flour samples are freshly ground at the time of product purchase, using a mill that does not heat the flour. The purpose of this practice is to ensure the highest nutritional value of the obtained flours. The flours were used immediately after milling in order to obtain different variations of sourdough.

2.2. Sourdough Fermentation

Spontaneous dough was formed by fermenting a mixture of flour and water without the addition of an external starter culture. The four types of flour, together with plain Borsec water, were mixed to prepare seven different doughs. Mixing was carried out using a dough blender in a ratio of 1:1 to form a dough. The dough was kneaded for 4 min for homogenization and left to ferment at 25 °C and 35 °C, respectively, for 24 h. Every 24 h, the refreshing procedure (backslopping) was carried out by adding 100 g of fermented dough, 100 g of white wheat flour 650, and 100 mL of plain Borsec water (ratio 1:1:1). Four refreshment steps were carried out to obtain mature doughs according to the traditional type I acid dough scheme.

2.3. Assessment of the Nutritional Profile of Dry Raw Materials

2.3.1. The Moisture Content

The moisture content was achieved via a gravimetric method that involved drying the samples at 130 °C for 90 min until a constant mass was reached, the method described by ISO 712:2010 [8]. Approximately 5 g of the sample was weighed into a vial, which was dried to a constant mass in an oven (MRC DK-500WT, MRC Ltd., Holon, Israel). The analysis was performed in duplicate. The moisture content (M , %) was determined using the following formula:

$$M (\%) = (M_0 - M_1) / M_0 \times 100 \quad (1)$$

where M_1 —mass of vial with cap (g); M_0 —sample mass.

2.3.2. Acidity

The method consisted of titration of the aqueous flour extract with a 0.1 N sodium hydroxide solution in the presence of phenolphthalein as an indicator. Two parallel determinations were performed, the result being the arithmetic mean of the two determinations, if the difference between them does not exceed 0.2 degrees of acidity per 100 g sample. The analysis was performed in duplicate.

Acidity (degrees) is calculated using the following formula:

$$\text{Acidity} = (V \times 0.1) / m \times 100 \quad (2)$$

where

V —volume of 0.1N NaOH solution (mL);

m —mass of the working sample (g);

0.1—the normality of the NaOH solution.

2.3.3. Protein Content

Total protein content was determined according to ISO 20483:2013 [9]. The protein content was analyzed by the Kjeldahl method with a FOSS Kjeltac 2300 analyzer (FOSS Group, Hillerød, Denmark) after acid hydrolysis in an auto-digester (Behrotest InKjel, 450 P, Behr—Labor Technik GmbH, Dusseldorf, Germany). According to the classical

Kjeldahl method, samples were digested using concentrated sulfuric acid. The ammonium sulfate salt and alkali generated ammonia, which was trapped in boric acid via steam distillation. Titration was performed using a hydrochloric acid 0.2 N solution. The analysis was performed in duplicate.

2.3.4. Determination of Ash

The sample to be analyzed (2–3 g) is precalcined at a temperature of 750 °C by adding ethanol, and the calcination is continued for another 4 h. After the calcination is completed, the crucible with the sample is removed from the oven, placed in the desiccator until it reaches room temperature and weighed quickly with an accuracy of 0.1 mg.

The ash content is calculated as follows:

$$w_{a,d} = (m_2 - m_1) \times 100 / m_0 \times 100 / (100 - W_m) \quad (3)$$

where

m_0 —mass of the working sample (g);

m_1 —mass of the calcination crucible (g);

m_2 —mass of calcination crucible and calcined residue (g);

W_m —sample moisture in mass percent.

As a result, the arithmetic mean between two determinations is taken. The results are expressed with an accuracy of 0.01%. The analysis was performed in duplicate.

2.3.5. Fat Content Determination

The determination is carried out with the Soxtec equipment from FOSS, in the presence of petroleum ether. The working steps are boiling step (40 min), rinsing (150 min), solvent recovery (15 min), solvent evaporation (10 min at 105 °C), and weighing of the glass vials.

The fat content is calculated according to the formula

$$M (\%) = (M_2 - M_1) / M_0 \times 100 \quad (4)$$

where

M_1 —mass of the empty glass bottle (g);

M_2 —mass of glass bottle with fat (g);

M_0 —mass of the working sample (g).

The results are expressed with an accuracy of 0.01%. The analysis was performed in duplicate.

2.3.6. Determination of Fiber Content

The determination is carried out with the Fibertech equipment by treating the sample, placed in the FiberBag, with boiling sulfuric acid (acid digestion), rinsing with water, which is followed by alkaline digestion with sodium hydroxide and again rinsing with water. The FiberBag is wrapped and placed in the crucible brought to constant mass (in the oven at 750 °C for 1 h). The crucible with the FiberBag inside is dried in an oven at $105^\circ \pm 1^\circ \text{C}$ for a minimum of 4 h and then left to cool in a desiccator for 30 min and weighed. The sample is then calcined at 600 °C for 4 h, after which it is weighed.

The crude fiber content is calculated as follows:

$$w_f = (m_3 - m_1 - m_4 - m_5) / m_2 \times 100 \quad (5)$$

$$m_5 = m_7 - m_6$$

where

m_1 —FiberBag table, g;

m_2 —initial mass of the sample, g;

m_3 —mass of the calcination crucible with the dry FiberBag, g;

m_4 —the mass of the calcination crucible and the residue obtained after calcination, g;

m_5 —mass of the empty FiberBag blank, g;

m_6 —mass of the calcination crucible, g;

m_7 —the mass of the crucible and the ash content of the empty FiberBag, g.

The result is rounded to the nearest 1 g/kg and expressed as a percentage. The analysis was performed in duplicate.

2.4. Assessment of the Physico-Chemical Properties and Microbiological Status for Spontaneous Sourdough Obtained from Different Flours

The maturity of the starter dough is evaluated by the stability of the pH, the acidity value, and its microbiota. Measurements of pH and total titratable acidity (TTA) were performed according to the Romanian standard methods 90/2007 [10]. The TTA value is defined as the amount of 0.1 N NaOH solution (mL) used to neutralize the 10 g sample weight.

Cell counts of LAB and yeast were performed using national and international food microbiology standards by using the viable cell count method on MRS agar and Potato Dextrose agar in duplicates using appropriate dilution of dough. The plates were anaerobically incubated at 30 °C for 72 h (LAB) and at 25 °C for 5 days (yeast). Incubation steps were performed using Panasonic and Memmert incubators. Results were expressed as CFU/g (colony forming units per gram dough). All the analyses were performed in duplicate.

2.4.1. Determination of Lactic Acid Bacteria (LAB) (According to ISO 15214/2001 [11])

Cell counts (expressed as CFU per gram of dough) were determined by mixing 10 g of sourdough with 90 mL of peptone physiological solution (made in a laboratory from ingredients—bacteriological peptone and NaCl (Oxoid, Ltd., Basingstoke, UK). Appropriate dilutions were made, and 1 mL inoculum was plated on MRS agar (Oxoid, Ltd., Basingstoke, UK), followed by incubation at 30 °C for 72 h. The colonies were numbered, and the interpretation of results was performed using the following Formula (6):

$$N = (\sum C) / (n_1 + 0.1 n_2) \times d \quad (6)$$

where N = number of CFU from two serial dilutions; $\sum C$ = sum of colonies counted in all retained plates; n_1 = number of plates retained at first dilution; n_2 = number of plates retained at the second dilution; d = first retained.

2.4.2. Determination of Yeast Count (According to ISO 21527-1:2009 [12])

The method implies dispersing 0.1 mL of sample inoculum onto the surface of DG-18 Agar (Dicloran Glycerol Agar, Oxoid, Ltd., Basingstoke, UK) using a Drigalski spatula followed by incubation at 25 °C for 5 days. After the incubation period, the colonies were counted and analyzed according to Formula (4).

3. Results

3.1. Assessment of the Nutritional Profile of Dry Raw Materials

The fermentation of spontaneous sourdoughs depends on the endogenous parameters, mainly represented by the chemical and microbiological composition/quality (microbiota) of the flour. In order to characterize the flour samples, the value of the parameters related to 100% product will be taken into account. Results for all tested samples are presented in Table 1.

The acidity of the flour expresses the degree of freshness, the wheat flour samples being freshly ground, and the flour being more acidic due in particular to acid phosphates. The acidity of normal wheat flours depends on the degree of extraction. The higher it is, the higher the acidity. The white flours of low extraction, which come from the endosperm, contain little mineral salts and fatty substances and therefore have low acidity (2–2.8 degrees).

Table 1. Quality and nutritional profile of raw materials (flours). (Results are presented as mean of two determinations \pm SD).

Samples	Moisture	Acidity	Fat	Protein	Ash	Fiber
	%	Grade	%	%	%	%
Einkorn wheat flour	10.04 \pm 0.02	8.33 \pm 0.29	2.59 \pm 0.03	19.18 \pm 0.18	2.45 \pm 0.00	2.93 \pm 0.00
Whole wheat flour	10.87 \pm 0.01	3.65 \pm 0.14	1.59 \pm 0.02	9.40 \pm 0.00	1.73 \pm 0.02	3.66 \pm 0.00
Corn flour	13.70 \pm 0.02	6.25 \pm 0.29	1.98 \pm 0.01	6.11 \pm 0.05	0.68 \pm 0.01	1.70 \pm 0.02
Rye flour	10.05 \pm 0.00	6.46 \pm 0.29	1.28 \pm 0.01	7.91 \pm 0.12	1.74 \pm 0.01	3.08 \pm 0.01

Some of the most important nutritional profiles of flours are their protein and fiber content. Wheat and rye are rich sources of fiber. Cereal fibers are rich in non-cellulosic polysaccharides, which have an extremely high capacity to bind water and quickly provide a feeling of satiety.

Einkorn wheat flour presented the highest protein value (19.18% \pm 0.18) while also having a fairly high fiber content. Whole wheat flour has the highest fiber concentration, 3.66% \pm 0.00, followed by rye flour and Einkorn wheat flour. Rye flour gliadin and gluten do not differ significantly in terms of structure and molecular mass compared to wheat proteins, but they have different colloidal properties (they do not form gluten, they do not form a continuous protein network in the dough, a structure which in the case of wheat flour obtain even for poor quality flour).

Regarding the content of mineral substances (included in the ash content), the two types of wheat flour but also rye flour presented the highest values, representing a rich growth substrate for lactic acid bacteria.

3.2. Assessment of the Physico-Chemical Properties and Microbiological Status for Spontaneous Sourdough Obtained from Different Flours

3.2.1. pH and Total Titratable Acidity

All sourdough variants showed, during the fermentation period at 25 °C and 35 °C, respectively, a decrease in pH values and an increase in acidity as a result of the production of lactic and acetic acid by the lactic bacteria from the spontaneous microflora of the tested flours. In the case of both variants of the fermentation temperature, the maximum acidity values were identified at 48 h of fermentation. The sourdough made from 100% Einkorn wheat flour presented the highest TTA value (13.4 \pm 0.05 degrees) at 48 h of fermentation at 25 °C, which decreased considerably until the last day of testing, reaching 10.7 \pm 0.12-degree acidity. This course was also observed in the case of the other variants of sourdough, less so in the case of the sourdough made of 100% corn flour and the mixture of corn flour 50% + rye flour 50%, the acidity constantly increasing until the last day of fermentation, reaching 11.7 \pm 0.01 degrees and 12.4 \pm 0.34 degrees, respectively.

Figure 1 shows that the acidity variations of different variations of spontaneous sourdough were influenced by the fermentation temperature used. A dough should be warm enough, at the optimum temperature for yeast growth, usually 29–32 °C, to encourage massive spontaneous fermentation and flavor creation. The highest values of TTA were determined at 48 h of fermentation at 35 °C, with the 100% Einkorn wheat dough having the highest value (27.4 \pm 0.0 degrees), followed by the 50% Einkorn + rye flour dough 50%, respectively 24.8 \pm 0.29 degrees.

One of the major characteristics of sourdough fermentation is a decrease in pH proportional to the maturation of the LAB community that produces lactic and acetic acid, eventually reaching a pH of about 4.0. The pH of the dough changes depending on the stage of fermentation it is in; the values determined in the case of the two fermentation temperatures are presented in Table 2. Since changes in pH can induce stress on cultures of lactic acid bacteria, evaluation of pH conditions is necessary to understand and control the evolution of microorganisms in dough. In addition, pH can influence the degree of lactic

acid fermentation in the dough. The optimum pH of the dough should be between 4.2 and 4.5 [2], and therefore, the fermentation time required at 25 to 35 °C will be somewhere between 6 and 24 h.

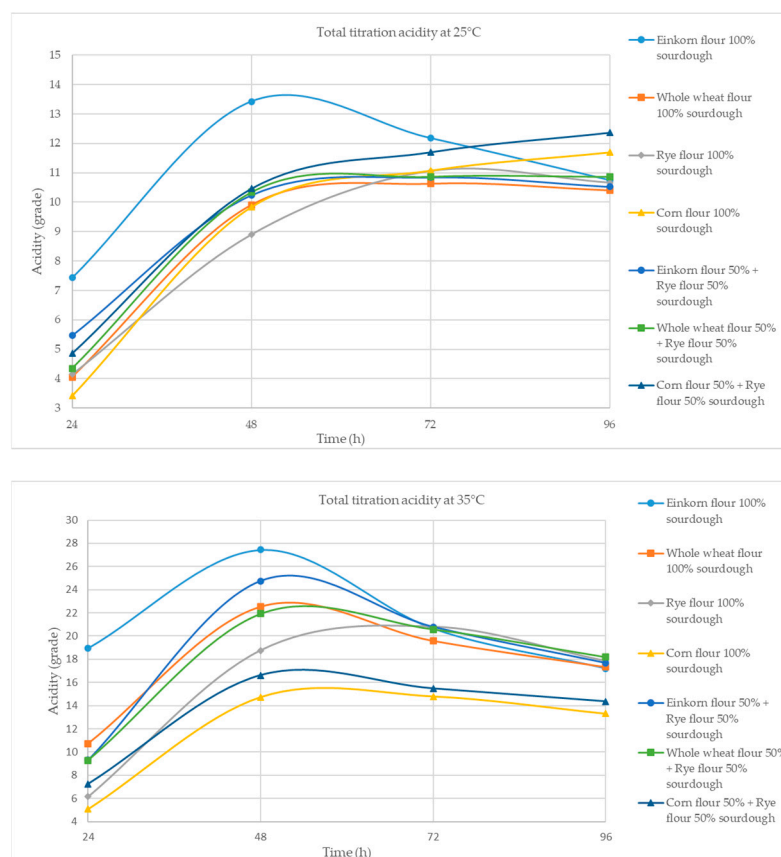


Figure 1. Evolution of total titration acidity values (TTA) for sourdough samples fermented at 25 °C and 35 °C (Results are presented as the mean of two determinations \pm SD).

Table 2. Evolution of pH values during the fermentation period at temperatures of 25 °C and 35 °C (Results are presented as mean of two determinations \pm SD).

Samples	24 h		48 h		72 h		96 h	
	25 °C	35 °C	25 °C	35 °C	25 °C	35 °C	25 °C	35 °C
Einkorn flour 100%	6.36 \pm 0.11	4.98 \pm 0.11	4.16 \pm 0.21	3.52 \pm 0.08	4.0 \pm 0.00	3.46 \pm 0.11	3.92 \pm 0.10	3.47 \pm 0.02
Whole wheat flour 100%	6.35 \pm 0.14	5.05 \pm 0.10	3.98 \pm 0.10	3.47 \pm 0.11	3.85 \pm 0.21	3.41 \pm 0.21	3.78 \pm 0.10	3.43 \pm 0.10
Rye flour 100%	6.48 \pm 0.12	6.1 \pm 0.15	4.15 \pm 0.15	3.67 \pm 0.21	3.86 \pm 0.25	3.39 \pm 0.05	3.78 \pm 0.21	3.39 \pm 0.16
Corn flour 100%	5.97 \pm 0.10	5.14 \pm 0.21	3.98 \pm 0.10	3.59 \pm 0.10	3.82 \pm 0.08	3.55 \pm 0.15	3.74 \pm 0.12	3.56 \pm 0.18
Einkorn flour 50% + Rye flour 50%	6.44 \pm 0.12	5.89 \pm 0.20	4.2 \pm 0.13	3.54 \pm 0.16	3.98 \pm 0.21	3.43 \pm 0.19	3.89 \pm 0.09	3.44 \pm 0.10
Whole wheat flour 50% + Rye flour 50%	6.38 \pm 0.15	5.16 \pm 0.18	4.1 \pm 0.18	3.57 \pm 0.18	4.0 \pm 0.10	3.41 \pm 0.20	3.93 \pm 0.02	3.41 \pm 0.21
Corn flour 50% + Rye flour 50%	6.21 \pm 0.12	5.33 \pm 0.10	3.93 \pm 0.20	3.69 \pm 0.21	3.84 \pm 0.05	3.52 \pm 0.31	3.72 \pm 0.10	3.54 \pm 0.11

Surveying the changes in the pH until it reached a value of about 4.3 allowed us to discover the optimal fermentation period for each of the two temperatures. It was observed that samples fermented at 35 °C reached a pH of 4.2 after 24 h, while samples maintained at 25 °C took 48 h to reach a pH of 4.1. It was also possible to find an increase in the volume of dough held at 35 °C that was higher than that occurring in dough fermented at 25 °C. This can be attributed primarily to a higher level of fermentation activity, producing more gas, which in turn decreases the density of the dough. From 48 h, the volume increase was stabilized.

3.2.2. Evolution of Lactic Acid Bacteria and Yeast Cultures of Spontaneous Fermentation Sourdough

The population of lactic acid bacteria for the sourdough fermented 24 h at 25 °C had an average of 6.29 log UFC/g. These values increased to 8.97 log CFU/g after 72 h of fermentation, respectively, 8.91 log CFU/g at 96 h. These values increased to over 8 log CFU/g after 48 h of fermentation at 25 °C, with value reached in 24 h of fermentation at 35 °C for all doughs tested. The lactic acid bacteria population was much more productive at 24 h of fermentation at 35 °C for all types of sourdough, but the growth during the fermentation period was very low (Figure 2). The population of lactic acid bacteria present in the sourdough obtained with whole wheat flour showed an increase throughout the fermentation period up to 96 h for the dough fermented at 25 °C, while for the dough kept at 35 °C, the maximum number was reached after 24 h, after which there was a slow decline until 96 h. The same pattern was also identified in the case of rye and corn flour, but also for the combinations of whole wheat flour 50% with rye flour 50% and corn flour 50% with rye flour 50%.

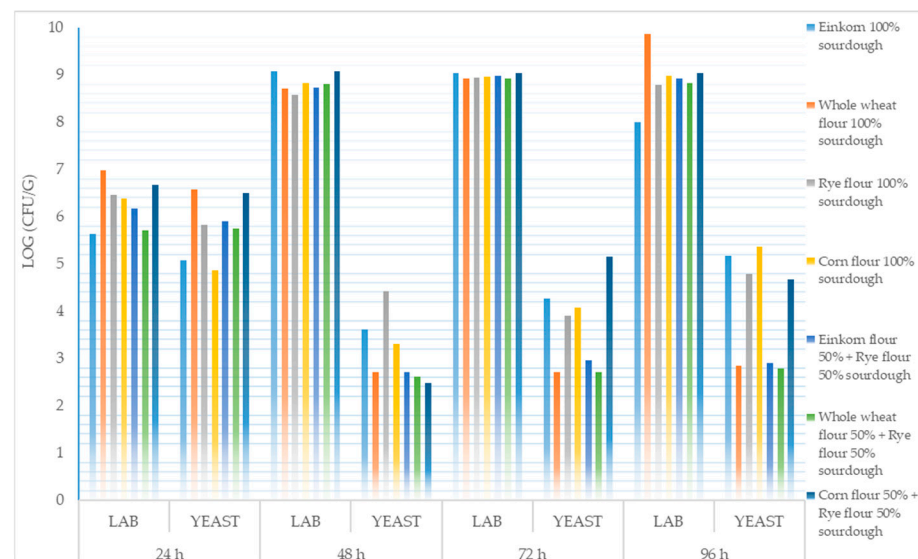


Figure 2. Evolution of lactic acid bacteria and yeast populations compared to pH values in the case of doughs fermented at 25 °C (Results are presented as mean of two determinations \pm SD).

Lactobacilli constitute a well-adapted population to the physical and chemical conditions in the dough, competing with the remaining microbial life until they become the dominant microbiota [13]. This allows the dough to spread over long periods if water and flour are added at appropriate intervals. Therefore, it can be said that the LAB population increases as the pH decreases and finally both reach a plateau when the dough ripens.

The dynamics of the yeast population of the fermented doughs at the two established temperatures generally revealed a stable growth of approximately log 5.78 CFU/g (25 °C) and log 7.21 CFU/g (35 °C) at 24 h of fermentation. After 24 h of fermentation, the yeast population showed a constant decrease until the end of the fermentation period, less in the case of sourdoughs from 100% corn flour and 50% rye flour with 50% corn flour. At 48 h of fermentation, the yeast population decreased dramatically in all doughs tested and for both fermentation temperatures.

4. Discussion

The baking process is a complex process based on a large number of physical, chemical, colloidal, biochemical, and microbiological processes. Some of these cannot occur without others, some follow each other, and often, they condition each other so that they cannot be treated individually.

The complexity of the baking process is largely due to the development and fermentation of the dough, which is a viscoelastic semi-finished product with a certain consistency imposed by the technological process and which can hardly be acted upon from the outside. Consumer interest in the last 20 years has led to the re-invention of the technological process by increasing the production of sourdough, a fermented semi-finished product, and also the production of selected microbial strains for the preparation of sourdough [14].

The use of the sourdough-based technological process as the basis of fermentation and leavening is one of the oldest biotechnological processes in the production of cereal foods [15]. Each sourdough is a different natural ecosystem that can produce a different quality of bread, the microbial metabolism being specific to the strain, the intermediate metabolites formed, especially flavor compounds but also antibacterial compounds, conferring a sensory and hygienic quality to bakery products based on yeast in comparison to conventional bread. Type I sourdough can be maintained for years by continuous refreshment using the previous batch as inoculum.

The type of flour that is used during starter maintenance can affect the culture's ecology, technical efficiency as a leavening agent, and unique sensory qualities. Flour helps introduce microorganisms into the dough environment [16]. Also, flour provides different nutrients (carbohydrates and amino acids) and non-nutrients (phenolic acids, amylase, ash), whose presence and concentrations can influence the survival of bacteria and yeast species [17–20]. Phenolic compounds and ash are naturally present in flour in varying amounts and have been shown to affect the acidification rate of starter cultures, thereby influencing microbial succession. Similarly, the presence or absence of the bran portion of cereals may also influence microbial ecology in ways that are not yet clear [21].

The study followed the chemical and microbiological characteristics of different variants of spontaneous sourdoughs obtained by combinations of wheat flour, corn flour, and rye flour, fermented at different temperatures, 25 °C and 35 °C, over a similar period of time (96 h). Thus, seven variants of spontaneous fermentation sourdoughs were tested. The flours used to obtain them were purchased from local producers and were from organically certified crops. Sourdough production is relatively simple and does not need complicated installations and can obtain different products with different characteristics just by varying the ratio between raw materials. By changing the rations and recipe, different sourdough can be obtained to promote diversity and different microbial ecosystems.

At the beginning of fermentation, the dough fermented at 25 °C had higher pH values than those observed in the batches of dough fermented at 35 °C and a slower increase in acidity. In parallel, the number of LAB was much higher in the dough fermented at 35 °C at the beginning of fermentation, but after 24 h, the batches of dough fermented at 25 °C had a higher number, which remained more or less constant until the end of fermentation. In the case of sourdough samples fermented at 25 °C, the pH values started from an average of 6.31 and decreased to an average of 3.82, and in the case of sourdough fermented at 35 °C, the pH value varied from an average of 5.37 to 3.46. The optimal pH values for the dough samples fermented at the two experimental temperatures determined that the optimal fermentation period was 48 h. The acidification rate of the dough was faster at 35 °C than at 25 °C. This fact became evident via the pH values obtained in the first 24 h. However, from this point, the pH values were lower in the samples kept at 25 °C, showing that a cooler fermentation temperature allows the acidification activity of the microorganisms to be prolonged for a longer time.

An optimal frequency of backslopping promotes great diversity and fermentative activity. Intermediate fermentation times achieve a balance between pH and acid-tolerant microorganisms. The longer the fermentation time, the more acid-tolerant species of lactic acid bacteria (LAB) and yeasts will predominate. The ideal fermentation time allows both lactic acid bacteria and yeast to reach an optimal growth rate and cell density. In the study carried out, the ideal fermentation time for the population of LAB and yeasts is 72 h at a temperature of 25 °C, and the most productive sourdoughs were the dough with 100% Einkorn wheat flour and the dough obtained from the 1:1 combination of rye and corn

flours. A stable number of LAB was obtained in all sourdough variations during the tested period, but the yeast population presented a decrease after 24 h backslopping, possibly because of the competitiveness of the LAB compared to the yeast. LAB numbers increased in all sourdough samples at the end of fermentation for 96 h. The results obtained based on the LAB population are interesting due to the effect of different flours used. For example, the flour of Einkorn wheat and corn in combination with rye flour showed important stimulating proprieties in the development of the LAB population during the tested period (96 h), while a different pattern was observed in the case of rye and corn flour was the maximum number was reached after 72 h, after which there was a slow decline until 96 h.

Sourdoughs fermented at 25 °C showed an increase in volume characteristic of a good fermentation, all samples showed some level of bubbling, and all smelled distinct from each other. The characteristic smell was that of acid and earth and was pungent. Only the sample of 100% corn flour in combination with rye flour 50/50 had a pleasant, sweet smell. These two sourdoughs and the technological parameters (fermentation temperature of 25 °C for a duration of 72 h) presented the best results and were selected for future experiments that imply phenotypic identification of isolated strains of lactic acid bacteria from selected sourdoughs and highlighting of strains with biotechnological potential for the bakery industry.

5. Conclusions

Using sourdough in bread making brings value to the final products, and one of the main advantages is the rich microbiota that grows spontaneously during fermentation time and daily backslopping. The microbial populations of a sourdough during the initial propagation of a starter and during continued maintenance are unique based on the flour types used and technological factors. Based on the results obtained in this study, future research implies phenotypic identification of isolated strains of lactic acid bacteria from selected sourdoughs and highlighting of strains with biotechnological potential for the bakery industry, but also to connect microbial ecology to the sensory and technical qualities of sourdough products.

In conclusion, this study showed that the use of different flours, rations, recipes, and fermentation temperatures has an important influence on microbial population, thus obtaining an active food ingredient with high potential to improve the technological and nutritional properties of bakery products.

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