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The Influence of Germinated Hull-less Barley Sordough Fermentation Conditions on the Microbiota Development

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Abstract. Sourdough plays an important role in quality assurance, especially during developing wholegrain wheat bread products. The value of barley and hull-less barley grains is determined by the low lipid and high carbohydrate (starch and resistant starch) content, balanced amino acid profile, dietary fibre and phenolic compounds. During germination (24 h) the amount of sugar, amino acids and vitamins increase in the grains. Therefore, it could be optimal environment for microbiota growth in sourdough. The goal of the research was to find optimal technological parameters and to study microorganism growth dynamic during the fermentation of sourdough with germinated hull-less barley. The study was performed using three steps of fermentation, with different temperature and time parameters. At each step pH, dynamics of lactic acid bacteria (LAB), yeast-like fungi and total plate count (TPC) growth were determined using standard methods. The results of the study showed that the optimal fermentation conditions for the first stage are 48 h and 26 ± 1 °C, for the second stage 8 h and 26 ± 1 °C and for the third stage 20 h and 28 ± 1 °C. During fermentation of the sourdough, the pH 3.91 is reached, LAB 8.8 $\log_{10}$ CFU g^{-1}, and the yeast-like fungi 8.7 $\log_{10}$ CFU g^{-1} was determined. Obtained data show that more than 80 bacteria and 50 microscopic fungal species were detected in hull-less barley flour, but after germination their diversity decreased and in the germinated hull-less barley sourdoughs dominated species were *Pediococcus pentosaceus*.
Keywords: spontaneous sourdough, germinated hull less barley, lactic acid bacteria.

Introduction

Barley, as a cereal crop, ranks fourth after wheat, rice and corn. In recent years, barley cultivation and processing in the world has been gradually increased and in the years 2020/2021, it reached 159.74 million metric tons (Arendt, & Zannini, 2013, Giraldo, 2019, Shahbandeh, 2021). For food production, barley (*Hordeum vulgare*) and hull less barley (*Hordeum vulgare L.*) are used traditionally. Hull less barley have higher nutritional value – they contain more proteins, lipids and soluble dietary fibres (Pejcz *et al*., 2017). Barley grains contain about 65–68% starch, 8–17% protein, 2–3% lipids and 1.5–3% minerals. The total amount of dietary fibre in grains varies from 11 to 34%; amount of soluble fibre – from 3 to 20%, including 3.6–9% of β-glucans. Barley also contains significant amounts of vitamins, as B1 (thiamine), B2 (riboflavin), nicotinic acid, B6 (pyridoxine), pantothenic acid, biotin, and folic acid (Arendt, & Zannini, 2013, Sterna *et al*., 2015, Mariotti *et al*., 2014). Currently, there is a growing interest about barley as high nutrition ingredient for development of new products (Robles - Ramirez *et al*., 2020). Also, they are a source of phenolic compounds (Arendt, & Zannini, 2013).

Due to a higher protein and β-glucan content, hull-less barley has attracted attention for bread production and also for sourdough fermentation (Çakır, Arıcı, & Durak, 2020).

Research data demonstrate that germinated grains are healthy product, they improve metabolism, strengthen immunity, compensate vitamins and minerals, and normalize the acid-base balance when used in food (Punia, 2020). During germination, the activity of enzymes (α- and β-amylases) increases, proteolytic enzymes are activated. The results obtained by researcher Rakcejeva (2006) reported that the content of vitamin B2 in germinated hull-less barley increased by 88.9%, the niacin content increased 2.1 % in comparison with hull-less barley grains. Vitamin C was not identified in non-germinated hull-less barley grains, after the germination it was synthesized till 26.9 mg kg^{-1}. Vitamin E content increased 1.7 times after germination. The content of essential amino acids increased by 18.8%. The amount of fibre increased from 1.64 to 2.18 % (Rakcejeva, 2006).

The sourdough bread technology has become a traditional and potential worldwide (Sterna *et al.,* 2015). Sourdough is a complex dough system made of tap water and

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wholegrain flour, which contains complex fermentation ecosystem from enzymes and microorganisms as lactic acid bacteria (LAB) and yeast-like fungi (Abedfar, & Sadeghi, 2019, De Angelis *et al*., 2019). The most important factors influencing the quality of sourdough during fermentation are flour composition (protein, carbohydrate, minerals) and fermentation conditions (time and temperature) (Abedfar, & Sadeghi, 2019). Temperature is a vital parameter in sourdough fermentation, it affects LAB and yeast-like fungi growth and production of microbial metabolites. The optimum growth temperature of LAB is 32–37 °C, for yeast-like fungi 28–30 °C, which ensures efficient fermentation (Xu *et al*., 2020). According to a Greek scientist, fermentation is also influenced by the enzymes in the sourdough, the number of stages of sourdough renewal, or the degree of generation, and the fermentation time between sourdough renewal (Mantzourani *et al*., 2019).

Spontaneous sourdough in Latvia traditionally consists of three phases or steps, more often in their preparation rye and wheat flour are used. Studies show that preparing sourdough from germinated rye flour, the first phase temperature is 26 °C, time – 24 h and the flour water ratio is 1:2, the second phase temperature is 32 °C, time – 8 h and the flour, water and sourdough ratio is 1:1:1 and in the third phase temperature is 28 °C, time – 16 h and the ratio of flour, water and sourdough is 1:1:1 (Kozlinskis, & Skudra, 2011).

The study by Turkey scientists (Çakır, Arıcı, & Durak, 2020) describe the ecosystem of lactic acid bacteria isolated from spontaneously fermented hull-less barley sourdough. Nine species were identified as *Latilactobacillus curvatus*, *Levilactobacillus brevis*, *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum*, *Companilactobacillus musae*, *Companilactobacillus paralimentarius*, *Leuconostoc mesenteroides* and *Limosilactobacillus equigenerosi*. *Pediococcus* species were dominant in sourdough from barley. The study concludes that hull-less barley sourdough provide microbiota, which is important for the development of sourdough technology using biologically activated hull-less barley.

Also, scientists from Poland (Pejcz *et al.,* 2017) reported successful use of barley flour for sourdough preparation, which resulted in increased bread volume and taste, aroma and structure properties. The beneficial effect of adding barley to wheat bread was significantly increased by the fermentation of barley sourdough, which preserves valuable β-glucans. Barley sourdough also contributed to higher concentrations of fibre and specific starch polysaccharides. Similarly, a study on barley (Çakır, Arıcı, & Durak, 2020) reports that hull-less barley is a healthy cereal especially in bran and flour, thus increasing the nutritional value of the products. However, there are few studies on the use of germinated hull-less barley in sourdough preparation.

The goal of the research was to find optimal technological parameters and to study microorganism development dynamic during the three step sourdough fermentation with germinated hull-less barley.

Materials and Methods

Materials

Hull-less barley (variety Kornelija, harvested in 2020), obtained from Latvia Institute of Agricultural Resources and Economics, Stende Research Centre was used in the present study.

The research was carried out in the scientific laboratories of the Faculty of Food Technology of the Latvia University of Life Sciences and Technologies in 2020 – 2021.

The germination of the grains was performed in a climatic chamber (Memmert HPP110) at the temperature 35 ± 1 °C, and constant relative air humidity 80±2 % for24±1 h (Rakcejeva, 2006), in the Faculty of Food Technology. Germinated grains were dried in the Memmert universal oven (Memmert UF55) at the temperature 60±1 °C for4±1 h, until moisture of grain was reached till 12 ± 1 %. Grain flour for experiment was obtained by milling the hull-less barley grains and germinated and dried hull-less barley grains in a Hawos laboratory mill (Hawos Kornmühlen, GmbH, Germany). Flour was sieved through AS 200 sieves (Retsch GmbH, Germany), particle size 160–710 μm, among which 50% were about 450 μm.

Sourdough fermentation

The study was performed using three step of sourdough fermentation process, renewing the ripe sourdough twice, adding a new portion of water and flour (Table 1).

Table 1

Technological parameters of germinated hull-less barley and hull-less barley sourdough (control sample)

No.	Technological parameters	Germinated hull-less barley sourdough			Hull-less barley sourdough, control*		
		I step	II step	III step	I step	II step	III step
1.	Germinated hull-less barley flour, g	100	100	100	-	-	-
2.	Hull-less barley flour, g	-	-	-	100	100	100
3.	Ripe sourdough, g	-	100	100	-	100	100
4.	Tap water, ml	200	100	100	200	100	100
5.	Temperature °C	26	32	28	26	32	28
6.	Time, h	48	8	20	24	12	16

* Hull-less barley (variety Kornelija) sourdough was used as a control sample.

Sourdough was prepared in beaker with graduation and mixed with spoon. Fermentation was performed under controlled laboratory conditions using Constant climate chamber HPP110 (Memmert, Germany).

Organoleptic parameters and physicochemical analyses

Organoleptic parameters – colour (dark to light brown), aroma and taste (light sour to sour), volume, pore shape was determined in each step of sourdough fermentation.

pH determination was performed for all experimental sourdough samples during fermentation using the standard method AACC 02-52 using a pH meter Mettler Toledo (Giessen, Germany) at temperature 21 ± 1 °C.

Before measurements, the electrode (InLab Expert Pro, Mettler Toledo, Giessen, Germany) was calibrated using three point buffer solutions (pH7; pH4; pH10). pH determination for each sample was performed in triplicate.

Microbiological analyses

Test samples for microbiological analyses were taken in accordance with the standard LVS EN ISO 6887-1: 2017 "Microbiology of the food chain. Preparation of test samples, initial suspensions and decimal solutions for microbiological examination". Initial suspension of test samples was prepared by 10^3, 10^4, 10^5, 10^6 dilution ratios.

The following parameters have been determined:

1) Total plate count microorganisms (TPC), according to the standard LVS EN ISO 4833-1: 2013. Cultivation was performed using Plate Count Agar (Scharlau, Ref.01-161) medium, under anaerobic condition at temperature 30 ± 1 °C, 72h;

2) Lactic acid bacteria were cultivated on sterile Petri plates with MRS media (Scharlau, Ref.01-135) and incubated under anaerobic condition at temperature 37 ± 1 °C for 72h;

3) Yeast-like fungi (yeast) was cultivated on Petri plates and sterile Malt Extract Agar (Scharlau, Ref.01-111) medium was used. Incubation was realised under anaerobic condition at temperature 27 ± 1 °C for 48h.

Identification of microorganisms

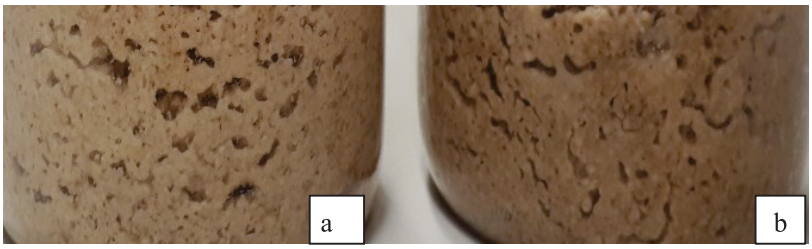
For DNA extraction, 5g of freeze-dried samples were weighted and placed in sterile 50-ml tubes under the laminar flow cabinet; the sterile hypotonic solution was added and cells were isolated using gradual centrifugation. After the cells collection, gDNA was extracted using ZR Quick DNA Fungal/Bacterial Miniprep Kit (Lot: ZRC206392). Concentration of DNA samples was measured using Qubit™ 3.0 fluorometer (Thermo Scientific). All samples were qualified for sequencing (OD 260/280>1.8 and 260/230>1.7) and used for library preparation according to the in–house sequencing protocol, with V4 (F515/R806) primer pair for 16S sequencing (Caporaso *et. al.*, 2011), and ITS1 (multiplex primers set, include LAB) (Koljalg *et al.,* 2013) and ITS 2 primers for fungi identification (Taylor *et al*., 2016). Sequencing was performed using iSeq (SN FS10000643, Illumina) and iSeq 100i1 Reagent kit v2by 2x150bp and dual index setup. ITS rRNA gene sequence data were analysed using QUUME2 platform.

Data analyses

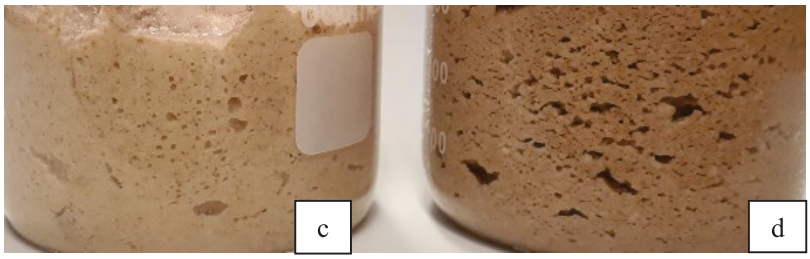
Mathematical data processing was performed using the computer program Microsoft Office 365 Excel. One-way analysis of variance ANOVA was used for data processing. Measurements were performed in triplicate, for which the standard deviation and means were calculated.

Results and Discussion

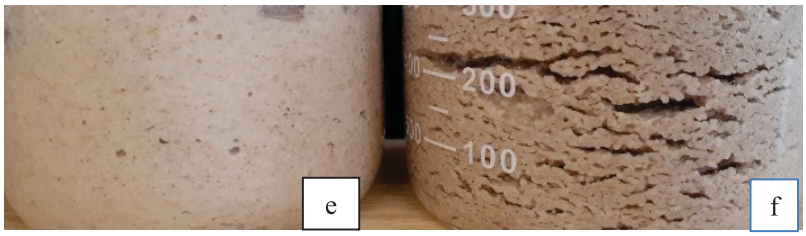
During germination, the amount of nutrients in grains changes, which is an excellent environment for the development of microorganisms during fermentation of sourdough. With the increase of reducing sugars, and biologically active compounds in the hull-less barley flour, the development process of microorganisms is expected to be different. Significant is to determine the optimal fermentation time and temperature in each step of germinated hull less barley spontaneous sourdough fermentation. Quality of sourdough characterizes pH – 3.5 – 4, amount of LAB (10^7 – 10^8 CFU g^{-1}) and yeast (10^6 – 10^7 CFU g^{-1}) (Gobbetti *et.al,* 2012). However, it is important not only to develop the number of microorganisms during fermentation, but also the diversity in order to achieve the diversity of the taste and aroma. Organoleptic characteristics of sourdough (colour, aroma, volume, pore size) show that there are visual differences between the control and germinated hull less barley sourdough in the fermentation time (Figure 1).



1. Hull-less barley sourdough after I step fermentation (24 h)
2. germinated hull-less barley sourdough after I step fermentation (48 h)



3. hull-less barley sourdough after II step fermentation (8 h)
4. germinated hull less barley sourdough after II step fermentation (8 h)



5. hull-less barley sourdough after III step fermentation (16 h)
6. germinated hull less barley sourdough after III step fermentation (20 h)

Figure 1. Hull-less barley and biologically activated hull-less barley sourdough after I, II and III steps of fermentation.

In the first step of fermentation, formation of air bubbles, which are irregular in shape, is observed (Figure 1, samples a and b). The formation of air bubbles indicates producing of CO_2. Germinated hull-less sourdough is darker comparing with hull-less barley sourdough. Both samples have sour taste and aroma, indicating the activity of lactic acid bacteria and yeast due to fermentation process.

In the second step of fermentation, germinated hull-less barley sourdough has pronounced air bubbles (Figure 1, samples c and d), the sourdough volume is larger that indicates a more active nature of yeast. The sourdough shows an acidic, fruity aroma and taste. Starting from glucose, homofermentative LAB mainly produce lactic acid from hexoces while heterofermentative LAB also produce, besides lactic acid, CO_2, acetic acid, and/or ethanol and also esters and aldehydes using hexoces and pentoses, thus enriching the taste and aroma bouquet in the sourdough (Reddy *et al.*, 2008).

In the third step (Figure 1, samples e and f) of fermentation, the samples volume is the same as at the first and second step; however, the germinated hull-less barley sourdough has more pronounced visual changes, the consistency is weaker comparing to the hull-less barley sourdough, which is more homogeneous, with round-shaped air bubbles. This can be explained by the active release of CO_2. Sourdough has a sour taste and aroma, which means that the activity of yeast and lactic acid bacteria continues.

In the study, the analysis of microorganism development dynamics was performed, to determine the technological parameters of germinated hull-less barley sourdough.

Step I

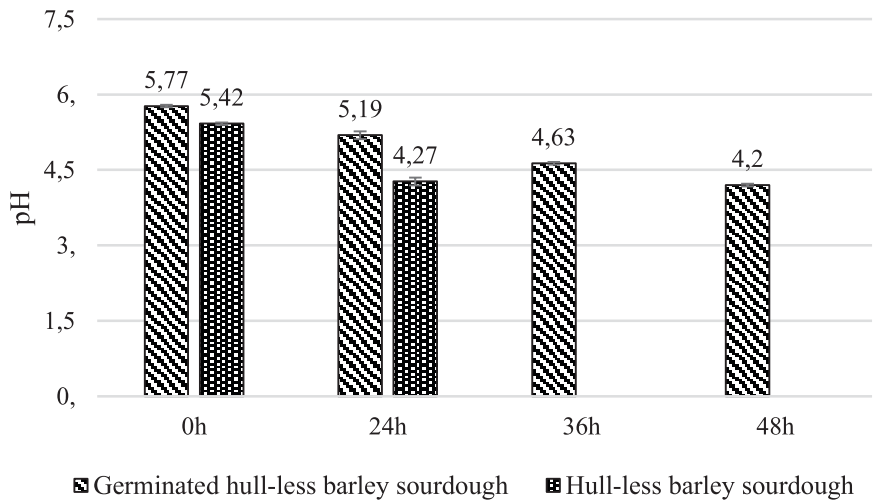


Figure 2. pH changes in germinated hull less barley and hull less barley sourdough fermentation in Step I.

Comparing the pH level changes (Figure 2) in the samples in step I, it can be concluded that the pH decreases in hull less barley sourdough from 5.42 to 4.27 after 24 h of fermentation, while in the germinated hull less barley sourdough the lowest point of pH is reached after 48 h of fermentation, 4.2 respectively. After 36 hours of fermentation it was 4.63. No significant differences ($p > 0.05$) were observed between the 36 h and 48 h fermented samples. Similar results have been reported in a study conducted by Arora *et al*. (2021). It was shown that depending on the type of flour and the method used, the average pH of the sourdough was 4.1 and the most common range was 3.4 to 4.9 (Arora *et al.,* 2021). Changes in pH can be explained by the development dynamics of the microorganisms, shown in Figure 3.

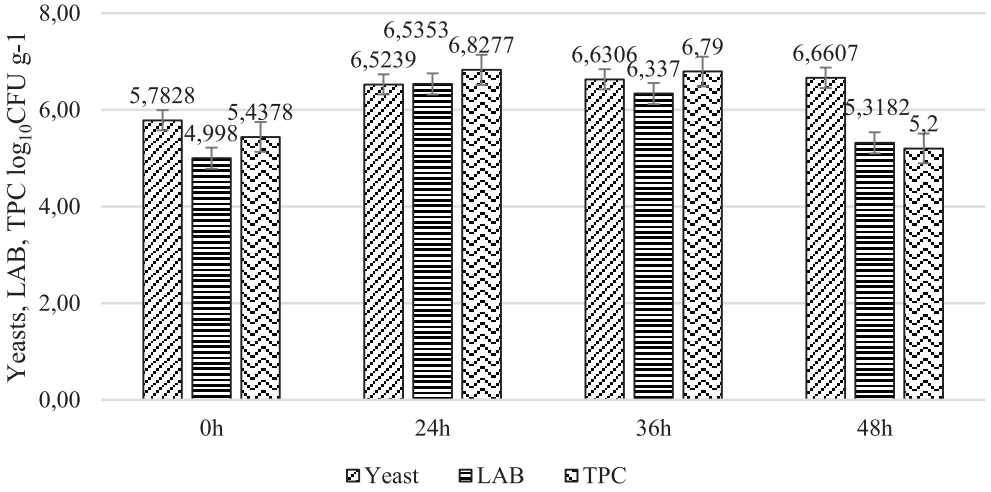


Figure 3. Yeast, LAB and TPC development dynamics in germinated hull-less barley sourdough fermentation. Step I.

In the first step, sourdough was fermented at 26 °C that is optimal for the yeast-like fungi development. As the number of yeast cells gradually increases, intensive alcoholic fermentation processes take place, resulting in an increase in the amount of ethyl spirits and the amount of CO_2. As a result of lactic acid bacteria, not only organic acids are formed but also pH is decreased . These metabolites reduce TPC and inhibit their development. The obtained results (Figure 3) show that during 48 h of fermentation the number of yeast cells increased till 6.66 ± 0.28 $\log_{10}$ CFU g^{-1}, while TPC decreased till 5.20 ± 0.22 $\log_{10}$ CFU g^{-1}. Changes in the control sample occurred after 24 hours of fermentation.

The development of dynamics of microorganisms in hull-less barley sourdough (Figure 4) in step I is lower, in comparison with germinated hull-less barley sourdough (Figure 3). Comparing the initial number of microorganisms in both samples, it can be seen that there are more of them in the germinated sourdough, which is related to the germinating, during which the microorganisms in the grain multiply.

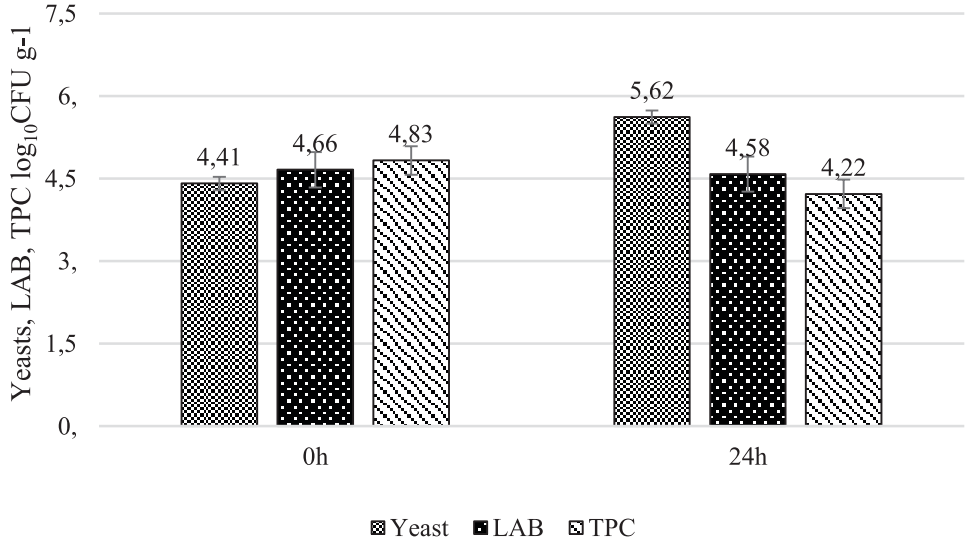


Figure 4. Yeast, LAB and TPC development dynamics in hull - less barley sourdough fermentation. Step I.

The obtained results show that the number of yeast cells increases to 5.62 ± 0.22 $\log_{10}$ CFU g^{-1} already in 24 h fermented samples and TPC decreases to 4.22 ± 0.34 $\log_{10}$ CFU g^{-1}. As a result of Step I, it is important to obtain a superiority of yeast and LAB over TPC, so that lactic acid fermentation processes can take place intensively during

further fermentation. The interaction between LAB and yeast is determined by environmental fluctuations that affect nutrient availability, organic acid synthesis, and pH decline.

The identification of microorganisms in the samples indicates that germinated hull-less barley flour and hull-less barley flour have different diversity of microorganisms (Figure 5).

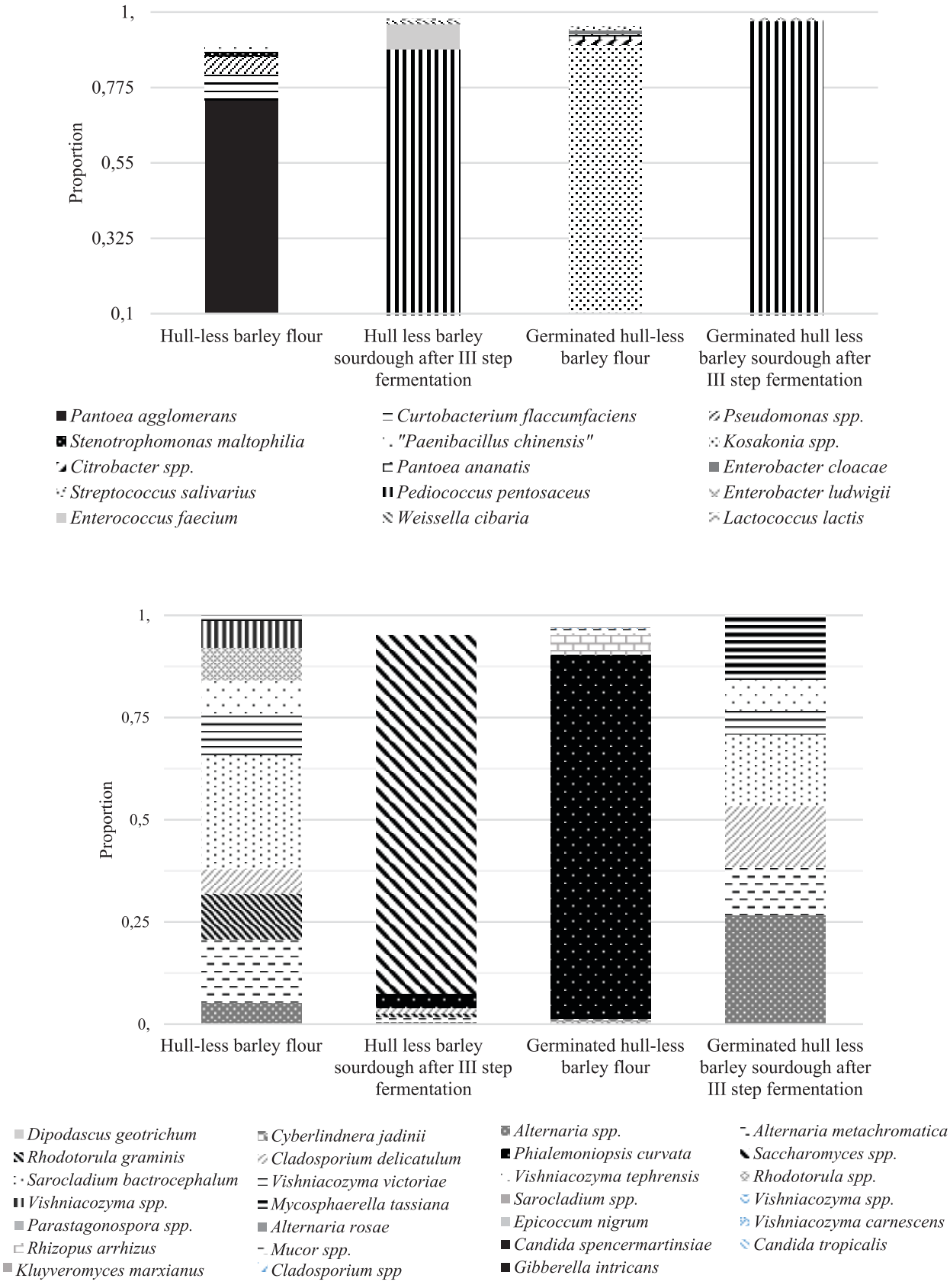


Figure 5. Composition of dominated microbiota in the flour and sourdough samples.

More than 80 bacterial and 50 microscopic fungal species were detected in hull-less barley flour, but after germination their diversity decreased in 70 bacterial and 10 microscopic fungal species, respectively *Pantoea agglomerans and Sarocladium bactrocephalum and Alternaria metachromatica* are predominant in the sample of hull-less barley flour. During the germination time, the diversity of dominated microorganisms' increases and as the dominant *Kosakonia cloacae*, *Pantoea agglomerans* cultures have also been identified. A study by scientists reported that the genus *Kosakonia* is part of the *Enterobacteria* complex most of which is isolated from plants and produces fucose-rich EPS with higher productivity and higher fucose content than previously reported in the studies (Roblero, *et al*., 2020). Diversity of fungal population in flour was also determined and *Phialemoniopsis curvata* is highly dominant. The highest yeast diversity has been identified in hulled barley flour, as it has not undergone any treatment that would reduce its amount, as is observed in germinated hulled barley sourdough. The predominant yeasts and mycelial fungi in the malting barley flour are *Alternaria* spp. and *Rhodotorula* spp. (Felšöciová *et al*., 2021). The predominant yeast species in germinated hulled barley flour are *Alternaria* spp. (26%), *Mycosphaerella tassiana* (24%), *Sarocladium bactrocephalum* (17%) and *Cladosporium* spp. (17%).

During under controlled three-step fermentation conditions, the microbiota of sourdough changes due to temperature and environmental conditions. Similar results obtained in the sourdough microbiota study (Kozlinskis & Skudra, 2011) shows that initially the yeast multiplies more actively, because the chosen fermentation temperature – 26 °C, is optimal for their development, which can also be seen in the results obtained in this study. Analysing the mutual indicators of LAB, yeast, TPC and pH, it was found that in step I the optimal fermentation time of germinated hull-less barley sourdough at 26 °C is 48 h.

Step II

Determining the optimal parameters for step II fermentation time pH, TPC, LAB and yeast changes dynamics considered during fermentation. To ensure the further fermentation process of sourdough, the growth of yeast -like fungi and LAB bacteria are essential. Active metabolism of LAB results in increased metabolites and decreased pH. The best results for germinated hull-less barley and hull-less barley sourdough during step II are observed for 8 h fermentation.

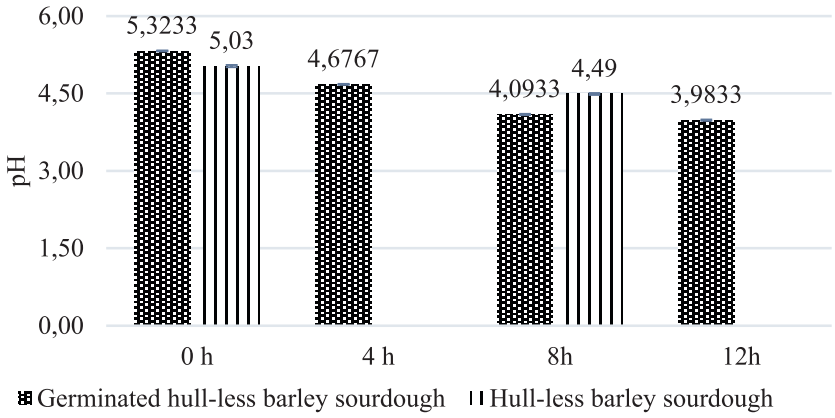


Figure 6. pH changes in germinated hull-less barley and hull-less barley sourdough fermentation Step II.

After sourdough refreshing (Figure 6), pH increased to the addition of fresh barley flour and water. After 8 h of step II fermentation germinated hull less barley sourdough pH = 4.09 as well as control sourdough pH reached 4.49. In this step, the LAB is still in the Lag phase and there is no rapid increase in the number of microorganisms; however, they are actively involved in metabolic processes and produce organic acid. No significant differences ($p > 0.05$) were observed between the 8 h and 12 h fermented samples; therefore, an 8 h fermented sourdough was selected in the following study.

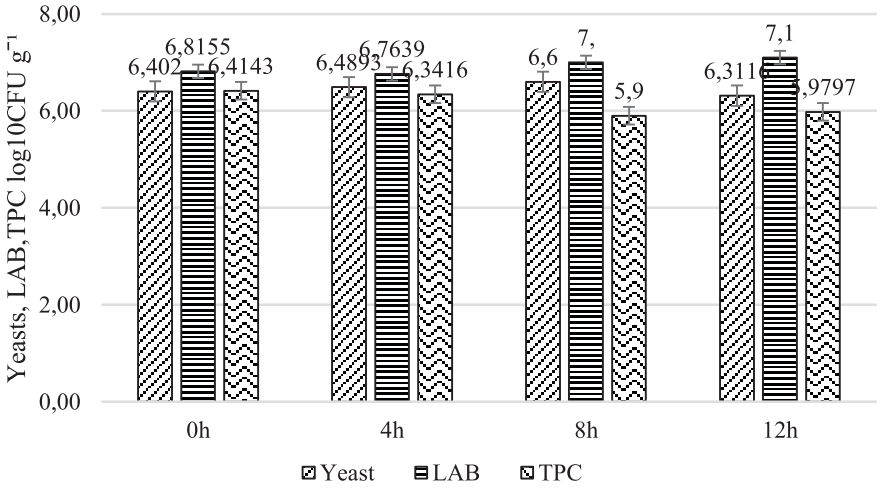


Figure 7. Yeast, LAB and TPC development dynamics in germinated hull-less barley sourdough fermentation Step II.

Analysing the development of microorganisms in step II (Figure 7), the maximum number of LAB is reached during eight-hour fermentation, 7.00 ± 0.14 $\log_{10}$ CFU g^{-1}. In turn, the number of TPC decreases from 6.41 ± 0.26 to 5.98 ± 0.21 $\log_{10}$ CFU g^{-1} throughout the fermentation, which could be explained by a decrease in pH to 4.49 during fermentation.

Yeast cell counts remain stable throughout step II fermentation. Similar results have been reported in a study (Kozlinskis & Skudra, 2011), where the number of yeasts of rye flour sourdough with added germinated rye grain after 48 hours (2 phase or step) of fermentation is 6.83 $\log_{10}$ CFU g^{-1}.

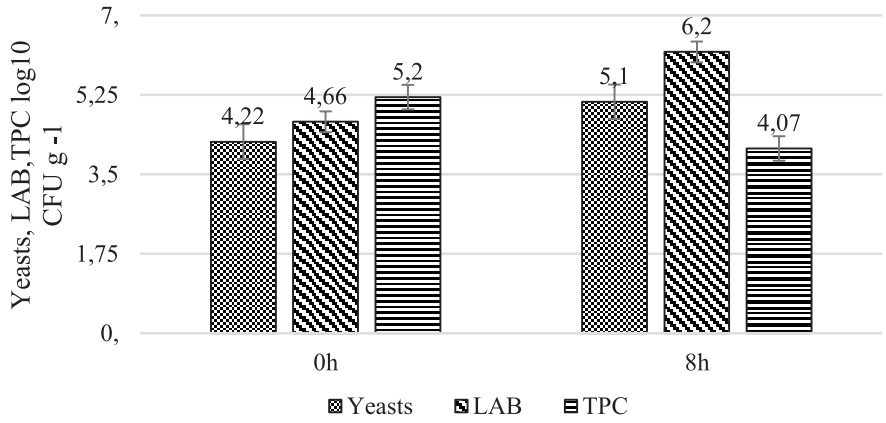


Figure 8. Yeast, LAB and TPC development dynamics in hull - less barley sourdough fermentation Step II.

The development dynamics of hull-less barley sourdough microorganisms (Figure 8) shows similar results as that of germinated hull-less barley sourdough. As can be seen from the obtained data, the amount of TPC significantly decreased ($p < 0.05$) from 5.20 ± 0.27 to 4.07 ± 0.22 $\log_{10}$ CFU g^{-1}, while the number of LAB and yeast increased from 4.66 ± 0.23 and 6.20 ± 0.19 $\log_{10}$ CFU g^{-1} to 4.22 ± 0.38 and 5.1 ± 0.29 $\log_{10}$ CFU g^{-1}, respectively.

Analysing the mutual indicators of LAB, yeast, TPC and pH, it was found that in step II the optimal fermentation time for germinated hull-less barley sourdough at 32 °C is 8 h.

Step III

pH of quality sourdough for bread making should be in the range 3.5 – 4 and LAB count in the sourdough should reach $10^7 – 10^8$ $\log_{10}$ CFU g^{-1} and yeast count - $10^6 – 10^7$ $\log_{10}$ CFU g^{-1}, respectively (Gobbetti. *et.al,* 2012, De Vuyst *et.al.,* 2016).

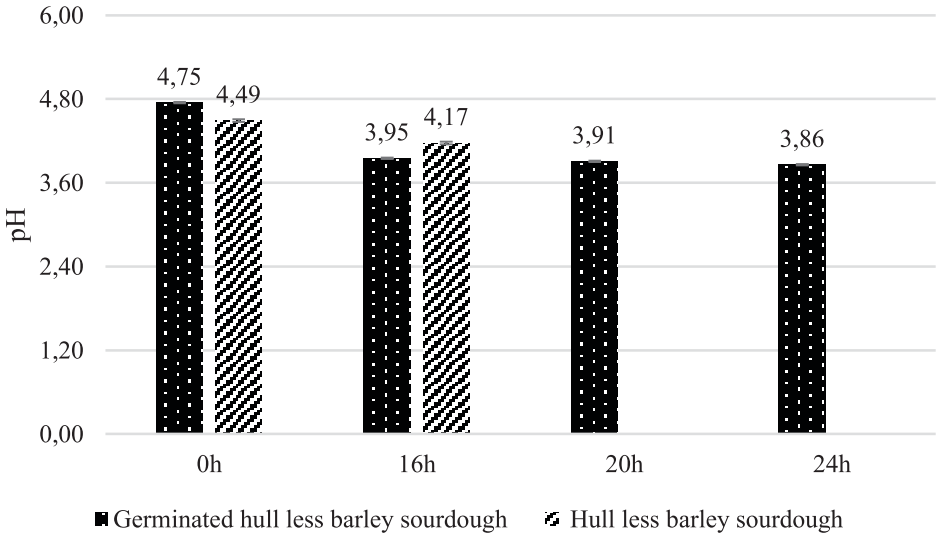


Figure 9. pH changes in germinated hull - less barley and hull - less barley sourdough fermentation Step III.

Analysing the pH level changes in the fermentation step III (Figure 9), it can be concluded that it decreases during the whole fermentation period. The optimal level of step III for germinated hull-less sourdough sample was reached in 16 h of fermentation; however, by analysing the dynamics of microorganism development in germinated hull-less barley sourdough (Figure 10), the optimal fermentation time was determined to be 20 h.

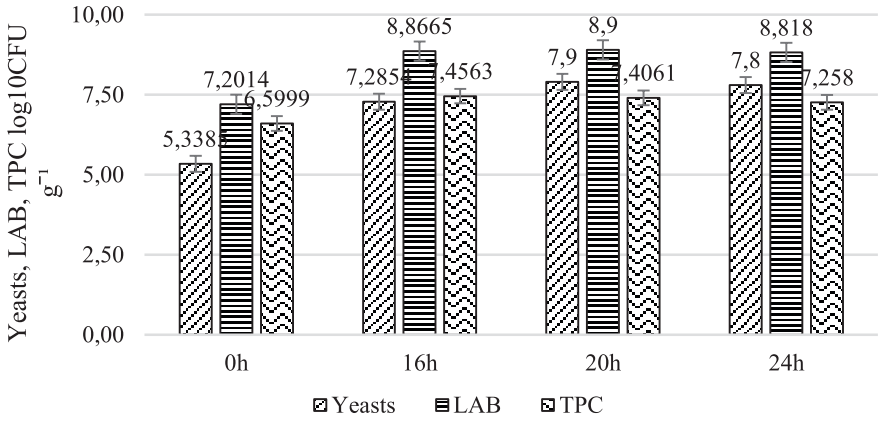


Figure 10. Yeast, LAB and TPC development dynamics in biologically activated hull-less barley sourdough fermentation Step III.

The obtained data show that (Figure 10) after sourdough refreshment, until the fermentation 20 h, the number of yeast and LAB increases. In the fermentation step III, the highest number of yeast and LAB was reached in 20 h of fermentation, respectively, yeast 7.90 ± 0.12 and LAB 8.90 ± 0.18 $\log_{10}$ CFU g^{-1}. Similar results were reported in a study by Austrian scientists (Fraberger *et al.,* 2020), where the number of LAB colonies during the fermentation of wheat flour sourdough was <5.00 to 9.59 $\log_{10}$ CFU g^{-1}, and the number of yeasts was lower, from <5.00 to 7.90 $\log_{10}$ CFU g^{-1}. In contrast, a scientific study conducted by Arora *et al*. (2021) indicated that the mean cell density observed after spontaneous sourdough fermentation of LAB and yeast was 8.5 and 6.5 $\log_{10}$ CFU g^{-1}, respectively. Similar results were observed in step III sourdough fermentation.

The control sample, the best results are achieved after 16 hours, but it is not optimal.

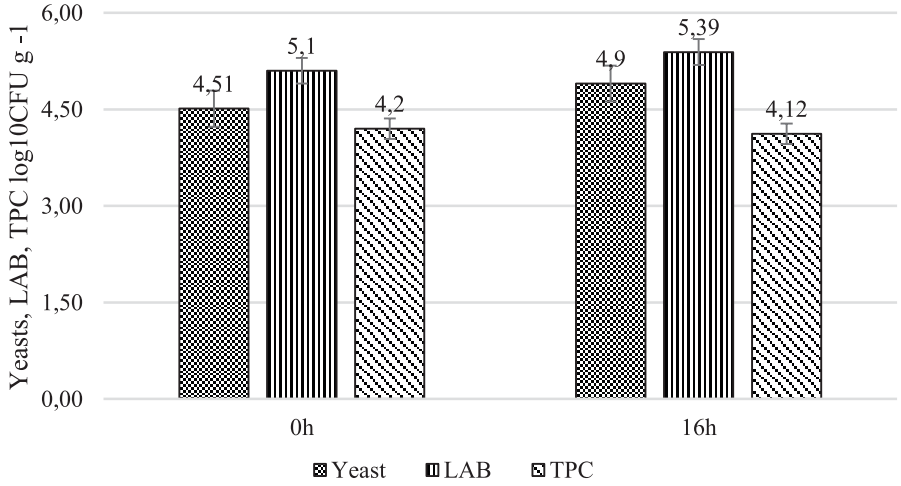


Figure 11. Yeast, LAB and TPC development dynamics in hull-less barley sourdough fermentation Step III.

For hull-less barley sourdough, the fermentation time in step III is 16 h. The results show (Figure 11) that the number of TPC decreases insignificantly ($p > 0.05$) from 4.20 ± 0.21 to 4.12 ± 0.23$\log_{10}$ CFU g^{-1}, while the number of LAB and yeast increases from 5.10 ± 0.18 to 5.39 ± 0.22 $\log_{10}$ CFU g^{-1} and 4.51 ± 0.26 to 4.90 ±0.34$\log_{10}$ CFU g^{-1}, respectively. Scientists Dertli and Yilmaz (2016) reported that the positive effects of sourdough are usually based on the metabolism of LAB, their activity ensures the formation of volatile, antibacterial and anti-fungal mould compounds.

Analysing the mutual indicators of LAB, yeast, TPC and pH in germinated hull-less barley sourdough, it was found, that in step III the optimal fermentation time at 28 °C is 20 h.

Less than 60 bacteria and fungi species were detected in the germinated hull-less barley sourdough. Obtained results of controlled fermentation show (Figure 5) that hull-less barley and germinated hull-less barley sourdoughs are dominated by LAB species *Pediococcus pentosaceus*, 82% and 94%, respectively. Similar data were obtained in a study by scientists (Çakır, Arıcı, & Durak, 2020), which aimed to characterize the biodiversity of LAB isolated from spontaneous hull-less barley sourdough and to determine its technological properties. According to the results of polymerase chain reaction (PCR), a group of scientists from

Turkey found nine different species detected in the sourdough samples of the study: *Pediococcus* spp. (dominant species). Also, scientists (Suo, Chen, & Wang, 2021) indicate that *Pediococcus pentosaceus* has been identified in various sourdough. *Pediococcus pentosaceus* has become increasingly attractive in fermentation of sourdough due to its ability to produce antioxidant exopolysaccharides, its antibacterial and antifungal properties, and its ability to reduce acrylamide content in baking. As can be seen, the predominant yeast species in hull-less barley sourdough is *Saccharomyces* spp. (87%). The scientific literature indicates that yeasts, including *Saccharomyces cerevisiae*, mainly promote the fermentation of sourdough (Gobbetti *et al.,* 2019). Similar data have been reported in a study indicating that *Kazachstania humilis* and *Saccharomyces cerevisiae* are the most common yeast species in sourdough (Wang *et al.*, 2020).

Conclusion

The optimal technological parameters of germinated hull-less barley sourdough, suitable for bread production, in the three-step fermentation are 48 h (26 °C), 8 h (32 °C), 20 h (28 °C).

The diversity of the microflora decreases during the germination of hull-less barley and during the preparation of sourdough. In hull-less barely flour up to 90 species, germinated hull-less barley flour up to 70 species of bacteria and fungi were detected. Germinated hull-less barley sourdough has a higher diversity of microorganisms, obtained less than 60 species, but in hull-less barley sourdough less than 55 species of bacteria and fungi. The predominant microorganisms in hull-less barley sourdough were *Pedicoccus pentosaceus* and *Saccharomyces* spp., but in germinated hull-less barley sourdough – 94% of *Pedicoccus pentosaceus* were established.

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