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ORIGINAL RESEARCH PAPER

QUALITY EVALUATION OF THE SOURDOUGH RYE BREADS

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Sourdough fermentation is a biotechnological process that has been reported to improve dough properties, to increase bread flavor and taste, to enhance nutritional value and to extend shelf life of sourdough bread. The quality of rye breads prepared with 20 and 40% sourdough, fermented with different mixed starter cultures was investigated in this study. The bread quality was evaluated in terms of specific volume, humidity, total titratable acidity, crumb characteristics and sensory profiles. Digital image analysis revealed that rye bread with 40% sourdough had a considerably denser crumb structure. Rye bread with 20% sourdough maintained superior texture characteristics over the storage period, while increasing the sourdough content to 40% had a negative effect on the texture. The sensory profiles of the bread highly depended on the type of starter cultures used for fermentation.

Keywords: sourdough, mixed starter cultures, lactic acid bacteria, bread quality, sensory profile

Introduction

Sourdough is a mixture of flour and water fermented by lactic acid bacteria (LAB); the levels of the LAB are usually higher than 5×10^8 cfu/g and the LAB:yeast ratio is generally 100:1 (Hansen, 2006). It has been previously shown that the use of sourdough can improve dough and bread properties by increasing flavor and taste of the bread and enhancing nutritional value through increasing mineral bioavailability, reducing the phytate content, lowering the postprandial glucose level and providing some exopolysaccharides with prebiotic behavior.

The positive effect of the sourdough on the bread quality resides on the specific metabolic activities of the LAB and yeast which are able to produce different organic acids, exopolysaccharides and enzymes (Arendt *et al.*, 2007) therefore extending the shelf life of sourdough bread (longer mold-free period, prevention of rope in bread, antistaling).

The interest in using sourdough for rye bread production increased considerably in the North, Central and Eastern European countries including the Baltic states, where rye bread is the most popular type of bread. The sourdough is essential in rye bread making (Hansen, 2006; Arendt *et al.*, 2007). One of the main functions of sourdough in rye bread making consists in the inactivation of α-amylase activity. Hansen et al. (2002) showed that the activity of α-amylase in the bread dough prepared with 20% sourdough was halved after resting (pH 4.5) compared to the initial activity in the flour. Moreover, the rye dough acidification is essential for achieving bread with suitable physical properties in terms of elasticity and extensibility and for conferring the acid flavor notes characteristic to the rye breads (Arendt *et al.*, 2007).

The sensory profile of the bread determined through descriptive analysis is mainly based on attributes like texture and flavor (Crowley *et al.*, 2002; Katina *et al.*, 2006). Type and amount of starter culture, ash content of flour, dough yield (DY, mass of dough/mass of flour x 100), temperature and time of fermentation, the amount of the sourdough added in the dough have been reported to influence bread flavor (Hansen and Schieberle, 2005; Hansen, 2006; Arendt *et al.*, 2007). In sourdough bread, LAB produce lower concentrations of volatile compounds than yeast; LAB are mainly responsible for dough acidification, while yeasts for the production of flavor compounds. Compared to the single starters containing *Lactobacillus brevis* and baker's yeast, the use of mixed starter cultures allow obtaining more aroma compounds and, as determined by bread sensory analyses, higher sugar-, acid- and bitter-type flavors (Meignen *et al.*, 2001). Moreover, the mixture of yeasts and LAB proved to be self-protecting and self-regulating (Plessas *et al.*, 2008).

According to Katina *et al.* (2006), the increase of the ash content and of the fermentation time enhanced the intensity of overall flavor, pungent flavor, roasted flavor and aftertaste. They also showed that the increase of fermentation temperature enhances the intensity of the pungent flavor in sourdough breads fermented with LAB and yeasts.

The nowadays developments in the field consist in engineering new starter cultures with improved sourdough fermentation properties, therefore leading to better bread quality. In addition, the starter culture must ensure easier control and economic viability of the sourdough making process (Plessas *et al.*, 2008).

The present study was designed (1) to determine the influence of different starter cultures on the rye sourdough and bread characteristics; and (2) to establish the influence of various amounts of sourdough on the quality of rye breads fermented with different associations of LAB and yeasts.

Materials and methods

Materials

Commercial whole rye flour (ash content of 1.64%, falling number of 250 s) retailed on the local market (Galati, Romania) was used in the study.

The inoculum used for preparing the rye sourdough consisted of four lactic acid bacteria (LAB) strains from MIUG collection, Faculty of Food Science and Engineering, Galati, Romania: *Lactococcus lactis* ssp. *lactis* (UGAL2), *Weissella confusa* (UGAL1), *Lactobacillus plantarum* (15GAL), *Lactobacillus brevis* (16GAL), and the commercial strains *Lactobacillus plantarum* and *Lactobacillus brevis* (DI-PROX MTTX), *Lactobacillus helveticus* (LH-B02) and *Kluyveromyces marxianus* subsp. *marxianus* (LAF-4).

Sourdough fermentation

Sourdoughs were prepared by well mixing in a large beaker the rye flour with tap water and inoculum to get the dough yield (DY, mass of dough/mass of four x 100) of 300. After covering with aluminum foil, the beakers were placed in an incubator at 37°C (40°C in case of the samples prepared with *Lactobacillus helveticus* and *Kluyveromyces marxianus* subsp. *marxianus*) for 20 hours. The control sourdough (DY 300), prepared without inoculum, was incubated for 20 hours at 37°C.
The inoculum size was 3-5 x 10^8 ufc/100 g dough in case of LAB from MIUG collection, while for the commercial strains, the producer is (EDR Ingredients Romania and Chr. Hansen) recommendations were followed. The leavener used for dough preparation was the compressed yeast *Saccharomyces cerevisiae* (9 g/kg dough), except for the samples with *L. helveticus* and *K. marxianus*.

Bread-making

The dough was prepared at 28ºC by mixing, in a laboratory mixing device, all ingredients: rye flour, water (total water in the recipe formulations was 550 ml), salt (2%), compressed yeast (1.5%), sourdough 20 and 40% (based on 1 kg flour). After fermentation at 28ºC for 30 min in a laboratory proofer, the dough was divided in two pieces which were molded and placed in baking trays. The proofed dough was obtained after a final leavening of 45 min; the trays were afterwards introduced into the oven. The samples were baked at 260ºC for 40 min (the steam tap was turned on 10-15 s before placing the samples into the oven).

pH and acidity

pH measurements were made according to Romanian standard methods 90/2007 by means of a Hanna digital pH-meter. The total titratable acidity (TTA) value was determined as the amount of 0.1 N NaOH solution (ml) used for neutralization of 10 g sample (Romanian standard methods 90/2007).

Biochemical analyses

The lactic acid and acetic acid were quantified by enzymatic analysis UV method (R-Biopharm, Boehringer Mannheim).

Rheological measurements

Sourdough samples were gently stirred before rheological analyses. Rheological measurements were carried out in triplicate using a controlled-stress rheometer (AR 2000, TA Instruments, New Castle, DE) attached with a computer control software (Rheology Advantage Data Analysis Program, TA, to New Castle, DE).

All steady-shear rheological measurements were done using a 40 mm 2° steel cone plate geometry and a gap of 1000 μm was used. For each test, approximately 2 g of sourdough sample was poured on the bottom plate of the rheometer.
The controlled shear-rate measurement technique was employed by progressively increasing the shear-rate from 0.1 up to 100s^{-1} and decreasing it to obtain viscosity vs. shear-rate data. All rheological measurements were carried out in triplicates at constant temperature of 20^{o}C.
The area under the upward (S_1) and downward (S_2) flow curves (shear stress vs. shear rate) were estimated through Rheology Advantage Data Analysis software v5.5.0 (TA Instruments Ltd.) and were used to calculate the thixotropy surface (ΔS) as the difference in area under the upward flow curve and the downward flow curve and the thixotropy index (T) as:

$$T = \left[\frac{S_1 - S_2}{S_1}\right] \cdot 100 \qquad (1)$$

Bread evaluation

Bread specific volume, humidity and total titratable acidity were assessed according to the Romanian standard methods 90/2007.
The porosity of the bread was estimated by analyzing the scanned images of the vertically halved breads by means of the Image J software (Data et al., 2007) that uses the contrast between the two phases (pores and solid part) in the image. The scanned color images were first converted to gray scale. Using bars of known lengths, pixel values were converted into distance units. The largest possible rectangular cross-section of the bread halves was cropped. After adjusting the threshold, the pores areas as fraction of total area were determined using the software.
Crumb firmness was measured at days 1 and 2 to assess the potential shelf-life of the breads. Bread crumb firmness during storage was determined as maximum compression force (40% compression, AACC method 74-09) using the texture profile analysis test (Texture Analyser). The measurements were carried out on two slices taken from the centre of the loaf. A crust penetration test was carried out on 10 mm deep and 25 mm wide crust pieces from the top of the loaf using the Stable Micro Systems blade set.
The sensory profiles of the bread samples were determined using the descriptive analysis. The most relevant descriptors for bread samples were considered: degree of the roasted flavor of the bread crust, intensity of pungent flavor, degree of fresh flavor, intensity of overall flavor and aftertaste of the bread crumb (Katina et al., 2006). The intensity of the attributes was rated on the scale from 0 (lowest intensity) to 10 (highest intensity). The descriptive panel consisted of ten trained assessors with proven skills. All sensory work was carried out at the sensory laboratory of Faculty of Food Science and Engineering (Galati). The panelists were particularly familiarized with the sensory descriptors and the attribute intensities.

Statistical analysis

Statistical analysis was carried out using the software Statistica 6.0 for Windows. All experiments were carried out in triplicate and the average values were reported. The analysis of variance (ANOVA) was performed between different bread types at each analysis time and within each bread type over the total storage time.

Results and discussion

Rye sourdough characterization

The pH of sourdough obtained through spontaneous fermentation was much higher (pH 4.88), compared to the fermentation with starter culture, when the pH varied between 3.56 and 3.88.

Analyzing the results in Table 1, one can see that the TTA of the sourdough, as well as the lactic and acetic acid contents, highly depended on the type of inoculum.

Table 1. pH, TTA values, lactic and acid acetic content of sourdough

Fermentation type	pH	TTA	Lactic acid, g/100 g whole flour	Acetic acid, g/100 g whole flour	Lactic acid/ Acetic acid
Control sample	4.88	9.89	1.11	0.97	1.15
LH-B02+ LAF-4	3.81	14.85	1.76	0.25	7.11
DI-PROX MTTX	3.81	13.86	1.61	0.31	5.17
15GAL+16GAL	3.75	15.41	1.79	0.39	4.55
UGAL1	3.56	16.14	1.89	0.32	5.82
UGAL2	3.88	12.55	1.46	0.34	4.34

* ml NaOH 0.1N/10 g dough

The TTA values of the sourdoughs obtained with starter cultures were much higher compared to the control sample (TTA of 9.89 and pH of 4.88). The highest and lowest acidities were found in case of the sourdough fermented with UGAL1 (TTA of 16.14 and pH of 3.56) and UGAL2 (TTA of 12.55 and pH of 3.88), respectively. The most important compounds responsible for pH decrease are lactic and acetic acids. As it is produced through homo- and heterolactic fermentation by LAB, the lactic acid content was always reported to be higher compared to the acetic acid (Vuyst and Neysens, 2005).

The lactic acid content in the sourdough decreased in the order UGAL1 > LH-B02+ LAF-4 > 15GAL+16GAL > DI-PROX MTTX > UGAL2 > Control, in agreement with the TTA values.

Even if *Lactococcus lactis* is a homofermentative LAB, it generated rather small quantities of lactic acid in case of rye sourdough fermentation, only 1.47 times higher compared to the control sample (1.11 g/100 g whole flour). *Lactococcus lactis* strains are mainly used in dairy industry; the suitability for sourdough fermentation was showed previously by Petrov *et al.* (2008), who isolated from spontaneously fermented rye sourdough *L. lactis* subsp. *lactis* B84 capable of full hydrolysis of starch and its conversion into lactic acid. The *Lactococcus lactis* strain (UGAL2) used in our study was isolated from rye epiphyte microbiota (Barbu *et al*., 2010).

The concentration of acetic acid in the rye sourdoughs fermented with starter cultures, ranged from 0.25 to 0.39 g/100 g whole flour. The acetic acid contents in the sourdough were in this order: Control > 15GAL+16GAL > UGAL1 > DI-PROX MTTX > UGAL2 > LH-B02+ LAF-4.

The lactic and acetic acid contents from sourdough, as well as their ratio, are considered important factors in the overall flavor perception during bread consumption.

The use of mixed starter cultures containing the *Kluyveromyces marxianus* yeast (LH-B02+LAF-4) allows obtaining sourdough with significantly higher lactic acid/acetic acid ratio (7.11) compared to the other investigated starter cultures with ratios ranging from 4.34 to 5.82. Both *Lactobacillus helveticus* and *Kluyveromyces marxianus* have been identified in the natural sourdough microflora, but there are only few available experimental results concerning the controlled use of these microorganisms for sourdough bread making (Plessas *et al*., 2008a,b). *L. helveticus* can adapt to different growth conditions, is able to utilize various carbohydrates and have a high ability to produce lactic acid. *K. marxianus* has been successfully used in bread making as baker's yeast (Dimitrellou *et al*., 2009).

In order to study the influence of starter culture on rheological properties of the rye sourdough, shear thinning tests were carried out by varying the shear rate from 0.1 to 100 s^{-1}. Analyzing the shear stress- and viscosity-shear rate relationships for different sourdough samples (Figure 1a and b), one can see the control sample is more viscous compared to the samples fermented with starter cultures. In case of the sourdoughs prepared with starter cultures, the differences in terms of viscosity are more obvious for lower shear rates (<20 s^{-1}).

In all studied cases, the sourdough samples displayed specific behavior to pseudo-plastic fluids, with time-dependent structural viscosity. The viscosity – shear rate rheogram presented in Figure 1b shows that sourdough samples exhibit shear thinning behavior characterized by viscosity decrease when shear rate increases. The apparent viscosity decreased to a constant value, when the destruction rate of the structure matches the reformation rate of the aggregates (Ionescu *et al*., 2008).

The rheological properties of the sourdoughs were recorded at increasing and decreasing shear rates (upward and downward flow curve) allowing to identify the hysteresis loop. The thixotropic behavior of each sample was quantified through the thixotropy index estimated with Eq (1).

The differences within thixotropic index of the samples depend on the metabolic activity of the starter culture used for sourdough fermentation (Table 2) and reflect the energy needed to destroy the structure responsible for flow time dependence (Gonzalez-Toma *et al*., 2008). The highest thixotropy index (12.24%) was found in case of sourdough fermented with *Lactobacillus helveticus* (LH-B02) and *Kluyveromyces marxianus* subsp. *marxianus* (LAF-4). The control sample had the highest values of the shear stress for the entire shear rates domain (Figure 1a), and was characterized by a fast reconstruction of the complex structure when decreasing the shear rate (thixotropy index of 6.42%).

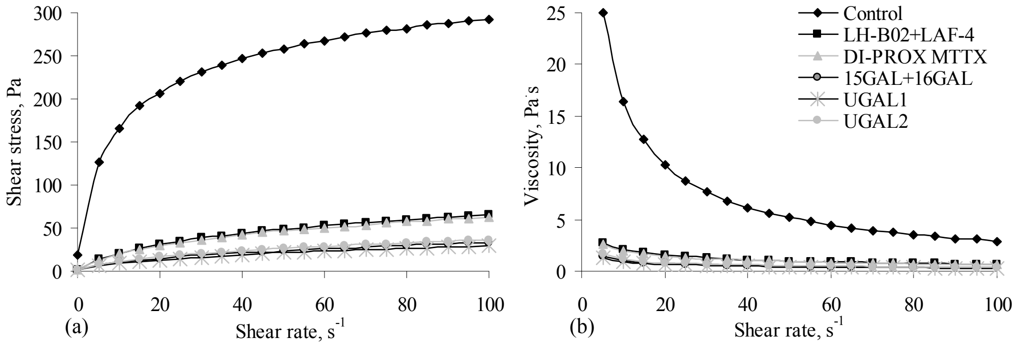

Figure 1. The influence of starter cultures on rheological behavior of sourdoughs. a) Shear stress vs shear rate rheogram b) Apparent viscosity vs shear rate rheogram

Table 2. Thixotropy index of the sourdough samples

Fermentation type	T, %
Control sample	6.42
LH-B02+ LAF-4	12.24
DI-PROX MTTX	11.05
15GAL+16GAL	8.55
UGAL1	10.07
UGAL2	9.78

Rye bread characterization

The increase of the sourdough level in the bread formulation from 20% to 40%, caused the increase of bread acidity. The highest TTA values were obtained in case of breads prepared with UGAL1, and the lowest value in case of control samples (Table 3).

According to our results, various additions of sourdough influence the quality characteristics of the rye bread. In all studied cases, the specific volume of the bread samples containing 20% sourdough was higher compared to the samples with 40% sourdough (Table 3). The highest specific volume was obtained in case of DI-PROX MTTX (1.58 cm^3/100 g). Changes of the specific volume of the bread containing different levels of sourdough were reported by Crowley *et al.* (2002), and the optimum level was linked with reduced staling of the product. A slight increase of the crumb moisture was observed in breads containing 40% sourdough compared to breads containing 20% sourdough (Table 3).

Digital image analysis revealed a significantly higher cell-to-total area ratio in crumb of the control sample compared to the sourdough bread fermented with starter cultures (Table 4).

Table 3. Physico-chemical characteristics of the rye bread containing 20 and 40% sourdough

Fermentation type	TTA		Crumb moisture, %		Specific volume, cm^3/100 g	
	20%	40%	20%	40%	20%	40%
Control sample	5.11	6.08	46.01	46.52	1.37	1.35
LH-B02+ LAF-4	5.21	6.26	45.22	45.62	1.53	1.41
DI-PROX MTTX	5.35	6.30	45.44	45.78	1.58	1.42
15GAL+16GAL	5.49	6.46	45.44	46.15	1.52	1.38
UGAL1	5.74	6.52	45.78	46.29	1.51	1.39
UGAL2	5.19	6.18	45.77	46.02	1.44	1.33

The crumb of the bread samples containing 40% sourdough had lower cell-to-total area ratios compared to with the samples containing 20% sourdough. These results suggest that the breads containing 40% sourdough have considerably denser crumb structure.

Table 4. Cell-to-total area ratio of the crumb rye bread containing 20 and 40% sourdough

Fermentation type	Cell-to-total area ratio	
	20%	40%
Control sample	1.159	0.955
LH-B02+ LAF-4	0.841	0.532
DI-PROX MTTX	0.923	0.646
15GAL+16GAL	0.781	0.519
UGAL1	0.732	0.592
UGAL2	0.429	0.399

The evolution of the rye bread during storage at room temperature for 48 hours was estimated in terms of crumb firmness. After 2 hours of storage no significant differences could be noted within bread samples prepared with 20% and 40% sourdough (Figure 2).

In all studied cases, the firmness increases during storage and the crumb became harder. Bread containing 20% sourdough maintained superior texture characteristics over the storage period, the crumb firmness varying with the starter culture used for fermentation (Figure 2a). The increase of the sourdough content to 40% had a negative effect on bread texture, no significant differences could be seen within samples (Figure 2b). The crumb firmness of the rye bread, after 48 h of storage ranged from 1300.13 to 1740.85 g in case of the samples containing 20% sourdough and from 1739.63 to 1905.11 g in case to the samples with 40% sourdough.

Descriptive analysis was used to determine the sensory profiles of the rye sourdough breads. Sensory attributes related of bread crumb were considered (Table 5).

Depending on the metabolic profile, the type of LAB can influence the production of certain volatile compounds in sourdough and bread. The highest intensity of aftertaste of bread crumb was obtained in the case of rye bread with DI-PROX MTTX and 15GAL+16GAL. Our previous results (Banu *et al.*, 2010) on whole rye flour sourdough fermented with the same starter cultures as in the present study, indicated the highest concentration of total phenolic content in the case of the samples with DI-PROX MTTX, and 15GAL+16GAL (590.8 and 457.8 mg ferulic acid/100 g d.w., respectively). Similar observations are provided by Liukkonen *et al.* (2003) who considered that the development of intense aftertaste and bitter flavor attributes in rye bread can be explained by the increased level of free phenolic compounds in sourdough fermented with mixed culture (LAB and yeast).

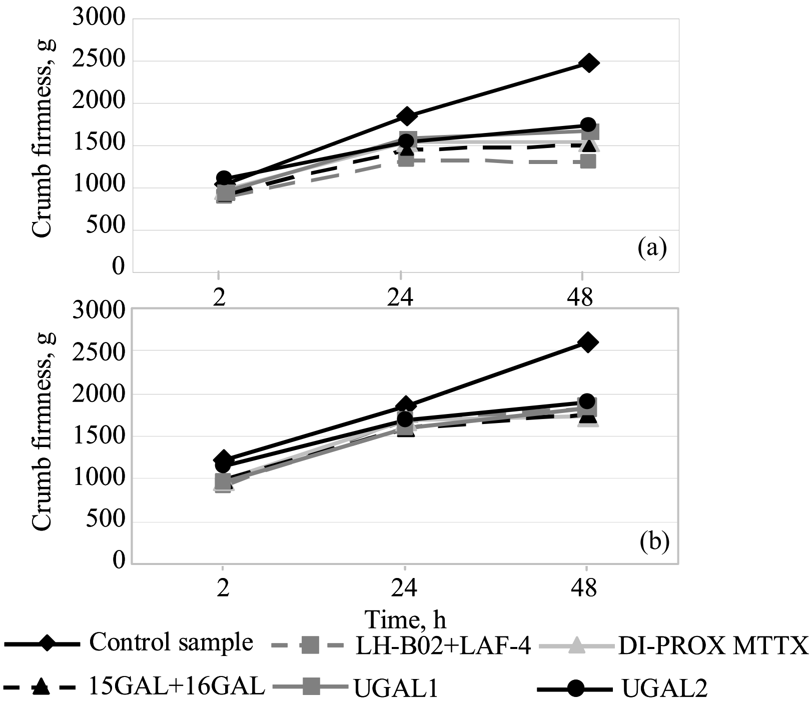

Figure 2. Evolution of crumb firmness values during storage of the rye bread containing 20% (a) and 40% (b) sourdough

Compared to the bread samples prepared with starter cultures, the intensity of pungent flavor of the bread crumb was higher and the degree of fresh flavor of bread crumb was lower in control samples. Within the samples fermented with pure starter cultures, the highest intensity of pungent flavor of bread crumb, and the lowest degree of fresh flavor were found in bread made with DI-PROX MTTX, 15GAL+16GAL and UGAL1 (Table 5), probably due to the higher content of acetic acid in sourdough (Table 1).

The production of ethyl acetate and hexyl acetate is higher in rye sourdoughs fermented with heterofermentative LAB compared to sourdoughs fermented with homofermentative LAB, whereas the content of aldehydes (e.g. hexanal) and diacetyl are higher in rye sourdoughs fermented with homofermentative LAB compared to heterofermentative LAB (Hansen and Schieberle, 2005). In our study the intensity of overall flavor of bread crumb was higher in the case of bread made with DI-PROX MTTX and 15GAL+16GAL, while the lowest score was obtained in the case of bread with UGAL1 (Table 5).

Table 5. Sensory profiles of the rye bread containing 20% (a) and 40% (b) sourdough

Fermentation type	Degree of roasted flavor of bread crust	Intensity of pungent flavor of bread crumb	Degree of fresh flavor of bread crumb	Intensity of overall flavor of bread crumb	Intensity of aftertaste of bread crumb
(a)					
Control sample	5	8	4	4	4
LH-B02+ LAF-4	7	3	9	9	7
DI-PROX MTTX	9	4	7	10	9
15GAL+16GAL	8	4	7	10	8
UGAL1	7	4	7	9	6
UGAL2	7	3	9	8	6
(b)					
Control sample	6	9	4	3	5
LH-B02+ LAF-4	7	3	9	8	7
DI-PROX MTTX	9	5	7	9	9
15GAL+16GAL	8	4	7	9	9
UGAL1	7	4	7	8	7
UGAL2	7	3	9	7	7

Our observations comply with the results of Hansen and Hansen (1994), who showed that bread made from sourdough fermented with the heterofermentative LAB had a pleasant, mild, sour odor and taste, whereas bread made from sourdough fermented with the homofermentative LAB had an unpleasant metallic sour taste. But, when the sourdough was supplemented with the sourdough yeast *S. cerevisiae*, the bread received a more aromatic flavor. Sourdough made with mixed starter cultures had a higher content of alcohols (ethanol, methylpropanol, 2- and 3-methylbutanol), esters (ethyl acetate and diacetyl) and some carbonyl compounds as compared to sourdoughs without added yeast (Hansen and Hansen, 1994).

The degree of roasted flavor had the same trend as the intensity of aftertaste and the intensity of overall flavor of bread crumb. Thus, the degree of roasted flavor was higher in the case of bread made with DI-PROX MTTX and 15GAL+16GAL (Table 5).

The proteolysis has a very significant role during sourdough fermentation in enhancing subsequent bread flavor (Katina *et al.*, 2006). Proteolytic activity of whole rye flour is, generally, very intense and the proteolysis leads to the

accumulation of amino acids into the sourdough. Amino acids (e.g. leucine and proline) are well-known flavor precursors in yeast fermentation during dough proofing and in the Maillard reaction during baking (Thiele *et al*., 2002).
According to Katina *et al.* (2004) the degree of roasted flavor was slightly more intense in sourdough fermented with *Lb. brevis* and correlated highly with the level of proline. In the case of the sourdough fermented with *Lb. plantarum*, the roasted flavor correlated with formation of ornithine and with reduced level of arginine in sourdoughs, which may indicate the ability of this strain to produce ornithine from arginine.

Conclusions

The amounts of sourdough used for rye bread preparation influenced the quality of the final products. The bread containing 40% sourdough had inferior quality compared to the bread containing 20% sourdough. Bread prepared with 20% sourdough maintained superior texture characteristics over the storage period, while the increasing of the sourdough content to 40% had a negative effect on crumb firmness. The overall sensory profiles of the bread samples were influenced by the type of starter cultures used for sourdough fermentation.

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