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EFFECT OF SOURDOUGH ON THE FERMENTATION OF DOUGH PIECES AND QUALITY OF BREAD MADE WITH RYE FLOUR

Dragutin Djukic[1*], Milorad Radović[2], Leka Mandic[1], Slavica Veskovic Moracanin[3]

[1]Faculty of Agronomy Cacak, University of Kragujevac, cara Dusana 34, 32000 Cacak, Serbia
[2]Branko Perišić Town Bakery, Kruševac, Jasički put nn, 37000 Kruševac, Serbia
[3]Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Serbia

[*]e-mail: lekamg@kg.ac.rs

Abstract

Flour quality, flour type and particularly the fermentation step in dough preparation are among major factors that contribute to high quality bread-making. Short fermentation steps have some advantages. However, they generally produce bread that has a short shelf life and is markedly crumbly and almost neutral in aroma and flavour. Therefore, a recent tendency in bread making has been to allow long fermentation when working with sourdough which is used in bread dough and bread making. The objective of this study was to analyse the effect of sourdough on the fermentation of dough pieces and quality of bread made with a mixture of rye and wheat flours (TYPE 500 wheat flour and whole-grain rye flour - 60 : 40) and determine the advantages of sourdough over the direct bread dough mixing method.

Three bread dough mixing methods were used: I - indirect mixed rye/wheat bread making method using flour steaming (including the yeast dough preparation step - steaming with warm water and resting for about 90 minutes, mixing and fermentation of yeast dough for 4 hours, and the bread dough preparation stage – mixing the yeast dough with flour and other components); II - indirect mixed rye/wheat bread making method without flour steaming (sourdough preparation without flour steaming); III - direct mixed rye/wheat bread making (directly without sourdough - mixing all the components at once). The study involved monitoring of the following: microbial characteristics of flour and dough (total counts of bacteria, yeasts and lactic acid bacteria) and of bread (presence of *Enterobacteriaceae*, yeasts and moulds); dough yield; chemical properties of dough and bread (pH and degree of acidity); organoleptic characteristics of bread (volume, porosity according Dallman, elasticity of the medium, pore uniformity, pore structure fineness, crust colour and glossiness, physical properties of the crust, bread crumb total score, external appearance, crumb appearance, odour and flavour of both the crust and the crumb) and bread yield.

Results show the highest counts of lactic acid bacteria and yeasts in the indirect bread dough mixing method using rye flour steaming. Mixed rye/wheat bread made with sourdough had a mild sourish flavour, an intense aroma, a prolonged shelf life and reduced crumbliness. This bread making method prevented the growth of the causal agents of ropiness and mouldiness in bread, thus prolonging its shelf life. The bread made using the direct method was better only in terms of bread volume.

The study suggests that the technological process of mixed rye/wheat bread making with sourdough is an important requirement in improving bread quality and assortment and can be used in any bakery facility.

***Key words**: Fermentation, Bread, Sourdough, Quality, Bread dough mixing.*

1. Introduction

The high proportional contribution of bread to the human diet in the Republic of Serbia (satisfying over 50% of energy requirements) necessitates that particular attention be given to bread quality considerations. Moreover, bread as an extremely important commodity is subject to daily assessment by consumers as the largest and most competent jury. Therefore, the technology used in making this bakery product is receiving increasing attention. The main characteristics of the technology of bread making in the bakery industry are short fermentation steps that significantly increase bread volume. Unfortunately, the newly developed, faster bread making procedures have resulted in quite the opposite - producing bread that has an increased staling rate, a markedly crumbly texture and an almost neutral flavour and aroma.

The consumer expects a distinct flavour and aroma from bread, giving less importance to bread volume. Therefore, the further development of the technology of bread making using sourdough to confer specific flavour and other improved organoleptic properties to bread is gaining importance. Research is largely based on sourdough preparation using starter cultures of yeasts and lactic acid bacteria (Arendt *et al*. [1], Clarke *et al*. [2], Vogel *et al*. [3], Corsetti *et al*. [4]). In this way, fermentation and sourdough preparation processes become faster and goal-directed.

Enhanced bread flavour and aroma are essentially the result of the components obtained during dough fermentation and bread baking (Hansen and Hansen [5], Rehman *et al*. [6]). In addition, lactic acid bacteria and yeasts in sourdough produce a number of metabolites that have a positive effect on its texture and freshness, prevent the growth of pathogenic microorganisms and inactivate toxic compounds in the dough. In this respect, particular importance is given to organic acids, exopolysaccharides and enzymes which, among other things, can be used as alternatives to bread additives (Leroi and De Vuist [7], Ravits and De Vuist [8], Petrulakova *et al*. [9]; Cagno *et al*. [10]). However, the use of sourdough in making bread from wheat flour and, in particular, rye flour does not always lead to expected improvements in bread quality; hence, special focus should be placed on further development of the technological procedure employed in the preparation, storage and use of sourdough (Lund *et al*. [11], Rehman *et al*. [6]).

The objective of this study was to examine the effect of sourdough on the fermentation of dough pieces and quality of bread made with a mixture of wheat and rye flours, and determine the advantages and disadvantages of using sourdough in rye/wheat bread making over the straight dough process.

2. Materials and Methods

Rye/wheat bread was made with Type 500 wheat flour (moisture - 13.4%, ash - 0.486%, acidity level - 1.8, water absorption capacity - 55.7%, quality number - 59.1) and whole grain rye flour (moisture – 11.07%, acidity level - 3.88, ash content on a dry matter basis - 1.79%) at a ratio of 60 : 40% (/w/w).

Three bread dough mixing methods (including the use of sourdough) were employed:

I - Indirect rye/wheat bread dough mixing method using flour scalding;

II - Indirect rye/wheat bread dough mixing method without flour scalding;

III - Straight rye/wheat bread dough mixing method (direct mixing of all ingredients at one time).

The bread dough mixing procedure is outlined in Table 1.

Table 1. Bread dough mixing procedure and stages (I- indirect bread dough mixing method with flour scalding, II - indirect bread dough mixing method without flour scalding, III - straight bread dough mixing method)

Bread dough mixing method	Stages/steps and bread dough preparation method
I	***Sourdough preparation*** *Steps:* - rye flour scalding: 2 kg of wholegrain rye flour was immersed in 2.3 L water at a scalding temperature of 70 ^{0}C and mixed until incorporated. The temperature of the scalded flour after mixing was 48 ^{0}C. The dough was allowed to rest for 90 minutes, with the temperature decreasing to 30-32 ^{0}C thereafter; - yeast dough mixing and fermentation: the scalded flour was mixed with 100 g malt flour (Progress Company, Novi Sad, Republic of Serbia) and 50 g compressed baker's yeast using a spiral mixer (MATING-200, Niš, Republic of Serbia), for 3 minutes at 105 rpm, followed by mixing for 5 minutes at 250 rpm. Yeast dough temperature was constantly checked and kept at 30 ^{0}C. Dough fermentation time was about 4 hours. ***Bread dough preparation*** *Steps:* - bread dough mixing: the yeast dough was mixed with 3 kg Type 500 wheat flour, 100 g salt, 20 g Pob Digo additive (PIP Novi Sad, Republic of Serbia) and 800 mL water. The ingredients were mixed for 3 minutes at 105 rpm and for 5 minutes at 250 rpm. Then, the dough was allowed to rest for 30 minutes; - re-kneading - (1 minute); - dividing into 590 g pieces; - rounding; - final moulding; - proving in a proving chamber (at a temperature of 30^{o}C and relative humidity of 70 - 80%) for 90 minutes - baking (for 45 minutes at 250 ^{0}C) and cooling.

II	***Sourdough preparation:*** - yeast dough mixing and fermentation: 2 kg rye flour was mixed with 100 g malt flour and 50 g fresh compressed yeast. The ingredients were mixed in a spiral mixer for 5 minutes at 105 rpm with the addition of 2.2 L water (44 ^{0}C), and then for about 8 minutes at 250 rpm. Yeast dough fermentation time was about 4 hours. ***Bread dough preparation:*** as in bread dough mixing method I.
III	***Bread dough preparation*** *Steps:* - straight bread dough method of mixing all ingredients at one time without the sourdough pre-step: Type 500 wheat flour (3 kg), wholegrain rye flour (2 kg), fresh baker's yeast (125 g), malt flour (100 g), table salt (100 g), additive (20 g) and water (2.9 L). The ingredients were mixed in a spiral mixer for 5 minutes at 105 rpm and, then, for about 8 minutes at 250 rpm. The other steps were the same as in the preceding two bread dough mixing methods.

The following dough and bread characteristics were evaluated:

- microbial properties of flour and bread dough, yield and chemical properties of bread dough,

- microbial properties of bread and major sensory attributes of bread.

Microbial properties of flour and bread dough were determined at the Laboratory of Microbiology, Faculty of Agronomy, Čačak. The analysis involved determination of the counts of yeasts and lactic acid bacteria using selective culture media. Yeast and lactic acid bacteria counts were determined on Sabouraud dextrose agar (Torlak, Belgrade) and MRS agar (Torlak, Belgrade), respectively. Microbial counts were expressed in terms of Colony Forming Units per gram (CFU/g) of the test sample. The significance of differences in microbial counts was assessed by the Lsd test (Statsoft.Inc [12]).

Bread dough acidity (pH) was determined by a pH metre upon fermentation (just before baking), and pH of the bread was measured by a potentiometer. The acidity level (AL) of dough and bread (Kaludjerski and Filipovic [13]) was calculated according to the formula: $AL = A \times C \times 100/M$, where A - the number of millilitres of 0.1 mol/L of NaOH solution consumed, C - amount concentration of the NaOH solution (mL/L), M - mass of sample in 25 mL extract (1/2 of the measured bulk sample).

Microbial properties of bread were determined at the Institute of Public Health, Kruševac. The standardised methods ISO 21528-2:2009 and ISO 21527-2:2011 were used to quantify *Enterobacteriaceae*, and yeasts and moulds, respectively.

Sensory (organoleptic) attributes of the bread were evaluated after its cooling (8 hours after removal from the oven), with 10 loaves evaluated per trait. The evaluation method was defined by the Regulation on the Quality of Grains, Mill Product, Bakery Products, Pasta and Quick Frozen Doughs (Official Gazette of the [14]). The following properties were evaluated: bread volume, porosity according to Dollman, bread crumb score (BCS) on a 7-point scale, crumb elasticity, pore uniformity, pore structure fineness, crust colour and glossiness, physical properties of the crust, external appearance, crumb appearance, and aroma and flavour of both crust and crumb on a 5-point scale.

3. Results and Discussion

3.1 Results

Yeast and lactic acid bacteria counts in the rye flour were within the expected limits reported in the literature (Table 2). Mixing and fermentation significantly increased these counts, particularly in the indirect dough mixing method using flour scalding (bread dough mixing method I). Differences in yeast counts between the dough mixed indirectly without flour scalding (method II) and the straight mixed dough (method III) were not statistically significant. In contrast, lactic acid bacteria counts were significantly lower in the straight dough process compared to the other two dough mixing methods.

Table 2. Yeast and lactic acid bacteria counts in samples of bread dough (bread dough mixing methods I, II, III) and rye flour (see Table 1 for a detailed description of bread dough mixing methods)

Bread dough mixing method/flour	Yeasts, $^{a)}$CFU/g	Lactic acid bacteria, CFU/g
I	4×10^8 a	17×10^3 a
II	14×10^7 b	3×10^3 b
III	9×10^7 b	6×10^2 c
Rye flour	9×10^3 c	2×10^2 d
ANOVA	**	**

Values followed by different small letters within columns are significantly different ($P \geq 0.05$) according to the LSD test; F test significant at $P < 0.01$.

$^{a)}$ CFU - Colony Forming Unit.

The acidity level and pH of dough are indicators of the fermentation activity of lactic acid bacteria and yeasts, and they play a significant role in determining the sensory properties of bread. The results of the present study show that indirect bread dough mixing with flour scalding gave the lowest pH and the highest acidity level of dough, which can be associated with the increased lactic acid bacteria counts in this test. The values of these parameters in the bread are consistent with those in the bread dough (Table 3).

Table 3. Effect of rye/wheat bread production method (bread dough mixing methods I, II, III) on the pH and acidity level of bread dough and bread (see Table 1 for a detailed description of bread dough mixing methods)

	Bread dough mixing method		
	I	II	III
dough			
pH	4.89	5.08	5.50
Acidity level	4.56	3.92	3.10
bread			
pH	6.19	6.28	6.55
Acidity level	3.50	3.10	2.40

The values for the indicators of the microbial safety of the breads produced (counts of *Enterobacteriaceae*, yeasts and moulds) were within the allowable range (Table 4).

Table 4. Counts of microorganisms as indicators of the microbial safety of bread, depending on the type of production (dough mixing method I, II, III) (see Table 1 for a detailed description of dough mixing methods)

Bread dough mixing method	Microorganisms	Measured value, [a] CFU/g	Reference values, CFU/g
I	*Enterobacteriaceae*	< 10	$10 - 10^2$
	Yeasts and moulds	< 10	$10 - 10^2$
II	Enterobacteriaceae	< 10	$10 - 10^2$
	Yeasts and moulds	< 10	$10 - 10^2$
II	Enterobacteriaceae	< 10	$10 - 10^2$
	Yeasts and moulds	< 10	$10 - 10^2$

[a] CFU - Colony Forming Unit.

The use of sourdough in rye bread making is aimed at improving the sensory attributes and freshness of bread. The sensory evaluation of breads made by different dough mixing methods is given in Table 5. The results show that breads made by the indirect dough mixing method had a dark rose, shiny and visually appealing crust. The loaves of breads from all dough mixing methods had an irregular shape. The crust of straight mixed breads cracked on both sides of the loaf, whereas the indirect dough mixing methods resulted in no visible cracks in the bread. Bread volume was the highest in the straight dough mixing method (Table 6) and the lowest in the indirect dough mixing method with flour scalding. As regards crust thickness as an important bread quality characteristic, the straight dough process gave the thickest crust, in contrast to the desirably thin elastic crust obtained in the bread made by the indirect dough mixing method using flour scalding. Wall and pore structure fineness differed across bread production methods. The bread produced by the straight dough method had an extremely rough structure, whereas the bread made in the indirect dough process involving flour scalding had the most favourable pore and wall structure in the cross section.

The analysis of bread scores reveals that the bread produced by the straight dough method was superior only in terms of volume, whereas higher quality in terms of the other properties was exhibited by the bread from the indirect dough process involving flour scalding.

Table 5. Sensory scores (porosity according to Dollman on a 7-point scale, the other properties on a 5-point scale) for the rye/wheat bread made by different dough mixing methods (I, II, III) (see Table 1 for a detailed description of dough mixing methods)

Property	Dough mixing method		
	I	II	III
Porosity according to Dollman	6.3	5.9	5.2
External appearance	4.4	3.5	2.2
Crumb appearance	4.2	4.1	2.6
Aroma of crust and crumb	5.0	5.0	3.0
Flavour of crust and crumb	5.0	5.0	3.0

The quality of bread with respect to retaining freshness is assessed by bread crumb scoring (BCS). Points given for this property 8 hours after baking (Table 6) suggest that indirect dough mixing methods received considerably higher scores. Another evaluation of bread freshness was performed 48 hours after baking. The results confirmed the scores of the initial assessment, showing advantages of using sourdough in rye/wheat bread making (Table 6). The breads made by the indirect dough mixing process 48 hours after baking retained freshness, thus achieving high crumb scores. The bread made by the straight dough method also showed visual signs of staling, notably crumbly texture, loss of freshness, noticeable crust dryness and cracks in the crumb.

Table 6. Effect of different rye/wheat bread making methods (dough mixing methods I, II, III) on the volume and organoleptic attributes of the crumb (BCS on a 7-point scale, the other properties assessed on a 5-point scale) 8 and 48 hours after baking (see Table 1 for a detailed description of dough mixing methods)

Dough mixing method	Bread volume (cm³)	Organoleptic assessment of bread crumb					
		8 h after baking			48 h after baking		
		Elasticity	Pore fineness	[a] BCS	Elasticity	Pore fineness	[a] BCS
I	2 093.38	4.1	3.7	5.3	1.2	0.6	4.3
II	2 185.33	3.8	3.2	4.7	0.9	0.5	3.7
III	2 352.98	2.4	1.9	2.9	0.5	0.3	2.1

Bread appearance after 48 h	
I	Low volume, underdeveloped crumb, non-crumbly texture, pore size evenness, small-sized pores, well-defined aroma.
II	Slightly reduced volume, well-developed crumb, non-crumbly texture with uniform small pores, well-defined aroma.
III	High volume, satisfactory elasticity of the crumb, crumbly texture, distinct flavour of crumb and crust.

[a] Bread Crumb Score.

3.2 Discussion

Bread dough production relies on fermentation processes conducted by lactic acid bacteria and yeasts originating from flour or starter cultures incorporated into the dough (Vogel *et al*. [3], Arendt *et al*. [1]). The counts of lactic acid bacteria and yeasts in flour are dependent on flour type, agroenvironmental conditions during the grain production stage and grain storage. Stolz [15] and Kline and Sugihara [16] reported counts of about 2×10^3 CFU/g for yeasts and about 2×10^2 CFU/g for lactic acid bacteria, as also confirmed by the results of the present study. Given the fact that natural populations include yeasts that have poor fermentation ability, yeasts should be added to flour during the dough preparation stage (Corsetti *et al*. [17], Galli *et al*. [18]). The abrupt increase in the counts of yeasts after fermentation in the dough produced by the indirect mixing method with or without flour scalding indicates their high metabolic activity and ability to degrade sugars, primarily glucose and fructose, which is in agreement with the results of Gobbetti *et al*. [19]. The significantly higher counts of lactic acid bacteria in the indirect dough method involving flour scalding, compared to the other two dough mixing methods, can be attributed to more favourable ecophysiological conditions during the preparation of dough using scalded flour. Flour scalding as a pre-fermentation method leads to a significant increase in the hydrolytic activity of amylases and other enzymes. This ensures an increase in the activity of lactic acid bacteria, as reported previously by Stolz *et al*. [20] and Hammes *et al*. [21]). Consequently, there is an improvement in lactic acid production and an increase in total acidity or a decrease in pH of the dough. The somewhat lower than reported total acidity of the dough in the present study is due to the activation of natural strains of lactic acid bacteria, whose metabolism is lower than that of commonly used selected strains (Ehrmann *et al*. [22]). The strains of selected yeasts used in this study may compete with natural strains of lactic acid bacteria for the same substrate, thus causing a reduction in both the fermentation capacity of these bacteria and lactic acid production, which is in agreement with the results of Ottogalli *et al*. [23] and Gobbetti and Corsetti [24]. Brandt [25] reported that a decrease in dough pH leads to a decrease in a-amylase activity, with undegraded starch binding all the moisture from the dough, thus preventing the crumb from becoming soggy, particularly in rye breads. Increasing acidity induces peptisation and swelling of rye flour proteins, thus increasing the consistency and, hence, air- holding capacity of the dough (Korakli *et al*. [26]). Rehman *et al*. [6] also stressed the importance of increasing acidity in inactivating undesirable microorganisms in the dough and bread, which prevents bread spoilage.

The rye/wheat bread produced by the indirect method of mixing with sourdough showed improvement in sensory properties and freshness. The results comply with the findings of Clarce *et al*. [2] who reported an important contribution of sourdough to bread-making, particularly in terms of volume, aroma and flavour. Bread flavour is associated with the ratio of lactic acid to acetic acid produced during fermentation. Good aromatic properties come from volatile compounds, primarily aldehydes, alcohols, ethers, ketones, etc. (Thiele *et al*. [27], Katina *et al*. [28], Loponen *et al*. [29]). Martinez - Anaia [30] and Gobbetti *et al*. [31] lay stress upon the important role of proteolytic enzymes synthetised by lactic acid bacteria in creating free amino acids as precursors of good flavour and rheological attributes of bread (Thiele *et al*. [32]). The improvement in the volume, texture and shelf life of the sourdough type bread is, among other things, associated with the increased production of exopolysaccharides by lactic acid bacteria (Korakli *et al*. [33], Tieking and Ganzle

[34]). Sourdough pre-fermentation control (degree of acidification) is the starting point in obtaining a high bread volume, as found by Barber *et al*. [35] and as confirmed by the results of the present study.

4. Conclusions

- The sourdough-type rye/wheat bread had a range of advantages over the bread made by the straight dough process. The most favourable fermentation-related characteristics of the bread dough expressed through the counts of lactic acid bacteria and yeasts, acidity level and pH were found in the dough produced by the indirect method involving rye flour scalding. The sensory analysis of the bread samples shows that the sourdough-type rye/wheat bread is characterised by mild sourish flavour, intense aroma and prolonged freshness. Pore structure fineness and crumb elasticity were better in sourdough-type breads than in the bread made by the straight dough process. The improvement of these properties helped sourdough-type breads become less crumbly in texture. The bread resulting from the straight dough process was superior only in terms of volume.

- This study suggests that the technological process of rye/wheat bread making using sourdough is an important requirement in improving bread quality and assortment and can be used in any bakery facility.

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