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EFFECT OF BREAD DOUGH MIXING METHOD ON RYE BREAD QUALITY

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The objective of this study was to evaluate the effect of sourdough (indirect bread dough mixing method) on the quality of rye/wheat bread (TYPE 500 wheat flour and whole grain rye flour – 60:40) and determine its advantages over the straight dough method.

Three bread dough mixing methods were used: I – indirect bread dough mixing using flour scalding; II – indirect bread dough mixing without flour scalding; III – straight dough mixing. The study involved the monitoring of the following: microbial characterristics of the flour and dough (yeasts and lactic acid bacteria) and of the bread (presence of Enterobacteriaceae, yeasts and moulds); chemical properties of the dough and the bread (pH and degree of acidity); organoleptic attributes of bread (volume, porosity according to Dallman, crumb elasticity, pore structure fineness, bread crumb score, external appearance, crumb appearance, flavour of both the crust and the crumb).

The results showed the highest counts of lactic acid bacteria and yeasts in the indirect bread dough mixing method using rye flour scalding. The rye/wheat bread made with sourdough had a mild sourish flavour, an intense aroma, a prolonged shelf life, and reduced crumbliness. The study suggests that the technological process of sourdough-type rye/wheat bread making is an important requirement in improving bread quality and assortment that can be used in any bakery facility.

KEY WORDS: fermentation, bread, sourdough, quality, bread dough mixing

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 should be given to bread quality. 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 bread making technology in the bakery industry are

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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, further development of bread making methods 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 (LAB) (1-4). 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 the dough fermentation and bread baking (5, 6). In addition, LAB and yeasts in the 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 (7-10). 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 (6, 11).

The objective of this study was to evaluate the effect of sourdough (indirect bread dough mixing method) on the quality of rye/wheat bread (TYPE 500 wheat flour and whole grain rye flour – 60:40) and determine its advantages over the straight dough method.

EXPERIMENTAL

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 using flour scalding; II-indirect rye/wheat bread dough mixing without flour scalding; III-straight rye/wheat bread dough mixing (direct mixing of all ingredients at one time). The bread dough mixing procedure is outlined in Table 1.

The following dough and bread characteristics were evaluated: a) microbial properties of flour and bread dough, yield and chemical properties of bread dough, and b) microbial properties of bread and major sensory attributes of the bread.

Microbial properties of flour and bread dough were determined at the Laboratory of Microbiology, Faculty of Agronomy, Čačak. The analysis involved the determination of the counts of yeasts and LAB using selective culture media. Samples of flour and dough (25 g) were each transferred to a sterile stomacher bag. Then, 225 mL of saline peptone water (8 g/L of NaCl, 1 g/L of bacteriological peptone, Torlak, Belgrade) was added and mixed for 3 minutes in the stomacher. Further decimal dilutions with the same diluents

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were made and the following analyses were carried out on duplicate agar plates: (i) counts of yeasts on Sabouraud-dextrose agar (Torlak, Belgrade) incubated under aerobic conditions for 72 h at 25°C, and (ii), LAB count on MRS agar (Torlak, Belgrade), incubated in a double layer for 48 h at 30°C.

Table 1. Bread dough mixing procedure and stages

Method	Stages/steps and bread dough preparation method
I	**Sourdough preparation** *Steps:* ***Rye flour scalding***: Two kg of wholegrain rye flour was immersed in 2.3 L of water at a scalding temperature of 70ºC and mixed until incorporated. The temperature of the scalded flour after mixing was 48ºC. The dough was allowed to rest for 90 minutes, with the temperature decreasing to 30-32ºC 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 (MAT-ING-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°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 30ºC and relative humidity of 70-80%) for 90 minutes; baking (for 45 minutes at 250ºC) and cooling.
II	**Sourdough preparation** *Steps:* ***Yeast dough mixing and fermentation***: Two kg of 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 of water (44ºC), 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) 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.

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 (12).

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

Microbial properties of the bread were determined at the Institute of Public Health, Kruševac. The standardised methods SRPS ISO 21528-2:2009 (36) and SRPS ISO 21527-2:2011 (37) were used to quantify *Enterobacteriaceae* and yeasts and moulds, respectively.

Detection and enumeration of *Enterobacteriacea*. A portion of the bread (25 g) was added to Buffered Peptone Water, BPW, (Oxoid, UK) at a ratio of 1:9 and mixed for 30 s in the stomacher. For direct enumeration, the pour plate method (SRPS ISO 21528-2: 2009) was used, with one millilitre sample volume (in duplicate) in Violet Red Bile Glucose agar, VRBG, (Oxoid, UK), incubated in a double layer at 37°C for 24 h. Confirmation of *Enterobacteriaceae* was done using negative oxidase reaction and glucose fermentation.

Detection and enumeration of yeasts and moulds. A 25 g sample was transferred to a sterile stomacher bag and 225 mL of saline-peptone water was added and mixed for 30 seconds in the stomacher. Dichloran 18% Glycerol agar, DG18, (Oxoid, UK) was used as a medium for the enumeration of osmophilic yeasts and xerophilic moulds in food and animal products. Decimal dilutions of the sample (with saline-peptone water) were made and one millilitre sample volume (in duplicate) was transferred to the agar plates. The detection and enumeration of yeasts and moulds were performed by the colony count technique at 25°C ± 1°C for 72 h (SRPS ISO 21527-2:2011).

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

The organoleptic evaluation in this study was performed by 5 evaluators well qualified in bread making technology and characteristics and qualitative attributes of different types of bread.

The bread volume was determined by a tape measure; it is the product of the value of the loaf circumference along the long axis and that along the short axis. Porosity according to the Dallmann scale (score) was obtained by comparing the bread crumb with Dallmann's classification photographs. The crumb elasticity was estimated by monitoring the ability of the crumb to return to its initial position after light pressure to the crumb was applied by both thumbs. Pore structure fineness was assessed by monitoring pore wall thickness and through the impression gained by moving finger tips along the surface of the cross-section. The bread crumb score (BCS) is the sum of the crumb elasticity and the pore structure fineness scores. The external appearance of the bread was evaluated by observing the shape and colour of the bread, the shiny appearance of the crust, and bubble and crack formation. The crumb appearance was evaluated after carefully cutting the bread. The crumb colour and uniformity, and the link between the crust and the crumb were observed under a source of light. The crumb sogginess and presence of salt or flour lumps were determined by moving the fingers along the surface of the cross-section. The crust aroma and crumb aroma were measured separately, with the scores depending on the aroma intensity. The crust and crumb flavour was determined by chewing. The crust flavour, crumb flavour and the crust and crumb flavour combined were evaluated separately.

RESULTS AND DISCUSSION

The yeast and LAB counts in the rye flour were within the expected limits reported in the literature (Table 2). The mixing and fermentation significantly increased these counts, particularly in the indirect dough mixing method using flour scalding (bread dough mixing method I). The 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, the LAB 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 the samples of bread dough and rye flour

Bread dough mixing method/flour	**Yeasts**, CFU/g [a)]	**LAB**, 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	**	**

[a)] Colony Forming Unit per gram;
Values followed by different small letters within columns are significantly different (P<0.05) according to the LSD test; **F test significant at P<0.01.

The acidity level and pH of dough are indicators of the fermentation activity of LAB and yeasts, and they play a significant role in determining the sensory properties of bread. The results of the present study show that the indirect bread dough mixing with flour scalding gave the lowest pH and the highest acidity level of the dough, which can be associated with the increased LAB 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 on the pH and acidity level of the bread dough and bread

	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 the bread prepared by different bread dough mixing methods

Bread dough mixing method	Microorganisms	Count (CFU/g)	Reference count (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	$10 - 10^2$
III	*Enterobacteriaceae*	< 10	$10 - 10^2$
	Yeasts and moulds	< 10	$10 - 10^2$

The use of sourdough in rye bread making is aimed at improving the sensory attributes and freshness of the bread. The sensory evaluation of the breads made by different dough mixing methods is given in Table 5. The results show that the breads made by the indirect dough mixing method had a dark rose, shiny and visually appealing crust. The loaves of the 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. The 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. The 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 for the rye/wheat bread made by different bread dough mixing methods

Organoleptic characteristics	Bread dough mixing method		
	I	II	III
Porosity according to Dollman	7	6	5
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 the bread with respect to retaining freshness was assessed by bread crumb scoring (BCS). The points given for this property 8 hours after baking (Table 6) suggest that the 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, notable crumbly texture, loss of freshness, noticeable crust dryness and cracks in the crumb.

Table 6. Effect of different rye/wheat bread making methods on the volume and organoleptic attributes of the crumb 8 and 48 hours after baking

Method	Bread volume (cm^3)	Organoleptic assessment of bread crumb[a)]					
		8 h after baking			48 h after baking		
		Elasticity	Pore fineness	BCS[b)]	Elasticity	Pore fineness	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 (organoleptic characteristics)							
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 the crumb and crust.						

a) BCS on a 7-point scale, the other properties assessed on a 5-point scale;

b) Bread Crumb Score.

Bread dough production relies on the fermentation processes induced by LAB and yeasts originating from flour or starter cultures incorporated into the dough (1, 3). The counts of LAB and yeasts in flour are dependent on the flour type, agroenvironmental conditions during the grain production stage, and grain storage. Stolz (15) and Kline and Sugihara (16) reported the counts of about 2×10^3 CFU/g for yeasts and about 2×10^2 CFU/g for LAB, which was also confirmed by the present study. Given the fact that natural populations include yeasts that have poor fermentation ability, yeasts should be added to the flour during the dough preparation stage (17, 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 LAB in the indirect dough method involving flour scalding, compared to the other two dough mixing methods, can be attributed to the more favourable ecophysiological conditions during the preparation of dough using the 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 LAB, 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 the pH of the dough. The somewhat lower total acidity of the dough than reported in the present study is due to the activation of natural strains of LAB, whose metabolism is lower than that of commonly used selected strains (22). The strains of selected yeasts used in this study may compete with natural strains of LAB 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 the dough pH leads to a decrease in the λ-amylase activity, with undegraded starch binding all the moisture from the dough, thus preventing the crumb from becoming soggy, particularly in rye breads. The increase in the acidity induces the peptisation and swelling of rye flour proteins, thus increasing the consistency and, hence, the air-holding capacity of the dough (26). Rehman et al. (6) also stressed the importance of increasing acidity in the inactivation of 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. (27-29). Martinez-Anaia (30) and Gobbetti et al. (31) lay stress upon the important role of proteolytic enzymes synthetised by LAB in creating free amino acids as precursors of good flavour and rheological attributes of bread (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 LAB (33, 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.

CONCLUSION

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 LAB 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 showed that the sourdough-type rye/wheat bread is characterrised 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 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 the quality and assortment, and can be used in any bakery facility.

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УТИЦАЈ НАЧИНА ЗАМЕСА НА КВАЛИТЕТ ХЛЕБА ОД РАЖАНОГ БРАШНА

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Циљ овог рада је био да се проучи утицај киселог теста (индиректни замес) на квалитет хлеба од мешаног ражаног брашна (пшенично брашно ТИП 500 и интегрално ражано брашно – 60:40) и утврде предности његове примене у односу на директан замес. У истраживањима су коришћена три начина замеса: I - индиректни начин производње мешаног ражаног хлеба са запаривањем брашна; II - индиректни начин производње мешаног ражаног хлеба без запаривања брашна; III - директан начин производње мешаног ражаног хлеба. У истраживањима су праћене: микробиолошке карактеристике брашна и теста (број квасаца и бактерија млечне киселине), као и хлеба (присуство *Enterobacteriaceae*, квасаца и плесни); хемијске особине теста и хлеба (pH и киселински степен); органолептичке карактеристике хлеба (запремина, порозност по Dallmanu, еластичност средине, финоћа структуре зидова пора, боја и сјај коре, вредносни број средине хлеба, спољашњи изглед, изглед средине, мирис и укус коре и средине).

Резултати истраживања указују да је највећи број бактерија млечне киселине и квасаца утврђен код индиректног замеса са попаривањем ражаног брашна. Ражано мешани хлебови, произведени са киселим тестом, имали су благ киселкаст укус са израженом аромом, продуженом свежином и мањом мрвљивошћу. Хлеб произведен директном методом био је бољи само у погледу запремине хлеба. На основу изнетог може се констатовати да је технолошки поступак производње ражано мешаних хлебова са киселим тестом битан предуслов за побољшање квалитета и асортимана хлеба и може се применити у сваком пекарском погону.

Кључне речи: ферментација, хлеб, кисело тесто, квалитет, замес

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