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A study of factors which influence mould spoilage in flat (sourdough) bread

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Abstract. Bakery products are an excellent substrate for the development of microbial spoilage, especially mould spoilage and lime disease (otherwise known as chalk disease), because they have high levels of water activity a_w = 0.94–0.97 and pH 5.5–6.0. Sliced bread in its packaging is highly susceptible to moulds and lime disease during storage. The aim of this study was to investigate the effects shown by the microbial contamination of flour quality, and the type of sourdough and organic acid, especially acetic acid, on mould spoilage in wheat and rye wheat bread. Microbial contaminations were studied in two batches of wheat flour and three batches of rye flour which had been manufactured in Belarus and Russia and in sourdough bread which had been produced using this flour. Investigated here was the impact of the quality and type of sourdough with various starter cultures of micro-organisms and the impact of the content of organic acid, especially acetic acid, on mould spoilage in wheat and rye wheat bread. The content of organic acids, including acetic acid, in different types of sourdough which has been prepared using different starter cultures and in different kinds of sourdough bread which have been studied using liquid chromatography. It was found that, in spite of the presence in flour of spore-forming bacteria, yeasts, and fungi, microbial contamination of the finished product immediately after baking was absent. It was proven that the use of starter cultures and sourdough can slow down or prevent entirely the microbial spoilage of bread. It was found that the content of acetic acid which had been accumulated during the fermentation of various types of sourdough served to effect the presence of mould spoilage on sourdough bread.

Key words: sourdough, bread, microbial contamination, mould, microbial spoilage

INTRODUCTION

In Russia, Belarus, and Ukraine in recent years, a large volume of bread has been sliced, packaged, and wrapped. This is the precipitating in the microbial spoilage of bread due to the presence of bacteria (known as rope or ropy disease), and yeast (lime disease) or fungi (moulds). Packing and wrapping prevents the loss of moisture from the cut surfaces of the bread slices, allowing a humid atmosphere to form around the loaf. This provides a moist surface for moulds and yeasts on which to grow. Additionally, the bread has a relatively high moisture content and water activity (a_w = 0.94–0.97) and a pH around 5.5–6.0. These factors makes the bread an excellent substrate for the development of microbial spoilage, especially when caused by mould and yeast (de Blackburn, 2008; Cauvain, 2012).

At the same time there is no microbiological safety standard in place for bakery products in these countries, except for bakery products which are produced with a filling (such as pies). Standards which govern the content of spore-forming bacteria and moulds in grain and grain products are not in place, both in Russia and in other countries. The content of spore-forming bacteria in the wheat flour was determined by the minimum time required for the development of ropy disease in bread. There are also standards in place to control the content of mycotoxins in the flour, but not for mould. Therefore, any study of the effects of microbial contamination of flour on the microbiological safety of bread is of interest.

Mould contamination of bread occurs mainly during transportation, cooling, cutting, and packaging operations. The bread is infected by direct contact with contaminated objects (transportation and packaging tools, hands, or clothing), or through the air. The degree of microbiological contamination in the bread reveals the sanitary conditions of the bakery in question. Contamination of a businesses premises and equipment leads to the secondary contamination of raw materials, semi-finished products, finished products, and packaging, and encourages the development of microbial spoilage of bread (De Blackburn, 2008; Cauvain, 2012).

The development of tools and effective methods which will help to improve microbial safety and the storage stability of bakery products is a very real problem, one which includes a number of questions. In recent years, the bio preservation of bread has gained increasing interest thanks to rising consumer demand. Lactic acid bacteria as bio-preservation organisms are of particular interest. Studies generate a great deal of interest where they are related to the use of substances of a microbial origin which are produced by the fermentation microflora of dough and sourdough as a protective barrier against the microbial spoilage of bakery products. Lactic acid bacteria are able to produce different kind of bioactive molecules, such as organic acids, fatty acids, hydrogen peroxide, and bacteriocins. For example, the acetic acid which is formed in the fermentation process can have an inhibitory effect on the development of spore-forming bacteria and mould (Clarke et al., 2002; De Blackburn, 2008; Cauvain, 2012).

Most of the baking companies which operate in Russia, Belarus, and Ukraine work with rye and wheat sourdough and starter cultures of lactic acid bacteria and yeasts from the collection of the St Petersburg branch of the State Research Institute of the Baking Industry. Regarding this, the study of the influence of Russian sourdough with starter cultures on the mould spoilage of bread is of interest.

The aim of this study was to investigate the influence of the microbial contamination of flour, the quality and type of sourdough, and the content of organic acids, especially acetic acid, which is formed during the fermentation in sourdough on the mould spoilage of wheat bread and rye wheat bread.

MATERIALS AND METHODS

Starter cultures and sourdough

Used in this study were two types of rye sourdough with humidity levels of 50% and 68%, and two types of wheat sourdough with humidity levels of 42% and 65%. The sourdough was prepared in accordance with the official instructions for Russian baking companies with the use of micro-organism starter cultures from the collection of the St Petersburg branch of the State Research Institute of the Baking Industry (Kosovan, 2008).

Lactic acid and yeast bacteria starter cultures have been widely used for many years as symbiotic compositions in bakery companies in Russia (Afanasjeva, 2003; Kosovan, 2008).

The following starter microbial composition and sourdough were used:

Three strains of lactic acid bacteria - L *brevis 5, L brevis 78,* and *L plantarum 63* - and one strain of yeast - *C milleri* - were used for the dense rye sourdough with a humidity level of 50% (Kosovan, 2008).

Four strains of lactic acid bacteria - *L brevis 1, L plantarum 30, L casei 26,* and *L fermentum 34* - were used for the yeast-free liquid rye sourdough with a humidity level of 68%, and for liquid wheat sourdough with a humidity level of 62%. Three strains of lactic acid bacteria - *L brevis 8, L brevis 27,* and *Lplantarum* 6 - and two strains of yeasts - *S cerevisiae 90* and *S minor 7* - were used for dense wheat sourdough (Kosovan, 2008). The technological parameters for the various types of sourdough are shown in Table 1.

Table 1. Technological parameters of different types of sourdough

Biotechnological indicators	Dense rye sourdough	Yeast-free liquid rye sourdough	Dense wheat sourdough	Yeast-free liquid wheat sourdough
Mass proportion of moisture, %	50.0	68.0	42.0	62.0
Temperature, °C	30.0	40.0	20.0	39.0
The time of fermentation, h	6-00	19-00	24-00	2-00

Flour

Two batches of wheat flour and three batches of rye flour were used, all of which were manufactured in Belarus and Russia.

Bread preparation

The formulations for the rye wheat bread, 'Darnitsky', are presented in Table 2. A proportion of the rye flour was replaced with flour which followed the sourdough composition in accordance with the existing instructions (Kosovan, 2008*)*. A total of 25% of the quantity of rye flour in the recipe was replaced by dense rye sourdough with a humidity level of 50%, and 20% of the quantity of rye flour in the recipe was replaced by yeast-free liquid rye sourdough with a humidity level of 68%.

Table 2. The formulations of rye-wheat bread 'Darnitsky'

Raw materials, %	Bread 'Darnitsky'			
	Dense rye sourdough	Yeast-free liquid rye sourdough		
Rye flour	35	40	35	30
Quantity of rye flour in sourdough	25	20	25	30
Wheat flour	40	40	40	40
Total flour	100	100	100	100
Sourdough	40	49	61	73
Yeast	0.5	0.5	0.5	0.5
Salt	1.4	1.4	1.4	1.4
Water	until dough humidity of 43%			

The required quantity of sourdough was mixed with the rest of the flour in the recipe, along with the yeast, salt, and water, until the dough achieved a humidity level of 43%. After mixing, the dough samples were shaped into 400g loaves, placed in aluminium pans so that a moisture content test of 43% could be achieved, and leavened at 30 °C until the volume was twice that of the initial volume. The leavened dough samples were cooked in an oven at 210 °C for eighteen minutes.

The formulations for the wheat bread are presented in Table 3. A proportion of the wheat flour was replaced by flour which followed the sourdough composition in accordance with the existing instructions. A total of 10% of the quantity of wheat flour in the recipe was replaced by dense wheat sourdough with a humidity level of 42%, and 5% of the quantity of wheat flour in the recipe was replaced by yeast-free liquid wheat sourdough with a humidity level of 62%.

Table 3. The formulations of wheat bread

Raw materials, %	Bread 'Darnitsky'	
	Dense wheat sourdough	Yeast-free liquid wheat sourdough
Wheat flour	90	95
Quantity of rye flour in sourdough	10	5
Wheat flour	100	100
Total flour	15	12
Sourdough	0.5	0.5
Yeast	1.4	1.4
Salt	until dough humidity of 47.5%	

The required quantity of sourdough was mixed with the rest of the wheat flour in the recipe, along with yeast, salt, and water, until the dough achieved a humidity level of 47.5%. After mixing, the dough samples were shaped into roughly 400g loaves, placed in aluminium pans, and leavened at 30 °C until the volume was twice that of the initial volume. The leavened dough samples were cooked in an oven at 200 °C for eighteen minutes.

Flour quality assessment

An assessment was carried out on the quality of the flour by analysing the following properties: the mass proportion of moisture in the flour was determined by drying it at a temperature of 130 °C for a period of forty minutes, while the mass proportion of ash was determined by burning flour in a muffle furnace at a temperature of between 600 °C and 900 °C until complete ashing had taken place with subsequent a determination being made of the non-combustible residue; the 'Falling' value for flour was determined by using the Hagberg-Perten method (ICC Standard No 107/1 (1995)), gluten content in wheat flour was determined by the complete sifting of gluten from 25 g of flour, and weighing and acidity were determined by titration, using a 0.1 n. solution of NaOH.

Sourdough and dough assessments

An assessment was carried out on the quality of the sourdough and dough by making use of the following properties: the mass proportion of moisture of the flour was determined by drying it at a temperature of 130 °C for a period of forty minutes, while acidity was determined by titration, using a 0.1 n. solution of NaOH (State Standard of

the Russian Federation, 1996). The lifting capacity was determined by the rate at which it rose in a glass of water at a temperature of 32 °C for a 10g mass of dough shaped into a ball and with a humidity level of 45%. The increase in volume was calculated by the ratio between the final volume and the initial volume multiplied by 100%. The content of volatile acids was determined by neutralising the evaporated volatile acid using a 0.1 n. solution of NaOH. The alcohol content was determined by using the iodometric method which is based on the quantity of sodium thiosulfate spent in titration. The content of acetic and lactic acid in various types of sourdough which were prepared with different microbial starter cultures were studied using liquid chromatography.

Baked bread assessment

An assessment was carried out on bread and sourdough quality levels in relation to the following properties: the mass proportion of moisture in the flour was determined by drying it at a temperature of 130 °C for a period of forty minutes, while acidity was determined by titration, using a 0.1 n. solution of NaOH (State Standard of the Russian Federation, 1996).

The content of acetic and lactic acid in leavened bread was studied using liquid chromatography.

Determining the microbial contamination of flour

Microbial contamination of wheat and rye flour were studied. A study of the microflora of flour was carried out in the following way: 10 g of flour was added to 100 ml of sterile water and diluted to between 10^{-1}–10^{-6}. From each dilution a 0.1 ml suspension was added to the surface of the meat-peptone agar in a Petri dish, and 1 ml of the suspension was introduced into a Petri dish and poured on top of malt agar which contained a total of 8% of dry solids.

Determining the microbial contamination of baked bread

Microbial contamination of wheat and rye bread were studied. The bread was prepared for microbiological analysis in the following way: immediately after baking in the opening of the oven, the loaves were packed into sterile paper, placed in a sterile room, and cooled to a temperature of between 25–28 °C. After cooling, the bread was cut in sterile conditions. A total of 10g of bread was added to 100ml of sterile water and diluted to between 10^{-1}–10^{-6}. From each dilution a 0.1 ml suspension was added to the surface of the meat-peptone agar in a Petri dish, and 1ml of the suspension was introduced into a Petri dish and poured on top of malt agar which contained a total of 8% of dry solids.

Determining the effect of the technology behind bread-making on the rate of appearance of mould spoilage

The impact of the type and quality of sourdough upon various micro-organism starter cultures and the impact of the content of acetic acid on mould disease in bread were investigated. Descriptions of the variations in the technological parameters of sourdough are represented in Table 1. In order to determine the effect of the technology behind bread-making with different types of sourdough on the rate of the appearance of mould spoilage, model experiments were carried out by contaminating sterile slices of a pure culture of the mould, *Penicillium chrysogenum*. Immediately after baking in the

oven opening, the loaves were packed into sterile paper, placed in a sterile room, and cooled to a temperature of between 25–28 °C. After cooling the bread was cut in a sterile environment, with slices being taken at a size of 3.5×6.5 cm and at a thickness of 0.3–0.4 cm. The slices were placed in sterile Petri dishes.

An aqueous suspension of a pure culture of the mould, *Penicillium chrysogenum*, was prepared for the infection of slices of bread. The biomaterial of *Penicillium chrysogenum* was transferred from a tube containing a pure culture of mould grown on malt agar to 1ml of sterile water using 'Tween-80' and was thoroughly suspended. The suspension was inoculated into each slice of bread in five shots using a microbiological needle. Petri dishes with infected slices were incubated at a temperature of 25 ± 1 °C until the first signs appeared of a growth of mould colonies. *Penicillium chrysogenum* was used because the *Penicillium* species are by far the most common for bread (Legan, 1993; Lund et al., 1996).

In addition, a loaf of bread in its packaging after cooling to between 25–28 °C was placed in the storage at a temperature of 25 ± 1 °C until the first signs of the growth of mould colonies.

Statistical analysis of the data

All of the experiments were carried out a total of five times; data was processed by using methods for mathematical statistics at a theoretical frequency of 0.95.

RESULTS AND DISCUSSION

In order to determine the role of the microbial contamination of flour in the microbial spoilage of wheat and rye wheat bread, five batches of flour were studied which had been developed at mills in Russia and Belarus. Spore-forming and other bacteria, yeasts, and moulds, the number of which varied widely, were identified in the flour (Table 4).

Table 4. Microflora of flour

Parameters	Wheat flour		Rye flour		
	Russia		Belarus		Russia
	Michurinsk	Barnaul	Polotsk	Baranovichi	S-Petersburg
Mass proportion of moisture, %	14.3	11.9	12.6	13.0	13.1
Mass proportion of ash, %	-	-	1.27	0.40	1.45
Falling number, s	354	279	206	270	152
Gluten content, %	30.8	30.1	-	-	-
Acidity, degrees N	3.0	3.2	3.6	3.4	
Colour	white with a yellowish tinge		greyish-white interspersed with particles of shells		
Odour	characteristic of flour, without extraneous				
Taste	characteristic of flour, without extraneous				
Count of microbes, CFU g^{-1}					
- bacteria	1,700	2,500	124,000	170,400	210,500
- spore-forming bacteria	20	800	2,000	200	500
- yeast	20	30	50	50	80
- moulds	130	1,000	2,200	300	400

However, it was established that, after baking in the wheat and rye wheat bread which had been prepared with sourdough both in the laboratory and in the bakeries themselves, there was no microbial contamination. The mould, bacteria, and yeast has not grown in the Petri dishes (Table 5). The studies confirm that the bread, after leaving the oven, is sterile in terms of the contamination of filamentous fungi, and any mould is a secondary infection (Lund et al., 1996; Afanasjeva, 2003; Cauvain, 2012).

Table 5. Microflora of bread immediately after the exit from the oven

Number of micro-organisms, CFU g^{-1}	Rye-wheat bread with sourdough		Wheat bread with sourdough	Wheat bread without sourdough
	Dense rye sourdough (humidity 50%)	Yeast-free liquid rye sourdough (humidity 68%)	Dense (humidity 42%)	
Bacteria	not found			
Spore-forming bacteria	not found			
Yeast	not found			
Moulds	not found			

To be able to determine the effect of sourdough on the quality of rye wheat bread and its stability in relation to microbial spoilage, an analysis was conducted of the biotechnological indicators of dense rye sourdough and liquid yeast-free sourdough. It was found (in Table 6) that different conditions of fermentation (involving the temperature and humidity of the sourdough, and the composition of micro-organisms) were affected in terms of their biotechnological parameters.

Table 6. Biotechnological indicators of different type's rye sourdough

Biotechnological indicators	Sourdough	
	Dense rye sourdough	Yeast-free liquid rye sourdough
Acidity, degrees N	14.9	20.0
Lifting capacity, min.	23	-
Increase in volume, %	75.0	45.5
Quantity of alcohol, %	1.8	0.8
Volatile acids, %	27.9	16.3
Lactic acid, g kg^{-1}	6.0	11.0
Acetic acid, g kg^{-1}	1.4	0.5

Dense rye sourdough had a good ability to lift (over the course of 23 mins), increasing in volume by 75%, and quite an amount of alcohol was also registered (1.8%) when compared to liquid yeast-free sourdough. This is due to the development of yeast in the dense rye leaven, which was used in the starter (part of the initial sourdough preparation), in contrast to liquid yeast-free sourdough with only lactic acid bacteria being used in the starter. The increase in volume (45.5%) in liquid yeast-free fermenting in relation to the activity shown by the heterofermentative lactic acid bacteria, *L brevis 1* and *L fermentum 34*, in producing CO_2 and the development of yeast cells from the flour. Sourdough also had different levels of acidity. There was a total of 1.8 times more

lactic acid and 2.8 times less acetic acid in yeast-free liquid rye sourdough when compared to the dense rye sourdough. Obviously, the accumulation of acetic acid contributes not only to heterofermentative lactic acid bacteria, but also to yeast.

When sterile slices of the bread known as 'Darnytskyi' with its dense rye sourdough were infected with the mould, *Penicillium chrysogenum*, it was found (in Table 7) that evidence of mould deterioration was not observed over the course of a total of 168 hours. After 168 hours of storage, the slices of bread in the Petri dishes became dried and hardened. At this stage monitoring was stopped. That bread which had been packaged into bags in a sterile environment was not mouldy after 336 hours of observation. That bread which had been packed in non-sterile conditions in the laboratory has exhibited signs of mould disease after a period of 192 hours.

Table 7. Effect of type of sourdough and its dosage when mixing the dough resistance to moulding of bread 'Darnitsky'

Biotechnological indicators	Sourdough				
	Dense rye sourdough	Yeast-free liquid rye sourdough			
Dough					
Part of the rye flour that was replaced by flour in the composition of sourdough, %	25	15	20	25	30
Bread					
Acidity, degrees N	7.2	5.3	5.9	6.9	8.0
Mass proportion of moisture, %	47.0	46.8	46.8	46.8	46.8
Quantity of alcohol, %	0.8	0.6	0.6	0.4	0.4
Volatile acids, %	18.3	15.5	16.7	14.2	12.5
Lactic acid, g kg^{-1}	4.8	4.7	4.8	5.2	5.4
Acetic acid, g kg^{-1}	1.0	0.1	0.1	0.2	0.3
Storage time before mould growth, hours:					
- sterile slices infected by *Penicillium chrysogenum*	no growth within 168h	60	72	80	no growth within 168h
Bread packed in a sterile environment	no growth within 14 days				
Bread packed in a non- sterile environment	192	168	192	192	192

On those slices of bread with a 15% liquid yeast-free rye sourdough instead of the rye flour in the recipe, mould was discovered after a period of sixty hours (see Table 6). When the amount of yeast-free flour in the composition of the sourdough is increased in favour of rye flour, to a total of 20% and 25% of the total flour content, the period of the growth for the mould increased to 72 and 80 hours respectively. The mass proportion of moisture did not vary enough from one kind of bread to another to be able to affect the relatively mould-free shelf life appreciably. Bread differs in acidity levels and in the content of acetic and lactic acids. It should be noted that the replacement in the recipe to the same levels of quantity (25%) with a dense rye sourdough and a liquid yeast-free rye sourdough bread had a close acidity reading of 7.2 and 6.9 deg, but the content of acetic acid in bread which was produced with liquid sourdough was five times less than it was

in bread produced with dense sourdough. When 30% of the quantity of rye flour in the recipe was replaced by yeast-free liquid rye sourdough, the development of mould in the bread was not observed within a period of 168 hours (which was also the case in bread which was produced with dense rye sourdough. But the acidity of the bread known as 'Darnytskyi' was at 8.0 deg – the maximum permissible according to normative documentation (State Standard of the Russian Federation, 1986). The bread had a pronounced acidic taste, one which would not be liked by consumers. Therefore it was found that the content levels of acetic acid which accumulated during the fermentation process effected the rate of the development of fungi on the bread, and that the lactic acid does not produce an effect. But evidently that other unidentified mould inhibitor can present itself in the fermented bread.

The effect of this method of bread-making upon the resistance of wheat bread to moulds was established (see Table 8). It was found that in bread slices with a dense wheat sourdough which was contaminated by *Penicillium chrysogenum*, the growth of mould colonies was observed between 18–24 hours later than in samples which had been prepared without sourdough and between 10–12 hours later than in samples with liquid yeast-free sourdough.

Table 8. Influence of the method of bread making on mould of wheat bread

Biotechnological indicators	Without sourdough	Liquid yeast-free sourdough	Dense wheat sourdough
Sourdough			
Acidity, degrees N		5.7	6.3
Mass proportion of moisture, %	-	62	42
Bread			
Acidity, degrees N	1.0	1.2	1.4
Mass proportion of moisture, %	44.1	44.2	44.1
Quantity of			
lactic acid, g kg^{-1}	1	3.5	3.5
acetic acid, g kg^{-1}	0.1	0.1	0.3
The storage time before mould growth, hours/day:			
- sterile slices infected by *Penicillium chrysogenum*	30-36	40-48	50-60
- bread packed in a non- sterile environment	5-6	no growth within 6-7 day	no growth within 10 days

Bread without sourdough packed in non-sterile conditions had mould disease within a period of between 120–144 hours. Bread made with liquid yeast-free wheat sourdough had mould disease present within between 144–168 hours. Bread made with dense wheat sourdough did not exhibit mould disease within a period of 240 hours. The content of acetic acid in bread with a dense sourdough was higher than it was in other samples. Therefore, it can be seen that the use of wheat sourdough slows down the mould spoilage of wheat bread. Our research confirms other studies of the effect of a sourdough addition in relation to the development of bacterial and fungal spoilage, including variations of the percentage of sourdough inclusion (Denkova et al., 2014). The

application of the developed starters in terms of the production of wheat bread guarantees a longer shelf life.

CONCLUSIONS

Studies have shown that the stability of bakery products in regard to mould spoilage depends on the type and quality of the sourdough, the technology being used, and the sanitary condition present at the point of production. Spore-forming and other bacteria, yeasts, and moulds were identified in the flour; however, no microbial contamination was found immediately after baking in the wheat bread and rye wheat bread when using this flour.

It was found that different fermentation conditions (regarding temperature, the humidity of the sourdough, or the composition of micro-organisms) affect the biotechnological parameters of the sourdough.

It was established that the content of acetic acid, which accumulates in bakery products during the fermentation of the sourdough, has an impact upon the speed of mould spoilage in bread. The lactic acid content does not effect the rate of development of fungi on the bread. But it is evident that other, unidentified, mould inhibitors can be present in the fermented bread.

It was proven that the use of sourdough in the preparation of bakery products when using rye and wheat flour allows their microbiological stability during storage to be increased.

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