

Influence of sourdough use on rope spoilage of wheat bread

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Abstract

Rope spoilage is a bread disease which consists in bacterial decomposition of the bread crumb. Spoilage organisms are heat-resistant spores of bacteria *Bacillus subtilis*, *B. licheniformis* and *B. pumilus* which survive the baking process. The aim of this paper is to study the influence of sourdough addition on characteristics of wheat bread and on occurrence of rope spoilage after baking and storage. Ropiness occurs in non-acidified breads and consists in breaking down bread components and leaving behind a fruity, melon-type odour. Sourdough addition increases the acidity of dough from 2.0 acidity degree (control sample) to 4.3 acidity degree in this work. It improves bread quality in terms of microbiological safety but reduces bread volume and porosity as well as worsens organoleptic properties (sour taste, dense and thick crumb). The results showed the reduction of bread height from 140 mm (control sample) toward 102 mm and of bread porosity from 90.2 % (control sample) toward 70.7 % for the last sample. Ropiness was observed only in control sample after 72 hours from baking. The results confirmed the positive role of sourdough on rope spoilage prevention.

Keywords: Wheat bread, rope, spoilage, sourdough, rope spores, *Bacillus subtilis*, wheat bread, prevention.

1. Introduction

Rope spoilage is a bread disease consisting in bacterial decomposition of the bread crumb. Spoilage organisms are heat-resistant spores of bacteria belonging to *Bacillus* genera which survive the baking process. Most important rope formers are *Bacillus subtilis*, *B. licheniformis* and *B. mesentericus*, also known as *B. pumilus* [1-4]. These bacteria are part of the grains microbiota and reach the flour through grinding.

Sources of rope spores microorganisms in bread products are mostly present in the ingredients, especially flour and yeast. Unlike moulds that are destroyed when exposed to baking temperatures, rope spores microorganisms are able to form spores and survive the baking process, germinate, and continue to grow. Optimum growth conditions for rope formers are 35...45°C, humid environment (e.g. packed bread) and pH values higher than 5.3 [5].

Pepe et al. [6] isolated sixty-one *Bacillus* strains from ropy breads. These strains were characterized on the basis of their phenotypic and genotypic traits. All of the isolates were identified as *B. subtilis* by biochemical tests, but molecular assays revealed that besides strains of *B. subtilis*, *B. licheniformis*, *B. cereus* and isolates of *B. clausii* and *B. firmus* were identified.

In other research, Valerio et al. [7] examined the diversity of spore-forming bacteria isolated from raw materials and bread using molecular methods along with a rapid and innovative technology, the FT-NIR (Fourier transform near-infrared) spectroscopy. They found 13 bacterial species belonging to *Bacillus* (10) and *Paenibacillus* (3) genera. The most frequent species was *B. amyloliquefaciens*, found also in ropy bread. The screening test performed for rope production indicated that mainly *B. amyloliquefaciens*, together with *B. subtilis* and *B. pumilus*, could cause spoilage in bread, even if the last two species were represented by a low number of isolates. The *Bacillus cereus* group and *Bacillus*

megaterium showed a lower percentage of isolates potentially able to cause the rope. However, considering the high number of *B. cereus* group isolates detected in the study of Valerio et al. [7], this bacterial group should also be considered important in rope spoilage.

Ropiness occurs in non-acidified breads and consists in breaking down bread components and leaving behind a sticky, pasty, stringy mass which have a fruity, melon-type odour. Ropiness is referred to when the bread is pressed together and then pulled apart. If it is ropery then it will stretch into long, sticky, web-like strands. It is only visible for a short period of time; some strains do not even form ropes at all. The main characterization is therefore a fruity, melon-type odour. Discoloration of the crumb and a bitter taste may also be other symptoms. Packed wheat bread with a long shelf life such as toast bread is highly susceptible.

Ropiness prevention can be done through chemical or biological methods. Rope forming bacteria are very sensitive to low pH values, therefore their growth is inhibited when chemicals are added to dough. The most efficient chemicals used in bread making are propionic acid, calcium propionate, acetic acid, and calcium hydrogen phosphate. A dose of 1-5 g/kg of flour leads to a delay of ropiness of 3 to 21 days zile [1]. Biological methods consist in the use of starter cultures of propionic bacteria (e.g. *Propionibacterium shermanii*) which have antagonistic behaviour towards rope forming bacteria of *Bacillus* genera due to the synthesis of propionic acid and some substances with antibiotic effect. Other biological methods are the use of sourdough, liquid yeast, lactic-acid culture containing *Lactobacillus plantarum*, *L. brevis*, *L. fermenti* etc. [2].

The use of the sourdough process as a form of leavening is one of the oldest biotechnological processes in food production [8, 9]. Sourdough is dough made of cereal products (e.g. wheat flour), liquids (e.g. warm water), and microorganisms in an active state. It is characterized by a complex microbial ecosystem, mainly represented by lactic acid bacteria and yeasts [10]. This dough is subjected to continuous fermentation. During sourdough fermentation, lactic acid bacteria (LAB) produce different metabolites such as organic acids, exopolysaccharides (EPS) and enzymes [11, 12]. To facilitate continuous production, a portion

of ripe sourdough dough is saved and used to seed a subsequent dough batch.

In a review of Decock & Cappelle [13] it has been shown that there is a clear trend in the market towards tastier breads which can be obtained using stabilised sourdough products.

The substances produced through lactic acid fermentation of sourdough have a positive effect on the texture and staling of bread, improving also bread palatability and sensory quality [8, 10].

According to Clarke & Arendt [8], the use of sourdough confers a natural image on the product because lactic acid bacteria have a long history of use in food and are regarded as safe microorganisms. The newer developments on the biochemistry and physiology of lactic acid bacteria were studied in many researches from the point of view of carbohydrate and nitrogen metabolism, nutritional implications, responses to environmental stresses and production of anti-microbial compounds [14, 15, 16].

Ryan et al. [17] used *L. amylovorus* DSM19280 to produce a wide spectrum of antifungal compounds active against common bread spoilage fungi. Wheat doughs fermented with *L. amylovorus* DSM19280 had good rheological properties and the breads thereof were of high quality as shown by rheofermentometer and texture analyser measurements.

Valerio et al. [18] worked with four fermentation products of the lactic acid bacterium *Lactobacillus plantarum* ITM21B and obtained baked products with no ropy symptoms after three days and an enhancement of the *Bacillus*-free shelf life of yeast-leavened bread for seven days.

This paper has the purpose of studying the influence of sourdough addition on characteristics of wheat bread and on occurrence of rope spoilage after baking and storage.

2. Materials and methods

2.1. Materials. To determine the correlation between the acidity of bakery products and the occurrence of rope spoilage of bread, four samples of bread with different acidity were prepared according to GOST 27669-88 [19]. Wheat white flour, yeast, salt and water were used for bread samples preparation.

The acidity content was modified by adding different quantities of sourdough (6 acidity degree):

- Control sample – bread without sourdough addition;

- Sample 1 – bread with 100 g of sourdough addition to 1000 g of flour;
- Sample 2 – bread with 150 g of sourdough addition to 1000 g of flour;
- Sample 3 – bread with 200 g of sourdough addition to 1000 g of flour.

2.2. Methods. Baking test method was used to determine the degree of flour contamination with sporogenous bacteria of *Bacillus* genera according to STAS 90/1988 [20]. This method consists in cooling the bread for about 1 hour until the crumb temperature reaches 40...45°C. Each loaf of bread is then covered with a cloth towel moistened with water or packed in polyethylene bags. For control purposes, bread samples prepared in this way are stored at 37°C for several days and then checked for ropiness occurrence. The first bread sample is examined after 24 hours (one day). If the crumb is stretching into wires that stick to fingers when bread is broken and it has foreign smell (of hay, rotten fruits, honey or others) specific to rope spoilage of bread, a contamination of first degree with bacteria of *Bacillus* genera is assigned to the flour. If no one of the above defects is present, the second bread sample is examined after 48 hours (two days). The presence of rope spoilage at this stage is assigned to the flour as second degree of contamination, or slightly contamination. The absence of any sign of spoilage leads to the third examination after 72 hours (three days). If the bread sample shows no signs of rope spoilage after 72 hours, the flour is considered appropriate for bread making.

Organoleptic characteristics determined for bread samples were appearance, crust colour, taste, smell and crumb elasticity. They have been established visual, tactile and tasting the bread.

Physico-chemical properties determined for the bread samples were acidity, bread height and bread porosity.

Bread acidity was determined using the titration method [21] which consists in measuring the volume of sodium hydroxide solution 0.1 N that is used in the reaction of neutralization of acids present in food. Titration is carried out in the presence of phenolphthalein indicator until the appearance of pale pink coloration.

Bread height was determined using the device U1-EIH or the ruler [20].

Bread porosity was determined using the method of weighing [22] which consists of weighing a certain volume of crumb collected by the device Juravliov.

3. Results and discussion

In order to study the influence of sourdough addition on characteristics of wheat bread and on occurrence of rope spoilage after baking and storage, four bread samples were prepared using the classical dough fermentation method of two stages (leaven and dough fermentation) with a certain quantity of sourdough added to leaven, except the control sample.

Dough pieces of determined quantity were placed in bread trays, left to stand for final fermentation and then placed in an oven for baking. After baking, the bread loaves were removed from the trays and stored to allow cooling. A bread of each sample was used to evaluate the organoleptic and physico-chemical properties. Three bread of each sample were stored in an incubator at 37°C for rope spoilage determination.

3.1. Organoleptic quality characteristics

Figure 1 presents the pictures of bread samples obtained during experiments. The first two samples have the best organoleptic quality characteristics: regular shape, smooth surface without cracks, yellow-brown colour of crust, white colour of crumb, well developed porosity, uniform, pleasant taste and aroma.

The last sample (no. 3) consisting of bread with 200 g of sourdough addition to 1000 g of flour showed the lowest organoleptic quality characteristics: flat shape, uniform surface, with some small wrinkles, porosity less developed than in the other samples, slightly sour taste.

3.2. Physico-chemical quality properties

Variation of bread acidity is presented in figure 2. Control sample has the lowest acidity, only 2 acidity degrees. Addition of sourdough having 6 degree acidity in different quantities for each sample, excepting the control sample leads to the proportional increase of bread acidity. Thus, sample 3 has 4.3 acidity degrees which is a little bit more than the double of control sample acidity. The evolution of bread acidity is obvious: it increases along with higher sourdough dose added to the leaven.

Bread height variation is presented in figure 3. As expected, control sample has the highest value for bread height. Bread height varies inversely with sourdough dose addition to the leaven.

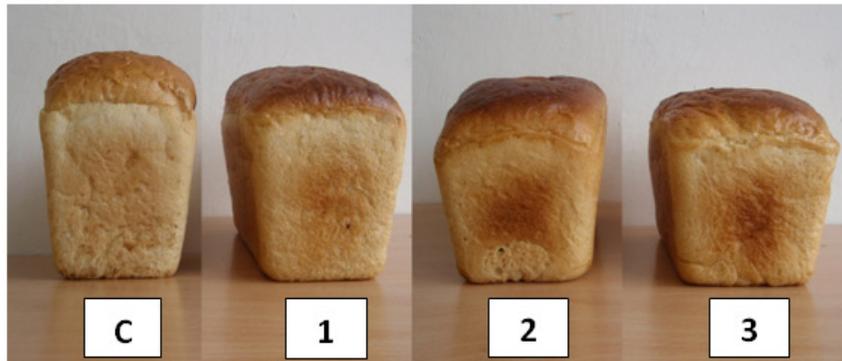


Figure 1. Pictures of bread samples obtained during experiments:

C – Control sample (bread without sourdough addition), 1 – Sample 1 (bread with 100 g of sourdough addition to 1000 g of flour), 2 – Sample 2 (bread with 150 g of sourdough addition to 1000 g of flour), 3 – Sample 3 (bread with 200 g of sourdough addition to 1000 g of flour).

Bread porosity has similar variation with bread porosity (figure 4) decreasing from the control sample (80.2%) to the last sample (70.7%).

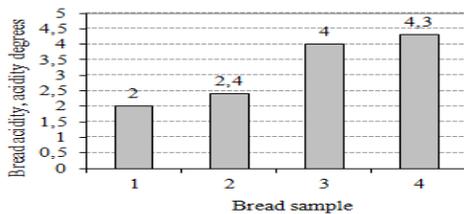


Figure 2. Variation of bread acidity for all bread samples

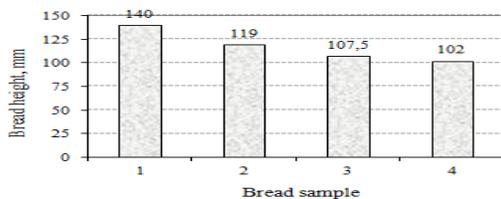


Figure 3. Variation of bread height for all bread samples

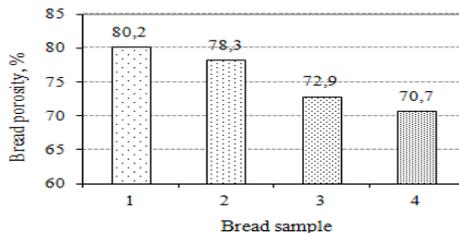


Figure 4. Variation of bread porosity for all bread samples

3.3. Occurrence of bread rope spoilage

Bread stored at 37°C in an incubator was observed after 24, 48 and 72 hours. The results are presented

in table 1. The first examination after 24 hours revealed no sign of bread rope spoilage. After 48 hours, the first signs of bread rope spoilage, in the form of an unpleasant odour of spoiled fruits, were observed in the control sample. The third examination after 72 hours showed an increase of bread rope spoilage signs for the control sample and again no sign for all the other bread samples prepared with sourdough addition.

Analyzing together the bread acidity and the occurrence of bread rope spoilage one could observe that when the bread acidity was higher, the first sign of rope spoilage appear later. Therefore, sourdough addition to the leaven has an inhibitory effect on the rope former bacteria. However, increasing bread acidity as a result of sourdough addition has a negatively influence on bread quality, the volume and porosity of bread.

Table 1. The occurrence of bread rope spoilage

Duration of incubation, hr	24	48	72
Control sample	–	±	+
Sample 1	–	–	–
Sample 2	–	–	–
Sample 3	–	–	–

Note: “–” no sign of bread rope spoilage detected; “±” the first sign of bread rope spoilage (unpleasant odour of spoiled fruits); “+” pronounced signs of bread rope spoilage (unpleasant strong odour of rotten fruits)

4. Conclusions

The experiments performed in this research showed that sourdough addition increases the bread acidity from 2.0 acidity degree (control sample) to 4.3 acidity degree (sample with the highest sourdough

added quantity). This improved bread quality in terms of microbiological safety but reduces bread volume and porosity as well as worsens organoleptic properties (sour taste, dense and thick crumb). The results showed the reduction of bread height from 140 mm (control sample) toward 102 mm and of bread porosity from 90.2 % (control sample) toward 70.7 % for the last sample. Ropiness was observed only in control sample after 72 hours from baking. The results confirmed the positive role of sourdough on rope spoilage prevention.

These results are consistent with the statements of Valerio et al. [7] saying that raw materials used to produce bread represent a rich source of spore-forming bacteria; therefore their microbiological quality should be monitored before use.

Other measures that can be taken in bread factories for preventing bread rope spoilage refers to fast cooling of bread after baking, correct packaging of cooled bread in clean packaging materials and keeping sanitary conditions along the entire production process.

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Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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