
**THE OCCURRENCE OF THE ROPE SPOILAGE OF BREAD
AT CAHULPAN BREAD-MAKING COMPANY**

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Abstract: *Rope spoilage is one of the most frequent diseases of bread, which is caused by the activity of bacteria of the genus Bacillus. The aim of this paper was to observe, determine and discuss the evolution of the occurrence of rope spoilage in bread at Cahulpan company during the warm months of 2011–2020 period. Seven types of bread have been chosen for the study. The most cases of the rope spoilage were detected in the long loaf breads during 2011–2016 period. The occurrence of the rope spoilage in these breads was primarily caused by the use of flour with a high degree of contamination with mesophilic aerobic spore-forming bacteria of the genus Bacillus, then by the titratable acidity under 2.0 degrees of acidity and/or the slow cooling of bread after baking. Due to several internal measures applied by the company (i.e., improvements of hygienic system and bread cooling after baking, rigorous check of suppliers, careful microbiological analysis of ingredients and use since 2017 of calcium propionat as a preservative against rope spoilage) based on previous research, the incidence of the disease decreased and disappeared after 2017.*

Keywords: *Bacillus subtilis, bread acidity, bread, rope spoilage, Ca propionate, cooling process, spore-forming bacteria, wheat flour.*

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1. Introduction

1.1. The rope spoilage of bread

The rope spoilage of bread is an undesirable disease consisting in the bacterial decomposition of the bread crumb. Such bread becomes unhealthy and improper for consumption [1; 15].

The disease is produced by bacteria of the genus *Bacillus*, primarily *B. subtilis* subsp. *mesentericus* and sometimes *B. licheniformis*, *B. cereus*, and *B. pumilus* [1, p. 356; 5, p. 402; 8; 12; 13; 14; 15, p. 441; 28]. Valerio et al. (2012) isolated, besides *B. subtilis*, another important species of the genus *Bacillus*, namely *B. amyloliquefaciens* [27] and a few species of the genera *Paenibacillus* [24]. Other species that are able to initiate the rope spoilage are *B. mycoides*, *B. polymyxa*, *B. firmus*, *B. clausii*, *B. megaterium*, etc., but with a less important role than *B. subtilis* [9; 12; 24].

The flour represents the main source of rope spoilage producing bacteria [12]. These bacteria are found in soil, in concentration of up to 10^5 C.F.U.¹/g soil, and the main path through which they reach the grain mass is represented by the dust particles that attach to the grains during wheat harvesting and processing. Additionally, when stored under improper conditions, wheat undergoes self-heating, and the number of bacteria increases considerably and can exceed $3 \cdot 10^4$ C.F.U./g [9].

Subsequently, these bacteria pass from the wheat into the flour during milling [7]. An insufficient cleaning of the wheat before milling contributes to obtaining a flour with a high level of contamination with microorganisms. The level of flour contamination also depends on the degree of flour extraction. Thus, the higher the extraction degree and the higher the yield in flour are, the higher the content of particles from the peripheral layers of the grains in flour will be. This means that this flour

¹ C.F.U. – colony forming units

will contain a higher concentration of microorganisms because the peripheral layers of the grains contain a larger number of microorganisms [7; 11]. As a consequence, brown breads obtained from flour with a high bran content, appear to be more susceptible to rope spoilage [7; 22]. The spores of *Bacillus* spp. may also be present in other raw materials such as baker's yeast and improvers [22], as well as in the bakery environment, e.g. the surfaces of processing equipment and the atmosphere [22; 24].

Bacteria producing the rope spoilage form thermoresistant endospores able to maintain their viability in the baking process. While during the baking of the dough the highest value of the temperature inside the bread is 98°C for several minutes, the spores are not destroyed. After baking and cooling the bread, surviving spores can reach the vegetative form, in certain conditions, and multiply, causing the spoilage of the bread [5, p. 402; 15, p. 441]. Optimum growth conditions for rope formers are 35...45°C, humid environment (e.g., packed bread), and values of pH above 5.3 (bread with low acidity).

The rope spoilage occurs more frequently in bread during the warm period of the year. The first signs appear after 24–48 hours of bread storage as a modification of the bread odour. Thus, initially, ropiness occurs as an unpleasant fruity odour, often described as similar to a strong scent of overripened pineapples [22] or melon, honey or valerian [5, p. 402]. It is then followed by patchy discoloration of the crumb in the central portions of the bread [22] as a result of enzymatic degradation of the crumb [12].

If the rope spoilage progresses, the smell intensifies and becomes rotten, and outbreaks of bacteria that appear as yellow-brown or pink-brown spots can be observed in the crumb [5, p. 402; 15, p. 441]. Also, the porous structure of the crumb deteriorates, gaps appear, and the crumb becomes soft and sticky because of the production of glutinous extracellular polysaccharides as a result of enzymatic activity [22]. These compounds contribute to the formation of thin strands or "ropes" when the bread loaf is broken or cut into slices and the pieces are taken apart giving, at the same time, the name of the disease as "rope spoilage" [5, p. 403; 15, p. 441; 22]. At the strong development of the spoilage, the crumb turns into a compact and dark mass, with a specific pungent and unpleasant smell, from which the name „potato disease" comes [5, p. 403; 15, p. 441; 29].

The basic factors leading to the occurrence of ropiness are [5, p. 403; 14; 29]:

- a) The degree of flour contamination with spore-forming bacteria of the genus *Bacillus*: the higher the number of spore-forming bacteria per C.F.U./g of flour, the more frequent the cases of the occurrence of the disease are [5, p. 403].
- b) The preparation and fermentation regime of the dough: the values of the pH of the dough has to be less than 5 to prevent the growth of bacteria.
- c) The conditions under which the bread is cooled and stored after baking. The rope spoilage occurs more often in summer when the cooling of bread after baking is slow, and it has temperature higher than 25°C for longer periods after removing it from the oven. The rapid cooling of the bread after baking at 18...20°C eliminates the occurrence of the disease.

2. Approaches to ropiness control and the aim of the paper

The bread with rope spoilage can cause illnesses to consumers if eaten by mistake, on the one hand, and it causes economic losses, on the other hand. Therefore, the implementation and enforcement of good manufacturing practices and food safety systems to reduce the proliferation of rope-causing bacteria and prevent the occurrence of ropiness are necessary. Some guidelines which may help in reducing the incidence of rope spoilage are the followings [3, p. 282, 357–359; 5, p. 403–404]:

- Effective inhibitors of microbial growth to lower the pH of the crumb to 5.4 or less [3, p. 90–92];
- Natural acidification of dough and bread through long fermentation times, sourdough use [3, p. 52; Mortazavi & Sadeghi, 2011; Sadeghi, 2008; Torrieri et al., 2014;] and whey addition that lower the pH of the dough below 4.6;
- Effective chemical acidulants as preservatives such as propionic acid, calcium propionate at levels of 0.1–0.5 % [3, p. 73–74];
- Proper heating, ventilation, and air conditioning (HVAC) systems to reduce the risk of air-borne contamination from outside the bakery [2; 3, p. 156–159];

- Proper sanitation practices such as hygienic design, cleaning, supplier check, microbiological analysis of ingredients [3, p. 357–359]
- Treatment of grain by infrared or other strategies that can reduce microbial load [2];
- Prevention of cross contamination by avoiding the contact between freshly baked bread with ingredients and/or stale bread [2; 3, p. 118, 359];
- Starter cultures of propionic acid bacteria that are antagonistic to spore-forming bacteria of the genus *Bacillus* producing propionic acid and some substances with antibiotic effect [5, p. 404];
- Starter cultures of lactic acid bacteria [4; 12; 21; 25; 26].

Several previous kinds of research have been aimed to address the control of rope spoilage of bread at the bread-making company Cahulpan located in Cahul, Republic of Moldova [16; 17; 18].

The first study was focused on the influence of sourdough addition on the characteristics of wheat bread and on the occurrence of rope spoilage after baking and storage [16]. The results confirmed the positive role of sourdough in preventing the ropiness occurrence, only the control bread being affected by the disease after 72 hours from baking. Also, the results were consistent with the literature, e.g., Valerio et al. (2012) showed that raw materials used for bread production are a rich source of spore-forming bacteria and sustained the need of monitoring the microbiological quality of raw materials, primarily the flour and yeast [24].

Further, the authors investigated the process of bread cooling after baking and determined the correlation between cooling and the occurrence of the rope spoilage in bread and bakery products [17]. The results showed that classical cooling was satisfactory only for the bread made from wheat flour that was not contaminated with spore-forming bacteria of *Bacillus* spp. The ropiness occurred after 48 hours in bread samples cooled in the bakery section and after 72 hours in the warehouse of delivery. The results highlighted the fact that the classical cooling at lower temperatures ($17.5 \pm 0.5^\circ\text{C}$) delayed the occurrence of the rope spoilage of bread as a result of a faster cooling of the crumb. However, because the shelf life of bread is 24–48 hours, the rope spoilage does not occur if bread is consumed to meet this term [17].

Another study [18] was focused on the use of calcium propionate to prevent the occurrence of rope spoilage in bread and the influence of the addition on the quality of bread. The levels of addition were between 0.1 and 0.4 % lower than the maximum 0.5 % indicated by Cauvain (2015) [3, p. 73–74]. The results showed that the first signs of ropiness were observed after 24 hours in the control bread (without addition of Ca propionate) while in bread with addition of Ca propionate only the sample with 0.1 % was affected after 72 hours. Therefore, an addition of 0.2 % Ca propionate could be sufficient to eliminate the hazard of ropiness occurrence in bread. At the same time, the properties of bread were determined. The results showed a decrease of the physical indicators of bread quality (total volume, height, porosity), while the acidity did not have a significant variation.

Given the results obtained in the successive researches presented above, Cahulpan applied several internal measures able to improve the cooling conditions of bread, and the hygienic system, to verify more rigorously the suppliers and to carefully perform microbiological analysis of the ingredients. Also, Cahulpan company decided to use the Ca propionate as a preservative against the rope spoilage. The use of Ca propionate started in 2017.

Therefore, the aim of this paper is to observe, determine and discuss the evolution of the occurrence of rope spoilage in bread at Cahulpan company during several years before the new measures were introduced and continued until the end of the year 2020 (period 2011–2020).

3. Materials and methods

3.1. Bread

Seven types of bread manufactured at Cahulpan bread-making company (Cahul, Republic of Moldova) have been chosen for the study: long loafs "Spicisor" (500 g), "Cahuleanca" (450 g), and "De Capitala" (350 g), bread in forms "Deosebita" (550 g), round shape "Wheat bread, quality I" (500 g), and "Wheat bread, quality II" (500 g), and elongated shape bread baked on the stove „Bread with milk and bran" (300 g).

3.2. Other materials and equipment

Digital thermometer, room thermometer, thermostat, balance, equipment to determine the loaf volume, special rulers, racks, Petri dishes, culture medium were used.

The Special Registers for registration of data related to the occurrence of the rope spoilage in bread obtained at „Cahulpan”, Cahul, Republic of Moldova for the periods 2011-2014 and 2015–present were also used.

3.3. Rope spoilage test

The rope spoilage test applied to bakery products manufactured at the Cahulpan was performed according to the requirements of the Instruction for the prevention of rope spoilage in bakery products [14]. Thus, the evaluation of the occurrence of rope spoilage was performed daily during the warm months of the year (May-September), each sample being analyzed after 24, 48 and 72 hours of thermostatisation at 37°C. The product was considered to be affected by rope spoilage if it had an unpleasant odor and a sticky core. The data obtained in rope spoilage tests were filled in a special Register for the evidence of the occurrence of rope spoilage in bakery products manufactured at Cahulpan company.

3.4. Number of spore-forming bacteria in flour

The standard method for determining the number of mesophilic aerobic spore-forming bacteria by culturing on Plate Count Agar medium (PCA – peptone from casein 5 %, yeast extract 2,5 %, glucose 1 %, agar-agar 14 %) was used to determine the degree of flour contamination. The inoculation was carried out after pasteurization of samples (aqueous flour suspensions) for the destruction of non-sporulated bacteria. For the microbiological analysis, 6 batches of flour of 2nd quality, received at the Cahulpan company in different periods of time during September-October 2013 were used.

3.5. Acidity of bread

The acidity of bread was determined with the aqueous extract method [20] which consists in determining the volume of sodium hydroxide (NaOH) solution 0.1 *n* necessary to neutralize the acids present in bread. The titration is carried out in the presence of phenolphthalein indicator until the appearance of pale pink and stable colour.

3.6. Calculation

Each experiment was carried out in duplicate and the results were provided as average ± SD (standard deviation). Excel programme of Microsoft Office 2010 software was used for calculations, plots and to analyse the data by one-way analysis of variance (ANOVA) then tested by least significant difference (LSD) for mean comparison when level $p \leq 0.05$.

4. Results and discussion

4.1. Detection of ropy bread, 2011-2020

The occurrence of the rope spoilage in the bakery products selected for the study was observed in the period 2011-2020, from May until Sptember, the warm months of the year. The analysis was based on the data recorded in the Special Register for the evidence of the occurrence of rope spoilage in bakery products manufactured at Cahulpan company [30; 31] and on several tests performed by the authors in co-operation with the Cahulpan employees in charge with the quality control of bread and bakery products.

Each type of bread was subjected to the rope spoilage test and observed for 72 hours, positive data being recorded in the Special Register [30; 31].

The results were then summarized and processed in Excel programme of Microsoft Office 2010. The results are presented in the form of a plot (Figure 1) as the occurrence of the rope spoilage in bread, in number of cases per year against the year. Where certain number of cases on ropy bread

per year were obtained a vertical bar is presented on the plot. Bars have different colours for each type of bread according to the legend of the plot.

The rope spoilage frequency varied in time and depended on the type of bread (Figure 1), the highest values being recorded in 2011 for both long loaf "Spicușor" and "Cahuleanca". High values were also recorded in 2013, 2014 and 2015, primarily for the long loaf "Spicușor" followed by the long loaf "De Capitală" and long loaf "Cahuleanca". The number of ropy bread cases per year decreased in 2016 due to the application of several internal measures intended to decrease the occurrence of the rope bread spoilage such as the improvement of the cooling conditions of bread and the hygienic system, the verification of the suppliers a.s.o. In 2017 and the years that followed, after the management of Cahulpan bread-making company decided to use the Ca propionate, as a result of previous results published in the same year [18], there was no more rope spoilage detected in any type of bread or baking product.

The occurrence of rope spoilage in the products presented in Figure 1 is explained primarily by the use of flour with high degree of contamination with mesophilic aerobic spore-forming bacteria. Whereas the determination of the degree of contamination of flour requires a long time (2-3 days), it makes impossible to detect the flour with a high degree of contamination at the moment of reception of flour at the company. Therefore, batches of flour are accepted without their initial microbiological control. That is why some of batches of flour can have a high degree of contamination with mesophilic aerobic spore-forming bacteria, which can lead to the occurrence of rope spoilage in bakery products. However, if such batches of flour are identified, the flour is mixed with flour free of contaminants or with flour with a low degree of contamination to „dilute” the spore-forming bacteria and reduce the risk of rope spoilage.

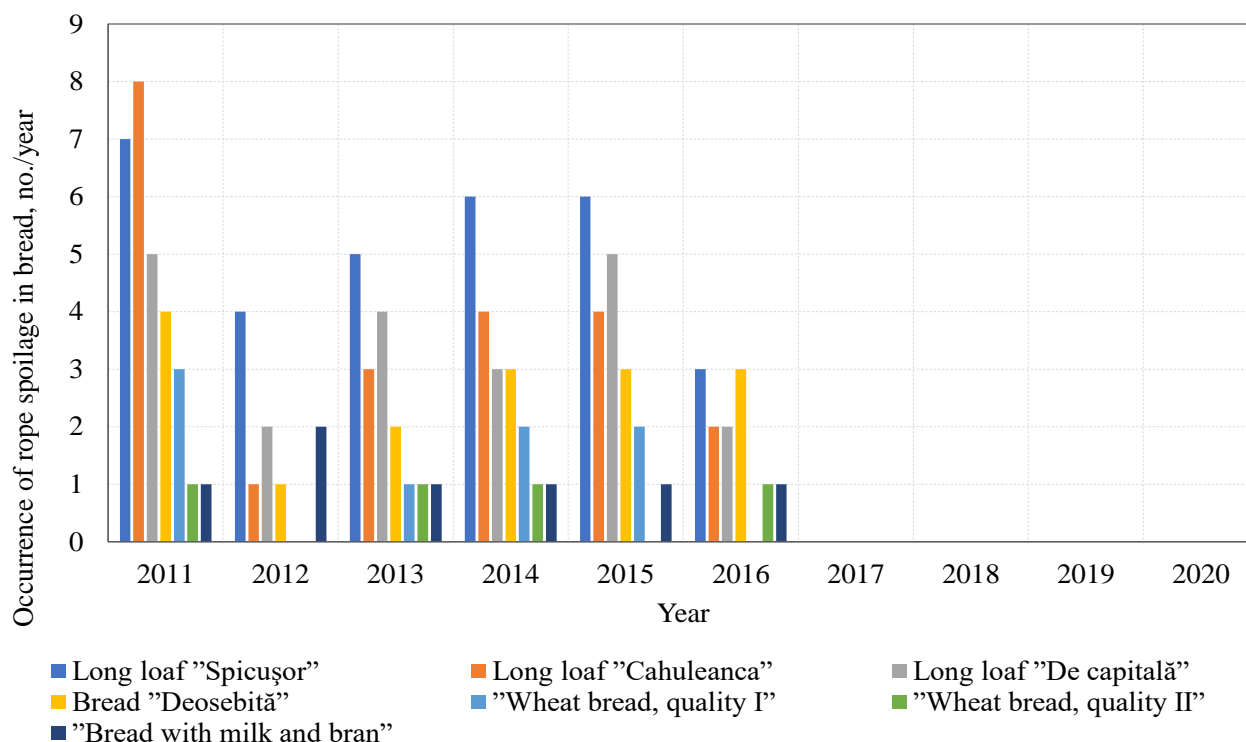


Figure 1. Frequency of occurrence of the rope spoilage in bakery products manufactured at the bakery company Cahulpan, Cahul, Republic of Moldova

Source: processing after data from Special registers [30, 31]

4.2. Microbiological analysis of flour

Previous statement was confirmed by the microbiological analysis of six samples of flour of second quality from different batches, received at the Cahulpan company during the period

September–October 2013. The content of mesophilic aerobic spore-forming bacteria in the analyzed flour samples, expressed in C.F.U./g, is presented in Figure 2.

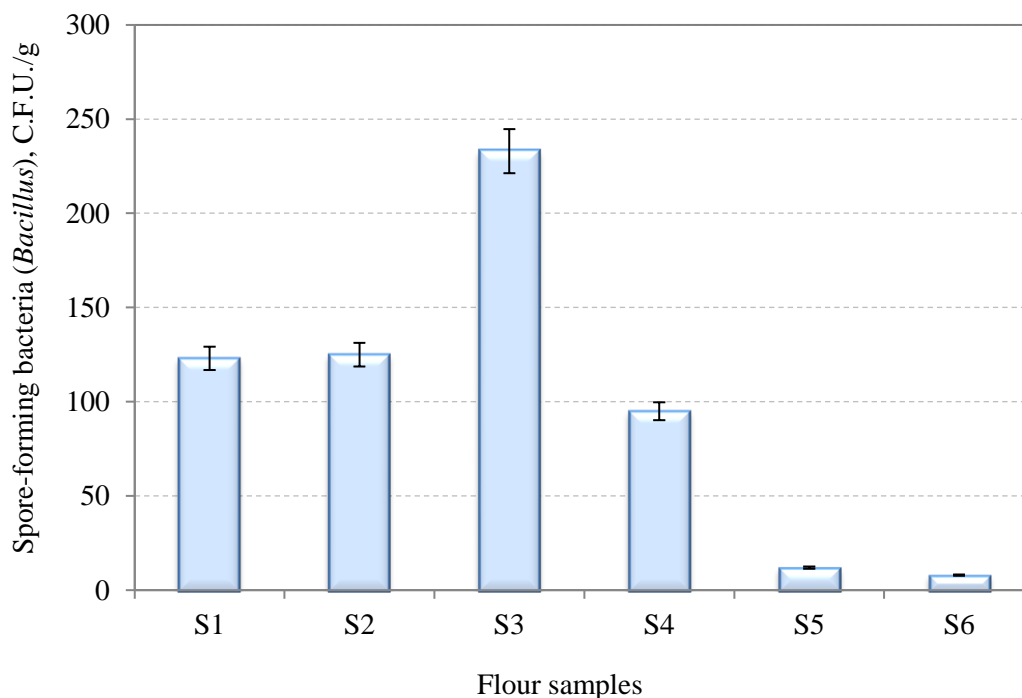


Figure 2. Degree of contamination of wheat flour with mesophilic aerobic spore-forming bacteria

Source: Authors' data processing

According to the data presented in Figure 2, the degree of flour contamination differed significantly ($p < 0.05$) in the case of specific batches, e.g., the flour sample S3 contained 230 ± 17 C.F.U./g and the flour sample S6 contained only 8 ± 3 C.F.U./g.

The difference in the degree of contamination of the flour received by Cahulpan company can be explained through the following statements:

- Non-compliance with the storage conditions of the wheat, which can lead to triggering of the biochemical and microbiological processes that take place in the mass of stored cereals, diminishing their quality (e.g., self-heating). The total number of microorganisms in the grain mass increases [5, p. 403] and, as a result, the degree of contamination of the flour obtained from this wheat also increases;
- Non-compliance with the parameters of the technological process of flour manufacturing – no washing or insufficient washing of the wheat grains before the grinding;
- Non-compliance with the hygienic-sanitary conditions during the wheat processing – improper disinfection of equipment, disinsection and rodent control of rooms;
- Non-compliance with the storage conditions of flour, which, like wheat, may undergo a self-heating process which leads to the increase of the total number of microorganisms.

4.3. Acidity of bread types

The acidity is determined for each batch and each baking product type, and it is registered in the official documents of the company. The acidity of the products affected by rope spoilage was also registered in the Special Registers. The values were selected from the documents and presented in Table 2 to see the values of acidity at which the rope spoilage was detected.

Table 1. Acidity of bakery products manufactured at Cahulpan company at which the rope spoilage was detected

No.	Product	Type of flour used to manufacture the products	Acidity, degrees of acidity		
			Minimum	Medium	Maximum
1.	Long loaf "Spicușor"	High quality flour	1.2	1.9 ± 0.3	2.2
2.	Long loaf "Cahuleanca"	Mixture: High quality flour – 65% + Flour of 1 st quality – 35%	1.2	1.7 ± 0.3	2.0
3.	Long loaf "De capitală"	High quality flour	1.2	1.7 ± 0.5	2.2
4.	Bread in forms "Deosebita"	High quality flour	1.0	1.5 ± 0.5	2.0
5.	Wheat bread, quality I	Flour of 1 st quality	2.0	2.5 ± 0.5	3.0
6.	Wheat bread, quality II	Flour of 2 nd quality	2.4	2.9 ± 0.5	3.4
7.	Bread with milk and bran	Mixture: Flour of 1 st quality – 65% + bran – 35%	2.6	3.2 ± 0.6	3.8

Source: processing data from Special Registers [30; 31]

5. Discussion

The discussion related to wheat flour contamination with spore-forming bacteria (point 4.1) can be used to explain the higher frequency of the occurrence of rope spoilage in long loaf types of bread (Figure 1). These baking products were made from flour with high extraction degree which had a lower acidity than wholemeal flour, for instance. The long loaf breads also had lower acidity, usually lower than 2.0 degrees of acidity, compared to bread made from flour of first and second quality, and/or with addition of bran.

According to GOST 27844-99 standard, the long loaf made from high quality flour must have at least 2.5 degrees of acidity [6]. Also, according to the requirements specified in the „Instruction for the prevention of rope spoilage in bakery products” [14], it is recommended to increase the acidity of bakery products made from wheat flour with one degree above the value indicated in the standard, which means that the long loaf bread during the summer period must have 3.5 degrees of acidity.

However, the increased acidity of the bread negatively influences its sensory properties (low volume, odour and sour taste, and brittle crumb) [17]. Therefore, according to the technological instructions established at Cahulpan, the maximum acidity during the summer period of the loaf should be 2.0-2.5 degrees, which in some cases (for example, when the degree of flour contamination is high) is not sufficient to prevent the development of the bacteria that cause the rope spoilage. The occurrence of rope spoilage in bread is possible especially if the acidity of the product is lower than recommended values, which can also be seen from the data presented in Table 1.

It is worth to emphasize that the values of acidity presented in Table 1 were determined for the ropy bread, meaning before 2017, and were discussed due to the influence on the occurrence of rope spoilage of bread. The acidity of bread measured for unspoiled bread was registered in ordinary registers and is not discussed in the paper.

To overcome the deficiency caused by low acidity of bread, the use of sourdough, whey or other natural acidulant can be applied during summer time. Also, the addition of Ca propionate was implemented in 2017 and the results are obvious: no ropy bread was detected since then.

Regarding the cooling of bread after baking, Rumeus & Turtoi (2016) showed its high influence on the occurrence of rope spoilage in bread and noticed that the faster the cooling and the lower the temperature of the environment were, the lower the incidence of ropiness was [17]. Thus, the goal of the delay or withdrawal of the rope spoilage of bread was reached due to the cross-contribution of discussed factors: type of bread, type of flour and its contamination with *Bacillus* spp., acidity, and of special measures of ropiness control such as cooling conditions after baking, especially time and temperature, and the use of Ca propionate as preservative.

6. Conclusions

Many consumers associate the quality of bakery products with their shelf life. The loss of the freshness of bread is due to several factors, one of the most important being the alteration caused by

microbial activity. Rope spoilage is considered one of the most common defects of bread caused by the activity of bacteria of the genus *Bacillus* from contaminated flour. If the degree of contamination of the flour is very high, then the first signs of rope spoilage (unpleasant odour, sticky crumb) can appear even after 6 hours of bread storage. Microbiological analysis to identify the batches of the contaminated flour with sporulated bacteria, at the reception in the company, requires a long duration (at least 48 hours). Therefore, the flour is usually received without initial microbiological analysis, which in some cases favours the occurrence of rope spoilage in bakery products manufactured at the Cahulpan company. However, the flour identified after the reception as highly contaminated with spore-forming bacteria, is mixed with flour free of contaminants or with reduced contamination for „diluting” the spore-forming bacteria and reducing the risk of rope spoilage.

Cases of the rapid spoilage of bakery products are very unwanted in businesses because they contribute significantly to the loss of consumer confidence in the quality of manufactured products and generate economic losses with immediate and long-term implications.

The acidity of bread and baking products lower than the recommended values that is 3.5 degrees of acidity during the summer period and the slow cooling process, at air temperature higher than 15°C and without forced circulation, increases the risk of rope spoilage. In 2017, Cahulpan company implemented the addition of Ca propionate as preservative and acidulant such as no rope bread was detected since then.

As a conclusion, Cahulpan bread-making company succeeded to delay or withdrawal the rope spoilage of bread as a result of cross-combination of several crucial factors such as the type of flour and its contamination with *Bacillus* spp., acidity, cooling conditions after baking and the use of Ca propionate as preservative.

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