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Effect of the Antimicrobial Agents Addition on the Stability of Part-Baked Bread during Refrigerated Storage and on the Sensory Quality of Full-Baked Bread

Alejandra Carrillo-Meza[1], Rossana Altamirano-Fortoul[1] & María E. Bárcenas[1]

[1]Departamento de Ingeniería Química y Alimentos, Universidad de las Américas Puebla. Cholula, Puebla 72810. México

Correspondence: María Eugenia Bárcenas Pozos, Departamento de Ingeniería Química y Alimentos. Universidad de las Américas Puebla. Cholula, Puebla 72810. México. Tel: -52-222-229-2126.
E-mail: maria.barcenas@udlap.mx

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Abstract

Part-baked bread (PBB) is a product which offers advantages to producers and consumers. The lower cost of refrigeration in comparison with freezing makes it an interesting option to preserve the PBB. The main problem of PBB stored in refrigeration is the mold occurrence on its surface. The addition of an antimicrobial agent in the formulation of PBB capable to delay the proliferation of mold would help to extend its useful life. The aim of this study was to determine the effect of potassium sorbate in comparison with calcium propionate on the number of microorganisms of PBB stored in refrigeration, as well as on the sensory quality of full-baked bread obtained from PBB. From the tests performed it was observed that there were no molds on the surface of PBB with antimicrobial agents during 28 days of storage at 5 ℃, while molds were on control PBB at 15 days of storage. It was also found that calcium propionate (0.16% flour basis) was more effective than potassium sorbate (0.16% flour basis) on delaying microorganisms growing. Addition of antimicrobial agents did not affect significantly the sensory attributes of bread obtained from PBB. However, the addition of propionate caused a decrease on the specific volume of bread.

Keywords*:* calcium propionate, part-baked bread, potassium sorbate, refrigeration

1. Introduction

Due to the important advantages for producers, two stages-baked bread has revolutionized the bread industry. Among the main advantages there are the reduction of economic losses caused by bread aging and the possibility to schedule the production at a more convenient time. Moreover, this technology offers advantages to the consumers, who can have freshly baked bread at any time of the day and in a variety of points of sale or even at home. Because of this, during the last decade a big number of researchers from all over the world have become interested in studying different aspects of part-baked bread (PBB) and full-baked bread obtained from the first one. Among recent studies, it can be named those related to the effect of different ingredients on the quality of PBB and FBB (Almeida & Chang, 2012; Almeida, Chang, & Steel, 2013; Altamirano-Fortoul, Hernando, & Rosell, 2014; Jiang, Le Bail, & Wu, 2008; Majzoobi, Raissjalali, Jamalian, & Farahnaky, 2015; Ronda, Rivero, Caballero, & Quiles, 2012); on the physical properties (Altamirano-Fortoul & Rosell, 2011; Farahnaky & Majzoobi, 2008; Jury, Monteau, Comiti, & Le Bail, 2007; Hamdami, Monteau, & Le Bail, 2006; Le Bail et al., 2010; Novotni et al., 2011; Park & Baik, 2007; Primo-Martín et al., 2006; Ronda, Gómez, & Quiles, 2010), the sensory attributes (Poinot et al., 2007) and the aging (Bosmans, Lagrain, Ooms, Fierens, & Delcour, 2014; Le Bail, Leray, Perronnet, & Roelens, 2011; Ronda, Quiles, Pando, & Roos, 2014) of these products. Refrigeration as a PBB preservation method has also been studied in the recent years (Bárcenas & Rosell, 2006; Bárcenas & Rosell, 2007; Bosmans et al., 2014; Karaoglu & Kotancilar, 2006; Karaoglu, Kotancilar, & Gurses, 2005; Lainez, Vergara, & Bárcenas, 2008) because it represents an interesting alternative to freezing. Bread is a low-cost and frequent consumption product, which manufacturing cost rises with the inclusion in its production of an expensive technology, like freezing. In this aspect, Le Bail et al. (2010) calculated that the demand of required energy for bread freezing is similar to required energy for baking. Furthermore, the formation and growing of ice crystals during PBB freezing and frozen storage can damage the structure of the product (Bárcenas & Rosell,

2006).

On studying refrigeration as a PBB preserving method, an essential aspect to evaluate has been the capacity of this technology to control the proliferation of microorganisms in the product. In an experiment performed by Karaoglu et al. (2005) it was found that the counts of part-baked white pan bread stored at 4 ℃ were lower than those of the same type of bread stored at ambient temperature. On the other hand, Lainez et al. (2008) evaluated the microbiological quality and stability of PBB stored at 7 ℃. They found out that the factor that determined the useful life of the product was the occurrence of molds on its surface, which happened after 9 days of storage. The use of antimicrobial agents is an option to extend the useful life of PBB stored on refrigeration, because the presence of these agents would represent an additional obstacle for the proliferation of microorganisms (Leistner, 2000). Karaoglu et al. (2005) added calcium propionate (0.2% fb) to the part-baked white pan bread that was stored at 4±2 ℃ and found that the antimicrobial agent affected aerobic mesophilic bacteria, but not the molds and the yeasts. From this point, it was considered to evaluate other antimicrobial agent for the preservation of this type of bread, particularly one that prevents the molds growing, like potassium sorbate (Deuel, Alfin-Slatee, Weil, & Smyth, 1954). On the other side, it has been demonstrated that some microbial agents may affect the yeast responsible of the fermentation of the bread dough. Suihko and Mäkinen (1984) reported that both propionate and sorbate used in bread doughs (in concentrations of 0.16% and 0.08%, respectively) had an inhibitory effect on *Saccharomyces cerevisiae*. Some of the attributes of bread, like flavor, volume and texture, which depend on the efficacy of the fermentation, may be affected as consequence of the inhibition of the yeast. There is where the interest to do sensory evaluation to bread obtained from PBB added with antimicrobial agents stored in refrigeration comes from. Therefore, the aim of our study was to determine the effect of potassium sorbate in comparison with calcium propionate on the microbial count of PBB stored in refrigeration, as well as on the sensory quality of full-baked bread obtained from PBB.

2. Materials and Methods

2.1 Interrupted Breadmaking Process and Refrigerated Storage

A basic recipe with commercial wheat flour (14% moisture; 13% protein; 0.6% ash content; 34% wet gluten; 105 mm tenacity; 96 mm extensibility), compressed yeast (1.4% flour basis), salt (1.8% flour basis), sugar (1.8% flour basis), and water (to achieve optimum consistency of 500 Brabender units) were used in this study; a batch of 10 kg of wheat flour was prepared and an improver was not used. When antimicrobial were used (calcium propionate or potassium sorbate) these were added in a concentration of 0.16% (flour basis). The bread making equipment was semi-industrial scale and property of a bakery industry (PABISAN Company, Mexico). All the ingredients were mixed (12 min) into a dough, left for 10 min, divided (150 g portions), kneaded, and molded. Dough was proofed at 35 ℃ and 65% relative humidity during 30 min. Then, part-baking was performed at 240 ℃ for 10 min in a gas oven (Static 20, Gouet, Spain). Part-baked bread was cooled at room temperature until the center of the crumb reached 40 ℃. The bread was wrapped into non-sterile polyethylene pouches, placed in cardboard boxes and stored in a refrigerator at 5 ℃. Breadmaking was carried out following the good manufacture practices for bakery. Samples were taken after 0, 7, 14, 21, and 28 days of storage and used a) for pH, water activity, and microbiological determinations, and b) to complete the baking process, at 250 ℃ for 10 min. The full-baked bread was cooled at ambient temperature for 60 min and then subjected to specific volume and sensory evaluation.

2.2 Physicochemical Properties

Bread volume was determined by the rapeseed displacement method. An AquaLab Serie 3 equipment was used to measure the water activity (Decagon, USA). The 02-52 method of AACCI (2000) was applied to determine the level of pH.

2.3 Microbiological Analysis

Dilution 1:10 was prepared weighing 10 g of bread and homogenizing it with 90 mL of sterile water. With this solution, dilutions 10^{-3} and 10^{-5} were prepared. 1 mL of the corresponding dilution was added to plate count agar (Merck), incubated, and total aerobic plate count (APC) determined as the number of colony forming units (cfu) per gram of bread, using a colony counter (Erma Optical). Each experiment was made by triplicate.

2.4 Sensory Evaluation

Sensory evaluation was carried out by an untrained panel of 20 judges and scored on a scale of 1 (dislike extremely) to 9 (like extremely). The attributes evaluated were flavor, odor, appearance, texture and overall acceptance. For each one of these attributes, the average response was evaluated. Bread was considered acceptable if the mean response was above 5 (neither like nor dislike).

2.5 Statistical Analysis

In order to assess significant differences ($p < 0.05$) among samples, analysis of variance was performed using Minitab 14 (Minitab Statistical Software, Minitab Inc.). Fisher's least significant differences (LSD) test was used to describe means with 95% confidence.

3. Results and Discussion

3.1 Microbiological Tests

Part-baked bread (PBB) is a susceptible product for proliferation of microorganisms. This phenomenon is attributable to the pH and the water activity of PBB, which are propitious for microorganisms reproduction (pH=5.44 ± 0.03 and a_w=0.97 ± 0.01, for PBB freshly obtained, produced in this study). Therefore, to detect opportunely the presence of molds on the surface of bread, during storage in refrigeration, different types of PBB were examined daily. It was observed (Table 1) that neither the PBB with calcium propionate nor the one added with potassium sorbate presented molds on the surface during the 28 days of storage in refrigeration (5 ℃). Meanwhile, in the control PBB molds appeared at 15 days of storage. It means that the addition of antimicrobials avoided the occurrence of molds on the surface during the storage period. On the other hand, after comparing the results with the molds occurrence time on the surface of the product reported by Lainez et al. (2008) (for PBB without antimicrobials stored at 7 ℃ and with the same formulation and elaboration method of the product studied on this research), which was of 9 days, it was found that storage temperature has an important effect on molds occurrence.

Table 1. Antimicrobials addition effect on molds occurrence on part-baked bread (PBB) surface, stored in refrigeration (5 ℃)

Time (days)	Control	Calcium propionate	Potassium sorbate
0	Negative	Negative	Negative
7	Negative	Negative	Negative
14	Negative	Negative	Negative
21	Positive	Negative	Negative
28	Positive	Negative	Negative

Likewise, having the aim to observe the evolution of the microorganisms count on PBB with and without antimicrobials during the storage in refrigeration, the number of total aerobic mesophilic bacteria (TAMB), molds and yeast was registered. The results are set on Tables 2, 3 and 4. On delaying the growing of the three types of microorganisms, calcium propionate was more effective than potassium sorbate. The effect of this preservative was bigger on yeast and molds and lower on TAMB. After 28 days of storage, the yeast count of PBB with propionate was lower to 10 CFU/g, while molds and TAMB counts were 15 and 27 CFU/g, respectively. In the case of PBB with sorbate, at the end of storage 20, 20 and 133 CFU/g for yeasts, molds and TAMB counts, respectively, were found.

Table 2. Antimicrobials addition effect on the number of aerobic mesophilic bacteria (log CFU[a]/g) of part-baked bread (PBB) stored in refrigeration (5 ℃)

Time (days)	Control	Calcium propionate	Potassium sorbate
0	1.99	<1	<1
7	2.38	<1	<1
14	2.72	<1	1.30
21	ND[b]	1.48	2.01
28	ND[b]	1.43	2.12

[a]CFU: Colony forming units.

[b]ND: Not determined due to molds on the PBB surface were observed at day 15 of storage in refrigeration

Table 3. Antimicrobials addition effect on the number of molds (log CFU[a]/g) of part-baked bread (PBB) stored in refrigeration (5 ℃)

Time (days)	Control	Calcium propionate	Potassium sorbate
0	2.15	<1	<1
7	1.63	<1	<1
14	1.36	<1	1.3
21	ND[b]	<1	1.3
28	ND[b]	1.18	1.3

[a]CFU: Colony forming units.

[b]ND: Not determined due to molds on the PBB surface were observed at day 15 of storage in refrigeration

Table 4. Antimicrobials addition effect on the number of yeasts (log CFU[a]/g) of part-baked bread (PBB) stored in refrigeration (5 ℃)

Time (days)	Control	Calcium propionate	Potassium sorbate
0	1	<1	<1
7	1.89	<1	<1
14	1.40	<1	1.30
21	ND[b]	<1	1.48
28	ND[b]	<1	1.30

[a]CFU: Colony forming units.

[b]ND: Not determined due to molds on the PBB surface were observed at day 15 of storage in refrigeration

The microbial activity of both potassium sorbate and sodium propionate depends on pH. The maximum pH for microbial inhibition with potassium sorbate is 6.5 and with propionate is 6.0. Therefore, it is possible to say that pH of PBB produced in this study (5.44 ± 0.03) was appropriate for the correct operation of both antimicrobials. Likewise, it is well known that the efficacy of sorbate and propionate rises while the pH of the environment approaches to its pKa (Sofos & Busta, 1981). This could explain why propionate (pKa 4.87) had more antimicrobial activity than sorbate (pKa 4.75). It also could explain the difference between the results obtained in the present study and those reported by Karaoglu et al. (2005), considering that pH of PBB produced by those researchers was higher (5.81-5.95) than the one of our bread.

3.2 Sensory Quality

Sensory attributes of food products depend on the ingredients included in its formulation and on the conditions and processing methods used in its production. In breadmaking, attributes like flavor, smell and texture are very appreciated by consumers and depend in an important way on the fermentation of sugars by yeasts. Due to the results of the microbial tests showed that potassium sorbate and calcium propionate added to the part-baked bread (PBB) affected the yeasts proliferation, the effect of these antimicrobials on flavor, smell, texture, appearance and general quality of bread obtained from PBB, stored during different periods of refrigeration, was analyzed as part of the present study. The results of the sensory evaluation are on Fig. 1. There were no significant differences on any of the evaluated attributes, at any of the evaluated storage time, among the different types of bread. At most of the cases, the evaluations given by judges to the different breads varied from "like moderately" to "like extremely" at all the attributes. It is possible to affirm that the addition of the antimicrobials tested in this study had no negative impact on the acceptance of the bread obtained from PBB stored in refrigeration.

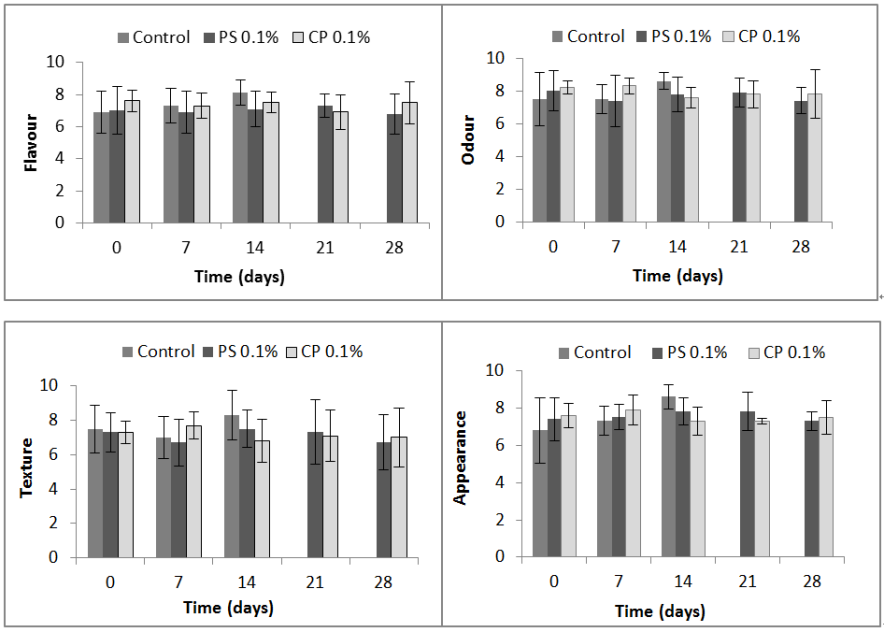

Figure 1. Effect of antimicrobial agent type (PS: potassium sorbate; CP: calcium propionate) and refrigeration (5 °C) storage time on sensorial properties of full-baked bread from part-baked bread

3.3 Specific Volume

Karaouglu and Kotancilar (2006), in their study about part-baked white pan bread, found that the specific volume of PBB with calcium propionate was lower than in control bread. Therefore, as complementation of the sensory evaluation, the effect of potassium sorbate and calcium propionate, added to the PBB formulation, on the full-baked bread specific volume was analyzed (results on Table 5). There were no significant differences among PBB with sorbate and control PBB specific volumes. However, PBB with propionate presented a lower specific volume in comparison with the other two types of bread. On yeast leavened bread the specific volume depends, among other factors, on the production of CO_2 by *Saccharomyces cerevisiae* during fermentation. A low specific volume indicates that yeasts on the dough did not produce the CO_2 necessary for a good expansion. Although it has been reported that *Saccharomyces cerevisiae* present in bread doughs (pH 5.9) is affected by both propionate and sorbate (Suihko & Mäkinen, 1984), our results suggests that the yeast used in our study for the production of PBB tolerated better sorbate than propionate.

4. Conclusions

Addition of antimicrobial agents to PBB formulation let to delay the proliferation of microorganisms without affecting sensory quality of full-baked bread obtained from PBB. Calcium propionate had more effect than potassium sorbate on microorganisms. It is probably that pH of PBB had influenced at some way on the effect of the antimicrobials. However, to confirm this point, more research would be necessary.

Table 5. Antimicrobials addition effect on specific volume (cm^3/g) of bread obtained from part-baked bread (PBB) stored in refrigeration (5 ℃)*

Time (days)	Control	Calcium propionate	Potassium sorbate
0	3.7 ± 0.44^b	4.0 ± 0.24^b	2.2 ± 0.58^a
7	3.9 ± 0.27^b	4.1 ± 0.09^b	3.0 ± 0.53^a
14	3.9 ± 0.13^b	4.0 ± 0.07^b	2.5 ± 0.09^a
21	ND**	3.6 ± 0.26^b	2.8 ± 0.20^a
28	ND**	3.2 ± 0.41^b	2.6 ± 0.09^a

*Measures on the same row followed by the same letter were not significantly different ($P<0.05$). Average of three replicas

**ND: Not determined due to molds on the PBB surface were observed at day 15 of storage in refrigeration

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