

Whole grain bread preserved with lemon juice: a comparative study**Pão integral conservado com suco de limão: um estudo comparativo**

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ABSTRACT

The use of natural substances for the replacement of chemicals in food conservation is a global trend. Thus, the aim of this study was to evaluate the effect of lemon juice on wholemeal bread conservation and determine shelf time. This is a quantitative, laboratory and explanatory study in which three formulas of the dough with the same composition of ingredients were tested. One formula had the addition of lemon juice at the concentration of 1.75%; other with industrial antifungal at 1.0%; and the last formula without any addition. Analysis of fibers, humidity, pH and titratable acidity were performed. Shelf life for all formulations was evaluated by the visual method until the formation of mold colonies for 5 days. The samples showed similarity in the variation in humidity level (32.4 to 37.9) in relation to time, having a reduction at the first moment and then in the increase of F1 and F2, and F3 remained constant. The fiber content of the formulations showed a discrepancy between the contents of the evaluated times. The pH of the three formulas was within the desirable range of 4.0 to 7.0 ensuring good quality for the product according to the technical pattern of baking. The acidity variation between formulas followed a common pattern. Shelf life was similar for formulas containing lemon juice and antifungal solution, however the formula without any addition had the shortest shelf time, confirming the efficiency of lemon in the conservation of wholemeal bread.

Keywords: natural substances; food conservation; self life.

RESUMO

O uso de substâncias naturais para substituição de produtos químicos na conservação de alimentos é uma tendência mundial. Dessa forma, o objetivo deste estudo foi avaliar o efeito do suco do limão na conservação de pão integral e determinar seu tempo de prateleira. Trata-se de um estudo do tipo quantitativo, laboratorial e explicativo. Foram testadas três fórmulas da massa do pão com a mesma composição de ingredientes, uma com adição de suco de limão a concentração de 1,75%, outra com antifúngico industrial a 1% e a última fórmula sem adição de conservante. Foram realizadas análises de fibras, umidade, pH e acidez titulável. A vida de prateleira para todas as formulações foi avaliada pelo método visual até a formação de colônias de bolores por 5 dias. As amostras revelaram semelhança na variação do teor de umidade (32,4 a 37,9) em relação ao tempo, tendo uma redução no primeiro momento e, em seguida, no aumento de F1 e F2, e o F3 manteve-se constante. O teor de fibras das formulações mostrou uma discrepância entre os conteúdos nos tempos avaliados. O pH nas três fórmulas ficou dentro da faixa desejável de 4,0 a 7,0 garantindo boa qualidade ao produto de acordo ao padrão técnico de panificação. A variação de acidez teve um padrão comum entre as fórmulas. Já a vida de prateleira, foi similar para as fórmulas contendo suco do limão e antifúngico, no entanto, a fórmula sem conservante apresentou menor vida útil, confirmando a eficiência do limão na conservação de pão integral.

Palavras-chave: substâncias naturais; conservação de alimentos; vida de prateleira.

1. INTRODUCTION

Bread is a food consumed all over the world and in different forms. Its basic formulation includes wheat flour, yeast and salt, but many optional ingredients are also included. As bread reaches a broad range of population and is very well accepted, it can be

used as a transporter of bioactive compounds or other nutritionally important components (SEHN; WALNUT; STEEL, 2015).

In 2018, Brazil produced 401,289 thousand tons of bread, generating more than 6 billion brazilian reais according to a research from Nielsen Company. In regard of world sales, it ranked 11th, representing the best position of a Latin American country (ABIMAP, 2019).

Nonetheless, the high consumption of carbohydrate-rich foods, especially with the use of refined flours used in industrial production, has favored population weight gain and consequently the development of Non-communicable Chronic Diseases - NCCD (BIELEMAN, 2015).

There are limitations in in vivo studies on the effects generated by food additives on the human body (KUMAR et al., 2019), but it is known that there are cases of allergies, cancer and disorders in the digestive system (BISSACOTTI; ANGST; SACCOL, 2015).

The search for products that offer health benefits is a consolidated trend, constituting a growing market demand as consumers become increasingly demanding. The increase in the interest of the population for products that offer health benefits, combined with the recommendation of a greater intake of foods containing fibres by national and international health agencies, has led the food industry to the development of new products enriched with these compounds.

In this regard, it is necessary to develop new food products with higher nutritional content, which provide increased satiety, facilitate weight control in addition to intestinal modulation and improvement of immune response (CAROCHO et al., 2016).

The objective of this study was to develop a formulation of wholemeal bread with the addition of lemon juice as a preservative and to evaluate the conservation of it by comparing with two other formulations (without adding the juice and with the addition of chemical preservative).

2. METHODOLOGY

The research was developed at the Laboratory of Dietary And Bromatology Techniques of the University Center of Technology and Sciences - UNIFTC. The study was quantitative, laboratory e explanatory.

The formulations presented in Table 1 were obtained based on previous studies to determine the best concentrations of tested inputs: zero addition of inputs; lemon juice; and industrial antifungal (calcium propionate 1%, Mauri, Brazil) (F1, F2 and F3, respectively). All ingredients were carefully separated and weighed by high-precision scale (0.1g to 1000g

capacity). The choice of ingredients for the base formulation was standardized and based on previously performed tests.

In this method, all dry ingredients were mixed at once, considering that yeast did not come into direct contact with salt. Then, the moist ingredients (eggs, olive oil, yam) were added and the cold water was gradually supplemented to the maximum hydration point and, subsequently, the lemon juice was added and the manual knead process began, which was performed for 10 minutes. To identify the ideal point of the dough, the veil point was used. Afterwards, the dough was beaded and covered with plastic film, for 15 min, resulting in the stage called table rest. This procedure induces the relaxation of gluten, promoting the modeling of breads. Finally, the fermentation of the dough was lowered and the processes of portioning and bileulation began which the quantity of 30 grams each portion was established (CANELLA-RAWS, 2003).

Then, the breads were stored in an oven for 30 minutes or until the duplication of volume, followed by the baking process. The estimated baking time depended on the portion of the bread, around 8 to 12 minutes at a temperature of 180 °C. After the total cooling of the breads, they were packed in portions of 5 units using low density polyethylene films (CANELLA-RAWS, 2003). Subsequently, the products were identified with batch, date and formulation.

The analysis of humidity, titratable acidity and pH were performed according to the methodology proposed by the Adolfo Lutz Institute (2008) and the determination of total fibers using the methodology recommended by Detman *et al.* (2012), and carried out in two batches, each one in three replicates. The breads were submitted to analysis at days: 1, 3, and 5 after production.

3. RESULTS AND DISCUSSION

The means and standard deviations of physicochemical determinations from the formulations of wholemeal bread (F1; F2; F3) elaborated are presented in Tables 2, 3, 4 and 5.

When analyzing the humidity content of the formulas with the storage period, it was observed that the behavior was similar both in F1 and F2, with reduction of humidity content on the third day and subsequent elevation on the fifth day. However, when analyzing F3, it was noticed that there was a balance in humidity content, which can be explained by the presence of chemical substances, called antiumectant, present in the antifungal, which aids in this control.

It is important to highlight that these products were easily influenced by climatic conditions. It is possible to consider that in formulations F1 and F2 were more susceptible to climate interference because it did not have the addition of chemical preservatives, compared to F3 which had the addition of industrial antifungal. It was taken into account that the product under study was packed in flexible packaging of low-density polyethylene film, the most suitable for packing breads.

In addition, Tejero (2004), states that cooking time and temperature are especially important in moisture determination. The oscillation of humidity content may have been influenced by the cooking process since no industrial furnace was used and the experiment was carried out on different days. It is worth mentioning that the temperature of the oven, in addition to the environment itself, may have undergone variations by modifying cooking time and interfering in the humidity content of the product.

Humidity is related to bread stability and quality as it influences the optimal processing and fermentation of the product. In addition, the high moisture content in breads can increase microbial activity, decrease shelf time and final product quality, as reported by Oliveira *et al.* (2011).

According to Guerreiro (2006), the desirable pattern for humidity is up to 35% in wholemeal breads. Considering the parameter in question, only F1 on the fifth day exceeded desirable standards for humidity content. Borges (2011) evaluated the humidity content in breads and found variation between $30.99\% \pm 0.66$ to $34.03\% \pm 0.20$, values very similar to those described in this study. The humidity contents found in the present study were lower when compared to the study of Oliveira *et al.* (2011), in which $34.85\% \pm 0.25$, to $35.21\% \pm 0.84$ humidity was found.

As can be seen in Table 3, there was an increase in the fiber content for the three formulations. Possibly, these differences in fiber content is due to whole wheat flour does not have a grinding pattern, i.e. containing different granumetry sizes. Thus, the homogenization of the dough does not happen equally, it is possible that the fibers may have concentrated more in a certain portion of the food, favoring the increase of fibers from the portion of the bread evaluated in the third and fifth days.

According to DRC n°. 54/2012 (BRASIL, 2012), a food to be classified as the holder of "high fiber content", must contain at least 5g per serving (100g) and to be defined as a "fiber source" must contain at least 2.5 g of fibers per serving.

Even if there is a discrepancy between formulas and between days, fiber content was still found in the three formulations, which allows classifying them as "sources of fiber" foods according to legislation. This suggests that the product could be used as a good option to increase the content of fibers in the daily diet of the population, because with the food transition and the high consumption of industrialized products there has been reduced fiber consumption (FAO; OPAS, 2016).

Daily fiber intake helps in reducing cardiovascular and metabolic diseases due to the ability to perform glycemic control, reduce serum lipid levels and act in the best functioning of the immune system. It is assumed that the fibers perform the rebalancing of the intestinal microbiota in addition to providing greater satiety and promoting better control of body weight (RODRIGUES; BERNAUD, 2013).

Within this reality, the pH values found in formulas F1, F2 and F3 remained balanced and close to the desirable pH range between the days observed, which may have collaborated to maintain the quality standard between the three formulas, in regard to the fermentation and conservation.

The pH of the three formulations ranged from 5.3 (± 0.16) to 7 (± 0.57). Technical standards for pH in roasted dough were not found in the literature. However, according to Quaglia (1991), the optimal conditions for the development of yeasts, during the fermentation of fresh dough, are established with pH values around 5.0 to 6.0 and should not exceed these values seeking to maintain the best fermentative capacity of bread.

When this variable reaches values above 6.0, fermentation may become impaired, favoring excessive production of glycerin and acetic acid, in addition to ethyl alcohol that decreases the final quality of the product. As a consequence, sensory quality problems can occur in the breads, such as unpleasant taste and reduced volume, which directly interferes with the texture of the product, according to Nunes (2006). Although these characteristics are not objects for this study.

Lemon juice contains ascorbic acid and citric acid in its composition, therefore, it can be used in baking as a natural substitute for chemical and industrial additives for having antioxidant actions and pH regulators, as reported by Canella-Rawls (2003). The similarity of pH values between formulas F2 and F3 suggests that an equivalent action of industrial antifungal and lemon juice may have taken place to maintain the quality of breads.

It is observed that the results found in F1, F2 and F3, in relation to the three observation times, there was a variation in acidity showing an increase on the third day of observation in

all formulas followed by a gradual reduction after the third day. According to Oliveira *et al.* (2011), these results can be attributed to the application of lemon juice and industrial antifungal, considering that its use may have had an acidity regulator effect, especially the values very close to acidity in formulas F2 and F3 on the fifth day. In fact, a proximity of values of F2 and F3 was observed that evidences equivalence in maintaining acidity, which possibly contributes to microbiological inhibition and with increased shelf time of the loaves.

According to Guerreiro (2006), the desirable acidity in whole breads is maximum 5%. In regard of this aspect, the results of the study had the formulations F1 and F2 above the standard only after the third day. According to Ordoñez (2005), acidity is an important parameter in the evaluation of the conservation status of a food product, because acidification perform an inhibitory function on microbial growth, which cooperates with the shelf life of the product.

After the daily analysis of the *shelf life* of the breads, it is observed that in the first and second days there was no visual modification related to the formation of colonies of molds and yeasts in the formulations F1, F2 and F3. In F1, the formula without the presence of preservatives, the occurrence of mold and yeast colonies were identified from the third day of observation, which made the product unnapropriate for consumption. In formulas F2 and F3, colony formation occurred only from the fourth day. It is assumed that the use of lemon juice in F2 exhibited antimicrobial effect in equivalence to the industrial antifungal, used in F3.

4. CONCLUSION

Considering the new market trends, more research is needed to contribute to the development of new products that value the use of natural additives. In this sense, a similarity was verified of the effect of lemon juice and industrial antifungal on the conservation of breads. This confirms that lemon juice could be a natural, healthier and cost-effective option in relation to the use of industrial chemical additives used in baking. By the visualization of the formation of colonies of molds and yeasts, the formulations with lemon juice and those with the chemical additive, had similar shelf life.

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TABLES

Table 1 - Formulations of breads.

INGREDIENTS	FORMULA ZERO (F1)	FORMULA WITH LEMON JUICE (F2)	FORMULA WITH ANTIFUNGAL (F3)
Whole wheat flour	100%	100%	100%
Rye flour	20%	20%	20%
Olive oil	5%	5%	5%
Honey	4%	4%	4%
Dry biological yeast	1,6%	1,6%	1,6%

Cooked yam	5%	5%	5%
Egg (yolk)	3,6%	3,6%	3,6%
Kitchen salt	1,6%	1,6%	1,6%
Water	90%	90%	90%
Lemon	0%	1,75%	0%
Industrial Antifungal	0%	0%	1%

Source: Research data.

Table 2 - Percentage of moisture of bread formulas at different times (%)

	1st day	3rd day	5th day
Formula 1	35.6 ± 1.01	32.45 ± 13.10	37.9 ± 1.47
Formula 2	31.46 ± 7.16	23.24 ± 0.44	33.74 ± 0.47
Formula 3	29.36 ± 0.93	29.32 ± 5.31	29.32 ± 5.31

Wholemeal bread; F1: bread without the addition of preservatives, F2: wholemeal bread at 1.75% lemon juice; and F3: wholewheat bread containing 1% industrial antifungal, according to the manufacturer's indication.

Table 3 - Fiber content in bread formulations at different times (%)

	1st day	3rd day	5th day
Formula 1	1.88 ± 0.15	2.71 ± 0.12	2.88 ± 4.54
Formula 2	1.66 ± 0.14	11.50 ± 0.47	11.5 ± 0.47
Formula 3	0.87 ± 0.93	1.89 ± 0.93	10.58 ± 5.31

F1: bread without the addition of preservatives, F2: wholemeal bread with 1.75% lemon juice; F3: wholewheat bread containing 1% industrial antifungal.

Table 4 - Determination of pH in the formulations of breads in the different times

	1st day	3rd day	5th day
Formula 1	5.3 ± 0.16	7 ± 0.57	7 ± 0.57
Formula 2	7.08 ± 0.03	6 ± 0	6 ± 0
Formula 3	6.98 ± 0	6 ± 0	6 ± 0

F1: bread without the addition of preservatives, F2: wholemeal bread at 1.75% lemon juice; and F3: wholewheat bread containing 1% industrial antifungal.

Table 5 - Percentage of acidity in bread formulations in different days after production

	1st day	3rd day	5th day
Formula 1	4.36 ± 1.21	5.86 ± 0.79	2.62 ± 1.03
Formula 2	3.56 ± 0.66	6.51 ± 0.13	3.43 ± 0.29
Formula 3	1.62 ± 0.65	4.91 ± 0.76	3.74 ± 0.76

Wholemeal bread; F1: bread without the addition of preservatives, F2: wholemeal bread at 1.75% lemon juice; and F3: wholewheat bread containing 1% industrial antifungal, according to the manufacturer's indication.