Freezing Technology of Ketogenic Bread as a Preventive Measure Against Mucilage (Potato Disease)

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Abstract: The pH of ketogenic bread is alkaline and as a result, favourable conditions are provided for the development of potato disease. The disease usually occurs when bread is stored under normal conditions at a room temperature within 20°C - 25°C temperature range. The first signs of potato disease occur within 36 to 72 hours after baking, due to the survival of spores of *Bacillus subtilis* and *Bacillus mesentericus* in the bread crumb. A number of preservatives added into bread effectively inhibits the growth of these bacteria. Yet, today consumers prefer safer bread products without synthetic additives, taking into consideration the nature of their eating habits and diet. In order to inhibit the development of pathogenic bacteria, it is recommended to acidify bread, yet it may have adverse effects as the product acquires undesirable taste characteristics. The article presents the production of a ketogenic bread with a mixture of seeds with and without 3% acetic acid from the total amount of linseed flour. Based on the results obtained, this level did not affect the suppression of potato disease. In the course of the research, various chilling/ freezing methods were used, including cooling in the blast freezer at -1 °C and keeping the keto bread at 0-4 °C, which proved to be the most suitable one. The bread produced applying the latter chilling/freezing method has the maximum 14-day shelf life and is the best solution in terms of preventing potato disease.

1 INTRODUCTION

According to the principles of a ketogenic diet, foodstuffs containing a minimum amount or without carbohydrates are used. An important change in the regular normal diet is the limitation of carbohydrates in the ketogenic diet, and foods such as sugar, honey, potatoes, sweet drinks and carbohydrate-based pastries cannot be included in this diet (Ненартович, 2020). This diet differs from others as it switches the body from burning glucose (sugar) to burning fats and ketone, thus transitioning into a state of ketosis. During this process, fat is burned as the main source of energy (Stroroschuk, 2020).

All foods have an appropriate shelf life due to a variety of spoilage, which are induced by physical, chemical and microbiological factors. The most common causes of food spoilage are extraneous micro-organisms, which can be introduced in a variety of ways: via the equipment, from the environment, due to a lack of hygiene of the food handlers, or via water or other basic or auxiliary materials used. The raw materials used in the production of keto bread such as milled linseed, various seeds are potential factors, affecting the development of potato disease (Peter, 2007).

Keto bread made with linseed meal is a mildly alkaline medium. In this medium the spore-forming bacteria *Bacillus subtilis* and *Bacillus mesentericus* develop and cause the mucilage of bread. These bacteria are resistant to high temperatures and persistent after the bread is baked at 180 °C. The first signs of the disease appear in 36 to 72 hours after bread is baked. Specific signs are easy to identify such as an increase in stickiness of the bread crumb, mucilage, softness (reminiscence of unbaked dough), after breaking bread crumb emits an unpleasant odour, threads tend to stretch, and storing bread longer, dark reddish-purple or yellowish-brown spots occur. In order to inhibit the activity of potato spore-causing bacteria, it is recommended to increase the acidity of bread (vinegar or lactic acid, lactic acid enzymes can be used), add calcium propionate (preservative E282) to the product, clean and disinfect the environment, reduce the moisture content of baked bread, cool rapidly to 10 ° C (in the presence of slow cooling and higher temperature, these bacteria proliferate rapidly) (Dong, Karboune, 2021; Valerio et al., 2012; Pater, 2007).

The best solution to inhibit spore-forming bacteria is refrigeration. Freezing is the process by which food is preserved at low temperatures. During freezing, the activity of microorganisms is stopped and the microorganisms enter a state of anabiosis (this is the suspension of the activity of microorganisms due to unfavourable physical or chemical factors). Low-temperature preservation can be further subdivided into freezing (temperature - 18 °C) and thawing (temperature - 0 °C). Yet, thawing only stops the activity of micro-organisms for a short period of time, and the product starts to deteriorate and the micro-organisms start to thrive after a while. Freezing increases the shelf-life of the product, whereas thawing increases the activity of microorganisms and the perishability of the product, that is why it is advisable to consume these products as soon as possible (Gaigalis et al., 2003).

It has been identified that freezing of the keto bread stops the development of spore-causing bacteria that cause potato disease; inhibits the activity of other microorganisms or destroys them; extends shelf-life (a shelf life of fresh bread is 4 days, when refrigerated 10 days, and once frozen up to half a year). Furthermore, the staling process of the bread is stopped (thawing of the bread does not change its sensory properties, so bread retains the flavour as a fresh one, except for the hardness of the crumb); frozen bread acquires resistance not only to biological agents but also to physical ones (the product is not damaged during storage, packaging, transportation) (Gaigalis et al., 2003).

Refrigeration is one of the best and most appropriate ways of preserving food, as it allows the nutritional value, taste, flavour, colour, vitamins and enzymes to be preserved for a sufficiently long time. By penetrating into the cells of refrigerated products, the cold stops all possible life processes, including the activity of micro-organisms (bacteria, yeasts, moulds) found in food. Properly frozen food will prevent spoilage and will not pose a risk of intestinal infections, as micro-organisms become inactive and goes into a resting state, when exposed to cold.

When a food product is exposed to low temperatures in the freezer, it starts to lose its heat due to heat transfer from the product to the environment. The temperature of the surface of the product changes rapidly compared to the temperature of the interior of the product. As the heat is removed from the water in the product, the temperature starts to drop and reaches the required value - below freezing. When the heat is removed from the food, water crystallises, changing phases and forming ice crystals (Muthukumarappan, Marella, Sunkesula, 2019).

The effect of refrigeration affects the ingredients in the food. Freezing a food reduces its water activity. Micro-organisms cannot reproduce and grow at low water activity and sub-zero temperatures. Slow freezing of food kills micro-organisms. Freezing does not significantly affect enzymes. Freezing or low temperatures affect some of the vitamins in the product: loss of vitamin C, loss of water-soluble vitamins, but vitamins A, B, D and E are not affected (Muthukumarappan, 2019; Aissa, Monteau, Perronnet, Roelens, Le Bail, 2010).

The use of refrigeration technology in food production is a useful and often important process that can help to control spoilage caused by micro-organisms, prolong the shelf-life of a product, and inhibit ongoing processes.

A recipe for bread with seed mixtures based on linseed meal has been developed for the ketogenic diet. The control products were baked at the Food Technology Department of the Technology Faculty at Kaunas University of Applied Science. A rope spoilage, a rare defect in practice, was detected in the finished product. the quality of the product and may spread in the production premises and contaminate other bread products.

Research problem: An innovative product adapted for ketogenic nutrition was produced at the Department of Food Technology of Kaunas University of Applied Sciences. Linseed bread produced under normal conditions, has a short shelf life due to potato disease caused by spore bacteria.

Aim of the study: to investigate the defect of potato disease present in the keto bread with seed mixtures, and to identify effective management measures while preserving the organoleptic properties and shelf-life.

2 METHODOLOGY OF THE STUDY

For the control of mucilage – potato disease in keto bread with seed mixtures, the cooling and storage conditions of the bread were changed and / or acetic acid was added to create an acidic medium to stop the development of microorganisms. The recipe was identical for all the bread samples tested, with the exception of the third and fourth samples, in which 3 % acetic acid was added to the total linseed meal content.

2.1 Production of ketogenic bread

Keto flaxseed bread recipe (Table 1):

Table 1. Recipe for flaxseed bread

No	Name of raw material	Raw material quantity, g
1	Ground flaxseed	0,160
2	Sesame seeds	0,015
3	Sunflower seeds	0,070
4	Poppy seeds	0,018
5	Olive oil	0,090
6	Caraway	0,010
7	Chia seeds	0,020
8	Egg mass	0,150
9	Egg whites	0,090
10	Cooking salt	0,001
11	Baking powder	0,002
12	Water	0,003
	Total	0,750

Keto flaxseed bread production: whisk the egg mixture and egg whites until smooth and add the water. whisk the egg mass and egg whites until homogeneous. The water is added. Then mix all the dry ingredients together, add the beaten egg mixture and pour in the olive oil. Mix all the products until homogeneous so that the ingredients are evenly distributed. The mixed dough is left to stand at room temperature for 10-15 minutes before production continues. The dough is then divided into 0,750 g semi-finished pieces, which are placed in the oven and baked at 180 °C for 30 minutes. This is followed by cooling and freezing according to the different test options.

2.2 Cooling, refrigeration and storage conditions for samples

The first sample(I): after baking, bread is cooled to temperature of 10 ± 2 °C and stored in a room at temperature of 20 ± 2 °C (control sample). The second sample (II): bread is cooled to 10 ± 2 °C and stored in the refrigerator at temperature of 0 to 4 °C. Third sample (III): bread is made with 3 % acetic acid, cooled to temperature of 10 ± 2 °C and stored in a room at temperature of 20 ± 2 °C. Fourth sample (IV): bread is made with 3 % acetic acid, cooled to temperature of 10 ± 2 °C and stored in the refrigerator at temperature of 0 to 4 °C. The fifth sample (V) - bread is cooled in the blast freezer which maintains temperature of - 1 ° C, cooling takes 30 - 40 minutes, this bread is kept in a room at temperature of 0 - 4 ° C. The sixth sample (VI): bread is cooled to temperature of 10 ± 2 °C, stored at a low temperature of between 18 °C and 18 °C, thawed at temperature of between 0 °C and 4 °C, and stored in a room at temperature of between 20 °C and 2 °C.

2.3 Sensory analysis of ketogenic bread

The sensory evaluation of the flaxseed bread was carried out by 9 respondents, assessing the bread's appearance, consistency, colour, aroma, taste, residual aftertaste and the overall evaluation of the bread. On a 5-point scale, where 1 is bad, 2 is unsatisfactory, 3 is satisfactory, 4 is good and 5 is very good. The sensory evaluation was carried out according to the methods of LST ISO 6658:2006.

2.4 Determination of the moisture content of bread

The moisture content is determined in accordance with LST (Lithuanian Standard Board) 1492:2013. The essence of the method is to dry the sample at 102 ± 2 °C to a constant weight. The loss in mass is calculated according to the appropriate formula:

$$\mathbf{W} = \frac{\mathbf{W1 - W2}}{\mathbf{W1 - B}} * \mathbf{100}$$

W - is the moisture content of the product to be analysed in %;

W1 - mass in grams of the analysed product before drying, g;

W2 - mass in grams of the analysed product after drying, g;

B - weight of the container, g.

2.5 Determination of pH

pH value was determined with a pH meter. The pH value was determined with a pH meter (PP-15, Sartorius AG, Germany) 16 hours after baking.

2.6 Texture of the product

The required pressure force of the product is determined using a texture analyser. The samples were compressed using a 20 mm diameter cylinder, a compression force of 5.6 N and a compression speed of 21 mm/min.

2.7 Determination of the total bacterial and yeast and mould fungi content of the product

The method consists of adding a prepared test sample to an empty Petri dish and mixing it with a nutrient medium of the dissolved agar (for total bacteria count PCA, yeast and mould fungi YEA). Other plates were prepared under the same conditions using 10-fold dilutions of the test sample. The prepared Petri dishes were incubated under aerobic conditions: for 72 hours for total bacterial counts at 30 ° C and for 72 hours to determine the content of yeast and mould at 25 ° C.

3 RESULTS AND DISCUSSION

The main raw material used in ketogenic breads is ground flaxseed, while sesame seeds and poppy seeds are also used to give the bread an unconventional taste. The nutritional value of the product is improved because flaxseed is rich in polyunsaturated fatty acids: omega-3 fatty acids and omega-6 fatty acids, which are indispensable for the human body (Danisha, Nizami, 2019). Sesame seeds are also rich in fatty acids, mainly linoleic and oleic acids. They are rich in folic acid, vitamins E and B_3, calcium and phosphorus (Yi et al., 2019). Poppy seeds are rich in vitamin E (Chelav, Khashaveh, 2013). The use of flaxseed meal is likely to reduce the acrylamide content of products (Kaur et al. 2017).

3.1. Results of the sensory analysis

The analysis of the sensory characteristics of the products is carried out by examining the crust, smelling the aroma, tasting the flavour, and examining the flesh and pressing with the fingers.

No signs of potato disease were observed when the products were tasted 16 hours after baking. The appearance of the linseed bread crumb in 16 hours after baking is shown in Figure 1, Figure 2.

Figure 1. Appearance of samples I, III, VI

Figure 2. Appearance of samples II, IV, V

The crust of the ketogenic bread was light and the surface was cracked due to the used-baking powder (Figure 3). The flavour was rich, with a fatty aftertaste, the porosity was low and the bread itself aromatic. 78% of respondents noticed differences between ketogenic bread with and without acetic acid. Respondents found that the addition of acetic acid gave the product an unpleasant taste, with a specific additive aroma.

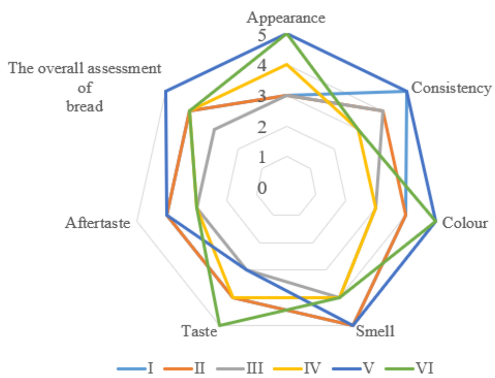

Figure 3. Sensory evaluation of flaxseed bread

A firmer crumb has been observed with the use of refrigeration technology.

Other researchers have also observed that chilled bakery products have delicate sensory and textural characteristics similar to traditional bread baked without refrigeration.

The main change in textural characteristics in frozen bakery products is the increase in firmness of the breadcrumb. The longer the bread is kept frozen, the harder the crumb becomes over time (Fik, Surówka, 2005).

Bárcenas, Rosel (2004) analysed the curing processes in the final baked product and found that storage at -18 °C resulted in a steady increase in retrogradation and in a change in the structure of the baked products. Studies have shown that crumb hardness can be reduced by modifying the formulation of the bread, for example, by using a higher content of vegetable fats or hydrocolloids, which increase the moisture content of the bread, thus reducing the hardness of the crumb and preserving all the advantages of the frozen product.

The sensory evaluation of the flaxseed bread showed that the bread with the best performance was sample V, i.e. the bread that had been chilled in a blast freezer (temperature -1 °C, stored at 0-4 °C).

3.2. Keto bread moisture results

Moisture content determination was carried out for all keto bread samples tested, monitoring the decrease/increase in moisture content during storage under different conditions after one day and after five days, respectively. For bread stored at 0 - 4 ° C, in addition, the test was performed after 12 days from the date of manufacture. The results obtained are shown in Figure 4.

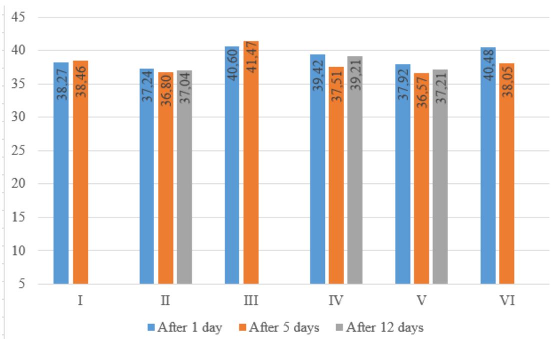

Figure 4. Moisture content of bread stored under different conditions (%)

The moisture content of bread showed that all the bread samples had an average moisture content of approximately 37 %, with the highest moisture content (40,60 %) for the bread made with acetic acid and cooled to 10 °C and stored at a temperature of 20 ± 2 °C (sample III). Moisture content was not determined for all bread samples tested 12 days after production, as samples I, III and VI were stored at temperature of 20 ± 2 ° C. The high temperature had an impact on the development of potato disease. The pathogens break down the proteins and carbohydrates in the bread dough (Dong and Karboune, 2021) and the product becomes unsuitable for human consumption. Furthermore, it can cause gastrointestinal and other disorders in the human body.

3.3. pH results

The pH of the ketogenic bread was alkaline 8,2 (options I, II, V and VI). This type of bread did not use sourdough starter and used baking powder for rising. The CO_2 gas released at high temperatures increases the volume and formation of porosity in the bread, which is not significant because the product does not contain raw materials containing gluten. This bread-making technology favours the development of bacteria of the Bacillus genus (Bacillus subtilis and Bacillus licheniformis), which cause potato disease (Mentes et al., 2007).

The test shows that after 6 days is develop potato disease, the crumb swells with slimy threads, has an unpleasant aroma and is unfit for consumption. Researchers are looking for effective ways to prevent bread potato disease. Usually by increasing the acidity and reducing the moisture content of the product, increasing the sugar and fat content of the recipe up to 15-20% by weight of the flour, or by the use of potato bread disease suppressants, inclusion of lysozyme, lowlands, propion, sorbic, benzoic, acetic acids and their salts in bread recipes (Zavorohina et al., 2018).

In this study, acetic acid was used at 3% of the total flour content. This additive changed the pH of the bread. It was found to have a mildly acidic pH of 6.7 (options I and IV), but did not stop the development of the disease and made the bread sensory unpalatable. It was not appropriate to increase the amount of acetic acid, as taste is a key factor for consumers and must be acceptable. The hypothesis that the growth of Bacillus genus bacteria is particularly inhibited by acidic pH was also refuted by Brožová et al. (2018). Studies have shown that Bacillus cereus can survive and grow for some time even at pH 4.2-4.8. Growth of Bacillus subtilis occurs in the pH range 5,5-8,5 (Logan and De Vos, 2015).

This is why the rapid cooling and freezing technology was used after baking the bread.

3.4. Results of texture analysis

The texture analyser was used to determine the compression force required for the product after one day, 5 days, 7 days, 12 days and 14 days from the day of manufacture, the results obtained are shown in Figure 5.

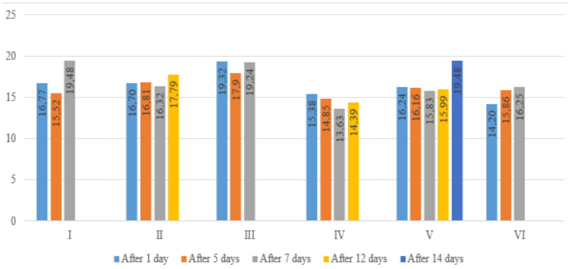

Figure 5. Results of texture analysis (N) for linseed bread

As seen, the compression force of all samples was high on average 16 N 1 day after baking. The highest compression force after one day was 19,32 N for sample III and the lowest was 14,20 N for sample VI. For all samples tested, a tendency was observed for bread to be the hardest before the expiration date as more compression force is required on the onset of the staling process, increased starch retrogradation (Elharïry et al., 2011) and the subsequent development of potato disease, except in sample V, when the bread was suitable to use for 14 days. The texture analyser test was carried out up to the date when the product was no longer suitable for consumption.

3.5. Microbiological test results

Micro-organisms hardly multiply and do not grow at sub-zero temperatures, but when the product is cooled, micro-organisms may become active or multiply rapidly (Lucas, Quellec, Le Bail, Davenel, 2005).

After determining the total bacteria count in all samples, the obtained results showed that after one day of production, all samples were good for consumption, as the total bacteria count and yeast obtained was 10^1 CFU / g. No mould was detected (Table 2).

Table 2. Number of micro-organisms in keto bread after one day

Keto bread	Total bacteria count, CFU / g	Yeast and mould, CFU / g
Sample I	$3,0 *10^1$	$4,9 *10^1$
Sample II	$5,6 *10^1$	$6,7 *10^1$
Sample III	$4,8*10^1$	$8,1*10^1$
Sample IV	$4,2*10^1$	$6,4*10^1$
Sample V	$3,5*10^1$	$5,1*10^1$
Sample VI	$5,2*10^1$	$7,2*10^1$

Tests were carried out daily until the results showed that the products were no longer suitable for consumption as the colony count exceeded 10^5 CFU/g. After 6 days sample I, sample III and sample VI, after 12 days, sample II and sample IV were no longer good for consumption. The shelf life of the sample V was the longest (14 days) compared to others.

All variants of the keto bread with seed mix, except variant V, developed potato disease after 6-7 days (Figure 6).

Figure 6. Signs of potato disease in keto bread

From the studies performed, it can be concluded that the shelf life is best for sample V, which has been refrigerated at -1 ° C and stored at 0-4 ° C.

CONCLUSIONS

After performing the research and analysing the results, it was found that the bread, which was cooled using a blast freezer and stored at 0 - 4 ° C, was evaluated the best. Based on the results, it can be stated that the shelf life of this bread is the longest in comparison to other storage conditions, that is, 14 days and it is the best solution for the prevention of potato disease.

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