

Starch transformations during freezing and refrigerating storage of hot dog rolls packaged in modified atmosphere

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Abstract: *Wheat hot dogs are an attractive food product, consumed throughout the world. As bakery products, they are subject to staling process and associated quality decrease, which limit their shelf life. One of the methods designed for bakery products durability prolongation are cooling and freezing processes in combination with the packaging system under modified atmosphere with a high concentration of carbon dioxide. Hence the aim of the study was to assess the rate of retrogradation process and starch structure changes in hot dog rolls packed by modified atmosphere system (MAP) during storage under refrigerated (1°C) and freezing (-18°C) conditions. Changes in moisture content, sensory, crumb hardness via TPA method and basic chemical composition of hot dog roll i.e. protein and lipids were analysed. Starch retrogradation processes were investigated by calorimetric method and spectrophotometrically. Comparative analysis of hot dog rolls storage variants indicated that the lower storage temperature is, the higher the level of maintenance of textural and sensory properties of the product. It has been shown that freezing hot dog rolls storage temperatures limited an increase in resistant starch content. In case of hot dogs storage under deep freeze conditions (-18°C), growth of RS was lowered by 46% compared to the refrigerated conditions. There was a significant increase in rolls hardness, whereby it was almost three times higher when hot dogs were refrigerated. It has been demonstrated that use of deep-freezing in combination with MAP significantly reduces the adverse effects caused by aging processes in hot dog rolls, especially RS formation.*

Keywords: *starch retrogradation, storage, hot dog rolls, freezing, refrigeration, modified atmosphere packaging.*

Introduction

Wheat hot dogs rolls, like other bakery products, are subject to staling process and associated quality losses. As a relatively unstable, hot dog rolls during storage may undergo adverse physical, chemical and biological changes. With the progress of aging process, bakery products lose the characteristic smell, crust

crispiness disappears, decrease in crumb elasticity and the increase of hardness is also observed, which is particularly evident in case of rolls [1, 2, 3].

Staling of bakery products mainly consist of processes associated with the migration of moisture, drying out the crumb and retrogradation of starch [4]. As a result of retrogradation observed from the moment of the bakery products cooling down after baking, resistant starch (RS) is appearing. According to set and applied definition, resistant starch is starch and its hydrolysis products, which are not absorbed in the small intestine of a healthy human [5]. Resistant starch reaches the large intestine where it is fermented with the involvement of bacterial microflora [6, 7]. The amount of RS present in the food products can be as high as 20% of the product weight. Its content in foods depends on certain parameters associated with food production and sometimes storage.

Eating of products containing RS leads to the improvement of the human body metabolism and lowers the amount of cholesterol, triglycerides in blood and liver, urea concentration in blood, glucose saturation in blood (and insulin). In the human digestive tract undigested starch - resistant starch is fermented in the colon. Formed by-products such as volatile compounds (methane, hydrogen) and short chain fatty acids (butyric), lead to a reduction in intestine pH and thus influences the development of a beneficial for health microflora [8, 9, 10].

One of the methods designed for extending the shelf life of bakery products is use of refrigerating and freezing processes, both at the production stage and during storage of the finished product. Freezing does not protect the product completely against processes inside it, but most of these reactions are slowed. Packaging of bakery products, including rolls, by MAP system also partially reduces staling and protects against adverse microbial changes due to the high concentration of carbon dioxide. The aim of the study was to assess the rate of retrogradation process and changes in resistant starch amount during storage of hot dog rolls packed by MAP system, under freezing and cooling. For the assessment of starch transformation the method of DSC thermal analysis was used.

Experimental

Materials

Wheat hot-dog rolls produced in industrial bakery, packed in polyamide/polyethylene (1 mm thickness) bags in a modified atmosphere containing 70% CO₂ and 30% N₂ were used for the study. Packaging in MA system allowed maintaining of a constant composition of atmosphere during the entire storage period. The composition of tested buns was as follows: wheat flour, yeast, water, salt, sugar, baking mix (gluten, glucose, soy flour, flour treatment agent: ascorbic acid, emulsifier: mono and diglycerides of fatty acids), calcium propionate. Rolls were divided into two experimental groups, which after cooling down (variant 1) or deep freeze (variant 2), were stored for 9 weeks in two temperature variants controlled with an accuracy to $\pm 1^{\circ}\text{C}$: variant 1- $t = 1^{\circ}\text{C}$;

variant 2 – $t = -18^{\circ}\text{C}$. Immediately after baking, cooling down and packaging and then at intervals of one week, the hot dog rolls thermal and physicochemical properties were evaluated. Before performing analysis, refrigerated hot dogs were warmed up to room temperature, and frozen hot dogs were thawed slowly in order to be heated uniformly and to avoid moisture condensation on the cold surface, and then warmed to room temperature.

Methods

Besides the basic sensory indicators (appearance, crust color and thickness, crumb elasticity and structure, the aroma and taste) [11] analysis included the water content of the crumb by mass [11], total protein content - by the Kjeldahl method [11], lipid content – according to Weibull-Soxhlet [11], blue value - spectrophotometric method [12] total acidity [11], the content of resistant starch – using Differential Scanning Calorimetry (DSC) [13], rolls hardness – texture profiling method, using texturometer TA.XT – [14].

Sensory evaluation

The sensory properties of the rolls were assessed on fresh and then once each week during 9-week storage. Each attribute of the crumb (taste, smell and elasticity) and crust (taste, smell and crispness) was assessed using a 5-point scale (Polish Standard). Each evaluation was carried out by group of 15 panelists appropriately trained and tested for sensory sensitivity.

Chemical composition

The moisture content in the crumb was determined according to Polish Standard. Samples of rolls (3 g, weighed with the precision of 0.0002 g) were put in vials and dried to constant mass for 3 h at 105°C . The moisture content was reported as a percentage. The protein content in rolls was determined using the Kjeldahl method. In order to calculate the total protein content, the nitrogen content should be multiplied by 5.7. Total protein content was reported as g of protein per 100 grams of the product. Lipids were quantified according to Weibull-Soxhlet method. Samples of rolls (8 g) were treated for 30 min with 25% HCl to release lipid substances, which were separated from the soluble fractions by filtration and extracted with petroleum ether for 1.5 h using a Tecator unit. Lipid content was calculated based on the decrease in the sample's dry mass. The total lipid content was reported as g of lipids per 100 grams of the product.

Blue value determination

The complex of amylose with iodine produces a blue color. Its intensity can be used to indicate the level of free amylose in a soluble fraction, which informs about starch retrogradation. 10 g of the crumb was homogenized with 50 cm³ of water for 2 min. Then the homogenate was mixed at room temperature for 20 min using a magnetic stirrer. The slurry was centrifuged (6140 g, 10 min). 2 ml of Carrez I and 2 ml Carrez II solutions were added to precipitate the protein matter. The whole sample was carefully mixed, allowed to stand for 10 min and finally

centrifuged (6140 g, 5 min). Afterwards, 4 ml of the supernatant was mixed with 25 ml of iodine solution (0.04%). The blue value was expressed as the absorbance measured at 580 nm using distilled water as a blank [12].

Determination of resistant starch content by DSC

Resistant starch (RS) fractions in the rolls were isolated with using α -amylase (Pancreatic α -Amylase, 3 Ceraalpha Units/g, Megazyme) and amyloglucosidase (3300 U/ml, Megazyme) [15]. Each sample of rolls (20 g) was incubated with 200 mL of α -amylase (0,02 g) and amyloglucosidase (0,16 ml) solution at 37°C for 16 h. The reaction was terminated with 20 mL of 99% ethanol. The samples were centrifuged for 10 min at 3074 g and the solids were suspended in 2 mL of 50% ethanol, mixed with 30 mL of 50% ethanol and centrifuged under the same conditions as before. This procedure was repeated. After the centrifuging, the solids (the RS fraction) were lyophilized. Differential scanning calorimetry measurements were carried out to determine the content of RS contained in the tested rolls using a Mettler Toledo Differential Scanning Calorimeter DSC-1. Samples of starch isolated from rolls (3 mg) were placed in hermetically sealed aluminum pans, heated from 20°C to 220°C and then cooled to 20°C at a scanning rate of 3°C min⁻¹. An empty pan was used as a reference. The melting temperature and enthalpy of RS were found from the obtained curves. An endothermal peak characterized RS melting. The analysis of curves included determination of temperature corresponding to the onset temperature, minimum of the endothermal peak and offset temperature. Enthalpy of RS melting (ΔH mJ mg⁻¹) was calculated based on the surface area between the peak and baseline.

Crumb hardness measurements

Crumb hardness measurements were performed using a Texture Analyzer TA.XT Plus governed by Texture Exponent 32 software (Stable Microsystems). Hot dog rolls samples were subjected to texture profile analysis (TPA) [14]. Hot dogs were compressed twice with an aluminum cylinder-shaped plunger with a diameter of 100 mm to a depth of 50% strain. The plunger moved at a rate of 1 mm s⁻¹ and the time between strokes was 20 s. The apparatus recorded the force exerted by the plunger as a function of time, from which texture parameter - hardness was determined. Hardness was defined as the peak force during the first compression cycle. These analyses were performed in eightfold.

Statistical analysis

Mean and standard deviations were calculated from the results of the analysis performed. The data was subjected to analysis of variance (ANOVA) and the comparison of means was performed using Tukey's Honesty Significant difference Test at significance $p \leq 0.05$ and standard deviation was calculated using spreadsheet. All experiments were performed at least in triplicate.

Results

The results of chemical composition analysis of fresh wheat bread indicate that 100 g of product comprises 38 g of water, 10 g of protein, 2 g fat and 50 g

simple sugars. During freezing storage, protein and fat content did not change but there were little fluctuations of moisture content. The moisture content decreased slightly (around 8%), in case of refrigerated wheat rolls it changed from 32.15% to 29.31% after 9 weeks storage. The observed decrease was similar in frozen wheat rolls (from 32.15% to 29.40%). Thus, thanks to the high barrier properties of used packaging, the storage temperature had no significant effect on the loss of moisture in the rolls crumb. Similarly, a small moisture loss during storage of bakery products has been reported in studies conducted by Fik et al. [16] and Shaik et al. [17].

Table 1. Effect of storage time on overall sensory quality, TPA texture measures for crumb, and some physicochemical changes in refrigerated hot dog rolls

Parameter	Storage week									
	0	1	2	3	4	5	6	7	8	9
Dry mass [%]	32.15 ±0.2 ^a	32.01 ±0.15 ^a	31.85 ±0.25 ^a	31.06 ±0.2 ^a	30.98 ±0.30 ^a	30.68 ±0.25 ^a	30.27 ±0.40 ^a	29.87 ±0.35 ^a	29.51 ±0.30 ^a	29.31 ±0.25 ^a
Blue value	0.97 ±0.03 ^a	0.85 ±0.07 ^b	0.76 ±0.1 ^b	0.56 ±0.1 ^c	0.41 ±0.1 ^d	0.32 ±0.7 ^e	0.21 ±0.1 ^f	0.1 ±0.04 ^g	0.05 ±0.04 ^h	0.02 ±0.00 ⁱ
Acidity [°]	1.77 ±0.05 ^a	1.76 ±0.05 ^a	1.75 ±0.05 ^a	1.77 ±0.04 ^a	1.76 ±0.05 ^a	1.72 ±0.03 ^a	1.78 ±0.04 ^a	1.75 ±0.06 ^a	1.72 ±0.04 ^a	1.78 ±0.05 ^a
Hardness [N]	16.94 ±4.55 ^a	51.54 ±5.65 ^b	59.83 ±3.50 ^c	56.17 ±4.00 ^c	60.43 ±3.05 ^d	63.09 ±4.06 ^d	71.73 ±5.03 ^c	70.43 ±4.5 ^c	69.08 ±7.02 ^c	71.51 ±5.98 ^c
Overall quality	32 ±2 ^a	27 ±3 ^b	19 ±2 ^c	13 ±3 ^d	0 ±0 ^e	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0

^{a,b,c} Mean values in the same row designated with different letters are significantly different ($\alpha = 0.05$).

The quality of fresh hot dogs was rated as very good (32 points). This result was the sum of points received for various distinguishing organoleptic parameters (appearance, taste, smell, color of crust, crumb elasticity). Significant changes in the quality of frozen hot dogs rolls appeared in the fourth week of storage and were related to their appearance. Other observed changes in rolls quality were associated with an increase in the crumb crustiness and a decrease in its elasticity, while the crust crispiness was lost. Rolls got a total of 16 points. Despite these changes, hot dogs stored in a deep freeze retained acceptable quality until the end of the storage period (Table 2), as opposed to refrigerated hot dog rolls which quality deteriorated significantly due to the decrease in crumb elasticity, and changes in taste and smell. This resulted in their disqualification in the fourth week of storage (Table 1). Similar changes were observed in studies conducted by Fik et al. [16]. According to the Polish Standard, such bakery products should not continue to be stored. To sum up this part of the study, it can be confirmed that MAP and deep-freezing allows to extend the period of maintain good quality of the studied hot-dog rolls up to 9 weeks (Table 2).

Table 2. Effect of storage time on overall sensory quality, TPA texture measures for crumb, and some physicochemical changes in deeply frozen hot dog rolls

Parameter	Storage week									
	0	1	2	3	4	5	6	7	8	9
Dry mass [%]	32.15 ±0.50 ^a	32.02 ±0.30 ^a	31.95 ±0.25 ^a	31.83 ±0.15 ^a	31.21 ±0.42 ^b	30.86 ±0.38 ^b	30.38 ±0.48 ^b	30.01 ±0.54 ^b	29.74 ±0.50 ^b	29.40 ±0.23 ^b
Blue value	0.97 ±0.05 ^a	0.86 ±0.03 ^b	0.65 ±0.06 ^c	0.54 ±0.05 ^d	0.48 ±0.07 ^d	0.39 ±0.05 ^e	0.25 ±0.06 ^f	0.18 ±0.08 ^f	0.09 ±0.05 ^g	0.07 ±0.03 ^g
Acidity [°]	1.77 ±0.15 ^a	1.75 ±0.20 ^a	1.75 ±0.15 ^a	1.78 ±0.22 ^a	1.75 ±0.13 ^a	1.73 ±0.10 ^a	1.78 ±0.14 ^a	1.76 ±0.09 ^a	1.77 ±0.11 ^a	1.75 ±0.06 ^a
Hardness [N]	16.94 ±3.10 ^a	18.3 ±2.55 ^a	19.03 ±2.50 ^a	26.16 ±2.50 ^b	26.59 ±3.02 ^b	32.17 ±4.03 ^c	31.34 ±2.03 ^c	32.73 ±2.50 ^c	37.25 ±3.02 ^d	40.07 ±3.55 ^d
Overall quality	32 ±2 ^a	31 ±2 ^a	27 ±2 ^b	26 ±2 ^b	19 ±2 ^c	16 ±2 ^d	15 ±2 ^d	12 ±2 ^e	5 ±2 ^f	2 ±3 ^f

^{a,b,c} Mean values in the same row designated with different letters are significantly different ($\alpha = 0.05$).

One of the parameters that define bakery products quality is its acidity. In case of tested hot dogs, acidity value did not change during storage. It is believed that the elevated concentration of carbon dioxide in the protective atmosphere can increase the perceived taste acidity of the packaged product [16]. In studied hot dog rolls there were not any taste changes found.

During storage of bakery products, including rolls, an increase in crumb hardness is observed [18]. The hot dog rolls texture test showed that differences in hardness depended on storage conditions. Higher changes were observed in the rolls stored under refrigeration (300% increase) compared to freezing (150% increase) (Tables 1 and 2). Changes in the textural properties of wheat rolls during storage in the frozen state, were also confirmed in the literature [18].

One of the applications of DSC (differential scanning calorimetry) is the assay of starch structure changes and its content in bakery products. The presence of RS, is reflected by endothermic peaks appearing on the DSC curves during starch heating [19, 20]. Conducted thermal analysis showed its presence in stored hot dog rolls. Based on obtained results, it was found that retrogradation proceed at different rates depending on the hot dogs storage temperature (Tables 3 and 4).

Table 3. Melting temperatures and enthalpy of RS contained in the refrigerated hot dog rolls

Storage week	T _{onset} [°C]	T _{peak} [°C]	T _{offset} [°C]	Enthalpy ΔH [mJ/mg]
4	102.32 ± 2.50 ^a	110.78 ± 3.46 ^a	121.31 ± 2.68 ^a	32.50 ± 5.50 ^a
5	107.31 ± 3.05 ^b	112.48 ± 3.65 ^a	123.21 ± 3.14 ^a	32.85 ± 3.65 ^a
6	107.24 ± 4.50 ^b	114.45 ± 2.56 ^a	123.31 ± 4.12 ^a	53.14 ± 3.58 ^b
7	116.23 ± 3.25 ^c	127.65 ± 4.76 ^b	136.27 ± 4.12 ^b	63.08 ± 5.72 ^c
8	125.31 ± 2.50 ^d	133.76 ± 3.23 ^c	143.12 ± 4.15 ^c	82.22 ± 6.65 ^d
9	124.32 ± 3.85 ^d	134.29 ± 3.57 ^c	148.31 ± 3.54 ^d	86.10 ± 5.95 ^e

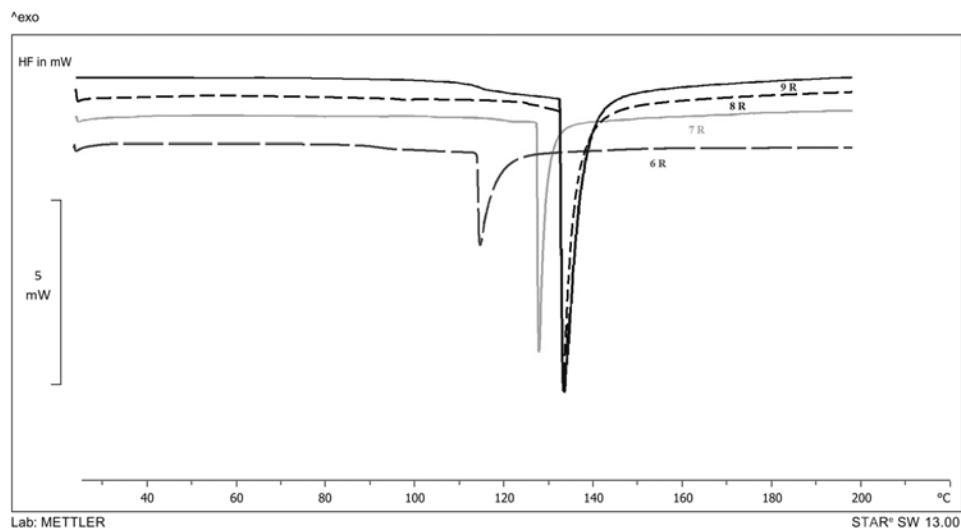
^{a,b,c} Mean values in the same row designated with different letters are significantly different ($\alpha = 0.05$).

Table 4. Melting temperatures and enthalpy of RS contained in the deeply frozen hot dog rolls

Storage week	T _{onset} [°C]	T _{peak} [°C]	T _{offset} [°C]	Enthalpy ΔH [mJ/mg]
4	107.23±2.15 ^a	115.32 _a ±2.65	124.31±2.12 ^a	38.14±2.01 ^a
5	109.25±3.55 ^a	117.12±3.58 ^a	128.4±2.55 ^b	42.29±2.22 ^b
6	111.35±2.56 ^a	119.23±3.07 ^a	131.52±3.05 ^b	48.49±3.14 ^c
7	118.23±3.12 ^b	125.12±3.21 ^b	142.32±3.86 ^c	54.26±3.18 ^d
8	121.31±3.76 ^b	128.32±3.65 ^b	145.32±2.98 ^c	61.03±3.54 ^c
9	122.8±3.54 ^b	130.21±2.43 ^b	148.23±3.72 ^c	72.24±3.76 ^f

^{a,b,c} Mean values in the same row designated with different letters are significantly different ($\alpha = 0.05$).

It was found that in both refrigerated (1°C) and deep freezing (-18°C) conditions the temperature minimum peak increased with storage time (from 110 to 135°C), as well as RS melting enthalpy. The observed increase in temperature in the minimum of endothermic peaks could be linked to the gradual transformation of starch structures into more ordered, crystalline forms. Examples of the DSC curves of RS starch isolated from rolls stored in refrigerated and frozen conditions are shown in Fig. 1 and 2.

**Figure 1.** DSC curves of RS from the refrigerated hot dogs kept for 6, 7, 8 and 9 weeks ($t = 1^\circ\text{C}$)

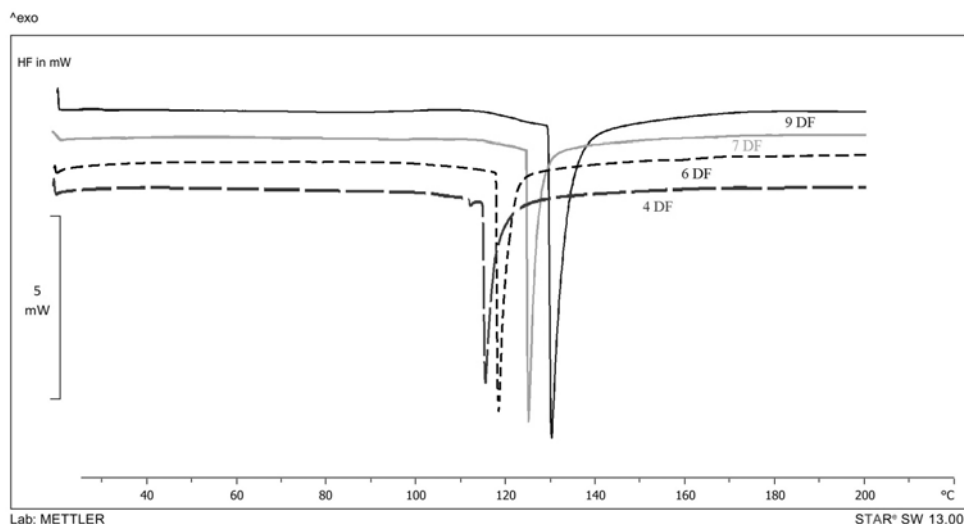


Figure 2. DSC curves of RS from the deeply frozen hot dogs kept for 4, 6, 7 and 9 weeks ($t = -18^{\circ}\text{C}$)

Under refrigeration conditions storage of tested hot dog buns, the RS melting enthalpy value increased by 164% in a period of five weeks. In the case of deep-frozen product, the enthalpy values doubled, indicating a smaller increase in RS starch content and less intensive staling processes (Tables 3 and 4). Based on the results, it was found that the process of starch retrogradation taking place in hot dog rolls progress at different rates, depending on the temperature of storage. Reported RS melting enthalpy values indicate that the freezing process reduces the speed of retrogradation, compared to the refrigeration storage temperatures. Research conducted by Fik and Surówka [21] confirm a gradual increase in RS during frozen storage of bakery products.

Determination of staling rate is an important parameter describing the stability of stored bakery products. The observed decrease in blue quantity (three-fold for frozen rolls, and ten-fold in chilled rolls), shows a steady increase in RS (Tables 1 and 2), just as the calorimetric analysis (Tables 3 and 4). These results are consistent with a study by Skotnicka and Palich [12].

Discussion

Staling of bakery products is a complex process that occurs in the structure crumb. It consists of various changes occurring during cooling and storage, such as moisture redistribution, resulting in crumb hardening and crust softening, starch retrogradation and others [22]. Loss of moisture is one of the parameters affecting bakery products quality, it causes that crumb becomes hard and dry, and the crust ceases to be crispy, it is, however, very difficult to avoid during long-term storage [23]. A huge impact on the amount of moisture loss comes from the barrier properties of packaging, as well as the water activity of stored product and the surrounding atmosphere humidity [24]. Decrease in bakery products water

content observed during storage is associated with progressive processes including starch retrogradation.

Starch retrogradation is one of the most important processes that contribute to bakery products staling. Transformation of digestible to resistant starch is accompanied by conversion from the amorphous to the crystal structure. The results obtained in this study indicate that, during storage, starch molecules reconnect and may obtain a more compact and ordered structure as evidenced by an increase in crumb hardness and a loss of its elasticity. In this process, a particular importance goes to amylopectin retrogradation, which branchings overlap each other and associate, causing an increase in the crumb stiffness [25]. Retrogradation occurs when the $-CH_2OH$ groups of starch chains are connected through hydrogen bonding with simultaneous disconnection of associated water molecules. Released water has characteristics of free water and contributes to the intensification of staling [26]. In stored bakery products at low temperatures, processes associated with water redistribution take place. Furthermore, there are interactions between proteins and starch, which can result in the formation of ordered structures of starch. Such structures require higher melting energies. Similarly, as in case of analyzed hot dogs, in tests conducted by Barcenas et al. [19], an increase in melting temperature of RS with elongation storage time was found. A similar trend was observed by Ribbota et al. [20], who analyzed retrogradation processes in frozen dough.

As a result of the retrogradation process the amount of RS in bakery products increase. From the point of view of nutritional value partial retrogradation of starch can be considered as an advantageous phenomenon, as RS as a component of dietary fiber, is necessary for the proper functioning of human digestive system [27, 28]. From health and wellness perspectives, resistant wheat starch reduces glycemic and insulin response, which has potential to help healthy individuals and those with type 2 diabetes manage blood glucose levels. It promotes gastrointestinal health through its prebiotic effect by selectively increasing the microbial communities of *Bifidobacterium adolescentis* in the large intestine [29]. Therefore, the chilled or frozen rolls with increased RS content may be considered even as a health-promoting product.

Staling of stored bakery products is associated with changes in the elastoplastic properties, causing deterioration of texture parameters. In frozen bakery goods they are mainly due to the structural changes of starch [21, 30]. Another reason for the increase of products hardness observed after thawing is ice crystals growth that may interfere with or alter the cross-linked structure of gluten, responsible for the crumb texture [19].

A useful indicator of bakery products freshness is its characteristic smell, which results from the presence of volatile compounds in the crust and crumb. When storing the compounds corresponding to taste and flavor form insoluble complexes with starch, are absorbed by proteins or oxidized. In the case of baked

bakery products due to evaporation during the cooling period, a lot of flavor – smell compounds moves to the surroundings [31].

It was demonstrated that bakery products like hot dog rolls, depending on the production technology and the use of packaging, are characterized by a varied consumption period. In order to increase the durability of products, they are packed under protective atmosphere. As shown by previously conducted studies, it has a beneficial effect on the stability of stored food products [32].

Obtained results show that the combination of a high concentration of carbon dioxide, oxygen elimination and temperature decrease, allow to maintain the high sensory quality of hot dog rolls for 4 weeks in the refrigerator and 6 weeks in freezers. Our analysis showed that freeze storage temperature and packaging in modified atmosphere slowed down the process of product staling, and reduced the effect of deterioration of flavor, aroma and texture of rolls. Comparative analysis of hot dog rolls storage variants indicates that in the lower storage temperature the textural and sensory qualities of the product are maintained at higher level. It has been shown that hot dogs storage in freezing temperatures limited the increase of RS starch content, and its amount, was lower by 19%, compared to the refrigeration conditions. It was found that changes in the rolls texture occurred in the first week of storage, expressed by a significant increase in rolls hardness, which was almost as twice high as in case of refrigerated bread. During the entire period of storage, hardness of frozen hot dog rolls increased by 136% and for refrigerated by 322%.

Generally, during the nine weeks of storage, the aging process of hot dog rolls was present, as evidenced by the significant changes of texture parameters, as well as the steady increase in the amount of RS starch demonstrated via DSC analyzes and measurement of blue quantity. However, these changes were significantly lower in case of rolls stored in deep-freeze conditions.

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