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SHELF-LIFE OF HOMOGENIZED SAUSAGE DEPENDS ON THE MOMENT IT WAS PLACED AT NEAR CRYOSCOPIC TEMPERATURE

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ABSTRACT

The aim of a study was to determine the impact of unfavorable storage conditions on physico-chemical, sensory and microbiological parameters of homogenized sausage kept at near cryoscopic temperature (n.c.t.). Post production the sausages were kept at 4°C for 12h (group I) or at 12°C for 48h (II) and 96h (III). Subsequently, all the products were stored at n.c.t. (-3.6±0.2°C) for 0, 7, 14, 21 and 28 days. Unfavorable conditions the sausages were exposed prior the cryoscopic storage, reflected to their increased acidity and free amino group content along with the decrease in free nitrates (III). They however changed neither the color nor the rheological parameters and total sensory value of the products studied. Moreover, the cryoscopic storage positively affected the microbiological quality of sausages analyzed.

Key words: homogenized sausages, unfavorable conditions of post-production storage, cryoscopic storage, quality.

INTRODUCTION

Increasing number of studies supports the idea of the storage of meat products at near cryoscopic temperature [24-29]. Microbiological safety, and consequently the prolonged shelf-life, are the main advantages of the storage of deeply-chilled products [27-28]. It is however not estimated if the extended storage at n.c.t. had not reflected to microbiological adaptation and subsequent decrease of consumer safety. According to Libelt [7] psychrotrophic organisms constitute up to 40% of microflora present in meat batter. It is reasonable that the potential risk of consumer would elevate by the high initial, i.e. pre-storage, microbiological contamination. An increased initial contamination in turn might be at least the consequence of improper conditions under which the product was kept before the application of n.c.t [1-2, 4-5, 7-11, 13]. According to Libelt, the shelf-life of steamed sausage stored at 4, 10 or 20°C amounts to 120, 96 and 48 hours, respectively [8]. Consequently, the important question is how cryoscopic storage will change the quality of meat product, when stored at n.c.t only during the part of its life cycle.

MATERIAL AND METHODS

One hundred-eighty samples of homogenized sausage manufactured under industrial conditions in HACCP-implemented plant were subjected to the study (Fig. 1). Chilled at 4°C, the sausages were vacuum-packed into PA/PE laminate bags, 6 pieces per package. The experimental material originated from three consecutive weeks of production. The manufacturer declared the shelf-life of product stored at 4°C for 14 days. Three groups (I, II and III) of equal quantity were distinguished among the experimental material. Group I products directly post 12-hour chilling were vacuum-packed and kept at n.c.t (-3.6±0.2°C). The sausages of groups II and III were chilled and vacuum-packed as well, but their storage was preceded by 48- (II) or 96-hour (III) incubation at 12°C. The samples of all the groups were subsequently stored at -3.6°C for 7, 14, 21 and 28 days, as well as the controls, which were put at n.c.t. after 12-hour chilling at 4°C.

Figure 1. The protocol of the experiment

<table>
<tr><td colspan="5">Homogenized sausage</td></tr>
<tr><td colspan="5">Experimental groups</td></tr>
<tr><td>I</td><td colspan="2">II</td><td colspan="2">III</td></tr>
<tr><td colspan="5">Chilling, 4°C, 12h</td></tr>
<tr><td colspan="5">Vacuum-packaging (180 packages)</td></tr>
<tr><td rowspan="3"></td><td colspan="4">Storage</td></tr>
<tr><td colspan="2">12°C, 48h</td><td colspan="2">12°C, 96h</td></tr>
<tr><td>I</td><td colspan="2">II</td><td colspan="2">III</td></tr>
<tr><td colspan="5">Storage at -3.6°C±0.2°C [days]</td></tr>
<tr><td>0 (K)</td><td>7</td><td>14</td><td>21</td><td>28</td></tr>
<tr><td colspan="5">Analyses (n=12 packages)</td></tr>
</table>

The quality of experimental sausages was assessed on the basis of their physico-chemical, rheological, sensory and microbiological properties.

The pH value, the minimal temperature of subcooling (m.t.sc.) and cryoscopic temperature (c.t.), free nitrate (III) and free amino group concentrations and the parameters of color (L*, a*, b*) and its stability (E6, E12, E24) were measured during the physico-chemical analyses. The total sensory value was determined as well.

Microbiological studies dealt with the total plate count, the numbers of coliforms and yeasts and moulds and the presence of *Escherichia coli*, *Salmonella* spp. (in 25g), coagulase-positive staphylococci (in 0.1g) and anaerobic spore-forming bacilli (in 0.1g).

The pH was measured with an aid of a MICROCOMPUTER CP-551 pH-meter. Thermographic method [27] was employed for the determinations of cryoscopic temperature and the minimal temperature of subcooling. Free nitrates (III) were quantified according to the Polish Standard PN-74/A-82114 [14], whereas free amino groups – as described by Kuchroo et al. [6]. The color of experimental sausages was expressed in L*, a*, b* scale with the measurements taken with an aid of a MINOLTA CR-200b reflection colorimeter. Color was determined directly after cutting into 10 mm thick slices while its stability (E) was assessed after 6-, 12- and 24-hour exposure to white glow light (250 lx). The following formula was employed:

$$\Delta E_x = \sqrt{\Delta(L^*_0 - L^*_x)^2 + \Delta(a^*_0 - a^*_x)^2 + \Delta(b^*_0 - b^*_x)^2}$$

where:
L^*_x, a^*_x and b^*_x – color parameters after x-hour (6-, 12- or 24-hour) exposure
L^*_0, a^*_0 and b^*_0 – the value of particular color parameter in non-exposed sausages.

The texture profile of experimental sausages was determined using a STEVENS QTS-25 analyzer. The 25 mm x 15 mm cylinders were cut along the sausage chubs. The samples were twice compressed by 70% deformation and 20-sec. relaxation time. Subsequently the hardness, springiness, cohesiveness, gumminess and chewiness of the sausage studied were determined.

The hardness was expressed by the maximal force [N] the sample was exposed during the first compression. The springiness was measured by the distance [mm] proceeded by the head of the analyzer during the second course of compression. The cohesiveness was calculated as the ratio of works done during the first (A1) and the second (A2) course of compression. The gumminess was determined as the product of hardness and cohesiveness [N], while the chewiness – as the product of gumminess and springiness [N].

The storage-related loss of mass was calculated as the difference between the weight of experimental sausages measured directly post production and after 7, 14, 21 or 28 days of experiment.

The total sensory value of experimental sausages was carried out by means of multiple comparisons according to Polish Standard PN-ISO 6564: 1999 with 5-point scale [20].

Microbiological determinations were done following the respective Polish Standards: total plate count – PN-EN ISO 4833: 2004 [18], coliforms – PN-ISO 4832:1998 [17], *Escherichia coli* – PN-ISO 7251: 2002 [19], *Salmonella* spp. – PN-EN ISO 6579: 2003 [21], coagulase-positive staphylococci – PN-A-82055-9: 1994 [16], anaerobic spore-forming bacilli – PN-A-82055-12: 1997 [15] and yeasts and moulds – PN-ISO 7954: 1999 [22].

Statistical analysis was done by means of univariate analysis ($P \leq 0.05$, ANOVA statistical package).

RESULTS AND DISCUSSION

The increase of acidity in experimental sausages, statistically significant in the products of group III, was observed after 14 days of cryoscopic storage (Table 1). Considerable decrease of pH appeared also in group II sausages after 28 days of experiment. Despite of the significant, storage-related changes in the pH of group II and III sausages, in none of the samples studied the pH decreased below the disqualifying level. The pH of group I sausage, which was kept under optimal conditions prior the cryoscopic storage, did not change significantly during storage and remained at assortment-specific level [3, 8, 12].

Table 1. The pH of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, $P \leq 0.05$)

Time of storage [days]	Experimental groups		
	I	II	III
0 (K)	6.07 b	6.10 c	6.07 c
7	6.02 aA	6.09 cB	6.04 cA
14	6.06 bB	6.06 bcB	5.98 bA
21	6.09 bB	6.03 bB	5.92 aA
28	6.06 abC	5.97 aB	5.89 aA

Pre-storage exposure to the unfavorable temperature promoted the formation of free amino groups in the sausages studied (Table 2). Consequently, the concentration of these compounds increased with cryoscopic storage. The degradation of proteins during the storage of meat products was also demonstrated in our previous studies [25].

Table 2. Free amino group contents (µg Gly/g) in homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, $P \leq 0.05$)

Time of storage [days]	Experimental groups		
	I	II	III
0 (K)	9378 a	9307 a	9416 a
7	9469 a	9616 a	9771 a
14	10007 b	10161 b	10368 b
21	10580 c	10621 c	10772 b
28	11158 d	11031 c	11363 c

On the other hand, the exposure of homogenized sausage to 12°C prior the cryoscopic storage reflected to the decrease of free nitrate (III) concentrations (Table 3). Either the storage-related changes or the differences in the parameter discussed observed among the groups I, II and III were found statistically significant at any period of storage. The phenomenon of a decrease in free nitrate (III) level observed in the stored products is generally consonant with the literature [24, 29].

Table 3. Free nitrate (III) concentrations [ppm] in homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, $P \leq 0.05$)

Time of storage [days]	Experimental groups		
	I	II	III
0 (K)	87.00 aC	73.94 aB	66.93 aA
7	76.30 bC	66.30 bAB	56.40 bA
14	63.50 cC	59.81 cB	49.57 cA
21	56.57dC	53.54 dB	43.83 dA
28	44.00 eC	41.97 eB	37.51 eA

The storage of homogenized sausages under unfavorable conditions before it was put at -3.6°C, resulted with the loss of their mass (Table 4). Although the time-related loss increased significantly with storage of group I, II and III sausages, any important differences between the particular groups were not demonstrated at any stage of experiment.

Table 4. Storage-related weight loss [%] of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, $P \leq 0.05$)

Time of storage [days]	Experimental groups		
	I	II	III
0 (K)	1.37 a	1.50 a	1.56 a
7	1.80 b	1.89 b	1.86 b
14	2.17 c	2.03 c	2.07 c
21	2.40 dB	2.17 dA	2.36 dAB
28	2.49 d	2.43 e	2.53 d

The environmental factors employed did not affect the outward appearance, color, smell, juiciness, texture, palatability, saltiness and the total sensory values of the sausages studied. A trend for decrease in the total sensory value of experimental products was observed during their cryoscopic storage but it was fund to be insignificant (Table 5). Consequently, the exposure of the sausages analyzed to 12°C prior their cryoscopic storage did not affect their sensory properties. Despite of unfavorable conditions before the cryoscopic treatment, the sensory value of homogenized sausages was high and amounted to *ca* 4.5 points for most of the parameters assessed, even if stored twice longer (for 28 days) than the producer-declared shelf-life.

Table 5. The total sensory value [points] of homogenized sausages stored at near cryoscopic temperature for 0-28 days

Time of storage [days]	Experimental groups		
	I	II	III
0 (K)	4.67	4.68	4.64
7	4.64	4.64	4.61
14	4.58	4.53	4.47
21	4.53	4.55	4.56
28	4.49	4.49	4.47

In the study of Libelt [8], most of the sensory parameters of scalded sausages remained within the lower limit of acceptance and amounted to 3.0 points after 96-hour storage at 10°C.

Instrumentally determined, the hardness of experimental sausages decreased with time, whereas the cryoscopic storage did not influenced their cohesiveness, springiness and chewiness (Tables 6-9). The unfavorable conditions of "pre-cryoscopic" treatment reflected however to the increased cohesiveness and gumminess (Tables 7 and 10).

Table 6. The hardness [N] of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, $P \leq 0.05$)

Time of storage [days]	Experimental groups		
	I	II	III
7	47.88 bc	48.34 c	47.46 b
14	45.91 b	45.44 b	45.03 a
21	49.38 cA	52.58 dB	49.08 bA
28	41.06 aA	41.99 aAB	43.40 aB

Table 7. The cohesiveness [N] of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, $P \leq 0.05$)

Time of storage [days]	Experimental groups		
	I	II	III
7	0.255	0.272	0.275 a
14	0.264 A	0.271 A	0.324 bB
21	0.260 A	0.269 A	0.312 abB
28	0.279	0.283	0.283 ab

Table 8. The springiness [mm] of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, $P \leq 0.05$)

Time of storage [days]	Experimental groups		
	I	II	III
7	7.618 a	7.650 a	7.509 a
14	7.402 aA	8.093 abB	8.338 bB
21	7.882 a	7.790 a	8.114 b
28	8.581b	8.47 b	7.963 ab

Table 9. The chewiness [N/m] of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, $P \leq 0.05$)

Time of storage [days]	Experimental groups		
	I	II	III
7	0.093 ab	0.101	0.098 a
14	0.090 aA	0.100 A	0.121 bcB
21	0.101 b	0.110	0.126 c
28	0.097 ab	0.096	0.099 ab

Table 10. The gumminess [N] of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, $P \leq 0.05$)

Time of storage [days]	Experimental groups		
	I	II	III
7	12.21 ab	13.13 bc	13.03 ab
14	12.13 abA	12.30 abA	14.59 bcB
21	12.84 bA	14.11 cAB	15.38 cB
28	11.36 a	11.63 a	12.28 a

The rheological properties of the sausages analyzed where similar as described in our previous studies [3].
The experimental conditions affected neither the minimal temperature of subcooling nor the cryoscopic temperature of the products studied (Tables 11 and 12).

Table 11. The minimal temperature of subcooling [°C] of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, *P*≤0.05)

Time of storage [days]	Experimental groups		
	I	II	III
0 (K)	-4.79 ab	-4.73	-4.55
7	-4.88 a	-4.79	-4.84
14	-4.67 ab	-4.74	-4.74
21	-4.61 ab	-4.84	-4.77
28	-4.50 b	-4.64	-4.62

Table 12. The cryoscopic temperature [ºC] of homogenized sausages stored at near cryoscopic temperature for 0-28 days

Time of storage [days]	Experimental groups		
	I	II	III
0 (K)	-3.52	-3.60	-3.61
7	-3.55	-3.57	-3.64
14	-3.64	-3.55	-3.71
21	-3.62	-3.56	-3.58
28	-3.67	-3.61	-3.55

The variable conditions, the sausages analyzed were exposed to prior the cryoscopic storage, did not affect the value of L* color parameter determined after 7, 14, 21 or 28 days of experiment (Table 13). Consequently the cryoscopic storage was proved not to change the brightness of homogenized sausages.

Table 13. The L* parameter of color of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, *P*≤0.05)

Time of storage [days]	Experimental groups		
	I	II	III
0 (K)	68.07 aB	67.31 aA	68.47 aB
7	69.50 c	69.40 d	69.29 c
14	69.18 bcB	68.76 bcA	68.93 bA
21	68.82 bA	69.10 cdAB	69.14 bcB
28	68.20 aA	68.62 bB	68.34 aA

The a*color parameter of control sausages kept at 12°C for 48 or 96 hours have tendency to increase (Table 14). The opposite trend was however observed after 7-day storage of group I, II and III sausages, i.e. the worse the conditions of "pre-cryoscopic" exposure were, the lower fraction of red was determined in the spectrum reflected.

Table 14. The a* parameter of color of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, *P*≤0.05)

Time of storage [days]	Experimental groups		
	I	II	III
0 (K)	11.10 aA	11.51 cdAB	11.57 cdB
7	11.40 bC	11.08 abB	10.75 aA
14	11.18 ab	11.33 bc	11.33 bc
21	11.39 bC	10.93 aA	11.18 bB
28	11.80 c	11.75 d	11.79 d

Four-day exposure of the controls to 12°C reflected to the significant decrease of yellow (b*) in the spectrum reflected compared to the products of experimental groups I and II (Table 15). Similarly, the yellow fraction was significantly decreased in the spectra of group I and II sausages stored for 7, 14, 21 and 28 days, whereas for group III it remained higher than in the controls.

Table 15. The b* parameter of color of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, $P \leq 0.05$)

Time of storage [days]	Experimental groups		
	I	II	III
0 (K)	13.56 cB	13.35 bB	12.43 aA
7	12.56 a	12.82 a	12.71 b
14	12.97 bB	12.91 aB	12.71 bA
21	12.84 abA	12.92 aAB	13.07 cB
28	12.95 bB	12.68 aA	12.90cB

The values of L* determined in our series were similar as measured in our previous study [3].

The experimental conditions did not influence the color stability of homogenized sausages exposed to glow light for 3, 6, 12 or 24 hours.

The 2- or 4-day exposure of sausages studied to 12ºC, before they were placed at n.c.t., reflected to the increase of microbiological counts. The 7-, 14, 21- and 28-day cryoscopic storage however, resulted in the decrease of microbiological contamination (Table 16). Characteristically, the less favorable the conditions of "pre-cryoscopic" storage were, the lower the contamination tended to be. It should be noted however, that the initial contamination was relatively low in all the samples studied. Total plate counts in group II and III sausages did not exceed 5×10^3 CFU x g^{-1} after 7-day storage, suggesting the satisfactory microbiological quality of the products examined [1, 11, 13, 23]. Neither coliforms, nor *Escherichia coli*, yeasts and moulds, *Salmonella* spp., coagulase-positive staphylococci and anaerobic spore-forming bacilli were demonstrated in any of the samples analyzed.

Table 16. Total plate counts [CFU/g] of homogenized sausages stored at near cryoscopic temperature for 0-28 days (values marked with different small letters are significantly different within the same column, values marked with different capital letters are significantly different within the same line, $P \leq 0.05$)

Time of storage [days]	Experimental groups		
	I	II	III
0 (K)	1.3×10^3	2.7×10^2	3.8×10^3
7	2.0×10^3	1.9×10^2	5.3×10^2
14	7.0×10^2 B	2.3×10^2 AB	8.0×10^2 A
21	1.1×10^2	1.6×10^2	2.2×10^2
28	1.3×10^2	5.3×10^2	3.3×10^2

The sanitary state of experimental sausages was very good compared to the bacterial contamination of meat products available in Polish market. According to Białasiewicz and Królasik [1], the coliforms were demonstrated in as much as 4.44% of perishable sausages examined.

CONCLUSIONS

1. The disobedience of post-production chilling affect neither the rheological properties nor the color of homogenized sausages stored at near cryoscopic temperature.
2. The exposure of homogenized sausages to 12°C prior their cryoscopic storage reflected to the increased acidity and the elevated free amino group content along with the decreased level of free nitrates (III).
3. Either the sensory or microbiological quality of homogenized sausages, kept at 12°C for 96 hours prior the cryoscopic storage, was high after 28-day (twice longer than the manufacturer-declared shelf-life) exposure to near cryoscopic temperature.
4. Cryoscopic storage positively affects the quality of sausages despite of unfavorable conditions they were exposed to directly post production.

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