EVALUATION OF TECHNOLOGICAL PROPERTIES AND OXIDATIVE STABILITY OF ORGANIC DRY FERMENTED PROBIOTIC SAUSAGES DURING LONG-TERM STORAGE

KAROLINA M. WÓJCIAK, MONIKA TRZĄSKOWSKA¹, DANUTA KOŁOŻYN-KRAJEWSKA¹, AND ZBIGNIEW J. DOLATOWSKI

Department of Meat Technology and Food Quality, Faculty of Food Science and Biotechnology, University of Life Sciences in Lublin, 20-704 Lublin, Poland
¹Department of Catering Technology and Food Hygiene, Faculty of Human Nutrition and Consumer Science, Warsaw University of Life Sciences - SGGW, 02-776 Warsaw, Poland

Received: April 5, 2012 Accepted: August 30, 2012

Abstract

The objective of this work was to study the oxidative stability of organic dry fermented probiotic sausages during long-term storage (6 months). Four test samples were prepared: sample A - control sausage, sample B - with 0.05% addition of sodium ascorbate, sample C - with addition of Lb. casei LOCK 0900 (2x10⁶ CFU/g) probiotic strain, 0.6% of glucose, and 0.05% of sodium ascorbate, and sample D - with 0.05% of sodium ascorbate, probiotics, and 0.6% of lactose. The study covered evaluation of the ripening process (21 d) by evaluating loss in the sausage weight. The pH value, oxidation-reduction potential, TBARS values, acid number, peroxide number, browning index after ripening (0) and after 2nd, 4th, and 6th month of chilling storage were identified. The total colour difference of sausages subjected to 3 h exposure to fluorescent light, texture parameters, and count of lactic acid bacteria (LAB) were identified after ripening (0) and after 6 months of storage. It was observed that an addition of probiotic bacteria and glucose (sample C) contributed to a significant decrease in the pH value and water activity in the sausage directly after ripening and during the entire chilling storage period. In samples with probiotics the option with glucose had a lower (P≤0.05) pH value by ca. 0.5 unit as compared to the sample with lactose. Significantly higher (P≤0.05) values of TBARS were observed in samples with probiotics as compared to control samples. The greatest oxidation stability during the entire chilling storage period was found in the sample with sodium ascorbate. Among samples with probiotic strain, the sample with glucose had the lowest peroxide number value (0.58–3.56 meqO₂/kg) and TBARS (1.10–2.08 mg MDA/kg) but also the greatest colour stability during exposure to light. The order of decline in oxidative stability was: sample B > sample C > sample D > sample A.

Key words: probiotics, dry fermented sausage, organic meat, oxidation stability, storage.

In the times when clients are more and more concerned about quality and health-promoting characteristics of meat products, they are not only looking for certified organic products but also expect confirmation of a greater nutritive value of such products, their prolonged life, and even an additional health promoting effects. Additionally, more customers are interested in more expensive products with longer shelf-life and higher quality. This group of products includes dry fermented products particularly popular in Europe, Asia, and USA, which are also gaining popularity in Poland. Dry fermented sausages are meat products made by mixing comminuted cured meat with fat, stuffed in casings, and subjected to preservation by fermentation, curing, cold smoking, and dehydration (19, 30). The quality of dry fermented sausages mainly depends on physico-chemical and microbiological characteristics of the meat ingredients and skillfully conducted technological process and storage conditions (19). The authors (11, 16, 33) claim that meat and fat from pigs bred in organic systems has better nutritive value than the same materials from mass production, which is proven by an improved composition of fatty acids. Kim et al. (16) and Hansen et al. (11) discovered a higher content of polyunsaturated fatty acids (C18:2n6, C20:3n6, C22:4n6) and lower content of saturated fatty acids (C14:0, C16:0) and monounsaturated fatty acids (C16:1n7, C18:1n9, C20:1n9) in meat from organic materials as compared to mass production meat. Using meat and fat from organic bred finishers for making dry fermented sausage may contribute to improving the profile of fatty acids and increasing health promoting characteristics of the product. Moreover, sensory quality of the meat, as evaluated by consumers, is higher for organic meat, mainly due to the content of intramuscular fat conditioning better taste and tenderness of the final product (10). The content of biologically active ingredients (carotenoids, tocopherols, dietary fibre,
CLA) is on a similar or higher level in organic meat (10, 11). Accelerated peroxidation of lipids, resulting from a modified composition of fatty acids, significantly shortening the best-before-date (11, 15, 25) is a certain problem marked in scientific publications on the quality of meat from organically bred finishers and products made from this kind of meat. A new concept assumes using probiotic bacteria for making dry fermented sausage, which can contribute to improvement of the general quality and increasing health-promoting characteristics and shelf-life of the products (15, 17, 23, 31). Studies by Jaworska et al. (15) and Neffe and Kołożyn-Krajewska (23) revealed that it is possible to make ripening loin by using appropriate probiotic strains as starter cultures (15, 17). The literature does not mention studies using organic meat and fat with probiotic starter culture for making potential probiotic dry fermented sausage.

The objective of this work was to study the oxidative stability of organic dry fermented probiotic sausages during long-term storage (6 months).

Material and Methods

Pork muscle ham and backfat were used as basic materials for making fermented sausage. The materials were obtained from Polish Large White purebred swine reared in an organic system. The life weight of the animals was 120-130 kg. The animals were fed feed from own farm, consisting of organic cereal (wheat, barley, oats, and peas), grain, and green matter.

Probiotic strain was prepared at the Chair of Food Hygiene and Quality Management of Warsaw University of Life Sciences according to method described by Jaworska et al. (15). The Lactobacillus casei LOCK 0900 strain was selected on the basis of results of in vitro studies (5), which comprised determination of resistance to the acidity of gastric juice and to bile, adherence to epithelial cells, and antimicrobial activity. All of these studies were carried out according to FAO/WHO recommendations (28, 29). This strain showed strong antagonistic activity against Gram-positive and Gram-negative pathogens (5).

Dry fermented sausage with the following composition: 80% of ham muscle and 20% of hard backfat was used as the test material. The meat was cured using a mixture with the following composition: 98.8%-99.0% of sodium chloride, 0.5%-0.6% of sodium nitrite, and 0.5%-0.6% of sodium nitrate. The backfat was diced and frozen at -19°C. Next, the meat was minced using 8 mm discs, mixed with minced frozen backfat and divided into four equal parts: sample A - control sausage, sample B with 0.05% addition of sodium ascorbate, sample C - with Lb. casei LOCK 0900 (2 x 10^6 CFU/g) probiotic strain, 0.6% of glucose, and 0.05% of sodium ascorbate, and sample D - with 0.05% of sodium ascorbate, probiotic strain and 0.6% of lactose. Fibre casings with a diameter of 58 mm were filled with the prepared mixtures. The products were then subjected to ripening for 21 d at 18°C and 70%-85% RH. Once the ripening was completed, the sausages were cold-smoked and vacuum packed. The samples were evaluated after ripening (0) and after 2, 4, and 6 months of chilling storage.

At ripening, the loss of sausage weight was evaluated by comparing the sausage weight before ripening (0) and after 3, 7, 14, and 21 d of ripening. Acidity was identified by measuring pH value with a digital pH/conductivity measuring device CPC-501 (Elmetron) and integrated electrode type ERH-111 (Elmetron).

The measurement of oxidation-reduction potential was done with a platinum integrated electrode, ERPt-13 type, with a digital pH conductivity measurement device CPC-501 (Elmetron) based on Ahn and Nam method (1). Intensity of the pink colour created as a result of oxidising fats with 2-thiobarbituric acid was measured with Nicols Evolution 300 (Thermo Electron Corporation) spectrophotometer at 532 nm wavelength (TBARS index). The TBARS value was expressed in milligrams of malonaldehyde per 1 kg of meat product (24).

The evaluation of oxidation indices was made on fat extracted from the product with the method reported by Folch et al. (7). Acid number was identified based on PN-ISO 660:1998 (27) and the peroxide number based on PN-ISO 3960:2000 (26).

The colour parameters were measured in the CIE L*a*b* system. L*, a*, b* values and reflectance values between 390 and 700 nm were measured. The measurement was done with a reflection method with 8200 Series (X-Rite) colorimeter using the D65 illuminant, 8 mm port size, and a 10° standard observer. The tests were conducted on samples with a diameter of ca. 55 mm and ca. 15 mm thick immediately after their cutting from the products and after 0.5, 1, 1.5, 2, and 2.5 h of exposure to fluorescent light with intensity of 250 lx. Total colour differences (TCD) were calculated using the following formula: TCD = [(ΔL^2 + Δa^2 + Δb^2)^1/2].

The browning index (BI) was calculated based on the obtained results of colour parameters, using calculations quoted by Homco-Ryan et al. (14) and Maskana (20).

The analysis of the lactic acid bacteria (LAB) count was conducted with an automatic system of microbe count measuring system TEMPO® (Biomerieux, France). The TEMPO® LAB test was composed of a bottle with a medium and a reading card. The reading card with the appropriate dilution of the tested sample was incubated for 40 h at 37°C. Proliferating lactic acid bacteria reducing substrates in the medium caused a formation of fluorescent signal. The signal was read with the TEMPO® reader and the count of lactic acid bacteria was calculated according to the Most Probable Number (MPN) method with the reading cards. The TEMPO® system used for the test helped to achieve reliability standards similar NF - ISO 15214 (23).

The texture of the meat product (2) was evaluated with a double deformation method of cylindrical sausage samples, with dimensions of 50x55
mm, making a part of the sausage bar, cut across, with T.A.XT.plus texture meter. The sample was compressed to 40% of the original height. The head movement speed was 10 mm/min. The following texture parameters were identified based on the relationship between the force and compression strain: hardness (N), springiness (mm), cohesiveness, and chewiness (N mm).

The tests were done for two series of dry fermented sausages, with at least two repetitions for each sample. A statistical characteristics of the samples was developed by identifying mean values (x) and standard deviations (SD). The obtained results were subjected to statistical analysis of variance. Significance of difference between the mean values was identified at the significance level P≤0.05 using the T-Tukey test.

Results

The analysis of the sausage weight loss at ripening revealed that the loss in all samples increased with the ripening time (Fig. 1). The greatest weight loss was observed on the 3rd d of ripening (ca. 10%). Weight loss of 10% was also observed between days 18 and 21 of ripening.

The lowest weight loss at ripening was observed in the control samples and the highest in the sample with probiotic and lactose (sample D). On the 21st d of ripening, all types of sausages had a similar water loss (29% - 33%).

The analysis of sausage acidity revealed that an addition of the probiotic strain and the type of applied carbohydrate had a significant impact on the dry fermented sausage pH value at storage (Fig. 2). In the samples without probiotic bacteria (samples A and B), a significant decrease (P≤0.05) in the pH value by ca. 0.5 unit was observed after the 4th month of chilling storage but after that period the pH value did not change significantly. In the case of sample D, a significant increase (P≤0.05) in the pH value was observed after the 2nd month of storage (pH 5.2) followed by a gradual decrease to the value of pH 4.9. In the sample with probiotic and glucose (sample C), a significant increase in the pH value was observed after the 2nd month of storage followed by a stabilisation of the pH value. Statistically significant (P≤0.05) pH values, lower by ca. 0.5-1.0 unit, were observed in samples with probiotic bacteria as compared to the control sample A and sample B with sodium ascorbate. pH values lower by ca. 0.5 unit (P≤0.05) were noted in the sample with glucose (sample C) as compared to the sample D with lactose during the entire chilling storage period.

A significant impact of the addition of Lb. casei LOCK 0900 strain and storage time on water activity in dry fermented sausage was observed (Fig. 3). Water activity during the entire chilling storage period was gradually decreasing. A significant drop by ca. 0.01 unit was observed in the samples after 2 months of storage. In the sample B, a significant reduction (by ca. 0.03) of the tested parameter occurred in the 4th month of storage. Water activity in samples with bacterial strain after the 4th and 6th month of chilling storage stabilised at the same level. In the case of sample with sodium ascorbate, after the 6th month of chilling storage, an increase in the water activity value was observed. Significantly (P≤0.05) higher values of water activity in the control samples as compared to probiotic samples were observed in all test periods. Significant (P≤0.05) differences in water activity between samples with probiotics were observed only immediately after ripening. A significantly lower (P≤0.05) value or water activity was observed in sample C as compared to sample D.

The addition of probiotic bacterial strain and the type of carbohydrate had a significant impact on the change in the oxidation-reduction potential in dry fermented sausage at storage (Table 1). The oxidation-reduction potential in the samples increased significantly (by ca. 30 mV) up to the 4th month of chilling storage and its decrease was observed after the 6th month. The only exception was observed in the sample with ascorbate, where the redox potential decreased by ca. 20 mV after the 2nd month of storage and was increasing up to the 4th month to stabilise at the end. Lower statistically significant values of the redox potential were observed in sample B as compared to other samples in the 2nd, 4th, and 6th month of tests. Higher (P≤0.05) values by ca. 20-60 mV of the oxidation-reduction potential in the control sample as compared to other samples were observed during the entire chilling storage period. The type of carbohydrates had a significant impact on the value of oxidation-reduction potential. Higher values (P≤0.05) by ca. 30 mV of the redox potential were observed in the sample D with lactose as compared to the sample C with glucose after 0 and 2 months of storage.

Analysis of the acid number in products during storage revealed a significant impact of probiotic addition and the type of sugar on the content of free fatty acids (Table 1). A significant increase in the acid number was observed after 2 months of storage followed by a drop after 4 months. No significant changes in the acid number value were observed in the final period of chilling storage. Significantly (P≤0.05) higher values of the acid number in the control sample as compared with the sample with sodium ascorbate were observed after 0, 4th, and 6th month of chilling storage. Significantly higher values of the acid number in samples with Lb. casei LOCK 0900 in comparison to samples A and B after 0, 2nd, and 6th month of storage were observed.

All sausage samples showed a significant (P≤0.05) reduction of peroxide content (0.70-1.81 meqO₂/g kg) up to the 2nd month of chilling storage, with the exception of the sample with sodium ascorbate, where the peroxide level did not change significantly. An increase (P≤0.05) in peroxide number in the 4th month of chilling storage was observed in all samples. In the case of control sample and the sample with LOCK 0900 strain and lactose, a further increase in the quantity of peroxides (sample B) or their statistically insignificant increase was observed.
Fig. 1. Weight loss (%) in dry fermented sausage during maturation.

Fig. 2. Changes of acidity in dry fermented sausage during chilling storage. Means followed by different capital letters A-D within the same sample or by small letters a-b within different samples are significantly different (P≤0.05).

Fig. 3. Changes of water activity in dry fermented sausage during chilling storage. Means followed by different capital letters A-D within the same sample or by small letters a-b within different samples are significantly different (P≤0.05).
Fig. 4. Total colour difference (TCD) during 2.5 h dry fermented sausage exposure to light on 0 d (a) and in 6 months (b) of chilling storage.

Fig. 5. Evolution of browning index (BI) of dry-fermented sausage during 6 months of chilling storage.
The sample with sodium ascorbate had the lowest statistically significant (P≤0.05) content of peroxides after 0 and 6th month of chilling storage - 0.83 and 2.38 meqO₂/kg, respectively, and the control sample revealed the highest content after 0, 4th and 6th month - 2.28, 4.12, and 5.58 meqO₂/kg, respectively. Significantly higher (P≤0.05) values of the ascorbate number after 0, 2nd, and 4th month of storage among all samples with an addition of probiotic strain were observed after 0, 2nd, and 4th month of storage in the sample with lactose as compared to the sample with glucose.

The analysis of oxidation processes revealed, for the majority of experiments, a significant impact of the storage period, addition of probiotics, and the type of applied sugar on the value of TBARS (Table 1). A significant increase in the TBARS index value (0.62-0.84 mg MDA/kg) was observed after the 2nd month of chilling storage and the lowest values of TBARS in the sample C with sodium ascorbate - ca. 0.85 mg MDA/kg.

The analysis of TCD values characterising the total change in colour revealed impact of time when the sausage was exposed to fluorescent light on the sausage colour fastness (Fig. 4 a, b). The analysis of TCD in sausages directly after ripening revealed that with the time of exposure to light the TCD values were gradually increasing, which indicated colour deterioration. With regard to the chilling storage time, the colour fastness was reduced. The maximum colour change observed after 2.5 h of exposure to light immediately after ripening was 4 units. Exposing the products to light for 0.5 h did not result in any visible changes in all tested samples. After 1 h of exposure to light, the greatest change in colour, described as recognisable by an inexperienced observer, occurred only in the control sample. After subsequent 30 min of exposure to light (1.5 h), the greatest change in colour was observed in sample B (3 units) and in sample C (2.5 units). After 2.5 h of exposure to light, it was discovered that the greatest change in colour (TCD) occurred in the control sample (4 units) and in sample C with Lb. casei LOCK 0900 and glucose, ca. 3.7 units. The authors describe such a deviation as a significant colour deviation.

The analysis of the colour change during exposure to light, conducted on the sausages at the end of chilling storage (6th month) revealed that the colour of all samples was more sensitive to fluorescent light (Fig. 4b). The greatest changes occurred even after 0.5 h of chilling storage.

### Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>Storage period/month</th>
<th>ORP (mV)</th>
<th>TBARS (mg MDA/kg)</th>
<th>AN (mg KOH/g)</th>
<th>PN (meqO₂/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>330.97 ± 1.14</td>
<td>1.20 ± 0.08</td>
<td>11.24 ± 0.18</td>
<td>2.28 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>363.23 ± 2.17</td>
<td>1.82 ± 0.19</td>
<td>17.41 ± 0.37</td>
<td>0.47 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>371.03 ± 0.07</td>
<td>0.73 ± 0.02</td>
<td>10.38 ± 0.32</td>
<td>4.12 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>337.17 ± 5.89</td>
<td>0.94 ± 0.02</td>
<td>8.58 ± 0.17</td>
<td>5.56 ± 0.02</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>321.57 ± 2.97</td>
<td>1.35 ± 0.09</td>
<td>12.36 ± 0.16</td>
<td>0.83 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>305.90 ± 1.28</td>
<td>0.85 ± 0.03</td>
<td>17.19 ± 0.06</td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>330.73 ± 1.43</td>
<td>0.67 ± 0.03</td>
<td>9.61 ± 0.09</td>
<td>2.76 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>328.07 ± 1.63</td>
<td>0.83 ± 0.01</td>
<td>7.87 ± 0.03</td>
<td>2.38 ± 0.02</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>297.47 ± 4.66</td>
<td>1.29 ± 0.02</td>
<td>12.21 ± 0.07</td>
<td>1.47 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>318.03 ± 2.84</td>
<td>2.08 ± 0.05</td>
<td>21.90 ± 0.18</td>
<td>0.58 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>353.40 ± 3.45</td>
<td>1.16 ± 0.01</td>
<td>9.73 ± 0.18</td>
<td>2.87 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>321.53 ± 2.43</td>
<td>1.10 ± 0.04</td>
<td>9.56 ± 0.04</td>
<td>3.56 ± 0.02</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>312.50 ± 1.63</td>
<td>1.40 ± 0.01</td>
<td>14.33 ± 0.37</td>
<td>1.72 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>351.47 ± 2.68</td>
<td>2.24 ± 0.04</td>
<td>21.06 ± 0.18</td>
<td>1.02 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>346.57 ± 0.63</td>
<td>1.16 ± 0.05</td>
<td>10.36 ± 0.04</td>
<td>3.02 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>334.07 ± 0.62</td>
<td>1.02 ± 0.01</td>
<td>9.78 ± 0.07</td>
<td>3.51 ± 0.00</td>
</tr>
</tbody>
</table>

Means followed by different capital letters A-D within the same sample or by small letters a-b within different samples are significantly different (P≤0.05); ORP – oxidation-reduction potential, AN – acid number, PN – peroxide number.
light activity, in reference to sample B (5 units) and C (ca. 6 units). The changes are described as a significant colour deviation. The smallest change in colour was observed in the samples with probiotics and lactose (ca. 2 units during the entire period of exposure to light). A change in colour within the range of 1-2 units is unrecognisable. Slower changes, from 0.5 to 1 unit, were observed in subsequent hours of exposing the products to light. The greatest changes during 2.5 h of exposure to light were observed for product B (with sodium ascorbate) and minor ones for products D (with Lb. casei LOCK 0900 and lactose).

The browning index (Fig. 5) was gradually increasing in all samples during 6 months of chilling storage. The greatest values after 2 months of chilling storage were observed in sample B (BI=20), in the 4th month of chilling storage in the control sample (BI=27.78) and in the 6th month in the sample with probiotics and lactose (BI=25). In the case of samples with probiotics the browning index was the most stable and the lowest as compared to other samples.

The analysis of lactic acid bacteria count (Table 2) revealed that during storage the bacteria count decreased by one logarithmic level in all test samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Count of lactic acid bacteria (log CFU/g) After ripening (0)</th>
<th>After storing (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8.23</td>
<td>7.29</td>
</tr>
<tr>
<td>B</td>
<td>8.41</td>
<td>7.04</td>
</tr>
<tr>
<td>C</td>
<td>8.79</td>
<td>7.85</td>
</tr>
<tr>
<td>D</td>
<td>8.70</td>
<td>7.32</td>
</tr>
</tbody>
</table>

Table 2
The comparison of the lactic acid bacteria count in dry-fermented sausage after ripening and storage process.

In samples with probiotics (C, D) the lactic acid bacteria count was higher by an incomplete logarithmic order as compared to the control sample and sample B with sodium ascorbate. The lactic acid bacteria count at the end of the chilling storage period was higher in samples of sausages inoculated with LOCK 0900 strain - 7.85 and 7.32 log CFU/g, respectively, as compared to other samples. The difference was lower than one logarithmic level. However, after 6 months of storage, samples with Lb. casei LOCK 0900 and glucose addition were characterised by better survivability of LAB than sausage with probiotic and lactose.

An addition of probiotic bacteria, the type of applied hydrocarbon, and storage time had a significant impact on the dry fermented sausage texture parameters (Table 3). Analysis of the results revealed that a statistically significant hardness occurred in the sample with probiotic and glucose directly after ripening (417.23 N). No significant difference in the hardness was observed for other samples. At the end of the chilling storage period, a significant increase was observed in the hardness of all tested samples except sample C, where hardness dropped by 22.5 N and chewiness by 7.93 N mm, but the drop was statistically negligible. The impact of LOCK 0900 strain addition on the product springiness, regardless of sugar addition, was also observed. The highest cohesiveness value immediately after production was observed in sample B (0.47) and the lowest in sample C (0.39). In the 6th month of chilling storage, the value of cohesiveness increased in two samples: control A and in the sample C with probiotic and glucose.

Table 3
Changes in textural parameters (TPA) of dry-fermented sausage at the beginning (0) and at the end (6 month) of chilling storage period

<table>
<thead>
<tr>
<th>Samples</th>
<th>Hardness (N)</th>
<th>Springiness (mm)</th>
<th>Cohesiveness (N mm)</th>
<th>Chewiness (N mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>367.22±A</td>
<td>0.63±A</td>
<td>0.41</td>
<td>92.24±A</td>
</tr>
<tr>
<td></td>
<td>26.27±A</td>
<td>0.02±A</td>
<td>0.02</td>
<td>14.13</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>356.30±A</td>
<td>0.64±A</td>
<td>0.47</td>
<td>105.60</td>
</tr>
<tr>
<td></td>
<td>26.27±A</td>
<td>0.01±A</td>
<td>0.01</td>
<td>6.60</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>417.23±A</td>
<td>0.71±A</td>
<td>0.39±A</td>
<td>123.51</td>
</tr>
<tr>
<td></td>
<td>97.12±A</td>
<td>0.07±A</td>
<td>0.07</td>
<td>14.50</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>343.99±A</td>
<td>0.77±A</td>
<td>0.45</td>
<td>120.18±A</td>
</tr>
<tr>
<td></td>
<td>42.34±A</td>
<td>0.09±A</td>
<td>0.01</td>
<td>15.28</td>
</tr>
</tbody>
</table>

Means followed by different capital letters within the same sample or by small letters within different samples are significantly different (P≤0.05)
Discussion

Developing the quality of organic meat products depends on the direction and speed of physicochemical and biochemical changes. Dry fermented sausages are subject to changes that can result in an increase or decrease in sausage quality. Changes caused by lipid oxidation are the major cause of adverse chemical and sensory changes, which result in reduced or impossible further storage of fermented meat products (4). Acidity analysis of the tested sausages revealed significantly lower pH values in samples with probiotics as compared to other samples, which was related to a higher level of lactic acid bacteria and simultaneously with an increase in the product acidity (3, 6, 13, 17, 18). Lactic acid also contributes to forming organoleptic features of the product. A significantly higher acidity observed after glucose addition (sample C), as compared to the sample D with lactose, could be the result of a faster use of monosaccharide than disaccharide in the lactic acid production (9) (Fig. 2, Table 2). The count of lactic acid bacteria was lower in the sample with lactose. A systematic significant increase in the pH value observed in all samples during storage resulted from a progressive proteolysis caused by the liberation of peptides and free amino acids leading to an increase of the pH value (3, 30). Based on the results of the sausage weight loss at ripening (Fig. 1) and pH value (Fig. 2) of the sample after production, a conclusion on its microbiological durability can be made. The weight loss in all samples was ca. 30% but only the samples inoculated with *Lb. casei* LOCK 0900 strain had pH values lower than 5.3 units, which classifies them as durable products under ambient conditions and microbiologically safe. The analysis of texture (Table 3) revealed that an addition of *Lb. casei* LOCK 0900 bacteria has a significant impact on the increase in sausage hardness. The observed relationship could have been the result of the pH value drop directed towards the isoelectric point of muscle proteins causing an increase in the protein solubility and, as a consequence, an increase in the product hardness and chewiness (9). A significant increase in hardness and chewiness observed in the reference study at the end of chilling storage period could be connected with a significant drop in pH value in samples A, B, and D observed during long-term storage.

A significantly lower (P≤0.05) water activity (Fig. 3) in samples with probiotics as compared to inoculated samples observed at the beginning of chilling storage could be the result of microbes producing organic acids during ripening, causing an increase in the protein structure denaturation and acceleration of water diffusion from the stuffing, which resulted in a faster and greater water loss (Fig. 1) and decrease in water activity in the product. The value of water activity was decreasing in parallel to the sausage weight loss during ripening (Fig. 1, Fig. 3). Most probably it resulted from a gradual diffusion of water mainly from the muscle tissue of the central part of the bar outwards. A decrease in water activity in food products results in decreasing the speed of adverse physico-chemical changes. A decrease in water activity by ca. 0.02 unit in samples tested during storage could have been attributed to proteolysis and peptidolysis and hydrolysis of triacylglycerols occurring in the stored product (3, 19, 30, 32), resulting from formation of low-molecular compounds. It is confirmed by the acid number count (Table 1) being the evidence of enzymatic activity of lipases of tissue and/or bacterial origin. Higher values of acid number and simultaneously lower water activity values (Fig. 3, Table 3) were observed in the samples with probiotics. Significantly lower values observed in samples with probiotics as compared to other samples could be the result of adding osmoactive substances (lactose, glucose).

Oxidation processes in fermented meat products are the main problem causing reduction of their quality and significantly shortening the best-before-date (3, 4, 6, 22, 30, 34). The products made as a result of oxidation change their colour, taste, and desired colour. Additionally, oxidation causes a decrease in the nutritive value of the product and sometimes may result in formation of compounds toxic to humans e.g. oxysterols (4, 6). According to McDonald and Hultin (21), the pH value of fatty acids, temperature, oxidation-reduction potential, exposure to light, iron content, and ion force have a key importance in lipid oxidation. A significantly higher TBARS values observed in samples with probiotics could be the result of *Lb. casei* LOCK 0900 strain being able to produce H₂O₂, which has a toxic action against pathogens, but on the other hand shows pro-oxidising impact of food ingredients. Analysing the obtained test results it can be stated that the value of TBARS index did not exceed 2.5 mg MDA/kg (Table 1) in the produced sausages in the entire period of chilling storage. Bozkurt et al. (3) and González-Fernández et al. (6) obtained similar results for dry fermented sausages. The latter authors studying dry fermented products confirm that higher values of TBARS (ca. 2.21 mg MDA/kg) do not necessarily mean poor quality of ripening products, since aldehydes identified with this method are the necessary component of taste and odour range formed in biochemical transformation of the fatty fraction occurring during the product ripening and storage. It should be emphasised that in all samples except the control sample, the peroxide level was below 4.0 meqO₂/kg (Table 1), which classifies the product as a good quality product (2-4 meqO₂/kg) according to Chizzolini et al. (4). Lipid oxidation was proven to cause adverse changes in the colour of meat products as a result of reaction occurring between secondary lipid oxidation products (4-HNE) and myoglobin (8). Free lipid radicals formed during lipid peroxidation process can catalyse oxymyoglobin oxidation to grey-brownish metmyoglobin (8).

The colour fastness criteria acquired by the International Lighting Commission (CIE) (12) was used in the analysis of the results. The criterion classified total colour differences (TCD) relevantly to the human perception of colours. It was assumed that the total colour difference between 0 and 2 is unrecognisable, and from 2 to 3.5 recognisable by an inexperienced observer,
whereas the value exceeding 3.5 constitutes a significant colour deviation for the observer. A reduced colour fastness at exposing the samples to natural light after 6 months of storage (Fig. 4b) could be the result of oxidation - under the impact of light - of red-coloured nitrosylmyoglobin (formed as a result of curing the meat) to grey-brownish metmyoglobin (8). Oxidation occurred faster in samples examined after 6 months of storage due to accumulation of lipid peroxides (Table 1) in the product, which accelerate adverse changes in haem pigments. Greater colour stability and lower browning index in samples with probiotics could be the result of the probiotics impact on acidity increase, which fosters the formation of nitrosylmyoglobin. Lactic bacteria proliferating in a larger quantity in samples C and D could impede the growth and development of saprophytic bacteria generating substances that could lead to the change of colour into grey-greenish. The obtained results suggest that it is possible to use ingredients from organic production to make potential probiotic dry fermented sausage with oxidation stability during long-term (6 months) chilling storage.

Acknowledgments: The study was supported by the Ministry of Agriculture and Rural Development (Grant RP-re-401-17-165/09). The authors are grateful to Professor Zdzisława Libudzisz from the Łódz Technical University (Poland) for the Lactobacillus casei LOCK 9090 loan. The batches of sausage for the tests were made in "Jasiołka" Meat Manufacture.

References


