Effect of the addition of pullulan on the quality of low-fat homogenized scalded sausages

Aneta Cegiełka\textsuperscript{a}, Małgorzata Gniewosz\textsuperscript{b}, Elżbieta Hać-Szymańczuk\textsuperscript{b} and Anna Chlebowska-Śmigiel\textsuperscript{b}

\textsuperscript{a}Faculty of Food Sciences, Department of Food Technology, Warsaw University of Life Sciences – WULS SGGW, Warsaw, Poland; \textsuperscript{b}Department of Biotechnology, Microbiology and Food Evaluation, Warsaw University of Life Sciences – WULS SGGW, Warsaw, Poland

**ABSTRACT**

This paper presents the study on determination of the effect of pullulan on the quality of low-fat homogenized scalded sausages stored in vacuum packages at 4 ± 1°C for 21 days. Control sausage (PC) contained 20% of pork jowl in recipe composition. In sausages P1, P2 and P3, respectively, 1/4, 1/2 and 3/4 of jowl content was substituted with pullulan. The chemical composition was determined, the color and texture were measured and sensory evaluation was performed in sausages. Microbiological analyses were performed in sausages after 0, 3, 7, 10, 14 and 21 days of storage. It was found that the use of pullulan did not lead to a deterioration in the microbiological quality of sausages during storage. However, no more than 1/4 of the jowl content should be substituted with pullulan due to pullulan-negative effect on the general appearance, taste and texture of scalded sausages with assumed recipe composition.

**Introduction**

Homogenized scalded sausages are popular meat products. Most of this type of sausages available on the European market contain 20–30% fat (Asuming-Bediako et al., 2014; Palka, Węsierska, & Niwecka, 2012). For several years, the market has also offered homogenized scalded sausages with approximately 15% or lower reduced content of this component. Reduction in fat content is achieved by modification of the recipe composition. This often relies on the substitution of a part of the fatty raw material with nonmeat ingredients, that is, polysaccharides used either alone or in different combinations. Due to their water-binding capacity, thickening and gelling properties, polysaccharides have a positive effect on the texture and sensory characteristics of scalded sausages with reduced fat content. Literature data indicate that starch, maltodextrin, carrageenan, locust bean gum, xanthan and fiber preparations may be used in the preparation of low-fat scalded sausages (Cierach, Modzelewskia-Kapitula, & Szacilo, 2009; Crehan, Hughes, Troy, & Buckley, 2000; García-Garcia & Totosaus, 2008; Hughes, Cofrades, & Troy, 1997; Lurueña-Martinez, Vivar-Quintana, & Revilla, 2004).

Homogenized scalded sausages are short shelf-stable products. They are characterized by relatively high pH and water activity (Korkeala & Björkroth, 1997), which are the most important factors affecting shelf stability. It should, therefore, be noted that modifications to the recipe composition of scalded sausages aimed at reducing fat content may affect not only the physicochemical and sensory characteristics of quality but also the microbiological quality of food (Delgado-Pando et al., 2011; López-López, Cofrades, & Jiménez-Colmenero, 2009).

Among substances of polysaccharide character, pullulan has relatively recently come to be used in food processing. This is an extracellular polysaccharide synthesized by Aureobasidium pullulans saprophyte. In the United States, pullulan was added to the Generally Recognized as Safe list in 2002 and it can be used in foods as a binder (GRAS Notice No GRN 000099). In Europe, the use of pullulan has been permitted since 2004, but only for dietary supplements in...
solid form, that is, as capsules and tablets (Commission Regulation (EU) No 1129/2011).

The properties and potential applications of pullulan are described in the literature. Study results indicate that pullulan exhibits thickening and binding properties (Cheng, Demirci, & Catchmark, 2011; Singh, Gaganpreet, & Kennedy, 2008). These studies suggest that pullulan could act as a substance which improves the texture of homogenized meat products.

Therefore, this paper presents a study on the determination of the effect of pullulan on the physicochemical and microbiological properties of low-fat homogenized scalded sausages. The results may contribute to a broadening of knowledge within the field of the use of this polysaccharide in food preparation.

Materials and methods

Preparation of sausages

Four formulations of homogenized scalded sausages were prepared differing in their recipe compositions in terms of pullulan and pork jowl content (Table 1). The basic recipe composition of sausages was 30.0% of beef trimmings, 30.0% of pork trimmings, 20.0% of pork jowl and 20.0% of water (added as flaked ice). The proportion of pork jowl substituted with pullulan – 1/4, 1/2 and 3/4 of jowl content – was established based on preliminary studies (results not published). The addition level of curing salt, mixture of phosphates and seasoning was calculated in relation to the total mass of the raw materials: beef, pork and pork jowl.

Beef and pork, as well as pork jowl, were purchased from a local manufacturer (Sokołów S.A.; Sokołów Podlaski, Poland) in amounts sufficient to conduct the experiment in three replicates. Immediately after delivery to the laboratory, the beef, pork and jowl were cut into pieces of approximately 2 x 2 cm and mixed thoroughly. Subsequently, each raw material was divided into three portions, each of which was vacuum packed in a multilayer film bag (polyethylene/polyamide [PE/PA], 75-µm thick) using a packaging machine (C200; Multivac Sepp Haggemüller GmbH & Co. KG, Wolferschwerden, Germany) and then frozen (−20 ± 1°C). Before each preparation of sausages, one portion of beef, pork and jowl was thawed (4 ± 1°C, 18 h). Then, the raw materials were ground separately in a meat grinder (AL2; Mesco, Skarżysko-Kamienna, Poland) through a plate with 5-mm diameter orifices. The process of sausage preparation was performed according to the procedure described below.

Sausage batters were prepared in a laboratory cutter (Hobart™ 84145; Hobart, Troy, OH, USA). In the first step (5 min), beef and pork were subjected to chopping and mixing with an addition of curing salt, flake ice and phosphate mixture. In the second step of chopping (5 min), pork jowl and pullulan (only P1, P2 and P3 sausages) were added. The temperature of batters after the chopping process did not exceed 12°C. Cellulose casings of a caliber of 22 mm (Nalo Faser®; Kalle GmbH, Wiesbaden, Germany) were filled with batter using a manual sausage stuffer (Royal Catering® RCWF-5L; Emaks Sp. z o.o., Zielenia Góra, Poland) followed by twisting sticks with a length of 12 cm. Sausages were hung on cooking racks at room temperature (about 18°C) for 30 min. Thermal processing of sausages was conducted in a smoking-scalding laboratory chamber (Jugema, Stalowa Wola, Poland). This involved the following steps: drying (40°C, 10 min), smoking (50°C, 7 min), scalding (until the center of the sausage reached the temperature of 68°C) and cooling with a cold water spray (10 min). Each preparation batch of sausages was weighed both prior to thermal processing and after its completion and cooling. The mass of each preparation batch of sausages per formulation was about 1800 g. Sausages within each formulation were divided into six portions (each about 300 g). Each of the separate portions of sausages was vacuum packed in a multilayer film bag (PE/PA, 75-µm thick) and stored in a refrigerator (4 ± 1°C) for 21 days.

Determinations of the basic chemical composition, instrumental measurements of color and texture and sensory evaluation of sausages were conducted about 24 h after their preparation. Measurements of pH and water activity (a_w), along with microbiological analyses of sausages, were performed after 0, 3, 7, 10, 14 and 21 days of storage.

Cooking loss and chemical composition analysis

Cooking loss in sausages was determined for each sausage formula according to Crehan et al. (2000). The weights of uncooked and cooked sausages were recorded. The sausages were hung on cooking racks at room temperature (about 18°C) for 30 min and weighed (uncooked weight). After thermal processing, during which time the core temperature of sausages reached 68 ± 1°C, the sausages were allowed to cool for 1 h, and weighed (cooked weight). The percentage cooking loss was calculated according to the following formula:

\[
\text{Cooking loss} = \frac{(\text{Weight of uncooked sausage} - \text{Weight of cooked sausage})}{\text{Weight of uncooked sausage}} \times 100
\]

Table 1. Recipe composition of homogenized scalded sausages with pullulan.

| Ingredient | Sausage formula (g/kg) |
|------------|------------------------|
|            | PC | P1 | P2 | P3 |
| Beef trimmings class II | 293.7 | 293.7 | 293.7 | 293.7 |
| Pork trimmings class II | 293.7 | 293.7 | 293.7 | 293.7 |
| Pork jowl | 195.8 | 146.8 | 97.9 | 49.0 |
| Pullulan (Carbosynth Ltd., Compton, UK) | 195.8 | 195.8 | 195.8 | 195.8 |
| Curing salt* (99.4% NaCl + 0.6% NaNO₂; Salino SA, Inowrocław, Poland) | 14.1 | 14.1 | 14.1 | 14.1 |
| Mixture of phosphates* (Tari K2, BK Giulini GmbH, Ludwigshafen am Rhein, Germany) | 2.3 | 2.3 | 2.3 | 2.3 |
| Seasoning for scalded sausages* (Moguntia Poland, Jelenia, Poland) | 4.6 | 4.6 | 4.6 | 4.6 |

*The amount of additive in relation to the total mass of beef/pork meat and pork jowl.

*La cantidades de aditivo en relación a la masa total de carne de ternera/cerdo y papada de cerdo.

Table 1. Composición de la fórmula de las salchichas escaldadas homogenizadas con pululan.
Cooking loss (%) = \frac{(\text{uncooked weight} - \text{cooked weight})}{\text{uncooked weight}} \times 100

The determination of water, protein and fat content in sausages was carried out in accordance with the requirements of the Association of Official Analytical Chemists (AOAC, 2007). The experiments used a FoodScan Lab near-infrared spectrometer (Foss Analytical A/S, Hillerød, Denmark) working in the spectral range of 850–1050 nm and using calibration based on the artificial neural network model. The content of salt (NaCl) in the sausages was determined using the potentiometric method with a Titrino 702 SM (Metrohm AG, Herisau, Switzerland) apparatus.

Measurement of color and texture

For the purpose of the instrumental measurement of color and texture, sausage sticks randomly drawn from packages were incubated at 20°C for 2 h.

Measurement of color on the surface of the longitudinal cross section of sausages was conducted using the CIE Lab*a*b* color system. For each formulation of sausage, five measurements were performed at different locations, and the results were averaged. A CM 2600d Chroma Meter (Konica Minolta Inc., Tokyo, Japan) colorimeter was used with a measuring head with a diameter of 8 mm, D65 illuminant (color temperature ~6500 K) and a standard two observer.

Measurement of sausage texture was carried out using a Zwicki 1120 (Zwick GmbH & Co.; Ulm, Germany) universal testing machine. The cutting test was performed with the use of a steel v-shaped blade of a width of 70 mm and 3-mm thickness at a constant speed of the measuring head of 50 mm/min. Sausage stick sections without casing (length 50 mm, diameter 15 mm) constituted samples for measurement. The shear force of sausages was expressed as the maximum force (N) reported during sample cutting. For each formulation of sausage, the test was performed in five samples, and the results were averaged.

Sensory evaluation

Sensory evaluation of sausages was conducted by a panel consisting of 10 individuals (aged 22–43) using a scaling method according to the Standard (PN-ISO 4121:1998). The sensory evaluation was carried out under standard conditions, including separate boxes, constant room temperature (18°C) and the same light intensity of about 500 lx.

The sensory panel was trained on the basis of the specificity of the product and the method of its evaluation. The assessed sensory attributes of sausages were general appearance, color in the cross-section, odor, taste and texture. For their assessment, a nonstructured 5-cm scale was used (0–5 arbitrary units) with defined border values: 0 – the total lack of acceptance, 5 – the greatest level of acceptance of a particular attribute.

Evaluators were provided with slices of sausages encoded and arranged in a random order on white plates. Prior to evaluation, sausage slices were conditioned at room temperature for 30 min. Evaluators were provided with weak tea without sugar to rinse the palate between the evaluation of each subsequent sample.

Measurement of pH and water activity

Measurement of the pH of sausages was conducted using a pH meter (CP-31; Elmetron, Zabrze, Poland) equipped with an Ag/AgCl (Metron, Czechanów, Poland) electrode according to the Standard (PN-ISO 2917:2001). The sample for measurement was prepared by mixing 10 g of finely ground sausage with 30 ml of deionized water.

Measurement of the water activity (a_w) of sausages was carried out using an Aqua Lab CX-2 (Decagon Devices Inc., Pullman, WA, USA) apparatus at a constant temperature of 25°C. The test sample was a 3-mm thick slice of sausage placed in a sample cell. For each formulation of sausage, pH and a_w measurements were performed in duplicate and the results were averaged.

Microbiological analysis

The packaging foil and casing of sausages were incised with a sterile scalpel and separated from the sausage with sterile tweezers. Then, the sausage was aseptically cut across the circumference and two samples, each of 10 g, were transferred to 90 ml of 0.1% sterile peptone water (bioMérieux, Marcy-l’Étoile, France). Each sample was homogenized in a stomacher blender (Lab Blender 400 Circulator, Seward Laboratory, London, UK) for 1 min at high speed and at room temperature (18°C). Further, a series of 10-fold dilutions in sterile peptone water were prepared. From each dilution, culturing on Plate Count Agar (BTL, Lodz, Poland) was performed for the total viable count (TVC; 30°C for 48 h) and the number of psychrotrophic microorganisms (7°C for 10 days) (Martinez, Djenane, Cilla, Beltrán, & Roncalés, 2005), on De Man, Rogosa, Sharp Agar (BTL, Łódź, Poland) for lactic acid bacteria (LAB; 30°C for 72 h), on Violet Red Bile Glucose Agar (BTL, Lodz, Poland) for Enterobacteriaceae (37°C for 24 h) and on Sabouraud with chloramphenicol Agar (BTL, Lodz, Poland; 30°C for 72 h) for yeasts and molds. The number of microorganisms was expressed as log_{10} of colony-forming units per gram (log CFU/g) (López-López et al., 2009).

Statistical analysis

Statistical analysis was performed using Statistica 12.0 (STATISTICA, 2014; StatSoft Inc., Tulsa, OK, USA). The significance of differences between examined parameters among samples was checked with the one-way ANOVA method and Tukey’s HSD test, with the significance level α = 0.05. The relationship between selected sensory attributes and instrumental results was evaluated using Pearson’s linear correlation at P < 0.05.

Results and discussion

Cooking loss and analysis of chemical composition

Cooking loss in homogenized scalded sausages did not significantly differ (P > 0.05) when pullulan was introduced into their recipe composition instead of jowl (Table 2).
In this study, cooking losses in sausages subjected to quality evaluation were slightly lower in comparison to the results obtained by Cierach et al. (2009) and Hughes et al. (1997) in scalded sausages containing 10–12% fat. Cierach et al. (2009) reported that weight losses in sausages with the addition of carrageenan were 10.97–11.26% being not significantly lower than in a control sausage (12.66%) of comparable fat content. According to Hughes et al. (1997), the introduction of carrageenan or oat fiber into the composition of low-fat sausages resulted in a significant reduction in cooking loss from 10.8% to 8.6% and 9.4%, respectively. Other researchers have shown that the introduction of polysaccharides into the recipe composition of scalded sausages enables a reduction of fat content of up to 15% or lower without a significant increase in cooking losses compared to their ‘full-fat’ counterparts. Such an effect has been obtained by Pietrasik (1999), who used modified starch. According to the author, a significant factor differentiating the amount of thermal loss in scalded sausage was the level of fat in the recipe composition. In contrast, the addition of modified starch had no significant effect on the value of this parameter, regardless of the fat content in the product.

In other studies, unlike in the present study, it was found that the amount of the thermal loss in scalded sausage can be reduced using polysaccharide ingredients. Yang, Choi, Jeon, Park and Joo (2007) stated that sausage containing hydrated oatmeal was characterized by a significantly lower cooking loss (5.75%) than control sausage (10.94%). Lurueña-Martínez et al. (2004) found that replacement of pork back fat with locust bean gum and xanthan gum in the composition of low-fat sausage caused a significant reduction in both the fat content in the sausage (from over 20% to below 10%) and cooking loss of 6.51% to even 1.50%.

Modification of the recipe composition had no significant (P > 0.05) effect on the protein and sodium chloride contents in homogenized scalded sausages (Table 2). However, significantly (P < 0.05) more water was found in those sausages with the highest content of pullulan (P3). In the composition of sausages in which pullulan was replaced by jowl in a proportion of 1/2 to 3/4 (P2 and P3, respectively), sausages contained significantly (P < 0.05) less fat in comparison to those with higher proportions of jowl (PC and P1).

In this study, the results of the basic chemical composition of sausages subjected to evaluation were similar to the results cited by other authors for scalded sausages of comparable fat content (Cierach et al., 2009; Crehan et al., 2000).

### Measurement of color and texture

The effects of the substitution of a part of the jowl with pullulan in the composition of sausage, regardless of the proportion, were not significantly different (P > 0.05) in terms of color lightness (L*), a* or b*; however, this did result in a significant (P < 0.05) increase in yellowness (b*) in terms of the color of the product (Table 2).

Similar to the basic chemical composition, color parameters of homogenized scalded sausages largely depend on their recipe composition. According to Hughes et al. (1997) and Crehan et al. (2000), a significant reduction in the values of L*, a* and b* parameters and an increase in the value of the a* parameter describing the color of homogenized scalded sausages are caused by a reduction in the amount of fat from about 30% to 12% or less. In other studies evaluating the quality of low-fat scalded sausages containing about 12% fat, García-García and Totosaus (2008) demonstrated that the color parameters L*, a* and b* may be affected by the addition of polysaccharide components, such as locust bean gum, potato starch and κ-carrageenan. Their effect on the color of a sausage is explained by their water-binding capability and the formation of a gel matrix which changes the amount of reflected light.

The introduction of pullulan to the recipe composition of homogenized scalded sausages affected the texture of the sausages, which was reflected by significant (P < 0.05) differences between average values of shear force (Table 2). Compared to PC products, the sausages containing the least amount of pullulan (P1) were characterized by

| Characteristic  | PC         | P1         | P2         | P3         |
|----------------|------------|------------|------------|------------|
| Cooking loss (%)| 6.5 ± 0.71 | 6.9 ± 0.55 | 7.2 ± 0.40 | 7.6 ± 0.78 |
| Moisture (%)    | 63.92 ± 0.31 | 63.27 ± 0.32 | 63.88 ± 0.72 | 66.14 ± 0.79 |
| Protein (%)     | 15.81 ± 0.56 | 15.76 ± 0.57 | 15.96 ± 0.86 | 15.25 ± 0.38 |
| Fat (%)         | 15.38 ± 0.28 | 14.54 ± 0.42 | 13.59 ± 0.47 | 11.91 ± 0.27 |
| Sodium chloride (%)| 1.85 ± 0.05 | 1.85 ± 0.05 | 1.90 ± 0.10 | 1.98 ± 0.10 |
| L*             | 60.50 ± 1.93 | 60.97 ± 2.43 | 59.65 ± 2.25 | 58.55 ± 1.96 |
| a*             | 14.29 ± 0.94 | 14.01 ± 1.33 | 13.42 ± 2.74 | 13.27 ± 1.47 |
| b*             | 6.13 ± 0.28 | 7.64 ± 0.39 | 8.65 ± 0.54 | 8.72 ± 0.55 |
| Shear force (N) | 26.11 ± 2.37 | 18.58 ± 1.45 | 11.23 ± 1.15 | 8.60 ± 0.58 |
| General appearance | 4.8 ± 0.29 | 4.6 ± 0.29 | 3.8 ± 0.58 | 2.0 ± 0.50 |
| Color           | 4.8 ± 0.31 | 4.5 ± 0.50 | 4.3 ± 0.15 | 4.2 ± 0.10 |
| Odor            | 4.5 ± 0.39 | 4.3 ± 0.35 | 4.2 ± 0.61 | 4.0 ± 0.50 |
| Taste           | 4.7 ± 0.31 | 4.2 ± 0.35 | 3.7 ± 0.35 | 3.5 ± 0.40 |
| Texture         | 4.7 ± 0.29 | 4.2 ± 0.61 | 3.2 ± 0.35 | 1.5 ± 0.20 |

PC: sausage with 20% jowl; P1: sausage with 15% jowl and 5% pullulan; P2: sausage with 10% jowl and 10% pullulan; P3: sausage with 5% jowl and 15% pullulan.

**Mean values in the same row with different letters differ significantly (P < 0.05).**
significantly \( (P < 0.05) \) lower values of shear force. An increase in the degree of substitution of jowl with pullulan in P2 and P3 sausages resulted in further significant \( (P < 0.05) \) decreases in the value of this texture parameter.

The attainment of an appropriate texture is one of the main problems in the preparation of scalded sausages with reduced fat content. The results of instrumental measurements of texture parameters indicate that if these sausages are prepared without the use of an animal fat substitute, their hardness is higher in comparison to their full-fat counterparts. This increase in hardness is explained by the higher content of protein in the product (Crehan et al., 2000; Pietrasik, 1999). The scalded sausages which were the research subject of the present study were characterized by a comparable protein content. Therefore, it is most likely that the cause of the gradual reduction of the shear force of sausages with increasing content of pullulan was the elimination from the recipe composition of some of the tissue raw material (pork jowl) and its replacement with a hydrocolloid. Hydrated pullulan demonstrate low viscosity as compared to other polysaccharides (Leathers, 2003). The aqueous solutions of pullulan are stable to heating and do not form gels (Oğuzhan & Yangilir, 2013). It can be assumed that the pullulan molecules did not support the interactions within the meat protein network. However, the texture of low-fat scalded sausages containing about 15% fat or less can be modified by the introduction of polysaccharides into their recipe. Functional properties of polysaccharides, such as water binding capability, thickening and/or gelling properties and interactions with other components of the product, help in obtaining a firm but sensorily desirable texture for this type of sausages (Cierach et al., 2009; García-García & Totosaus, 2008). The effectiveness of polysaccharides in shaping the texture of scalded sausages, however, depends on their type, the amount of additive and also the basic raw material composition of the sausages. In contrast to the results obtained in this study, Cierach et al. (2009) found that the addition of \( k \)-carrageenan resulted in a significant increase in the instrumental hardness of homogenized scalded sausages containing about 10% fat. The results of the instrumental measurements of texture showed that the hardness of control sausage was 2.05 N/cm², and that of sausages with 0.5% and 0.7% of carrageenan: 8.08 and 9.85 N/cm², respectively. Increases in the hardness of scalded sausages of a comparable fat content were also found by García-García and Totosaus (2008), who used various combinations of three different polysaccharides. The authors found that the highest level of hardness of the sausage (54.9 N) was achieved when it contained the combinations of starch, locust bean gum and carrageenan, and the lowest (38.4 N) for sausage with the addition of locust bean gum and carrageenan.

Sensory evaluation

The use of pullulan, regardless of its proportion in the recipe composition, had no significant \( (P > 0.05) \) effect on the color or odor of homogenized scalded sausages (Table 2). However, based on correlation analyses, it was found that sensory perception of the color of sausages evaluated in the cross section was significantly dependent \( (r = -0.71) \) on yellowness \( (b^* \) parameter value). This could indicate a negative impact of pullulan on this sensory quality attribute. Sausage with the lowest proportion of pullulan (P1) was also not significantly different \( (P > 0.05) \) from the control sausage (PC) in terms of the other quality attributes under study: general appearance, taste and texture. However, an increase in the degree of the substitution of jowl with pullulan caused a significant \( (P < 0.05) \) deterioration in the general appearance, taste and texture of P2 and P3 sausages in comparison to PC sausage. In the opinion of the sensory panel, lower scores in the evaluation of general appearance of P2 and P3 sausages were caused by the weaker adhesion of the casing to the batter and the visible jelly clusters under the casing. One cause of taste deterioration for P2 and P3 sausages was the less perceptible taste of the meat. The use of pullulan in homogenized scalded sausages differentiated their texture to the greatest extent. In the evaluation of this attribute, P2 sausage was given a significantly lower \( (P < 0.05) \) score in comparison to PC sausage. Increasing the degree of jowl substitution with this polysaccharide in the P3 sausage resulted in a further significant \( (P < 0.05) \) deterioration of texture. According to the panelists, P2 and P3 sausages were too soft and did not exhibit the firmness typical for this type of sausages. A strong correlation between the sensory evaluation of texture and the value of shear force \( (r = 0.87) \) indicates that substitution of a part of jowl with pullulan had a negative impact on the texture of sausages.

The results obtained in this study confirm the findings published by Tobin, O’Sullivan, Hamill and Kerry (2013); according to whom, sensory acceptance of scalded sausages with reduced fat content is determined by appropriate arrangement of recipe composition. However, in contrast to the results obtained in this study, other studies demonstrate that the use of polysaccharides can have a significant positive effect on selected sensory quality attributes of homogenized scalded sausages containing 15% fat or less. According to Cierach et al. (2009), in order to improve the sensory perception of color, hardness, juiciness and taste of this type of sausages, the addition of 0.5–0.7% \( k \)-carrageenan was sufficient. In another study, Lurueña-Martínez et al. (2004) found that the use of a mixture of carob and xanthan gum in the homogenized scalded sausages simultaneously enabled a reduction in the level of animal fat and the substitution of 50% pork fat with olive oil, with no significant deterioration of their overall sensory acceptance.

Changes in pH and water activity

Measurement of pH and \( a_w \) can be used to predict the shelf life of meat products (Tilkens, King, Glass, & Sindelar, 2015). Changes in average pH and \( a_w \) values in sausages with the addition of pullulan during storage are presented in Table 3.

Substitution of jowl with pullulan at 1/4 did not significantly change \( (P > 0.05) \) the pH of P1 sausage in comparison to PC sausage. Increasing the degree of substitution of jowl with pullulan caused a significant \( (P < 0.05) \), although slight decrease in pH value in P2 and P3 sausages directly after preparation (day 0). These results are consistent with Korkeala and Björkroth (1997), who report that the pH of homogenized sausages is usually in the range of 6.0–6.5. During 21 days of refrigerated storage, the pH of P2 and P3 sausages was stable. In PC and P1 sausages, only on day 21 was a slight but statistically significant \( (P < 0.05) \) reduction reported in the pH value in relation to the initial pH.
### Table 3. Changes in pH and $a_w$ (mean ± standard deviation) in homogenized scalded sausages with pullulan during storage at 4 ± 1°C.

| Day of storage | PC | P1 | P2 | P3 |
|---------------|----|----|----|----|
| pH            |    |    |    |    |
| 0             | 6.15 ± 0.03 | 6.14 ± 0.04 | 6.11 ± 0.04 | 6.11 ± 0.03 |
| 3             | 6.12 ± 0.10  | 6.09 ± 0.04 | 6.10 ± 0.10 | 6.09 ± 0.11 |
| 7             | 6.08 ± 0.09  | 6.06 ± 0.08 | 6.09 ± 0.09 | 6.07 ± 0.09 |
| 10            | 6.08 ± 0.08  | 6.07 ± 0.08 | 6.07 ± 0.08 | 6.08 ± 0.09 |
| 14            | 6.06 ± 0.08  | 6.05 ± 0.06 | 6.06 ± 0.08 | 6.06 ± 0.08 |
| 21            | 6.03 ± 0.04  | 6.02 ± 0.03 | 6.02 ± 0.02 | 6.02 ± 0.03 |

PC: sausage with 20% jowl; P1: sausage with 15% jowl and 5% pullulan; P2: sausage with 10% jowl and 10% pullulan; P3: sausage with 5% jowl and 15% pullulan.

$^a$Mean values in the same row with different letters differ significantly ($P < 0.05$) – effect of pululan proportion in recipe composition of sausages.

$^b$Mean values in the same column with different letters differ significantly ($P < 0.05$) – effect of storage period.

The average $a_w$ values of sausages after preparation were within a range from 0.962 to 0.969 (Table 3). Sausages containing pullulan were characterized by slightly higher initial $a_w$ values in comparison to the control sausage. However, the $a_w$ value of the sausage with the highest content of pullulan (P3) was significantly ($P < 0.05$) higher in comparison to the control sausage (PC). The increase in water activity could have been caused by the ability of pullulan to bind water and its retention in the matrix of the product. During refrigerated storage, $a_w$ of sausages increased to 0.978–0.980. In PC, P1 and P2 sausages, a slight but statistically significant ($P < 0.05$) increase in $a_w$ value was found on days 14 and 21 in relation to the preparation date. Only the P3 sausage, that is, containing the highest proportion of pullulan in its recipe composition, had a stable $a_w$ during 21 days of storage. Tilkens et al. (2015) found that meat products with a combination of pH ≤ 5.1 and $a_w < 0.96$ can be considered stable, even if they are stored in a non-refrigerated environment. Sausages investigated in this study had higher pH and $a_w$, which suggests a relatively short shelf life. Changes in $a_w$ value in sausages during storage, however, were slight, and this was mainly affected by the vacuum package, which reduced drying.

### Microbiological analyses

Changes in the TVC and in the number of psychrotrophic bacteria in sausages during refrigerated storage are presented in Figure 1 and Figure 2. The initial TVCs in sausages were 2.86–3.00 log CFU/g on average and this resulted from the thermal processing of sausages. The results are consistent with the results obtained by other authors (Delgado-Pando et al., 2011; Jo, Lee, Lee, & Byun, 2001; López-López et al., 2009). During 21 days of storage, a gradual increase was observed in the TVCs in sausages. In PC, P1 and P2 sausages, the TVC increased by 1.57, 1.48 and 1.40 log, respectively, compared to initial TVCs. The smallest change in TVC during the entire storage period, that is, an increase of 0.67 log, was found in sausage containing the highest content of pullulan (P3).

Immediately after preparation, the number of psychrotrophic bacteria in sausages was within the range of 2.35–2.61 log CFU/g. During 21 days of refrigerated storage, the most intense growth of this group of microorganisms was found in the PC sausage, wherein the number of psychrotrophic bacteria increased by 2.03 log relative to the initial number. The lowest increase in this group of bacteria was observed in the P3 sausage, wherein the number of...
psychrotrophic bacteria on 21st day was higher by 1.08 log CFU/g compared to the number on the date of preparation.

Throughout the entire storage period, no presence of LAB, bacteria of Enterobacteriaceae family, yeasts or molds (the number of these microorganisms was less than 1 log CFU/g) was observed in the sausages tested.

Based on the results obtained, it can be concluded that the absence or weak growth of native microflora in the sausages resulted from both the use of cellulose casing and vacuum packaging, which reduce the drying of batter, and the refrigerated storage of sausages. Partial substitution of pork jowl with pullulan had a small but beneficial effect on the reduction of microflora in sausages. It was found that the greater the proportion of pullulan in the recipe composition, the lower the increase in psychrotrophic microflora. Stable pH in sausages with pullulan (P2 and P3) during 21 days of storage constituted a confirmation of this phenomenon. The results obtained by other researchers suggest that the reason for pH decrease in vacuum-packed refrigerated meat products, including homogenized scalded sausages, may result from the growth of LAB (Korkeala & Björkroth, 1997; Nielsen & Zeuthen, 1985; Nowak & Krysiak, 2005). However, in those sausages which are the subject of this study, no growth of LAB was observed.

Similar results to those in the present study have also been obtained by other researchers. Jo et al. (2001) found no differences in the growth of microorganisms between vacuum-packed sausages with the addition of chitosan oligomers and controls during refrigerated storage for 21 days. In another study, López-López et al. (2009) demonstrated that substitution of 50% pork fat with olive oil and seaweed in homogenized scalded sausage had no effect on its initial microbiological quality. However, refrigerated storage of vacuum-packed sausages for 41 days resulted in a significant increase in the TVC and LAB.

Conclusions

The results obtained indicate that substitution of 1/4, 1/2 and 3/4 of the recipe content of pork jowl with pullulan in low-fat homogenized scalded sausages did not differentiate the cooking loss, but it affected the texture of sausages. The manifestation of a deterioration of the texture of sausages containing pullulan included a significant reduction of shear force value in comparison to the control sausage. Furthermore, the sausages in which 1/2 and 3/4 of the recipe content of pork jowl was substituted with pullulan received significantly lower scores in sensory evaluation of texture in comparison to the control sausage. The use of pullulan did not cause any deterioration of microbiological quality in homogenized scalded sausages stored under a refrigerated environment for 21 days. The results of sensory evaluation demonstrated, however, that only sausage in which 1/4 of the recipe content of jowl was substituted with pullulan did not differ significantly from the control sausage in terms of each of the evaluated attributes. Therefore, it can be concluded that, in the scalded sausages with a given recipe composition, no more than 1/4 of the jowl content should be substituted with pullulan.

Since the use of pullulan did not pose any technological difficulties, it seems reasonable to continue research into the possibility of expanding the application of pullulan in meat processing.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Figure 2. Number of psychrotrophic bacteria (log CFU/g) in scalded sausages during storage at 4 ± 1°C.

Sausage formula: full diamond: sausage with 20% jowl (PC); empty square: sausage with 15% jowl and 5% pullulan (P1); empty triangle: sausage with 10% jowl and 10% pullulan (P2); empty circle: sausage with 5% jowl and 15% pullulan (P3).

Figura 2. Número de bacteria psicrotrófica (log CFU/g) en las salchichas escaladas durante almacenamiento a 4 ± 1°C.

Fórmula de las salchichas: rombo relleno – salchicha con 20% de papada (PC); cuadrado vacío – salchicha con 15% de papada y 5% de pullulan (P1); triángulo vacío – salchicha con 10% de papada y 10% de pullulan (P2); círculo vacío – salchicha con 5% de papada y 15% de pullulan (P3).
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