Assessing the use of frozen pork meat in the manufacture of cooked ham

Francine Gomes Basso LOS1, Rosa Cristina PRESTES2, Daniel GRANATO1, Deise Rosana Silva SIMÕES1, Silvane Souza ROMAN3, Ivo Mottin DEMIATE1*

Abstract
This study aimed to evaluate the effects of slow (–18 °C) and fast freezing (liquid nitrogen) of pork meat, and the use of exudate released upon thawing, on the physicochemical, color, rheological, microbiological, histological, and sensory characteristics of cooked ham. The meat samples were frozen at –18 °C and thawed after 22 weeks for the production of cooked ham. No significant difference was observed regarding physicochemical, color and microbiological parameters or in sensory acceptance. The hardness and chewiness parameters showed significant differences when compared to the control sample (ham made from chilled meat). Light microscopy of cooked ham samples showed that changes in the tissues were caused by freezing and thawing the meat. The effect of exudate was significant on the sodium content and compression force parameters, but this difference was not perceived in the sensory analysis, confirming that frozen pork meat can be used to produce cooked ham without loss of quality.

Keywords: histology; sensory analysis; texture; freezing-thawing.

Practical Application: The use of frozen meat in cooked ham allows flexibility to producers.

1 Introduction
The quality of cooked ham depends on the quality of the raw materials, as well as the processing operations employed: brine injection, tumbling, curing and cooking (Casiarghi et al., 2007). Although usually cooked ham is produced with fresh meat, the use of frozen raw material shows the advantages of producing more homogeneous batches from previously selected frozen hams with similar physicochemical characteristics and better control of stock and production levels (Utrera et al., 2012).

Studies have been conducted to confirm the use of frozen/thawed raw material in cooked ham. Peña et al. (1998) compared physicochemical characteristics and protein profile to demonstrate the possibility of using frozen meat in Spanish cooked ham. The effect of freezing the raw material on color, texture and water holding capacity (WHC) in cooked ham was also studied by Utrera et al. (2012) and Estevez (2011).

The freezing rate determines the size of the ice crystals that are formed, which is a critical factor in minimizing tissue damage by frozen and fluid loss after thawing (Li & Sun, 2002). At an industrial level it is not common to use exudate thawing in the preparation of meat products, primarily because if the collection of this fluid is performed improperly it increases the risk of microbiological contamination. Most studies regarding the industrialization of meat products with frozen/thawed meat do not clarify the use of the exudate that is released during thawing.

The objective of the present study was to evaluate the effect of slow (–18 °C) and fast freezing (liquid nitrogen) on pork meat and the use of exudate released upon thawing, on the physicochemical, structural (rheological and histological), technological and sensory characteristics of cooked ham.

2 Materials and methods
2.1 Meat freezing

The pork meat was provided by an inspected slaughterhouse of Ponta Grossa city (Paraná State, Brazil). Hams from Topigs 20TM (Topigs Norsvin, Curitiba PR Brazil), aged 160 days and weighing approximately 100 kg, were deboned after 24 hours of chilling. The skin, bones, soft bones and excess fat were removed manually. The hams were cut into pieces of approximately 150-200 g, and packaged in bags of 0.05 mm thickness low density polyethylene (LDPE).

The slow freezing was performed in a commercial freezer (Metalfrio, Porto Alegre, RS, Brazil) at –18 °C for eight hours. For rapid freezing, the samples were submerged in a tank containing liquid nitrogen at –196 °C for about three minutes. After that, the samples were kept frozen at –18 °C for 22 weeks, time considered to be appropriate from an industrial standpoint to allow flexibility in the processes of preparation and use of raw materials.

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The samples were thawed at 5 °C (Brasil, 1995), in a refrigerator (Electrolux, Curitiba, PR, Brazil) until they reached 2 °C in the core (Xia et al., 2009). The volume of exudate released was measured in a graduated cylinder.

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1Departamento de Engenharia de Alimentos, Universidade Estadual de Ponta Grossa – UEPG, Ponta Grossa, PR, Brasil
2Departamento de Tecnologia e Ciência dos Alimentos, Universidade Federal de Santa Maria – UFSM, Santa Maria, RS, Brasil
3Universidade Regional Integrada do Alto Uruguai, Erechim, RS, Brasil
*Corresponding author: demiate@yahoo.com
2.2 Production of cooked ham

The formulation of cooked ham produced in the present work is shown in Table 1. The ingredients were homogenized in water at 1 °C by using an industrial blender (Siemens, Brusque, Brazil) and brine was added to the meat in a 27 L capacity tumbler (Sileveira, Chapeco, Brazil). The tumbler was performed for 6 h at 22 rpm under refrigeration, followed by curing for 12 h at 4 °C (Lachowicz et al., 2003). The samples were packed in polyamide bags (PA) in pieces of approximately 700 g, sealed under 90% vacuum (Jetvac, São Caetano do Sul, Brazil) and molded into 155 × 115 × 90 mm pieces. Samples were cooked in a water tank (Sileveira, Chapeco, Brazil) according to Terra (2000): 30 minutes at 60 °C, 30 minutes at 70 °C, 30 minutes at 80 °C, until reach 72 °C at the core. After cooking, the samples were cooled at 4 °C. Three replicates were conducted in February, July and September 2013, under the same conditions. The tests were performed by varying the meat freezing methods and the presence or absence of the exudate, according to Table 2. On the assays in which the exudate was used, it was added directly in the tumbler to complete the weight of the meat (meat + exudate = 70%). To produce the control treatment (C), chilled meat was collected and trimmed one day before processing.

2.3 Physicochemical analysis

The physicochemical composition of the samples of cooked ham was analyzed in triplicate according to the Association of Analytical Chemists (1990) for the following parameters: pH by the Kjeldahl method; lipid by the Soxhlet method; sodium nitrite content by spectrophotometric method and sodium nitrite content by nitric-perchloric digestion, followed by reading on a flame photometer, model 7000 (Tecnov, São Paulo, Brazil) (Association of Analytical Chemists, 1990). Water activity was determined at 25 °C by using an Aqualab device, model 3TE (Decagon Device Inc., Pullman, USA). All chemicals used were of analytical grade (Merck, Darmstadt, Germany).

2.4 Instrumental color

The color assessment was performed by reflectance according to the CIE L*a*b* system to measure the parameters L* (lightness), a* (redness), b* (yellowness), C* (saturation) and h* (hue). The samples were cut into 2.5 cm thick slices and seven measurements were performed on each slice using a Mini EZ Scan spectrophotometer, model 4500L (HunterLab, Reston, VA, USA) with D65 illuminant and 10° observation angle.

2.6 Technological properties

The technological properties in this study aimed to evaluate the behavior of the samples during processing and their subsequent use in prepared foods, such as frozen and reheated foods for consumption (Pedroso & Demiate, 2008).

To evaluate the reheating loss samples were cut in 2.0 × 2.0 × 6.0 cm, weighed and submerged for 6 min in 400 mL.
of boiling water in a beaker covered with a watch glass, and then drained on paper towels and cooled to 7 °C for 6 min. The percentage of reheating loss was calculated as the difference in weight percentage (Hachmeister & Herald, 1998).

The sliceability evaluation was performed using a commercial slicer (Arbel, São José do Rio Preto, Brazil); 10 slices of approximately 2 mm thickness were cut and evaluated for integrity using a 10-point scale, with 10 being excellent slicing, and 0 being extremely poor slicing (with fully brittle slices). The results were expressed as a percentage of sliceability (O’Neill et al., 2003).

2.7 Histological analysis

The histological analysis was performed to evaluate the damage caused by freezing and thawing, and visible changes in the product structure due to the presence or absence of exudate. Samples of ham were fixed in 10% formalin and prepared following the stages of gradual dehydration, diaphanization, infiltration and embedding in paraffin according to the conventional technique cited by Junqueira & Carneiro (2008). From each paraffin block, 4 μm thick histological slides were obtained for later staining by hematoxylin-eosin. The analysis of histological sections was performed using light microscopy (Lambda LQT– 3, ATTO Instruments Co., Hong Kong, China) and the images were captured by the Motic Images Plus 2.0 software (Motic, Hong Kong, China). The histological specimen from each sample was evaluated using a 4 to 10 fold increase in objective lens and 10 fold increase eyepiece, providing a final magnification of 40 to 100 times.

2.8 Sensory evaluation

After approval by the Ethics Committee on Research involving Humans at the State University of Ponta Grossa (UEPG) (protocol # 256 684), sensory evaluation was performed in individual cabins. Prior to the sensory analysis, products were analyzed microbiologically according to current legislation (Agência Nacional de Vigilância Sanitária, 2002) for thermotolerant coliforms microbiologically according to current legislation (Agência Nacional de Vigilância Sanitária, 2002) for thermotolerant coliforms. Prior to the sensory analysis, products were analyzed microbiologically according to current legislation (Agência Nacional de Vigilância Sanitária, 2002) for thermotolerant coliforms. Prior to the sensory analysis, products were analyzed microbiologically according to current legislation (Agência Nacional de Vigilância Sanitária, 2002) for thermotolerant coliforms.

The initial group of assessors participating in the sensory analysis was composed of 172 individuals, in which 85% women and 15% men, aged between 18 and 67. The assessors were previously selected using a ranking test, in which texture (softness) and salty taste were used to classify samples and to prove the evaluators’ ability in differentiating samples (International Organization for Standardization, 2012). The 68 selected individuals were than assess the samples using the Multiple Comparison Test to softness and salty taste attribute.

The samples were prepared in 2 cm cubes, identified by random three-digit codes and served at temperatures below 10 °C. The Multiple Comparison Test was carried out separately to the parameters softness and salty taste. A nine-point scale was used in each test, with extremes of (1) being extremely less soft/salty than the standard (control sample), and (9) being extremely softer/salty than the standard. The results were evaluated by two-factor analysis of variance (ANOVA) followed by the Dunnett’s difference test (Meilgaard et al., 2006).

2.9 Statistical analysis

The results were expressed as mean ± standard deviation. After undergoing testing for normality by the Shapiro-Wilk test and homogeneity of variance by Levene's test, the data were assessed by one-factor ANOVA followed by Fisher's test, when parametric, or Kruskal-Wallis test when non-parametric (Granato et al., 2014). The effects of the two freezing methods and the use of exudate were assessed by multiple regression analysis. For the statistical analysis, Statistica software version 7.0 (Statsoft Inc., Tulsa, USA) and Excel version 2010 (Microsoft, Redmond, WA, USA) were used (Nunes et al., 2015).

3 Results and discussion

3.1 Physicochemical analysis

The results of the physicochemical analysis of the cooked ham can be seen in Table 3. None of the evaluated physicochemical parameters showed a significant difference (p > 0.05) in relation to the proposed changes in the raw material. This result is

Table 3. Physicochemical results (mean ± sd) for cooked hams produced with meat frozen by different methods and for control sample.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SF1</th>
<th>SF2</th>
<th>FF1</th>
<th>FF2</th>
<th>C</th>
<th>p-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.25 ± 0.01</td>
<td>6.30 ± 0.07</td>
<td>6.35 ± 0.03</td>
<td>6.36 ± 0.03</td>
<td>6.34 ± 0.09</td>
<td>0.339</td>
</tr>
<tr>
<td>Moisture (g/100 g)</td>
<td>74.19 ± 1.57</td>
<td>73.67 ± 1.51</td>
<td>74.62 ± 1.81</td>
<td>75.50 ± 2.40</td>
<td>75.31 ± 1.98</td>
<td>0.743</td>
</tr>
<tr>
<td>Ash (g/100 g)</td>
<td>3.47 ± 0.59</td>
<td>3.55 ± 0.70</td>
<td>3.65 ± 0.44</td>
<td>3.58 ± 0.58</td>
<td>3.78 ± 0.23</td>
<td>0.977</td>
</tr>
<tr>
<td>Protein (g/100 g)</td>
<td>18.33 ± 1.39</td>
<td>18.69 ± 0.44</td>
<td>18.11 ± 1.03</td>
<td>18.62 ± 0.55</td>
<td>18.42 ± 0.81</td>
<td>0.936</td>
</tr>
<tr>
<td>Water/protein ratio</td>
<td>4.06 ± 0.30</td>
<td>3.94 ± 0.13</td>
<td>4.13 ± 0.32</td>
<td>4.06 ± 0.20</td>
<td>4.10 ± 0.29</td>
<td>0.919</td>
</tr>
<tr>
<td>Fat (g/100 g)</td>
<td>2.28 ± 0.39</td>
<td>2.42 ± 0.15</td>
<td>2.12 ± 0.35</td>
<td>1.63 ± 0.40</td>
<td>2.19 ± 0.24</td>
<td>0.093</td>
</tr>
<tr>
<td>Water activity</td>
<td>0.984 ± 0.003</td>
<td>0.980 ± 0.001</td>
<td>0.981 ± 0.005</td>
<td>0.982 ± 0.003</td>
<td>0.981 ± 0.003</td>
<td>0.627</td>
</tr>
<tr>
<td>Sodium (g/100 g)</td>
<td>1.19 ± 0.11</td>
<td>1.36 ± 0.05</td>
<td>1.19 ± 0.15</td>
<td>1.28 ± 0.12</td>
<td>1.18 ± 0.03</td>
<td>0.136</td>
</tr>
<tr>
<td>Nitrite (mg/kg)</td>
<td>45.28 ± 0.41</td>
<td>45.60 ± 0.50</td>
<td>45.63 ± 0.32</td>
<td>45.05 ± 0.23</td>
<td>45.32 ± 0.47</td>
<td>0.404</td>
</tr>
<tr>
<td>L*</td>
<td>66.11 ± 2.88</td>
<td>62.38 ± 3.19</td>
<td>64.29 ± 3.16</td>
<td>61.42 ± 1.11</td>
<td>65.55 ± 2.89</td>
<td>0.248</td>
</tr>
<tr>
<td>a*</td>
<td>11.20 ± 1.83</td>
<td>12.71 ± 0.51</td>
<td>11.61 ± 1.23</td>
<td>12.75 ± 0.42</td>
<td>11.32 ± 1.17</td>
<td>0.337</td>
</tr>
<tr>
<td>b*</td>
<td>11.00 ± 0.48</td>
<td>10.94 ± 0.32</td>
<td>10.90 ± 1.11</td>
<td>10.84 ± 0.42</td>
<td>9.67 ± 0.14</td>
<td>0.115</td>
</tr>
<tr>
<td>C*</td>
<td>15.78 ± 1.03</td>
<td>16.79 ± 0.60</td>
<td>16.01 ± 0.84</td>
<td>16.77 ± 0.10</td>
<td>14.92 ± 0.94</td>
<td>0.072</td>
</tr>
<tr>
<td>h*</td>
<td>45.16 ± 5.76</td>
<td>40.92 ± 0.19</td>
<td>43.67 ± 4.90</td>
<td>41.65 ± 2.96</td>
<td>40.80 ± 2.70</td>
<td>0.656</td>
</tr>
</tbody>
</table>

Note: SF1 – sample produced with frozen meat by slow process, without exudate; SF2 – sample produced with frozen meat by slow process, with exudate; FF1 – sample produced with frozen meat by fast process (N), without exudate; FF2 – sample produced with frozen meat by fast process, with exudate; C – sample produced with chilled meat. ** Probability values obtained by one-way ANOVA.
consistent with other studies on the effect of freezing on raw material in the production of ham. Peña et al. (1998) found no significant difference in water activity and moisture, and Utrera et al. (2012) observed no statistical difference in results related to moisture, protein, fat and pH.

The pH of the samples showed no significant difference. The pH values were similar to those reported by Tomovic et al. (2013) from 6.20 to 6.25 for cooked ham with 20% injection. Li et al. (2011) found a mean pH of 6.24 for ham tumbled for 6 hours, which was similar to the tumbling time of our study. On the other hand, Peña et al. (1998) concluded that the pH of cooked ham produced with frozen/thawed meat showed significantly lower values than those of ham made with chilled meat.

The moisture content ranged from 73.67 to 75.50 g/100 g. Utrera et al. (2012) found no significant difference in moisture content in cooked hams with 20% injection, which was produced with fresh and frozen/thawed meat. Casiraghi et al. (2007) found values of moisture content of 66.1, 68.6 and 70.6 g/100 g for ham with brine injections of 25, 30 and 35%, respectively. The protein contents ranged from 18.11 to 18.69 g/100 g, which were similar to the values reported by Casiraghi et al. (2007) for cooked ham with 25% injection, and by Tomovic et al. (2013) for ham with 20% injection.

The lipid content ranged from 1.63 to 2.42 g/100 g, which was consistent with values reported by Los et al. (2014) for cooked hams produced in Brazil (0.89 to 3.57 g/100 g). Vámková et al. (2007) found values ranging from 1.56 to 4.04 g/100 g, and the average value of fat content reported by Cheng et al. (2005) was 1.89 g/100 g. Utrera et al. (2012) reported that the fat content of cooked ham presented significant difference in relation to the leg muscle used for processing and not in relation to the use of frozen/thawed meat compared to fresh meat.

In the present study, the sodium content of the samples ranged from 1.18 to 1.36 g/100 g. Multiple regression analysis coupled with ANOVA showed that the exudate factor had a significant effect (p = 0.023) on sodium (Table 4), while the freezing and the combined effect of exudate and freezing had no impact on sodium level. Peña et al. (1998) reported that ham produced with frozen/thawed meat showed a lower salt content than ham produced with fresh meat.

The residual values of nitrite, which were analyzed 30 days after production, ranged from 45.05 to 45.63 mg/kg. According to Hustad et al. (1973), after the addition of nitrite to meat its concentration immediately decreases by 16% and during processing a further 51% is lost. At the end of the process only 33% of the amount that was initially added remains. The loss of nitrite is influenced by the composition of the product, processing, storage temperature and pH.

There was no statistical difference between the color parameters evaluated in the produced ham. The L* values that were found ranged from 61.42 to 66.11, similar to those reported by Vámková et al. (2007), which were from 61.57 to 65.79, and Li et al. (2011), which were 61.80.

### 3.2 Rheological analysis

Table 5 shows the results of the rheological characterization of the samples. The control sample (C) showed significantly lower value for hardness and was softer compared to the others. Among the samples that used frozen meat, it can be seen that the SF1 sample showed the highest value for hardness, followed by the FF1 sample, but these two samples did not differ significantly. The incorporation of exudate released upon thawing decreased the hardness value of the SF2 and FF2 samples when compared to samples in which exudate was not used, i.e. samples SF1 and FF1. This result shows that fresh meat provides softness in ham, but the use of frozen meat with the incorporation of exudate could also be an interesting alternative for processing. This result was expected, due to the fact that it is generally agreed that chilled meat shows better WHC because it has not suffered damage to muscle fibers from freezing, i.e. it has the ability to retain its own water and also absorbs brine more effectively than meat submitted to freezing (Wirth et al., 1981).

Chewiness, which is a parameter calculated from hardness, presented similar results. Utrera et al. (2012) demonstrated that, hardness, as well as chewiness, increased in ham produced with frozen and thawed meat compared to ham made with chilled meat.

The absence of significant difference in the hardness of the samples produced with meat submitted to slow freezing (SF1) and fast freezing (FF1) can be attributed to recrystallization during storage, i.e. the increase of small ice crystals formed by fast freezing (Bevilacqua & Zaritzky, 1982). Ngapo et al. (1999) studied the effect of different freezing rates in pork meat and concluded that the crystals formed reached a peak in size during slow freezing, however, small crystals formed during rapid freezing achieved this peak by recrystallization. According to Mortensen et al. (2006), small ice crystals formed by rapid freezing may undergo recrystallization and increase in size during storage at temperatures of −20 °C.

The compression force and shear force showed no significant differences between the samples by ANOVA. However, multiple regression analysis (Table 4) showed that the exudate (p = 0.036)
and the combined effect of exudate and freezing (p = 0.043) influenced negatively the compression force of hams.

Cooling losses showed significantly higher results for samples prepared from frozen meat. This can be explained by the fact that frozen meat retains less water in the protein matrix compared to chilled meat. This is due to shrinkage caused in the myofibrillar proteins, which reduces the capillary forces that maintain the water that is bound in the inter-filamentary spaces (Liu et al., 2000). The increase in solute concentration during freezing leads to denaturation of sarcoplasmic proteins, which influence the retention of water during the post-thaw process (Leygonie et al., 2012).

WHC as well as syneresis, freezing loss, heating loss, and slicing did not significantly differ between themselves (Table 5). Even with the loss of sarcoplasmic proteins in frozen and thawed meats (Savage et al., 1990) according to Schmidt (1994), this type of protein has less influence on the binding formation than myofibrillar proteins. According to Tornberg (2005), myofibrillar proteins, especially myosin, have a high gel forming ability even in low concentrations (from 0.5%), while sarcoplasmic proteins require a concentration of 3.0% to bind meat products. These parameters may also have been influenced by adding ingredients that improve the binding characteristics of the product (Pietrasik et al., 2007; Hsu & Sun, 2006), such as carrageenan and soy protein isolate, and by tumbling action which extracts myofibrillar proteins (Tornberg, 2005).

These results are interesting from the industrial and technological points of view because they demonstrate the possibility of using frozen meat (either by the slow or fast method) when it is thawed at 5 °C, in cooked ham production without resulting in a loss in product quality.

### 3.3 Histological analysis

Figure 1 shows photomicrographs of samples which demonstrate the changes in the tissues caused by freezing and thawing of the meat. The control sample (C) showed a more integral and cohesive protein matrix structure with intact muscle fibers, similar to the results reported by Prestes et al. (2013) for chicken ham.

The SF1 sample showed unstructured muscle tissue with cells dispersed in the extracellular matrix. Intact muscle cells could be seen next to the dispersed cells, but all were with cytoplasmic striations, indicating that, as expected, slow freezing caused changes in cellular structure. The SF2 sample showed organized muscle tissue with intact muscle cells of normal appearance alongside cells with cytoplasmic striations (edema).

The FF1 sample was characterized by large, well-connected, swollen muscle cells and cytoplasm with intense cross-striation, and the presence of unstructured connective tissue around the muscle tissue. It was expected that the meat frozen by the fast method would present small ice crystals and uniform cell structure, and this appearance was perceived in the FF1 sample. However, the appearance of large muscle cells may be explained by the storage of the meat at a higher temperature (–18 °C) than the freezing temperature by liquid nitrogen, causing recrystallization of ice crystals (Mortensen et al., 2006). The FF2 sample showed well organized muscle tissue in lobules of normal aspect. The muscle cells had distinct striations and slightly less intensity than the FF1 sample, with small areas of breakdown.

From these results it is possible to say that the control sample (C) showed better muscle structure and that there was a change in the cellular structure of the SF1 and FF1 samples, and to a lesser extent in the SF2 and FF2 samples. The latter was possibly due to the reincorporation of exudate and the effect of tumbling (Katsaras & Budras, 1993). The damage observed in the photomicrographs of the samples produced with meat subjected to freezing can be correlated to the higher degree of hardness, as shown in Table 5.

The use of exudate, which is composed mainly of sarcoplasmic proteins, represented largely by myoglobin, can also explain...
the deeper color in the photomicrographs of the SF2 and FF2 samples compared with the SF1 and FF1 samples.

### 3.4 Sensory evaluation

Softness and salty taste were the evaluated attributes, which were related to compression force and sodium content, respectively, for which there was a significant effect of using the exudate. Despite the fact that the results for instrumental texture presented significantly different values for hardness (Table 5), and the control sample showed a better structural aspect according to histological analysis (Figure 1), no statistical differences were perceived in the sensory evaluation between the treatments and the control, according to the Dunnett test (data not shown), both in terms of softness and salty taste.

Utrera et al. (2012) reported that the difference in hardness and chewiness of hams produced with frozen/thawed meat was perceived by trained evaluators in a sensory panel. In the present study, sensory analysis indicated that the substitution of chilled meat by frozen/thawed meat, with or without the incorporation of exudate, did not influence the perception of softness and salty taste.

**Figure 1.** Cooked hams’ samples photomicrographs, with details on the right. Note: A and B – control sample (C); C and D – SF1 – sample produced with frozen meat by slow process, without exudate; E and F – SF2 - sample produced with frozen meat by slow process, with exudate; G and H – FF1 – sample produced with frozen meat by fast process (N₂), without exudate; I and J – FF2 - sample produced with frozen meat by fast process, with exudate. tc = connective tissue; arrow = cell striation. *= normal aspect cell. Zoom: 40× e 100×.
4 Conclusion

Changes in the texture profile and the cooling losses were detected in the ham samples produced with frozen/thawed meat, whereas the water holding capacity (WHC), syneresis, freezing loss, heating loss and slashing of the ham samples did not differ significantly. By using light microscopy analysis it was possible to observe changes in the tissues due to freezing and thawing the raw materials. The effect of the exudate was significant only in terms of the compression force and sodium content parameters; however, these differences were not perceived by the sensory analysis. Considering the results obtained, the technical viability of using frozen/thawed meat in cooked hams was proved, regardless of the considered method of freezing. The possibility of using frozen/thawed meat in the production of cooked ham increases flexibility within processing plants without changes in expected product quality.

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References


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