International Food Research Journal 23(1): 55-60 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Eletrophoretic profile of exudate of chicken breast submitted to different thawing methods

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Article history

Received: 1 April 2015
Received in revised form: 12 June 2015
Accepted: 18 June 2015

Keywords

SDS-PAGE
Microwave
Proteins

Abstract

The objective of this study was to characterize the exudates resulting from the thawing of half chicken breasts that had first been submitted to fast freezing (-36°C for 2 hours) and then thawed using the following five different methods: REFRI (under refrigeration in a domestic refrigerator at ± 7°C for 22 hours); MICRO (in a microwave for 6 minutes); STOVE (in an oven with air circulation at ± 40°C for 2 hours and 15 minutes); BATH (packed in low-density polyethylene bags and placed in a cold water bath at ± 10°C for 2 hours and 15 minutes); and ROOM (ambient temperature at ± 17°C for 2 hours and 20 minutes). After thawing, protein concentration (mg/mL) was determined and polyacrylamide gel electrophoresis (SDS-PAGE) was performed. No significant differences were found ($p > 0.05$) in protein concentration present in the exudates for REFRI, STOVE, BATH and ROOM treatments but making the correlation with protein concentration and % of exudate released the MICRO and ROOM showed the greatest losses of proteins. The results indicated that the thawing methods affected the electrophoretic profile of the exudates that were generated and the percentage of exudate that was released. It was found that the samples with a higher percentage of exudate also had a higher quantity of bands and greater intensity, which proved the existence of greater damage to the meat proteins, generating fragmentation, loss of structure and the release of small peptides which affected the nutritional quality of the meat and its functionality. Thawing at low temperatures seemed to cause less damage to the meat structure and maintained the properties of the meat. Therefore, it was concluded that this was the best option for thawing. Exudates are often discarded, but they contain proteins that play important roles in the gelatinization process, and they have the potential to be used, which would reduce losses at the industrial level.

Introduction

The main aim of freezing is to inhibit microbial growth and to slow down metabolic activities. Even though freezing is one of the least aggressive methods of preservation it still results in some modifications in foods, especially the formation of ice crystals (Leygonie *et al*., 2012) which affect the characteristics of products. The extent of such damage is directly related to the speed of freezing (Xia *et al*., 2012).

The freezing process can be classified as slow or fast depending on its speed. A slow freezing speed is considered to be 0.05°C/minute and a fast freezing speed is 0.5°C/minute. According to Wirth *et al.* (1981), a rate of 0.2 to 1.0 cm/h may be considered to be a slow freezing rate and 1 to 5 cm/h can be considered to be a fast rate; a rate of > 5 cm/h can be defined as ultra-fast freezing. Fast freezing promotes the formation of small ice crystals that are distributed evenly both inside and outside the muscle cells. Slow freezing results in the formation of lesser quantities of large ice crystals in the extracellular region, which result in lesser damage to cells (Fellows, 2006; Damoradan *et al*., 2010).

To ensure a quality end product it is necessary to understand appropriate thawing methods. There are several methods to thaw meat but in general the criterion that pieces of meat should preferably be thawed more quickly at low temperatures still dominates (Okamoto and Suzuki, 2002). Thawing can also be performed with cold air circulation (domestic

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refrigerators or cold rooms), with slightly-heated air (oven), in a microwave oven, with cold water, or at room temperature (Xia *et al*., 2009; Leygonie *et al*., 2012; Gambuteanu *et al.*, 2013; Wang *et al*., 2015). Innovative thawing processes, such as high pressure, microwave, ohmic thawing and acoustic thawing can decrease the thawing time without causing an increase in exudate and loss of product quality (Li and Sun, 2002).

Despite the afore mentioned important factors there are few studies (Kim *et al*., 2013; Ali *et al.*, 2015) that have evaluated the differences in the exudates (meat juice) that are generated from thawing. Most of these studies were related to beef and pork (Xia *et al.*, 2012; Kim *et al*., 2013; Wang *et al.*, 2015) and there have also been a few studies of chicken meat (Ali *et al*., 2015) and ostrich (Leygonie *et al*., 2012), although these studies mainly focused on the raw material and not on the exudate. According to Savage *et al*. (1990), exudate mainly contains sarcoplasmic proteins, which are soluble in water and which include enzymes. Exudate may be related to the oxidation of muscle proteins, which can affect pH, color and hardness (Joo *et al*., 1999).

Although Brazilian legislation does not prohibit the use of exudate, in many cases the health authorities do not allow it to be re-incorporated during the industrialization of meat products, especially when its collection does not occur in satisfactory hygienic conditions. Due to a lack of information and knowledge, companies often end up discarding exudates, which can reach values > 10% of the weight of the raw material (Xia *et al.*, 2009). In addition, most studies of frozen/thawed raw material do not contain details about the incorporation or otherwise of the exudate released during thawing.

Polyacrylamide gel electrophoresis (SDS-PAGE) is a widely-used technique for purifying macromolecules that allows molecules to be separated according to their size, shape or load. It appears to represent a useful alternative to assist in the characterization and assessment of the effects of freezing/thawing methods on meat. Most of the studies reported in the literature have evaluated the electrophoretic profile of the proteins present in meat (Benjakul *et al*., 1997; Xia *et al*., 2012; Ali *et al*., 2015) however, there are very few studies reporting on the electrophoretic profile of exudate. This study aimed to evaluate the electrophoretic profile of different exudates of chicken breast subjected to five different thawing methods.

Materials and Methods

The experiments were performed in the laboratories of the Federal University of Santa Maria (UFSM) in Santa Maria, (RS, Brazil) and the Regional Integrated University of High Uruguay and the Missions (URI) in Erechim (RS, Brazil). The samples of ± 250 g half chicken breast *(Pectoralis major)* were donated by Cooperativa Central Aurora Alimentos (Quilombo, SC, Brazil).

The samples were frozen individually in the factory (quick freezing to -36°C for 2 hours) and then kept in a freezer (-18°C) until analysis. The five different evaluated methods were referred to as follows: REFRI (under refrigeration in a domestic refrigerator at ± 7°C for 22 hours); MICRO (in a microwave for 6 minutes); STOVE (in an oven with air circulation at ± 40°C for 2 hours and 15 minutes); BATH (packed in low-density polyethylene bags and placed in a cold water bath at ± 10°C for 2 hours and 15 minutes); and ROOM (ambient temperature at ± 17°C for 2 hours and 20 minutes).

For all the methods the temperature considered as the parameter for the completion of defrosting was 10°C inside the muscle. A PHILCO microwave (model PMS 35N, 900W power, Curitiba, PR, Brazil), an ALPAX (model 400-4ND, Diadema, SP, Brazil) oven with air circulation, and a CONSUL refrigerator (São Paulo, SP, Brazil) were used.

After thawing, the following determinations were performed: percentage loss of exudate, determination of the protein content in the exudate and polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

Exudate

To assess the exudate losses, the samples were weighed in a MARTE (model AS 2000C, São Paulo, SP, Brazil) semi-analytical balance before and after thawing, and the loss percentage was calculated based on the initial and final weight of the cuts using the method suggested by Northchutt *et al.* (1997) with modifications.

Determination of the protein concentration

The protein concentration (mg/mL) in exudate was determined by the method Bradford (1976) with bovine serum albumin (A3294, Sigma-Aldrich) as standard.

Electrophoretic profile of exudate

Electrophoresis was conducted under denaturing conditions according to the method described by Laemmli (1970) using a 15% concentration of

polyacrylamide gel. To prepare the samples, firstly, 40 uL of 60% trichloroacetic acid (w/v) (Synth, Brazil) was added to 100 uL of raw samples, which were placed in Eppendorf-type tubes and stored in a freezer (-15°C) overnight. The samples were subsequently centrifuged at 10,000 g, at 4°C for 30 minutes and the supernatant was removed.

Subsequently, 100 uL of a frozen aqueous solution of 90% acetone (v/v) (Química Moderna, Brazil) was added to wash the pellet without undoing it, and this was then centrifuged as in the previous step and then this step was repeated a second time. The precipitate was re-suspended in 50 µL of sample buffer [2.5 mL of 0.5 M Tris-HCl buffer, pH 6 (Sigma-Aldrich, Switzerland); 5 mL of 10% SDS solution (w/v) (Ludwig Biotech, Canada); 5 mL of 100% glycerol (Sigma-Aldrich, Switzerland); 0.5 mL of β-mercaptoethanol (Sigma-Aldrich, Switzerland); and 2.5 mg bromophenol blue, supplementing the volume to 25 mL with distilled water.

The preparation of the 15% resolving gel was achieved by adding together 4.925 mL of acrylamide/bisacrylamide (30:0.8) (Ludwig Biotech, Canada), 5 mL of Tris-HCl buffer solution (0.75 M, pH 8.8) (Sigma-Aldrich, Switzerland), 75 µL of 10% ammonium persulfate solution (w/v) (Merck, Germany) and 5 uL of tetramethylethylenediamine (TEMED) (Ludwig Biotech, Canada). All these components were blended and transferred to the electrophoresis cell, leaving approximately 2.5 cm of the cell free. The rest of the cell was completed with distilled water to form a straight line on the gel and it was left to polymerize for 40 minutes at 25°C; after the polymerization of the gel the water was removed.

The 12% stacking gel was prepared by adding 0.5 mL solution of acrylamide/bis-acrylamide (30:0.8) (Ludwig Biotech, Canada), 2.5 mL of Tris-HCl buffer solution (0.25 M, pH 6.8) (Sigma-Aldrich, Switzerland), 1.925 mL of distilled water, 75 uL of 10% ammonium persulfate solution (w/v) (Merck, Germany), and 7.5 µL of tetramethylethylenediamine (TEMED) (Ludwig Biotech, Canada). All these components were blended and transferred to the electrophoresis cell, on the resolution gel, which had already been polymerized. Before applying the stacking gel the comb was placed for the formation of the wells for the application of the samples. After the complete polymerization of the stacking gel (30 minutes at 25°C), the comb was removed.

The standard of molecular mass used as a marker was from 10 to 200 kDa (Thermo Scientific, USA): 15 uL of the standard was added to the first well and 20 uL of the samples to be analyzed were added to the other wells. Running buffer containing: 14 g of glycine (Sigma-Aldrich, Switzerland); 1 g of sodium dodecyl sulfate (SDS) (Ludwig Biotech, Canada); 3 g of Tris base (Sigma-Aldrich, Switzerland) and 1000 mL of distilled water was used. A constant current of 300 mA and a voltage of 250 V was utilized for about 1 hour in order for the gel to permeate the samples.

After the run, the stacking gel was removed. The resolution gel was then washed with distilled water and stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 (Sigma-Aldrich, Switzerland) dye for 24 hours. Subsequently, the gel was decolorized by heating in distilled water in a microwave until perfect visualization of the bands.

Three repetitions were performed for each experiment and the analyses were carried out in triplicate, at least. The results were submitted to analysis of variance (ANOVA) and Tukey's test with a significance level of 95% ($p< 0.05$), using Statistica® 9.0 (StatSoft Inc., Tulsa, OK, USA) software.

Results and Discussion

Exudate

Table 1 shows the results of the exudate (%) for the samples of half chicken breast subjected to various thawing methods. The REFRI, STOVE and ROOM samples showed no significant difference ($p <0.05$) between them, with values ranging from 3.55 to 4.84%. The BATH and MICRO samples showed the lowest and highest volume of exudate, 1.27% and 7.65%, respectively, and differed significantly from the other samples. The BATH treatment had the lowest percentage of exudate, which was a very interesting result because it presented a lower level of liquid that the method using refrigeration (REFRI), which is the most recommended method for thawing.

The greatest loss of exudate was found for the MICRO treatment, due to increased protein denaturation, which caused a greater loss of water. According to Leygonie *et al.* (2012) and Ali *et al.* (2015) in addition to moisture that is lost, thawed meat loses functional properties such as water retention capacity and proteins, which can jeopardize the gelling of the final product in which this raw material is used.

Kim *et al*. (2013) evaluated the loss of exudate in frozen pork (- 20°C) that was thawed at 1.0 °C for 24 hours. The results showed losses of up to 10.25%. No reduction was observed in the same study in terms of the final protein content for the different treatments. Pietrasik and Janz (2009) found exudate of 3.30% for beef thawed at 4°C. However, Leygonie *et al.* (2012) reported thawing losses at 4°C (air speed of 1.2 m/s and thawing to reach 0°C at the center) for ostrich

Table 1. Exudate (%) and protein (mg/mL) of the samples of half chicken breast submitted to different thawing methods

Treatments	Exudate (%)	Protein (mg/mL)
REFRI	3.60^{b}±0.97	1.19^{a}±0.01
MICRO	7.65^{a}±0.98	0.95^{b}±0.03
STOVE	3.55^{b}±0.36	1.13^{a}±0.08
BATH	1.27^{c}±0.52	1.15^{a}±0.01
ROOM	4.84^{b}±0.78	1.19^{a}±0.01

* Means with different letters in the horizontal differ significantly ($p < 0.05$) by Tukey's test.
** The treatments correspond to: REFRI – thawing in refrigerator; MICRO – thawing in microwave; STOVE - thawing in an oven; BATH – thawing in water; and ROOM – thawing at room temperature.
*** N=15.

meat in the order of 5.09 ± 0.21%. Wang *et al.* (2015) found values of exudate in beef thawed by different methods in the order of 2.99% to 7.04%, and of these values about 0.11% and 0.96% respectively corresponded to the level of protein.

The results found in these study were consistent with those found by Xia *et al.* (2012), who tested different thawing methods (under refrigeration at 4°C, room temperature to 20°C, immersion in water at 14°C and 9°C, and using a microwave) for pork loin and who produced results for exudate loss of 3.07%, 4.36%, 4.77%, 5.50% and 6.64%, respectively. The results found were also similar to those found by Pietrasik and Janz (2009) and Wang *et al.* (2015).

The formation of ice crystals during freezing leads to structural damage that is caused by solute concentration in the meat, which, in turn, leads to changes in the biochemical reactions that occur at the cellular level and influence the physical parameters of the meat. Large extracellular ice crystals disrupt the physical structure, breaking and separating the myofibrils. The formation of small intracellular ice crystals causes lesser damage. Freezing and thawing can change both the content and the distribution of water in the meat tissues because they release the water that is immobilized and bound to the proteins by the intracellular spaces. However, the aim is that the water released in thawing is redistributed to the extracellular and sarcoplasmic spaces (Leygonie *et al.*, 2012).

Ambrosiadis *et al.* (1994) reported that rapid thawing by submersion in water decreased exudates losses and that thawing in a microwave for 35 minutes to reach 0°C resulted in higher losses.

In the present study, the results of the exudates losses were lower for the REFRI and BATH methods, which was in line with literature reports that methods that use low temperatures and shorter times produce less damage to meat and lower exudates losses.

Determination of the protein

No significant differences were found ($p > 0.05$) in protein concentration present in the exudates for REFRI, STOVE, BATH and ROOM treatments (Table 1). Already MICRO treatment had the lowest concentration of protein in the exudate. These results can be explained by differences in thawing time for the MICRO treatment was only 6 minutes and the rest were over 2 hours and can also be explained by the effects of different thawing processes tested on the structure of the meat. However the correlation between protein content (mg/mL) with the exudate (%) released for every 100 g of meat we will have the amount of protein lost to REFRI, MICRO, STOVE, BATH and ROOM would 4.28 g/100 g , 7.26 g/ 100 g, 4.01 g/100 g, 1.46 g/100 g, 5.76 g/100 g respectively, and therefore the MICRO and ROOM remain the treatments had higher losses.

MICRO treatment presented greatest losses of exudate and protein probably because of the extent of protein denaturation and damage the structure (Leygonie *et al.*, 2012;. Ali *et al.*, 2015). The denaturation leads loss of water retention capacity by the protein which causes greater losses of exudate even with short thawing process time (6 minutes). Most studies only evaluate the protein content in the meat after thawing and not in the exudate which is difficult to compare.

Electrophoretic profile

Polyacrylamide gel electrophoresis (SDS-PAGE) was performed to determine whether there were differences in the electrophoretic profile for the exudates derived from the various tested thawing methods. The results are shown in Figure 1.

Although the protein content present in the exudate did not present significant difference for some treatments were observed difference between the bands for the different treatments. A higher or lower intensity and difference in the bands was observed for the treatments, indicating a variation in the size proteins present in the exudate from the different thawing methods (REFRI, ROOM, BATH, STOVE and MICRO).

The best treatments were those that showed the least amount of bands, and of lower intensity, which was observed for REFRI and BATH. The STOVE and MICRO treatments showed the worst results. The greater the intensity of the bands (and the greater

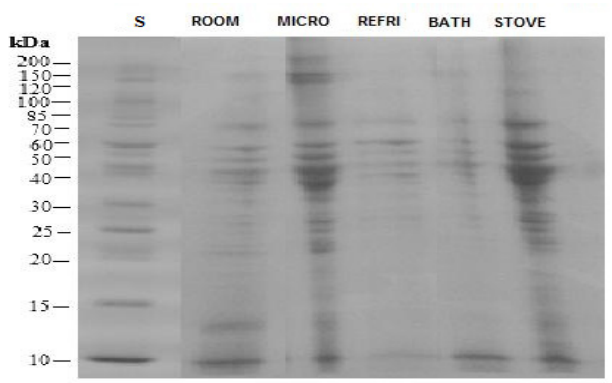

Figure 1. Electrophoretic profile corresponding to exudate samples obtained by the following different thawing methods: room temperature (ROOM); in a microwave (MICRO); in a refrigerator (REFRI); in cold water (BATH); and in an oven (STOVE).
Column S: molecular weight marker.

the presence of several larger bands) the greater the damage to the thawed meat, which resulted in greater loss of myofibrillar and sarcoplasmic proteins, which can cause fragmentation and release small peptides into the exudate.

The bands that are characteristic of the myofibrillar proteins are myosin in the range below 205 kDa (Xia *et al*., 2012), actin in the range of 45 kDa, tropomyosin and troponin in the range of 40 to 45 kDa, and short-chain myosin in the range of 25 kDa (Ali *et al*., 2015). The bands that are characteristic of sarcoplasmic proteins have a wide range of distribution between 19 kDa and 110 kDa and are basically enzymes (Ali *et al.,* 2015).

The electrophoretic profile that was obtained was general (for all the proteins that were present) and showed very characteristic bands near 200 kDa and 70 kDa and a greater presence of bands between 60 kDa and 30 kDa, as well as a band that was subsequently located in the 10 kDa range. The electrophoretic profile for the STOVE and MICRO treatments indicated the presence of myosin in the exudate that was derived from these methods, which was not observed for the other treatments.

All the treatments had bands of higher or lower intensity in the range between 30 kDa and 60 kDa. The band in the range of 45 kDa indicated the presence of actin, and between 40 and 45 kDa it indicated the presence of tropomyosin and troponin T. The MICRO and STOVE treatments also presented a band around 25 kDa, which was characteristic of myosin light. All the treatments showed a band at 10 kDa, indicating the presence of small peptides. Overall, the BATH and REFRI treatments showed weaker bands and a smaller amount of bands, indicating less loss of protein in the exudate and less degradation due to the thawing process.

To provide a comparison, the exudate electrophoresis results were compared with the results for meat that are available in the literature. Xia *et al.* (2012) performed the electrophoretic profile of pork loin after different thawing methods and observed no induction of protein aggregation and fragmentation that resulted from thawing. There was no increase or decrease in the myofibrillar proteins (actin and myosin), which correspond approximately to the bands of 205 kDa and 45 kDa, respectively. However, in another study by the same authors (Xia *et al*., 2009) of different freeze-thaw cycles the electrophoretic profile showed differences that indicated the aggregation and fragmentation of proteins.

Ali *et al*. (2015) performed the electrophoretic profile of protein extract of chicken breast that was subjected to freezing at - 20°C for one week and then six cycles of freeze-thawing at 4°C for 12 hours. Electrophoresis was performed for the myofibrillar and sarcoplasmic proteins separately. The electrophoretic profile showed no differences in the band corresponding to myosin (205 kDa), but several bands were visible between 231 and 250 kDa, and the intensity of the bands between 130 kDa and 86 kDa decreased in intensity as the number of cycles increased. In other words, part of the protein was loaded into the exudate (meat juice). These authors concluded that thawing can cause the degradation of myofibrillar proteins and proteolysis. There was a reduction of the intensity of the band of actin (45 kDa), suggesting a break with the release of small peptides. Bands from 5 to 80 kDa that were found in raw meat disappeared after the third freeze-thaw cycle. For the sarcoplasmic proteins, these authors found a reduction in the molecular weight bands of 19 kDa to 110 kDa as the number of cycles were increased, which is also related to protein denaturation and increases in exudate.

Based on the results the use of electrophoresis proved to be a viable technique to assess the exudates, in addition to the proteins in the meat. The results of the electrophoresis may have been related to the percentage of exudates because it was found that the samples with a greater percentage of exudate also showed a higher number of bands and greater intensity.

Conclusion

It was observed that the thawing methods significantly affected the exudates that were generated. Thawing in cold water was the method that presented the lowest loss of exudate and lower

protein losses in the exudate.

The use of electrophoresis proved to be a viable technique to assess the exudates, as well as the meat proteins. It was found that the samples with a higher percentage of exudate also showed a greater amount of bands, and greater intensity, which proves that those samples suffered more damage to the meat proteins, which in turn generated fragmentation, loss of structure and the release of small peptides, affecting the nutritional quality and functionality of the meat. Thawing at low temperatures seemed to cause less damage to the meat structure and allowed its properties to be maintained: it was therefore concluded that it is the best option for thawing. Exudate is frequently discarded but it contains proteins that play an important role in the gelatinization process that occurs in meat products, which suggests that if it were adequately collected it would have the potential to be used and losses at the industrial level could be reduced.

References

Ali, S., Zhang, W., Rajput, N., Khan, M.A., Li, C. and Zhou, G. 2015. Effect of multiple freeze-thaw cycles on the quality of chicken breast. Food Chemistry 173(2): 808-814.

Ambrosiadis, I., Theodorakakos, N., Georakis, S. and Lekas, S. 1994. Influence of thawing methods on the quality of frozen meat and drip loss. Fleischwirtschaft 74: 284-286.

Benjakul, S., Seymour, T.A., Morrissey, M. and An, H. 1997. Physicochemical changes in Pacific whiting muscle during iced storage. Journal of Food Science 62: 729-733.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram of protein utilizing the principle of protein-drye binding. Analytical Biochemistry 72: 248-254.

Damoradan, S., Parkin, K.L. and Fennema, O.R. 2010. Química de alimentos de Fennema. 4nd edn. p. 719-759. Porto Alegre: Artmed.

Fellows, P.J. 2006. Tecnologia do processamento de alimentos: princípios e praticas. 2nd edn. 602 p.Porto Alegre: Artmed.

Gambuteanu, C., Borda D. and Alexe, P. 2013. The effect of freezing and thawing on technological properties of meat: review. Journal of Agroalimentary Processes and Technologies 19(1): 88-93.

Joo, S.T., Kauffman, R.G., Kim, B.C. and Park, G.B. 1999. The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. Meat Science 52(3): 291-297.

Kim, G.D., Jung, E.Y., Lim, H.J., Yang, H.S., Seon, T.J. and Jeong, J.Y. 2013. Influence of meat exudates on the quality characteristics of fresh and freeze-thawed pork. Meat Science 95(2): 323-329.

Laemmli, UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277: 680-85.

Leygonie, C., Britz, T.J. and Hoffman, L.C. 2012. Impact of freezing and thawing on the quality of meat: Review. Meat Science 91: 93-98.

Leygonie, C., Britz, T.J. and Hoffman, L.C. 2012. Meat quality comparison between fresh/thawed ostrich M. iliofibularis. Meat Science 91(3): 364-368.

Li, B. and Sun, D.W. 2002. Novel methods for rapid freezing and thawing of foods – a review. Journal of Food Engineering 54(3): 175–182.

Northchutt, J.K. Factors affecting poultry meat quality. 1997. Bulletin 1157 of Cooperative Extension Service. The University of Georgia College of Agricultural & Environmental Sciences. Download from *http://pubs.caes.uga.edu/caespubs/pubcd/b1157-w.html* on 3/3/2011.

Okamoto, A. and Suzuki, A. 2002. Effects of high hydrostatic pressure thawing on pork meat, Progress in Biotechnology 19: 571-576.

Pietrasik, Z. and Janz, J.A.M. 2009. Influence of freezing and thawing on the hydration characteristics, quality, and consumer acceptance of whole muscle beef injected with solutions of salt and phosphate. Meat Science 81(3): 523-532.

Savage, A.W.J, Warris, P.D. and Jolley, P.D. 1990. The amount and composition of the proteins in drip from stored pig meat. Meat Science 27(1): 289-303.

Silva, J.A. 2000. Tópicos da tecnologia dos alimentos. 227p. São Paulo: Varela.

Wang, S., Lei, G., Qiu, X. and Li C. 2015. Intelligent thawing household refrigerator. Applied Thermal Engineering 77:101-105.

Wirth, F., Leistner, L. and Rodel, W. 1981. Valores normativos de la tecnologia carnica. 127p. Zaragoza: Acribia.

Xia, X., Long, B., Liu, Q. and Liu, J. 2009. Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze-thaw cycles. Meat Science 83 (2): 39-45.

Xia, X., Kong, B., Liu, J., Diao, X. and Liu, Q. 2012. Influence of different thawing methods on physicochemical changes and protein oxidation of porcine longissimus muscle. Food Science and Technology 46(1): 280-286.