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ORIGINAL PAPER

Effect of freezing-thawing process on some quality aspects of pork *Longissimus dorsi* muscle

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Abstract

The present study was aimed to investigate the freezing – thawing process performed on pork Longissimus dorsi muscle. Two thawing conditions were taken into consideration, air/slow thawing and ultrasound/fast thawing. Freezing process was conducted under two temperatures, -18 °C and -60 °C, on fresh unaged samples and on aged samples. Data showed that storage temperature and ageing period had a significant effect ($p<0.05$) on proteins quantity found in dripped liquid, highest values being recorded for aged samples stored at -60 °C, for both slow and fast thawing. Results obtained for water holding capacity were similar to those obtained for firmness, showing that aged samples frozen at -60 °C had a better capacity to retain free water and a lowest value for recorded firmness, with no differences for thawing conditions. Only rheological determinations showed existence of some structural differences for defrosting mode (slow/fast), samples thawed in ultrasounds being more elastic with a better structure.

Keywords: *Ultrasounds, air thawing, protein loses, water holding capacity, firmness, rheology.*

Introduction

Frozen storage is an important preservation method for meat and meat products. Utilization of frozen products instead of chilled ones provides the possibility of increased storage time, greater flexibility in inventory and greater product control. Quality changes in meat due to freezing and thawing processes has concerned researchers and industry workers for a long time. It is thought that freezing reduces the meat quality, although no clear conclusion can be drawn from the scientific literature [1]. Functional properties of muscle proteins are closely associated with the structural integrity of proteins. Factors that can influence the specific structure and functionality of muscle proteins includes animal species, freshness of meat, and whether or not meat had been previously frozen or stored under frozen conditions Due to freeze-induced changes in myofibrillar proteins, processed products prepared from frozen meat tend to lose functional properties, such as water-holding capacity, emulsification and gelation capacity [2]. The effect of freezing and thawing processes on meat quality has been reported in several studies, most of them focused on fish meat and some on red meats [3-7]. Frozen and thawed meat quality can be affected by several factors including freezing temperature, rate of the freezing and thawing process together with temperature fluctuations [8, 9]. GONZALES-SANGUINETTI & al. [10] stated that by decreasing thawing time the exudate quantity was higher. On the other hand SAKATA & al. [11] reported that no correlations were found between freezing temperature (-20°C and -80°C) and WHC of meat and lipid oxidation during one month of frozen storage. NGAPO & al. [12] demonstrated that drip loss was lower, when a shorter thawing period was used. NGAPO [12] & NGAPO [13]

found no significant differences between protein concentrations from drip liquid obtained after different freezing treatments (frozen -thawed and frozen- stored - thawed) and the one obtained from fresh meat. In addition, NGAPO & al. [12] by using differential scanning colorimetry to study protein denaturation in pork found no differences in the DSC profile of fresh and thawed meat. However, freezing and thawing processes may result in protein and lipid oxidation affecting meat protein functionalities [7]. Understanding the flow and deformation behavior of thawed meat is important in choosing the process parameters of freezing and thawing. Rheological techniques can be used to predict the performance of thawed meat during mixing, tumbling, cooking, etc. Although many studies showed that the quality of the fresh meat is closely related to the quality of thawed meat, there are limited data regarding the influence of the freezing temperature, meat ageing and thawing method on meat characteristics.

The present study aimed to investigate the influence of freezing, ageing and thawing processes on proteins lost in dripped liquid. Also rheological behavior of thawed pork meat treated under different conditions was reported.

Material and methods

Materials

Longissimus dorsi muscles were obtained from eight carcasses with the same weight 24 h after slaughter. Any seen fat or conjunctive tissue was removed and muscles were cut in cuboids of approximately 100 g and wrapped in polyethylene film.

The experiment was divided in two parts, a freezing process and a thawing process, resulting a total of eight samples per replicate. Data were collected from six replicates.

The freezing part of the experiment was carried out by varying four factors, as following:

A frozen process carried out at -18 °C, on unaged samples (24 h postmortem);

A frozen process carried out at -60°C, on unaged samples (24 h postmortem);

A frozen process carried out at -18 °C, on aged samples (three days at +2°C);

A frozen process carried out at -60°C, on aged samples (three days at +2°C).

After 1 month of frozen storage, all the samples were thawed. Two types of thawing processes were used:

(1) Air thawing at +16 °C, further considered as slow thawing (Time needed to achieve the temperature range from -7 °C to -1 °C was 55 min);

(2) Ultrasound-assisted thawing (Clangsonic equipment) (the set intensity of 0.6 W/cm^2) in a water bath at +15°C, further considered as fast thawing (Time needed to achieve the temperature range from -7 °C to -1 °C was 8 min).

Soluble proteins

After cooking, the soluble proteins from the liquid expelled were determined using the Lowry method [14](Lowry *& al*., 1951). The quantity of proteins (mg/l) from the solution obtained was determined using a T80+UV/VIS Spectrometer (PG Instruments Ltd) at a wavelength of 726 nm and the soluble protein was expressed as BSA [15].

Expressible moisture (EM)

Samples of known weight (3.0±0.5g), with 2.5 cm in diameter and 1cm length were individually placed in 250 ml centrifuge tubes fitted with thimbles of filter paper supported by

a metallic mesh and centrifuged (Refrigerated Centrifuge TGL-16M) at 880 g for 20 minutes at 4 °C. EM was calculated as the percentage of moisture lost during centrifugation [16, 17].

Texture analyses

After thawing process, raw uncooked samples were supposed to texture analyses. Samples were cut in cuboids of 2×2 cm. Every sample was let in resting conditions for 30 minutes, at +15 °C, covered with a polyethylene film to avoid air drying. The textural characteristics of samples were analyzed using a TA.XT.*Plus* Texture Analyzer. The technical parameters of the apparatus were: Compression Test Mode; Test Speed of 1.5 mm/s. Maximum force recorded during the test was reported as *Firmness (Kg Force)* [15].

Rheological analyses

After each thawing process a slice of 2 cm from the exterior of sample was removed, then another slice of 2 mm thick was cut perpendicular to muscular fiber distribution. From the center of that slice was removed a circle with 40 mm in diameter using a circular drift. Samples were assessed rheologically by creep-recovery test using a control–stress rheometer (AR2000ex, TA Instruments, Ltd). The temperature was set at 20 ˚C using a Peltier temperature control system. The procedure was conducted using parallel plate geometry with a 40 mm diameter and a gap of 2000μm was used. In faze of creep testing a stress of 5 Pa was applied (ensured to be in the linear viscoelastic domain, by using a stress-sweep test previously) maintained for 300 s. For the recovery step, the stress was set at 0 Pa, allowing sample to recover for a period of 600 s. The mathematical models were also determined using the Rheology Advantage Data Analysis Program (TA, New Castle, DE) by applying an equation which combines Voigt and Burger models:

$$J(t) = J_0 + sum\{J_k[1-exp(-t/\lambda_{ret})]\} + t/\eta \qquad (1)$$

where J_0 ($=1/G_0$) is the instantaneous and fully recoverable elastic compliance (1/Pa), J_k from this equation defines the retarded compliance from Kelvin-Voigt model J_1 ($=1/G_1$) (1/Pa) together with the equilibrium compliance J_e ($=1/G+t/\eta$), after BARNES [18], λ_{ret} ($=\eta_0/G_1$) is the retardation time from Kelvin-Voigt model (s); η_0 is the Newtonian viscosity (Pa×s), and t is time (s) [19, 20].

Statistical analyses

Statistical analyses were carried out using Microsoft Excel Software applying Anova Single Factor. Each experiment was carried out in six replicates and the results were reported as mean values. The Fisher`s least significant difference (LSD) test was used to determine differences between treatment means with Statgraphics Centurion XVI.I software.

Results and discussions

Soluble proteins

Soluble proteins quantity found in dripped liquid resulted after thawing processes are shown in figure 1. Statistical analyses showed significant differences ($p<0.05$) between frozen treatments. Thus, it was observed significant differences ($p<0.05$) between protein quantity resulted from samples frozen at -18 °C and those frozen at -60 °C, regardless ageing treatment or thawing process (fast or slow). The highest protein quantity lost in dripped liquid was found in samples frozen at -60 °C, regardless ageing or thawing processes, leading to the idea that freezing temperature can be thought as primary factor affecting protein loses. Although SAKATA & al. [11] and BERTRAM & al. [21], found no effect of freezing temperature (-80 °C) on quality of thawed meat, we do not know in what proportion low freezing temperatures,

such as –60 ÷ –80 °C, are responsible for protein loses, especially that RAHELIC & PUAC [22] stated that ice crystals location are not definitely determined by freezing temperature.

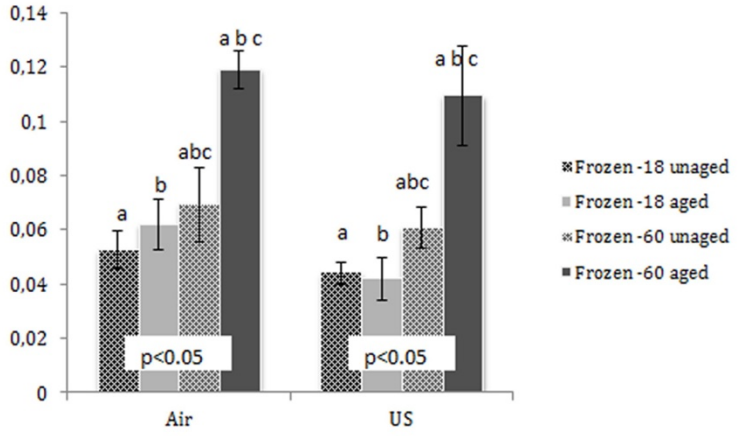

Figure 1. Soluble proteins quantity found in dripped liquid after thawing period. Values represent means of six replicates ± Standard Deviation. Values marked with same letter within same block of columns differ significantly ($p<0.05$). **US** = thawing process performed with ultrasounds; **Air** = thawing process performed in air.

The highest values of lost proteins (0,119 and 0,109 mg/L) from this study resulted from samples aged and frozen at -60 °C, for both thawing procedures. This can be due to both the number and position of ice crystals (intracellular or extracellular) formed at that temperature. Also an increase in number of lost proteins can be due to the fragmentation of myofibrils, a phenomenon that appears in aged meat under refrigerated conditions.

Regarding meat frozen at -18 °C it was observed that for all the samples, regardless ageing process or thawing time (55 or 8 minutes) difference between protein quantity was insignificant ($p<0.05$), as shown by LSD test. These results are similar to other studies. ANON & CALVELO [23] using freezing temperatures of -20 °C, found no significant differences between concentration and composition of lost proteins, values being independent by freezing rate (time needed for a sample to move from -1 °C to -7 °C). More, NGAPO & al. [12, 13], using a freezing temperature of -18 °C freezing rates (12, 30, 60, 120, 240 and 900 minutes) and different thawing rates (12, 60 and 180 minutes), found no differences in data regarding composition and concentration of total proteins lost with dripped liquid.

Expressible moisture (EM)

Water holding capacity of thawed samples under different conditions is presented in figure 2. It can be observed that values are significantly different in both cases (fast and slow thawing). In the case of slow thawing aged samples stored at – 60 °C presented the lowest values of expelled moisture with significant differences between aged and unaged samples for both storing temperatures (-18 and – 60 °C). The same situation was observed for fast thawed samples (in ultrasounds). No significant differences ($p>0.05$, Fisher LSD test) were registered between EM values of unaged samples stored at different temperatures for both thawing conditions (slow and fast thawing).

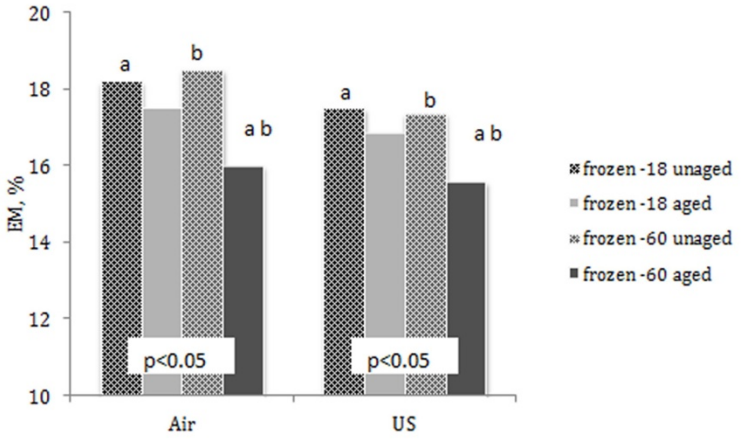

Figure 2. Samples` expressible moisture after thawing period. Values represent means of six replicates. Values marked with same letter within same block of columns differ significantly ($p<0.05$). **US** = thawing process performed with ultrasounds; **Air** = thawing process performed in air.

Regarding slow thawing process, our data are in agreement with results reported by SAKATA, & al. [11], who found no significant differences regarding water holding capacity for meat samples stored at –20 °C and those stored at –80 °C, both previously unaged. According to OFFER & TRINICK [24] statements, water location can be influenced by modifications which appears in rigor phase, when the structure formed between thick and thin filaments, lead to reduction of disposable space for existing water.

Texture analyses

Figure 3 presents values obtained after measuring samples` firmness, after each thawing process. It can be observed that in both cases of slow and fast thawing, texture was significantly influenced by varied factors ($p<0.05$). Regarding slow/air thawing data showed similar data (Fisher LSD test) for unaged samples, frozen at – 18 and – 60 °C. The only existence difference was recorded between unaged samples and those aged, stored at – 60 °C.

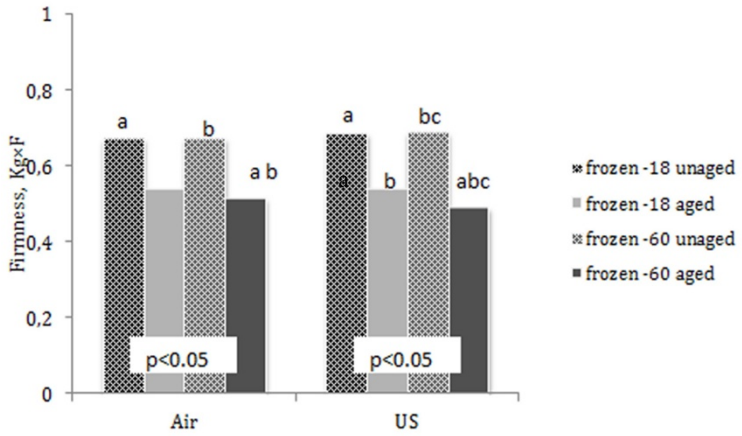

Figure 3. Texture variation of thawed samples under different conditions. Values represent means of six replicates. Values marked with same letter within same block of columns differ significantly ($p<0.05$). **US** = thawing process performed with ultrasounds; **Air** = thawing process performed in air.

For samples subjected to fast thawing, there were recorded differences both for aged and unaged samples frozen at – 18 and – 60 °C, as can be seen from letters distribution. Considering these results it can be said that aging of meat before freezing at low temperatures such as – 60 °C are the most influential, having as a result decrease in firmness, lowest value being recorded for samples thawed under fast conditions (ultrasound thawing). There are not

many existing data regarding effect of freezing-thawing treatments on firmness of raw thawed meats. However, in our case it seems that low freezing temperatures had a positive effect on aged meat regardless chosen thawing method. A possible explanation is that formation of small intracellular ice crystals in case of a fast thawing process could increase ageing rate by increasing the enzymatic activity [25].

Our data showed an existing correlation between firmness of raw meat and EM. So, the lowest values in both cases were recorded for aged samples frozen and maintained at – 60 °C regardless thawing condition (slow/fast thawing). These results came in agreement with HUFF-LONERGAN, & al. [26] results, who stated that there exists a correlation between exudate loses and meat texture and that samples with high amounts of exudate loses tends to be less tender.

Rheological analyses

The increase of strain in time when a stress of 5 Pa was applied to the samples showed the existence of creep and then recovery phenomena, after removing stress, as shown in figure 4. From data it can be seen that unaged samples before freezing showed a weaker structure comparing to aged ones (increased strain). More, curves resulted from aged samples, regardless frozen-thawing process were smoother compared with those unaged, and after applying Burger model equation, standard error values resulted for unaged samples were higher (data not shown).

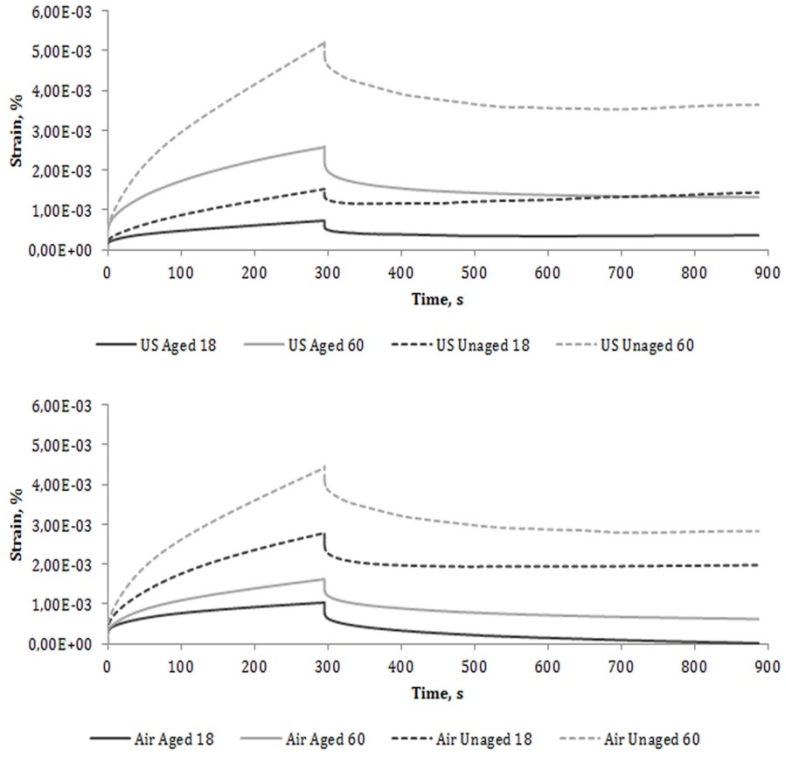

Figure 4. Effect of thawing processes on creep-recovery curves for meat samples frozen in different conditions.

Regarding freezing process it can be seen that samples stored at a lower temperature (- 60 °C) replied with a higher value for strain during stress applying period. Comparing with other studies carried out on fresh refrigerated meats, samples coming from frozen meats were weaker, showing a damaged structure. In our study the stress acceptable to respect the viscoelastic condition showed to be not higher than 5 Pa, when CHATTONG &

APICHARTSRANGKOON [19], used a 30 Pa stress value for ostrich-meat "yor" (a commercial ostrich meat product).

When speaking of thawing conditions, strain values obtained after 300 s of applied stress differed. Values were higher for samples thawed in air previously stored at -18 °C, aged or unaged (7.97×10^{-4} and 24.8×10^{-4} Pa) comparing to those thawed in ultrasounds and stored at -18 °C (5.85×10^{-4} and 0.13×10^{-4} Pa). Contrary, for samples frozen at -60 °C, strain recorded after 300s of applied stress was higher for samples thawed in ultrasounds (22.16×10^{-4} and 48.58×10^{-4} Pa) compared with those thawed in air (13.16×10^{-4} and 41.17×10^{-4} Pa).

Applying Burger`s equation (Table 1 & 2) showed no statistical differences between compliance`s parameters determined ($p>0.05$) for both thawing conditions used in the study (slow/air or fast/ultrasounds), aging period or storing temperature having no significant effect on values. Under both thawing circumstances viscosity values were higher for samples stored at -18°C aged, being the only cases indicating significant differences in Fisher `s LSD test.

Table 1 Creep compliance parameters with Burger`s model for creep curves of samples thawed in ultrasounds

Treatment type	$J_0\times10^{-6}$ (1/Pa)	$\eta\times10^{-6}$ (Pa×s)	$J_1\times10^{-4}$ (1/Pa)	$J_e\times10^{-4}$ (1/Pa)
1	1.348±1.45	4.711±2.37[a]	0.391±0.05	0.783±0.02
2	0.372±0.07	1.781±0.21	2.212±1.40	3.641±1.85
3	4.643±2.94	1.895±0.92	0.721±0.09	1.242±0.19
4	0.146±0.03	0.511±0.22[a]	2.709±1.68	3.963±2.28
p value	**0.1456**	**0.1134**	**0.242**	**0.204**

Values represent means of six replicates ± Standard Deviation.
Means with same superscript within same column differ significantly ($p<0.05$).
1=Thawed in ultrasounds, aged, stored at -18˚C; **2**= Thawed in ultrasounds, aged, stored at -60˚C; **3**= Thawed in ultrasounds, unaged, stored at -18˚C; **4**= Thawed in ultrasounds, unaged, stored at -60˚C;

Table 2 Creep compliance parameters with Burger`s model for creep curves of samples thawed in air.

Treatment type	$J_0\times10^{-6}$ (1/Pa)	$\eta\times10^{-6}$ (Pa×s)	$J_1\times10^{-4}$ (1/Pa)	$J_e\times10^{-4}$ (1/Pa)
1	1.561±1.87	4.543±2.13[a]	0.651±0.09	1.426±0.00
2	0.279±0.10	1.427±0.55[a]	1.493±0.26	2.342±0.04
3	1.246±0.55	1.348±0.31[a]	2.104±0.40	3.363±0.51
4	2.412±0.03	1.335±0.22[a]	1.989±1.68	3.673±2.28
p value	0.4689	0.0758	0.255	0.282

Values represent means of six replicates ± Standard Deviation.
Means with same superscript within same column differ significantly ($p<0.05$).
1=Thawed in air, aged, stored at -18˚C; **2**= Thawed in air, aged, stored at -60˚C; **3**=Thawed in air, unaged, stored at -18˚C; **4**= Thawed in air, unaged, stored at -60˚C;

Conclusions

Our results in terms of protein loses are confirmed by literature showing that semi permeability of cellular membrane of meat in refrigerated conditions is mostly affected during postmortem period, and that freezing has no significant contribution, regardless of freezing rate. More in depth studies are necessary in order to determine the effects of lower freezing temperatures, such as -60°C used for storage on pork meat quality. Regarding the water holding capacity of thawed meat, our results indicate that both freezing temperature and aging

period influence EM values, a better water holding capacity being characteristic for samples previously aged and stored at lower temperatures (-60°C). However, thawing conditions (slow in air or fast by ultrasounds) had no significant influence on EM values. Texture of raw thawed samples showed to be correlated with EM values, samples with highest water holding capacity being most tender. Rheological measurements showed a better structure for aged samples thawed by means of ultrasounds (fast thawing).

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