

HISTOLOGICAL, PHYSICOCHEMICAL AND MICROBIOLOGICAL EVALUATION OF PORK SMOKED LOIN MADE OF INJECTED FROZEN MEAT

EVALUAREA HISTOLOGICĂ, CHIMICĂ ȘI MICROBIOLOGICĂ A MUȘCHIULUI FILE OBTINUT DIN CARNE INJECTATĂ ÎNAINTE DE CONGELARE

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ABSTRACT | REZUMAT

Meat processing industry largely uses frozen meat as raw material and applies the post-thawing processing. This situation often implies working with lower quality meat, when compared to using fresh, unfrozen meat and fresh fish. As brine injection is known to enable the retaining of more moisture during cooking, we aimed to evaluate whether brine injection before freezing raw meat, has any effect on the quality of the meat products obtained from previously injected thawed meat. Boneless pork loin samples (*Longissimus dorsi*) were subjected to freezing in two groups: fresh meat (control) and brine injected meat (test). After two weeks, they were thawed and further processed to smoked loin in a meat processing facility. Only control meat was subjected to brine injection after thawing. The resulted smoked loin was refrigerated for 2 weeks and subjected to quality evaluation, by histological assessment of product structure. Also, the shelf life was evaluated by monitoring moisture content, pH and aerobic plate count (APC) during storage. The histological evaluation of the structure revealed higher quality for ham obtained from the test meat. The moisture content was higher, while the pH and APC followed improved shifts during storage, for the test meat, compared to the control one. This study suggests that injecting pork meat prior to freezing might be correlated with higher quality processed products, which tend to have improved shelf life.

Keywords: brine injection, frozen meat, pork ham, quality

Industria de obținere a produselor din carne folosește pe scară largă carne congelată ca materie primă, aplicând decongelarea acesteia înainte de procesare. Această metodă presupune utilizarea unei materii prime de calitate inferioară în comparație cu produsul refrigerat, necongelat. Întrucât injectarea cărnurilor în cadrul tehnologiei de procesare la produse din carne, duce la reținerea apei în produs, studiul de față își propune să evalueze măsura în care injectarea cărnurilor înainte de congelare, ar putea avea un efect pozitiv asupra calității produselor finite, în comparație cu cele fabricate din carne injectată după decongelare. Probe de musculatură dezosată de porc (*Longissimus dorsi*) au fost supuse congelării în două grupuri: carne proaspătă ne-injectată (control) și carne injectată (test). După două săptămâni, ambele categorii de probe au fost decongelate și apoi procesate la mușchi file, într-o unitate de obținere a produselor din carne. Doar probele de carne din grupul-control, au fost injectate după decongelare. Produsul finit obținut a fost refrigerat pentru o perioadă de două săptămâni, pe parcursul căreia ambele categorii de produse finite au fost supuse testării pentru evaluarea calității și examenului histologic, pentru evaluarea morfologiei. Calitatea și valabilitatea produselor au fost evaluate prin testarea umidității, pH-ului și NTG-ului pe perioada depozitării. Umiditatea probelor din grupul-test a fost mai bună în comparație cu cea a probelor din grupul-control, iar pH-ul a înregistrat o evoluție mai bună pentru grupul-test. Valorile NTG au indicat o calitate microbiologică superioară a probelor din grupul-test. Acest studiu sugerează faptul că injectarea cărnii de porc înainte de congelare ar putea fi corelată cu o calitate mai bună și o valabilitate superioară a produselor finite.

Cuvinte cheie: injectarea saramurii, carne congelată, mușchi file, calitate

The food industry widely embraces the benefits of enhancing meat flavor and texture by using injected brines and marinades which brings added value recog-

nized by consumers, while also increasing marketability by improving sensory properties and shelf life (3, 6). Meat processing industry largely uses frozen meat as raw material and applies post-thawing processing, thus working with lower quality grade meat, when compared to using fresh, unfrozen meat (2, 7).

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Food business operators in the meat industry have already largely embraced the "enhanced meat technology", which assumes injecting whole muscle meat with solutions of water, salt, phosphates and other ingredients (5). Enhanced meat brings many benefits in comparison the regular meat, in terms of quality (tenderness, moisture, flavour), consumer convenience, extended shelf-life and improved food safety, sensory quality and consumer acceptance, increased profitability and even new product development (4, 5). As meat enhancement is already acknowledged as efficient quality amplifier for raw meats (3, 11), there is a need to understand how meat products manufactured from enhanced meat are different from products obtained from regular meat.

As brine injection is known to enable the retaining of more moisture during cooking, this study aimed to evaluate whether brine injection before freezing raw meat, has any effect on the quality of the meat products obtained from previously injected thawed meat.

MATERIALS AND METHODS

Boneless pork loin samples (*Longissimus dorsi*), provided by a private slaughterhouse operator in Buzău County, were subjected to slow freezing (-18°C) in two groups: fresh meat (control) and brine injected meat (test) (Fig.1). After two weeks of frozen storage, they were thawed, at 5°C, in a refrigerator and further processed to ham in a meat processing facility. Only control meat was subjected to brine injection after thawing (Fig. 2). The resulted smoked pork loin was refrigerated for 2 weeks and subjected to quality evaluation, microbiological parameters were tested at days 1, 3, 6, 9, 14 of refrigeration, to monitor the pattern of modifications taking place during storage, in the control and test groups:

- In order to evaluate the influence of brining-freezing sequence order on the muscle structure, the histological assessment of the product structure was performed at the end of the storage period.

- The shelf life was tested by monitoring moisture content, pH and aerobic plate count (APC). The physical, chemical and microbiological analyses were performed at days 1, 3, 6, 9 and 14 of final product refrigeration.

Smoked pork loin production

Prime material preparation. The pork loin is preserved by wet salting, after the trimming, skinning and cooling of fresh meat. Brine injection is done at 10% of the weight of the muscle pieces. Brine injection is performed on fresh meat, before freezing, for the test group samples, while for the control samples, it is performed on thawed meat samples, resulted from meat which was frozen for 2 weeks. The injected pieces are immersed in brine tanks, for 4 full days. Draining is allowed for 24 hours. Warm water rinsing is repeated

twice and then trimming is performed once again. The pieces of meat are tied with rope at one of the ends (using a stainless-steel needle). The pieces are hanged on sticks for smoking (15).

Brine production. For 100 kg of pork loin, the brine is prepared of 10 l iced water, 1.81 kg nitrite - salt mixture and 0.013 sugar. The injection brine was prepared at 15 °Bé, while the brine for tank immersion was prepared at 14°Bé (20°Bé corresponds to 21.2% NaCl).

Smoking. The pork loin pieces are subjected to cold smoking for 16 hours for final product.

Histological analysis.

Slices of 1-2 cm in diameter were fixed for 24 hours in 10% formalin solution. The histological processing was performed according to the routine procedure, by inclusion in paraffin, microtome cutting of blocks, yielding of 4.5 µm thick sections to be displayed for drying in the thermostat at 37 °C. The slices were routinely stained by the HE (haematoxylin - eosin) method. Light microscopy analysis of the meat product samples was performed under an Olympus BX 41 microscope with an integrated computerized photography system. The histological evaluation of the samples was focused on revealing modifications of the muscular, connective, adipose, cartilage, bone and lymphatic tissue structure of the meat products, and on the comparative assessment of the two groups of samples included in the study. Histological assessment was performed for the injected and uninjected meat of the test group, before freezing, as well as for the final products of the two batches.

Physico-chemical evaluation.

Two parameters were chosen for the assessment of the sample shelf life and quality. The moisture content was determined using the oven-drying method, in a mechanical-convection oven, according to the Romanian standard SR ISO 1442:2010 (13). The grinded sample material was weighed to 2 g and air dried at approx. 125°C, in a 50 mm diameter and 40 mm deep aluminium dish. The sample was covered, cooled in a desiccator and weighed until constant weight. Loss in weight was reported as moisture, according to AOAC 950.46 (1). The pH measurement was performed by the potentiometric method, according to SR ISO 2917:2007 (14).

Microbiological evaluation

Analysis of APC was performed according to the method described by SR EN ISO 4833-1/2014 (12).

Data analysis

The study design included two batches of boneless pork loin samples, as prime material for smoked pork loin manufacturing: fresh meat (control) and brine injected meat (test). These were further processed into smoked pork loin as final product, divided accor-

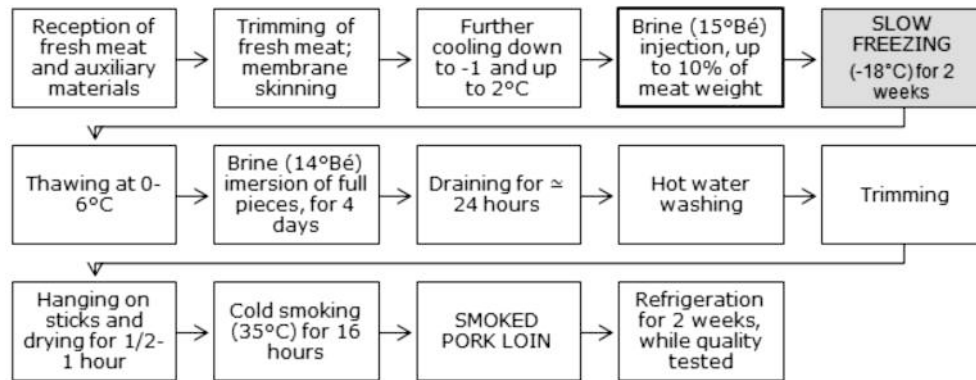


Fig. 1. Smoked loin production – test group samples

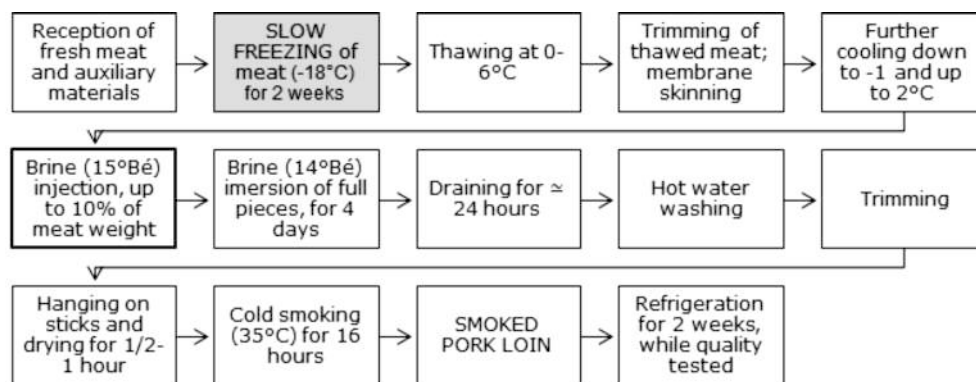


Fig. 2. Smoked loin production – control group samples

dingly into the control group and the test group. The two batches of samples were considered the treatments, which were analysed at days 1, 3, 6, 9, 14 of refrigeration. Each batch included 3 samples. Each parameter was reported as a mean for the three samples. Variance analysis of data was performed by One-way analysis of variance (ANOVA) using SAS (ANOVA v.9.1). The threshold of significance level between treatments was $p < 0.05$.

RESULTS AND DISCUSSIONS

Histological evaluation results

The histological evaluation of the test-group meat, before freezing was performed to compare the muscle structure of raw injected meat, with the regular architecture of raw, chilled muscle tissue. As expected, the samples of raw, chilled uninjected meat revealed the characteristic histological aspect of regular tissue components, with delimited muscle fibers with visible endomysium, striated nuclei located peripherally. The sarcoplasm revealed a fibrillar aspect. The interstitial space revealed rare, flattened blood vessels, discrete inflammatory cells infiltrate, mostly mononuclear cells. The occasional adipose cells exhibit regular cytomorphological characteristics (Fig. 3).

However, the injected meat (brine injected pork loin

test samples, examined before freezing) revealed slight alterations of the muscular tissue architecture. Even if the muscle fibers kept cellular integrity, with visible striations, euchromatic peripheral nuclei, mild dilaceration was visible due to liquid accumulation. The blood vessels reveal whole red cells which kept specific cytomorphological characteristics (Fig. 4).

The findings revealed in the first two photomicrographs demonstrate the slightly altered structure of the injected muscle, a mild disorganization of the muscle tissue with intact muscle cells of normal appearance alongside cells with cytoplasmic striations (resembling edema). By comparison of the two muscle samples, it may be possible to advise for the uninjected meat to be subjected to further processing by freezing, as the injected meat samples already present mild damage by intercellular fluid accumulation (dilaceration), which might increase with the freezing-thawing process, and possible causing severe muscle damage.

The histological evaluation was also performed on samples of finished product prepared from injected meat belonging to both groups, test-group and control-group. The histological aspect of the smoked loin manufactured from muscle injected after freezing and thawing (control-group), as revealed by the photomicrograph in figure 5, reveals that few muscular fibers kept the normal histologic appearance. The fibers in the

photomicrograph revealed in figure 5 are sectioned mostly longitudinally but also diagonally along the length of the fiber. The sarcoplasm allows visible striations to be seen and the nuclei are easily visible peripherally. However, numerous muscular fibers are missing the sarcolemma, so the sarcoplasm is conflated in an oxyphil mass. Other fibers are fragmented and dilacerated. The connective tissue and the adipose tissue reveal mostly normal histological appearance; however, some of the adipose cells are fragmented.

The ruptured muscle fibers are mostly due to the freezing-thawing process to which the fresh meat has been subjected prior to brine injecting and further processing. It is widely known that ruptured fibers are associated with valuable proteins being washed out from the meat during the purge process. Since in industrial processes, meat must be fully thawed before brine injection, at the point of injection, meat has already lost structure and nutrients from ruptured muscle fibers. Brine injection will replace lost meat juice, with brine. The histological aspect of the finished product (Fig. 5) produced by this common method is supportive evidence for the above explanation.

The histological aspect of the smoked loin manufactured from meat injected before freezing (test-group), as revealed by the photomicrograph in figure 6, reveals oxyphil striated muscular fibers.

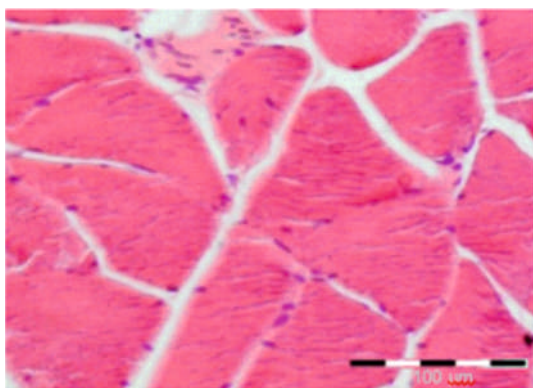


Fig. 3. Pork loin – raw, chilled, uninjected meat

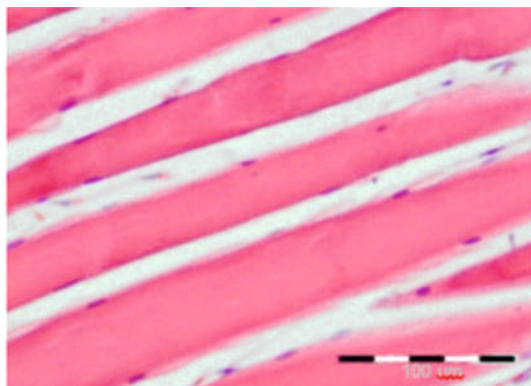


Fig. 4. Pork loin – raw, chilled, brine injected meat

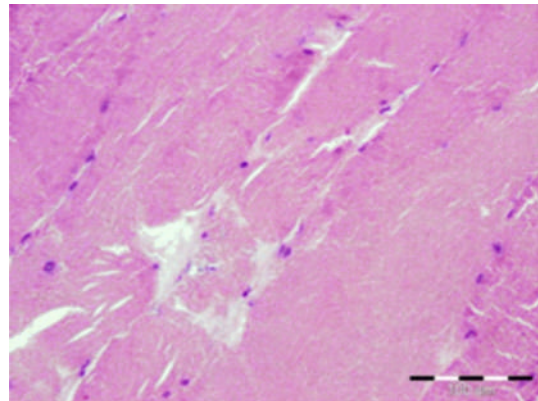


Fig. 5. Smoked loin – made of meat injected after freezing

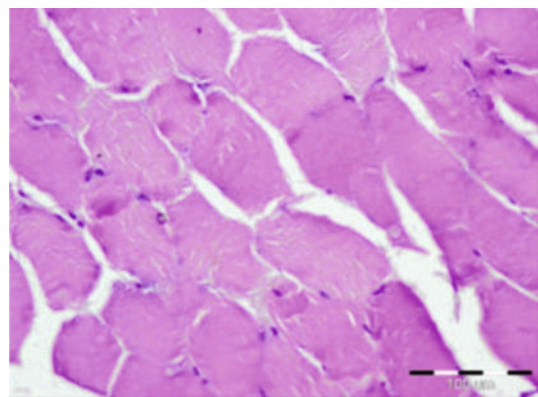


Fig. 6. Smoked loin – made of meat injected before freezing

The fibers in figure 6 are mostly sectioned transversally. The cellular components are strongly oxyphil, homogenous, some nuclei are not visible, while the interstitial connective tissue reveals dilated aspect with rare cells. The muscle fibers have kept their integrity and present regular histological structure. The sarcolemma seems intact, there are no fragmented fibers like in the previous figure, and the only marked modification aspect seems to be the slightly dilated intercellular space.

The histological aspect revealed in Fig. 6 is indicative of higher quality of the smoked loin, compared to the one presented in Fig. 5. The better muscle fiber quality revealed by histological assessment in the test group samples suggests that freezing meat after brine injection may be associated with a better preservation of the muscular structure and an obvious less muscle fiber rupture phenomenon in the final product.

Physico-chemical evaluation results.

Water content analysis revealed lower values for the control-group samples (the average water content is 63.264 ± 3.8072) than for the test-group samples (the average water content is 65.988 ± 3.1345), throughout the storage period, as illustrated in Fig. 7. Also, data reveals that water loss is more pronounced

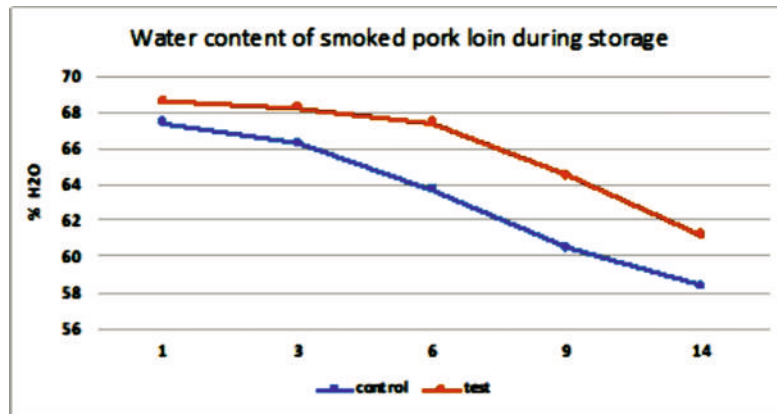


Fig. 7. The water content of smoked pork loin during storage – a comparison between control and test samples

and more rapid for the control samples (58.4% water at day 14), than for the test samples (61.17% at day 14). This indicates that the brine injection before freezing of meat could have a positive impact on the moisture of the product, preventing purge loss associated with thawing and moisture loss associated with storage. However, the statistical analysis of the data failed to reveal significance of these differences between the considered treatments (f-ratio value is 1.52555; p-value 0.251824. The result is not significant at $p < 0.05$).

The pH analysis results.

The pH testing revealed lower values for the test-group samples (the average pH value is 6.38 ± 0.0847) than for the control-group samples (the average water content is 6.29 ± 0.1087), throughout the storage period, as illustrated in Fig. 8. The faster pH fall is considered desirable in the meat products industry (3), and consequently the test-group samples prove the added value of brine-injection before freezing, from this point of view. Both batches of samples registered a drop of pH by day 6 and then an increase in pH for days 9 and 14 of storage. Even if there is an obvious better pH

trend associated with the test-group samples of smoked pork loin, there was no significant statistical difference according to the results of the data analysis (The f-ratio value is 2.1327. The p-value is 0.182315. The result is not significant at $p < 0.05$).

The increase in pH between days 10th and 15th of storage is in agreement with the findings reported in the literature (3; 10).

Microbiological evaluation results.

All analyzed samples were in line with the international recommendations and guidelines concerning APC of meat products, as the revealed values were lower than the threshold recommended in international guidelines (10^7 CFU/g, or 7 log CFU/g) (8; 9; 11), at all times throughout the storing period (Fig. 9). Analysis of aerobic plate counts reveals better microbiological quality of the test-group samples (with an average APC of 3.26 ± 0.5288 log CFU/g), in comparison with the control-group samples (with an average APC of 4.28 ± 0.8136 log CFU/g) (Fig. 9).

These results prove higher microbiological quality for smoked pork loin obtained from meat injected with

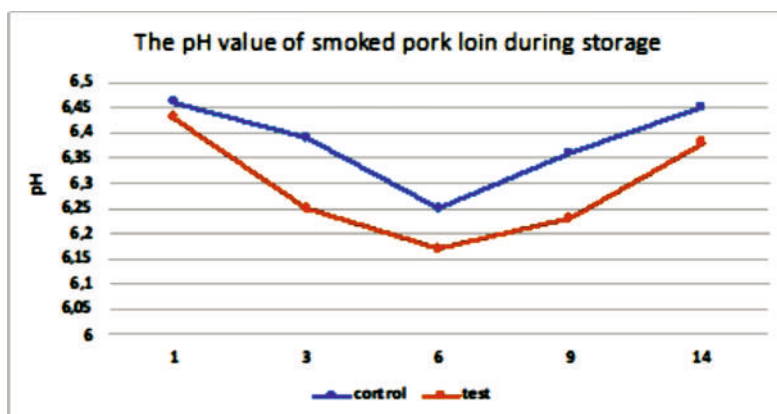


Fig. 8. The pH value of smoked pork loin during storage – a comparison between control and test samples

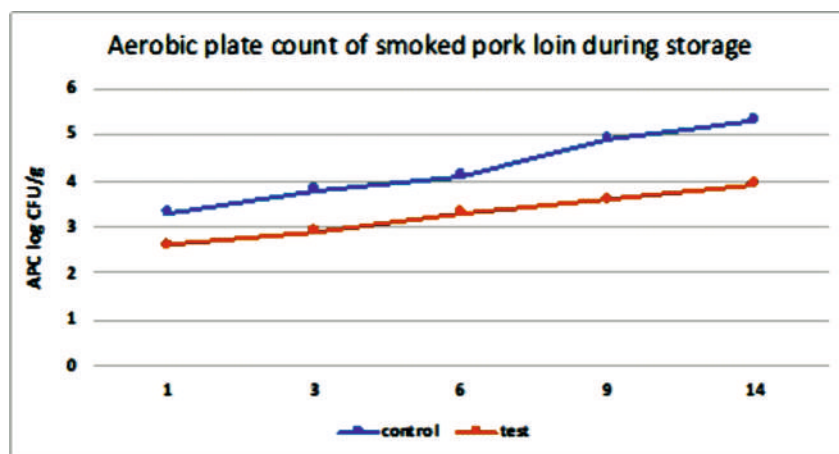


Fig. 9. The APC values of smoked pork loin during storage – a comparison between control and test samples

brine before freezing. The impact of these results comes from the statistical significance which is associated with the difference in APC values between the test-group samples and the control-group samples (The f-ratio value is 5.45937. The p-value is 0.047673. The result is significant at $p < 0.05$).

CONCLUSIONS

The histological evaluation of the structure revealed higher quality for smoked pork loin made of the test meat. The moisture content was higher but revealed no significant variation during the monitoring cold storage period. The pH followed improved shifts during storage, for the test meat, compared to the control one, as revealed by testing at days 1, 3, 6, 9, and 14 of refrigeration. The APC revealed significantly better microbiological quality for the test-group samples than for the control samples. This study suggests that injecting pork meat prior to freezing might be correlated with higher quality processed products, which tend to have improved shelf life. Further study of larger pool of samples is needed for statistical significance and including sensory evaluation of the final product in the research protocol may enhance the quality evaluation precision.

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