DISTINGUISHING BETWEEN FRESH AND FROZEN-THAWED POULTRY MEAT: HISTOLOGY TO DETECT FOOD ADULTERATION

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I. INTRODUCTION

Global meat production is increasing because of population growth, rising income, and urbanization. It is projected to be 14% higher by 2030, compared to the base period average of 2018-2020, with poultry meat as the primary driver of this growth, because it represents an affordable option, compared to red meats, in response to expanding global demand for animal proteins [1]. Since fresh meat is a perishable material, it quickly loses its desired sensory and microbial qualities. Long-term storage and transport between slaughterhouses, meat processors and consumers may take days or even several weeks, so freezing is an excellent way of extending the storage life of meat and makes transport easier. However, during freezing and thawing processes the meat loses moisture, which contains components contributing to the characteristic flavour and nutritional value. The texture of meat is also affected by the formation of ice crystals, which damage the muscle structure and increase the water activity on the meat surface [2, 3]. That is why the retail price of frozen or thawed meat is generally lower than the price of its fresh counterparts. Due to the perceived higher quality, consumers are still willing to pay a higher retail price for fresh meat. In many cases, the significant price gap between frozen and chilled products rises an incentive to deceive the consumer and perpetrate frauds. Additionally, in the case of poultry meat, Council Regulation (EC) No 1308/2013 defines “fresh poultrymeat” as “poultrymeat which has not been stiffened at any time by the cooling process prior to being kept at a temperature not below -2°C and not higher than +4°C” and prohibits the sale of previously frozen poultry meat as fresh. The EU Labelling Directive (Regulation No 1169/2011) requires a process or treatment of a food to be declared where it is misleading not to do so. Therefore, it is mandatory to indicate if the meat has previously been frozen.

The aim of this study is to evaluate the performance of histology to differentiate between fresh and frozen-thawed chicken meat in order to verify its feasibility as official test. To evaluate the performances and to study the applicability of histology, samples subjected to chilling treatment were included too, to investigate if technological hardening can invalidate final results.

II. MATERIALS AND METHODS

Ten chicken skinned carcasses were bought at the slaughterhouse in order to have fresh meat reference material (never frozen): breast and thigh muscles samples were isolated. From these muscles, samples were prepared and used as reference materials. The breast samples were randomly divided into three groups, plus two thigh samples groups. Group A (breast, n=36), and group D (thigh, n=10) consisting of fresh samples, were immediately fixed in 10% neutral buffered formalin; group B (breast, n=36) and group E (thigh, n=10), including frozen samples, were immediately frozen at -18°C for 48 hours, thawed at 4°C for 24 hours and then fixed in 10% neutral buffered formalin; group C (breast, n=40), including chilled samples, was hardened and sliced using conventional technological procedures, refrigerated at 4°C and then fixed in 10% neutral buffered formalin. Temperature controls were carried out through a data logger over time. After fixation both fresh and frozen-thawed samples were trimmed to a final standard size of 1x1x0,3 cm, placed into numbered plastic boxes and routinely processed for histological examination. Paraffin embedded blocks were cut on a microtome into 3-5 µm slices, stained with haematoxylin and eosin (HE) and observed by optical microscopy at increasing

magnification (X10, X20, X40), in transversal sense by two histopathologists. The histological evaluation aimed to identify microscopic changes related to the freezing-thawing cycle. Parameters considered were the following: presence or absence of vacuoles of various size, empty or filled with eosinophilic material, in muscle cells; increased separation of muscle fibres; presence of transverse breakage; fibres shrinkage and tearing. All microscopic alterations were semi-quantitatively scored on a scale ranging from 0 to 3 (absent, mild, moderate, severe).
The results of the microscopic examination were entered into a database, cleaned, and analysed using Stata/SE 14.0. The validity of the evaluation of the histopathological alterations was calculated for parameters that resulted significantly associated with freezing treatment. In addition to the test's performance, Cohen's K was calculated as a measure of agreement between the two observers.

III. RESULTS AND DISCUSSION

Regarding the grade of separation of muscle fibres, the presence of transverse breakage, fibres shrinkage and tearing, no differences were observed between the groups of samples. The only parameter statistically associated with freezing treatment was the presence/absence of vacuoles.
All fresh breast samples showed no presence of vacuoles, as well as all fresh thigh samples. On the other hand, in all frozen-thawed samples vacuoles were presents, as result of intracellular rupture or damage by formation of ice crystals. Those vacuoles, of variable size, appeared as optical empty spaces and were characterized by sharp margins. In the frozen thigh samples vacuoles were less evident but registered in all except one sample. Finally, none of the 40 chilled breast samples showed microscopic vacuoles. Overall, histology showed a sensitivity of 97,9% (95%CI: 88,9-99,9%) and a specificity of 100% (95%CI: 95,8-100%), Cohen's kappa was 1.
Sensitivity and specificity of the histological method were remarkably high and would justify the use of this technique for official control purposes. Furthermore, the combined Cohen's kappa of 1 indicates a perfect agreement between the readers and the intermediate repeatability of the test.

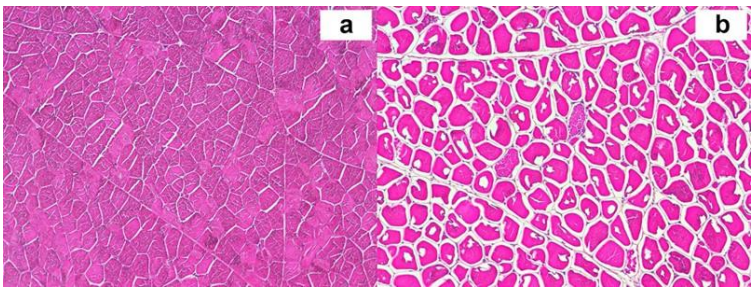

Figure 1. Chicken breast, HE stain (X10 magnification).
It is clear the presence of vacuoles in thawed sample (b), compared to fresh sample (a).

IV. CONCLUSION

The present study showed the suitability of histological test for the authentication of poultry meat as fresh or frozen-thawed. Overall, histology proved to be a simple and cost-effective control strategy that can detect freshness declaration frauds in chicken meat, aiming to the final goal represented by the protection of both consumers and honest food business operators.

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