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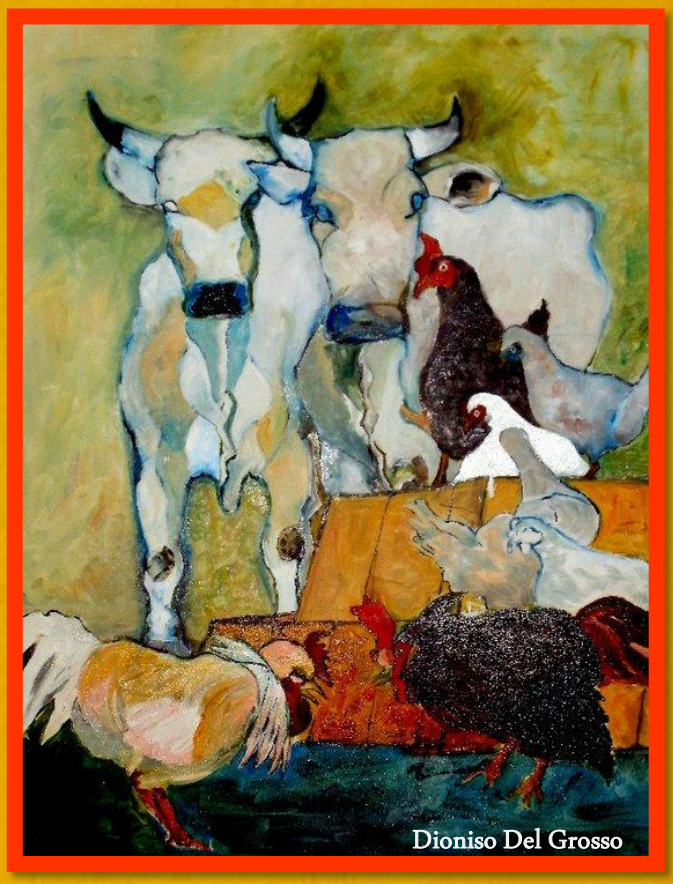

20-22 Giugno 2018

XVIII Convegno SICV

XVI Convegno SIRA

XV Convegno AIPVET

X Convegno ARNA

V Convegno RNIV

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MBC
Via Nizza 52
Torino

Con il patrocinio di

CITTA' DI TORINO

REGIONE PIEMONTE

72° CONVEGNO SISVET

Con il contributo di

Segreteria Organizzativa

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72° CONVEGNO SISVET

20 – 22 Giugno 2018

Università degli Studi di Torino

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I contributi presenti negli Atti del
72° Convegno SISVet 2018
potranno essere citati utilizzando il codice
ISBN 978-8890909214

A HISTOLOGICAL BASED METHOD FOR THE DISCRIMINATION OF FRESH FROM FROZEN THAWED FISH MUSCLE OF EUROPEAN HAKE (*Merluccius merluccius*)

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Freezing process is commonly applied to prolong the seafood shelf life. However, this preservation technique produces physical-chemical modifications against muscle tissue that may lead to product's quality alterations and expose the thawed product to higher perishability and spoilage process. [1]. Thus, freezing and thawing processes have to be mandatory declared in order to guarantee both fair business-to-consumer practices and consumer's safety [2,3]. Nevertheless, deliberate substitutions of fresh with thawed fish are reported as common fraudulent incidents and numerous analytical discriminating techniques (physiological, chemical and physical) have been developed [4]. Among them, the histological method has been demonstrated as an accurate, and relatively low time and cost-consuming technique [5]. The present study aimed at providing a standard histological procedure to discriminate fresh and frozen-thawed *Merluccius merluccius* (European hake), selected as possible model of white meat fish species. A preliminary analysis was performed on 8 fresh muscle tissue specimens to define the standard morphological characteristics and to select the sampling site among three different anatomical areas (lateral line, dorsal muscle, ventral muscle). Then, 15 fresh products, sampled at different shelf-life (24h, 72h and 120h), were analyzed to evaluate the microanatomical alterations related to fish spoilage. Ninety tissue samples (30 fresh, 30 experimentally frozen at -20° C and 30 industrially frozen at -80°C) were subsequently collected to select morphological and morphometric parameters to be included in a standard operational grid. After a statistical analysis, three morphological (muscle structural score corresponding to 0= fully destructured, 1= partially destructured, 2= well preserved; presence of vacuoles; presence of intermiofibral seroproteinaceous material) and one morphometric (vacuoles per field, for which a cut off value of freezing process corresponding to 1.12 vacuoles per field was set) parameters were ultimately included in the operational grid. Accuracy and repeatability of the procedure were calculated on the analysis carried out by one expert and one in training operators on 50 randomly selected samples among the groups of the previous experiment. The final validation was conducted, by the same operators, through a blind test on 30 (13 fresh and 17 industrially frozen) additional commercial products. The procedure showed high sensitivity (97-100%) and specificity (94-100%) and a high diagnostic concordance of the two operators was observed, regardless of their operating know-how. Thus, the proposed operational grid represents a simple, reliable and low-cost check tool against fraudulent practices.

[1] Pavlov, A. Changes in the meat from aquaculture species during storage at low temperature and attempts for differentiation between thawed-frozen and fresh chilled meat. A review. BJVM 10(2): 67–752, 2007. [2] Regulation EU No 1169/2011 OJ, L 304. [3] Regulation EU No 1379/2013. [4] Uddin, M. Differentiation of fresh and frozen-thawed fish. In: Leo et al. (Eds.), Handbook of seafood and seafood products analysis. CRC Press. pp.735-750, 2010. [5] Bozzetta, E., Pezzolato, M., Cencetti, E., Varello, K., Abramo, F., Mutinelli, F., Ingravalle, F., Teneggi, E. Histology as a valid and reliable tool to differentiate fresh from frozen-thawed fish. Journal of Food Protection, 75(8):1536-1541, 2012.