

## PREVENTING FRAUDULENT ADULTERATIONS: PRELIMINARY STUDY FOR THE SELECTION OF HISTOLOGICAL PARAMETERS FOR THE DISCRIMINATION OF FRESH AND FROZEN-THAWED OCTOPUS MANTLE

Lara Tinacci (1), Francesca Abramo (1), Giulia Scardino (1), Vincenzo Miragliotta (1), Daniele Nucera (2), Alessandra Guidi (1), Andrea Armani (1)

(1) Università degli Studi di Pisa, Dipartimento di Scienze Veterinarie. (2) Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari

The application of appropriate analytical techniques represents an important tool to prevent deliberate substitutions of fresh with thawed seafood. The histological method has already been validated to discriminate fresh from thawed fish [1,2], while it has never been applied to cephalopods [4]. The present study aimed at selecting histological parameters of freezing-induced structural alteration on Octopus (*O. vulgaris*), to be further used in the setting up of an operational grid for the discrimination of fresh and frozen-thawed samples. Ten whole fresh exemplars (F), maintained at 0-2°C, were sampled at different time (24h, 72h, 144h and 192h) within octopus shelf life [3]. obtaining longitudinal and transversal mantle samples. Samples were fixed in 10% formalin, embedded in paraffin and sections stained with H&E were observed to define fresh tissue morphological pattern and to highlight possible histological spoilage-related alterations. Then, 85 mantle tissue sections belonging to 20 fresh (F), 20 conventionally frozen in a laboratory freezer at -20 °C for 15 days (CF), 25 industrially frozen in bulk blocks at -80°C (IFB) and 20 curled and Individually Quick Frozen (IQF) exemplars, were analogously processed and screened to select histological parameters descriptive of freezing-thawing process. Overall tissue structural organization(4x magnification)gaping among muscle fiber bundles and white round-oval-saccular spaces or clefts between and within the muscle bundles,(10x magnification) were assessed on the central area of the longitudinal section, including radial, circular muscle bundles and a central connective tissue layer. Moreover, white spaces between and within muscles bundles were measured by morphometry and results expressed as percentage. Focal myofiber degeneration, shrinkage and swelling were histological modifications related to shelf-life. The increase of the overall tissue structure alteration, myofiber gaps and the presence of white spaces between and within muscle bundles were all statistically confirmed to be related to freezing as plausibly induced by the effects of water crystallization phenomena [4].The mean white space percentages recorded in F (22.97%), CF (43.09%), IF (46.57%), IQF (59.69%) highlighted significant differences between fresh and frozen tissue types. Thus, the study confirmed the suitability of all the aforesaid histological parameters as analytical indicators for the discrimination of fresh and frozen cephalopods. [1] Bozzetta E et al. Histology as a valid and reliable tool to differentiate fresh from frozen-thawed fish. Journal of Food Protection, 75(8):1536-1541, 2012; [2] Tinacci L., et al. Histological discrimination of fresh and frozen/thawed fish meat: European hake (*Merluccius merluccius*) as a possible model for white meat fish species ; [3] Barbosa, A. & Vaz-Pires, P. Quality index method (QIM): development of a sensorial scheme for common octopus. Food control 15, 161-168, 2004; [4] Ueng, YE. & Chow CJ. Textural and Histological Changes of Different Squid Mantle Muscle during Frozen Storage. Journal of Agricultural and Food Chemistry 46, 4728-4733, 1998.