



Physico-chemical, colorimetric, rheological parameters and chemometric discrimination of the origin of *Mugil cephalus*' roes during the manufacturing process of *Bottarga*

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ABSTRACT

The aim of this work was to measure the physico-chemical and the colorimetric parameters of ovaries from *Mugil cephalus* caught in the Tortoli lagoon (South-East coast of Sardinia) along the steps of the manufacturing process of *Bottarga*, together with the rheological parameters of the final product. A lowering of all CIELab coordinates (lightness, redness and yellowness) was observed during the manufacture process. All CIELab parameters were used to build a Linear Discriminant Analysis (LDA) predictive model able to determine in real time if the roes had been subdued to a freezing process, with a success in prediction of 100%. This model could be used to identify the origin of the roes, since only the imported ones are frozen. The major changes of all the studied parameters ($p < 0.05$) were noted in the drying step rather than in the salting step. After processing, *Bottarga* was characterized by a pH value of 5.46 (CV = 2.8) and a moisture content of 25% (CV = 8), whereas the typical per cent amounts of proteins, fat and NaCl, calculated as a percentage on the dried weight, were 56 (CV = 2), 34 (CV = 3) and 3.6 (CV = 17), respectively. The physical chemical changes of the roes during the manufacturing process were consistent for moisture, which decreased by 28%, whereas the protein and the fat contents on the dried weight got respectively lower of 3% and 2%. NaCl content increased by 3.1%. Principal Component Analyses (PCA) were also performed on all data to establish trends and relationships among all parameters. Hardness and consistency of *Bottarga* were negatively correlated with the moisture content ($r = -0.87$ and $r = -0.88$, respectively), while its adhesiveness was negatively correlated with the fat content ($r = -0.68$).

1. Introduction

The mullet (*Mugil cephalus*) is a fish species living in the coastal waters of the tropical, subtropical and temperate zones of all seas (Fishbase, *Mugil cephalus* Linnaeus, 1758). The eggs of the mullets, usually known as roes, are removed from the fish in their original ovarian sac and manufactured through a salting and a drying process to obtain a seafood which is known with different names depending on the geographical area it is produced, such as Greek *Avgotaracho*, Japanese *Karasumi* or Italian *Bottarga* (Piras, Scano, Locci, Sanna, & Marincola, 2014). Processed roes are commercialized as whole ovaries or grated in jars.

Most of the studies in literature about *Bottarga* regard the characterization of the lipid fractions: the fatty acid and the fatty alcohol

profiles (Bernasconi et al., 2007; Scano et al., 2008; Scano et al., 2010) together with the percentage of the different lipid classes in *Bottarga* (Scano et al., 2008) were determined on commercial dried products. Differences in the fatty acids, fatty alcohols and cholesterol levels due to the manufacturing process of *Bottarga* were studied in fresh and dried roes by Scano, Rosa, Locci, Dessì, and Lai (2009), Scano et al. (2010), and Rosa et al. (2009). Rosa et al. (2009) also studied the levels of Polyunsaturated Fatty Acids (PUFA) and conjugated diene hydro peroxide (HP) during the storage of *Bottarga*. Lipid oxidation at different storage times and temperatures was investigated in *Bottarga* also monitoring the modifications in the levels of phospholipid degradation products (choline, phosphorylcholine, and glycerophosphorylcholine) (Rosa et al., 2012). Moreover, to deepen the degradation extent of *Bottarga*, water soluble metabolites such as amino acids and organic

Abbreviations: PCA, Principal Component Analysis; LDA, Linear Discriminant Analysis; PUFA, Polyunsaturated Fatty Acids; PDO, Protected Designation of Origin; PGI, Protected Geographical Indication

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acids were determined on the final product (Locci, Piras, Mereu, Marincola, & Scano, 2011) and both on fresh and on dried roes, also investigating the effect of the frozen storage period of fresh roes (Piras et al., 2014). Rosa et al. (2012) studied the changes in the levels of total sugars and free amino acids such as lysine, methionine, and tryptophan to assess the presence of Maillard reaction compounds during *Bottarga* aging. Biogenic amines, caused by amino acids degradation, were determined on commercial dried roes (Kung et al., 2008) also during a 180 day storage period (Restuccia et al., 2015). The *Mugil cephalus* roe oil was found to have functional properties, being a potential bioavailable source of omega-3 PUFA (Rosa, Atzeri, Putzu, & Scano, 2016) with potential benefits in cancer prevention (Rosa et al., 2016; Rosa, Scano, Atzeri, Deiana, & Falchi, 2013).

On the other hand, few studies are present in literature about the physico-chemical characterization of the macro constituents of mullet roes, which were usually limited to the study of the finished product (Barra et al., 2008; Kalogeropoulos, Nomikos, Chiou, Fragopoulou, & Antonopoulou, 2008), and to the study of the differences between dried mullet roes from fishes of different geographical origin (Barra et al., 2008). The difference of the physico-chemical characteristics between fresh and processed roes were studied in mullet roes of Turkish (Çelik, Altunelatan, Dinçer, & Acarlı, 2012) and United States origin (Lu, Ma, Williams, & Chung, 1979). The results reported in the literature showed a great variability in the physico-chemical characteristics among roes of different geographical origin. Little information is present in literature about *Bottarga* produced in Sardinia from local fishes (Barra et al., 2008): the study is based on only 5 commercial dried roes samples of local origin, for which pH is reported to be 5.3 on average, moisture 29.5% whereas proteins, fat and salt 47.7%, 13.3% and 4.3% respectively, calculated as a percentage of dried weight.

Sardinia (Italy) is one of the *Bottarga* main productive areas in the Mediterranean Basin, with the manufacturing of mullet roes of local origin and of mullet roes imported as frozen mainly from the FAO 31 area (Western Central Atlantic area). Since 2002, there has been an average growth in *Bottarga* sales of about 5% per year; according to data updated to 2008, the manufacturing of *Bottarga* in Sardinia is about 400 tons per year, almost absorbed by the local market. Due to the limited spawning period of the mullets fished in Sardinia, that comes from the end of August to the beginning of October, only small amounts of the roes used for the manufacturing of *Bottarga* is of local origin, whereas the remaining part is imported mainly from the Atlantic ocean as frozen roes. Despite this, the local origin manufacturing of *Bottarga* has a great importance both in terms of traditional and economic value. *Bottarga* is considered one of the most representative seafood of Sardinia and it has had the recognition of Traditional Product of Sardinia (Decreto Legislativo 30 aprile 1998, n. 173, 1998; Decreto Ministeriale 8 settembre 1999, n. 350, 1999; Regione Autonoma della Sardegna RAS, 2016). Over the years, given the peculiarities of *Bottarga* produced in Sardinia, whose qualities are widely recognized by both producers and consumers, the interest in certification (Protected Designation of Origin, PDO and/or Protected Geographical Indication, PGI) of this product has increasingly accrued. For these reasons, it becomes important to deepen the analytical knowledge of the product, in particular the one of local origin.

In this work, the physico-chemical characteristics of mullet roes of fishes caught in the Tortoli lagoon (Sardinia, Italy) were studied during all the steps of the manufacturing process of *Bottarga*. This foodstuff was also characterized in terms of biometric, rheological and colorimetric parameters. The Linear Discriminant Analysis (LDA) multivariate approach paired with non-destructive, cheap, fast and real time techniques has been assessed to be a valid method in the identification of the origin of a food matrix (Caredda et al., 2017). With the aim of finding a method to establish the origin of *Bottarga* and considering that the origin discrimination is possible as only the imported roes are frozen, whereas local roes are processed after the catching of the fishes and their evisceration, we evaluated the capability of the colorimetric

parameters coupled with the LDA approach in the discrimination of fresh mullet roes which had or had not undergone a freezing process.

2. Materials and methods

2.1. Manufacture process

During the reproductive period of the mullets living in the Tortoli lagoon (Sardinia, Italy) (GPS coordinates: 39°56'48.8"N 9°41'14.3"E) corresponding to the end of August to the beginning of October, the fishes are caught in their way from the lagoon to the sea where they are instinctively driven to spawn, and immediately transported inside the plant of the "Cooperativa Pescatori Tortoli" where all females are eviscerated. Only fishes > 25 cm in length are caught; fishing is done by hand with the help of landing nets and evisceration is manually performed with proper knives. The roes are washed for removal of the blood and then weighted. The manufacturing process of *Bottarga* consists of two steps: a salting step, in which the roes are kept in trays and manually covered with sea salt for 2 h, and a drying step, in which the salted roes are kept in ventilated, dehumidified and thermostated chambers (5 × 3 m) at 14.5 °C for a period ranging from 7 to 10 days, turning them upside down twice a day. The end of the drying step is determined when a specialized operator perceives the "right" tactile feeling when pressing the roes with the fingers. Before marketing, *Bottarga* is packed under vacuum (whole roes) using a Lapack 550 S (Lavezzini S.r.l., Piacenza, Italy) vacuum machine, or grated and put into 40 g glass containers.

2.2. Sampling

In two different days during the third week of September 2014, about 150 female mullets per day were caught and eviscerated in the "Cooperativa Pescatori Tortoli". Among the total of the obtained roes, 54 gonads were chosen based on their weight (range: between 250 and 350 g, representative of the 75% of the total of the roes usually processed in these days from "Cooperativa"). The gonads were divided into three homogenous groups: 1) fresh roes (n = 18); 2) roes which underwent the salting step (n = 18); 3) roes which underwent both the salting and the drying steps (n = 18). The fresh, salted and dried roes were stored frozen at −20 °C until analysis. Fresh and salted roes were stored in single plastic bags in order to maintain the integrity of the fragile roes whereas the dried roes were stored in vacuum packages. All samples were analyzed after thawing for 24 h at 4 °C. After thawing, the external membrane enveloping the gonads was removed. Then, fresh and salted roes were homogenized by using an Ultra-Turrax (T25 Basic, IKA WERKE, Staufen Germany) for 3 min at 13.500 rpm under ice, whereas dried roes were grated with an electric grinder for 30 s.

2.3. Chemicals

For protein analysis, sulphuric acid (> 96%, RPE-ISO for analysis), boric acid (RPE-ISO-ACS for analysis), copper(II) sulphate pentahydrate (RPE-ACS, for analysis), hydrochloric acid 0.1000 N standard solution were from Carlo Erba Reagents (Milan, Italy), whereas potassium sulphate (purity ≥ 99%) was from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

For NaCl analysis, nitric acid > 65% (RPE-ISO-ACS, for Analysis) and silver nitrate 0.1000 N standard solution were from Carlo Erba Reagents (Milan, Italy). For fat analysis, diethylether (RS, anhydrous, for analysis, stabilized with BHT) was from Carlo Erba Reagents (Milan, Italy).

2.4. Chemical analysis

pH was measured by using a Lab 860 pH-meter (SI Analytics GmbH, Mainz Germany) on the central part of both gonads of all the thawed

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