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Physical-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 physical-chemical and the colorimetric parameters of ovaries from *Mugil cephalus* caught in the Tortolì 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).

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

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). 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, Rosa, Marincola, et al., 2008; Scano, Rosa, Mereu, 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, & 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 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, et al., 2016) with potential benefits in cancer prevention (Rosa, Scano, Atzeri, Deiana, & Falchi, 2013; Rosa, Piras, et al., 2016).

On the other hand, few studies are present in literature about the physical-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 physical-chemical characteristics between fresh and processed roes were studied in mullet roes of Turkish (Çelik, Altınelataman, Dinçer, & Acarlı, 2012) and United States origin (Lu, Ma, Williams, & Chung, 1979). The results reported in the literature showed a great variability in the physical-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 173, 1998; Decreto Ministeriale 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 physical-chemical characteristics of mullet roes of fishes caught in the Tortolì 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 Tortolì 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 Tortolì” where all females are eviscerated. Only fishes more than 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 2h, and a drying step, in which the salted roes are kept in ventilated, dehumidified and thermostated chambers (5 x 3 meters) 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 Tortolì”. 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 24h 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 samples, and averaged. Moisture was determined by heating 3 g of sample (weighted with the uncertainty of 0.0001 g) in a nickel-aluminum-steel capsule containing 40 g of quartz sand at 102±2°C for 4 h (ISO, 2004). Protein content was determined by using the Kjeldhal method (ISO, 2014). In particular, 0.5 g of sample (weighted with the uncertainty of 0.0001 g) were digested with a

mixture of H_2SO_4 96% (20 ml), $CuSO_4 \cdot 5H_2O$ (1 ml of a 5% water solution (w/v)), and K_2SO_4 (15 g) using the following temperature program: 140°C for 15 min, 190°C for 15 min, 200°C for 20 min, 220°C for 20 min, 240°C for 90 min, 290°C for 10 min, 320°C for 10 min, 420°C for 90 min. Ammonia contained in the limpid digested was steam distilled and collected in a solution containing H_3BO_3. Nitrogen content was finally quantified by titration with HCl 0.1000 N and converted to the protein content multiplying the value of N content by the conversion factor of 6.25. Fat content was gravimetrically determined on 3 g of sample (weighted with the uncertainty of 0.0001 g) by refluxing diethylether at 90°C for 6h on a Soxhlet apparatus (Soxhlet, 1879). The flask containing the extracting solvent was then heated in oven at 102±2°C to eliminate all solvent residues from fat extracted and weighted on an analytical balance. Salt content was determined by homogenizing 2.5 g of sample with distilled water and HNO_3 at 60°C for 30 min and titrating with a standard solution of $AgNO_3$ 0.1000 N on an automatic titrator (Mettler Toledo DL55 Titrator, Port Melbourn Australia). Protein, fat and NaCl contents were expressed as percentage of the dried weight.

2.5 Biometric analysis

All the samples were weighted in the different steps of the manufacturing process (as fresh roes, after the salting step and after the drying step) to calculate the weight loss due to the process. Length, width and thickness in different parts of the gonads (Fig. 1) were measured on the dried roes.

Figure 1 near here

2.6 Rheological characterization

The penetrometry test was carried out on the dried roes, using a TA-XT2 texture analyzer (Stable Micro Systems, Surrey, UK) with a 5 kg load cell, equipped with a 4-mm diameter cylindrical flat probe and sample support platform with hole plate 7-mm. After thawing and prior to the test, samples were conditioned at 15°C for 3 h. The pre-test, test and post-test speeds were 1.0 mm s^{-1} each; the depth of sample penetration was 10 mm. The registration began with the measurement of the force when the probe was going down into the sample and ended when the probe returned to its initial position. A force/displacement curve was recorded (Salvatore, Pes, Mazzarello, & Pirisi, 2014), measuring both positive and negative values. Four parameters were measured: 1) the maximum force on the curve, Fmax (N), corresponding to the hardness; 2) the positive area (N mm), corresponding to the consistency (internal strength of bonds within a dried roes); 3) the negative maximum force, Negative Fmax (N) corresponding to the adhesive force necessary to detach from the cylinder of the dried roes; 4) the negative area (N mm), corresponding to the adhesiveness (necessary work to overcome the attractive forces between the surface of the dried roes and the materials with which it came into contact). The cylinder probe was cleaned after each measure. Measurements were carried out in triplicate.

2.7 Colorimetric Analysis

Colorimetric parameters (CIE coordinates: L*, a*, b*; illuminant C) were recorded using a Chroma Meter CR-400 (Konica Minolta; Milan, Italy) colorimeter (diffusion illumination 0º viewing angle geometry). Data were recorded on the 18 fresh roes before freezing and after thawing, and on the 18 salted and on the 18 dried roes only after thawing. Parameters were recorded in the central part of each gonad and results were averaged.

2.8 Statistical Analysis

Data were analyzed through a one-way ANOVA ($p = 0.05$) (Minitab 16 Statistical Software (2010), State College, PA: Minitab, Inc.). The model included the effect of the three steps in the manufacture process (F,

3 levels; fresh, salted and dried roes). The comparison between means was performed using Tukey's significant difference test. Linear Discriminant Analysis (LDA) and Principal Component Analysis (PCA) were performed using a R-based software developed by the Group of Chemometrics of the Division of Analytical Chemistry of the Italian Chemical Society, freely downloadable from the site gruppochemiometria.it. LDA model was validated by using the Cross-Validation method with 5 deletion groups.

3. Results and Discussion

3.1 Chemical analysis

Before reporting the chemical results obtained in this work, some considerations have to be done regarding the data reported in literature about the chemical characterization of *Bottarga*: Table 1 shows the macro composition of fresh and dried mullet roes reported in literature.

Table 1 near here

To properly discuss data, also the differences in the manufacturing process used in the different countries to obtain *Bottarga* have to be considered. Unfortunately, not all the studies in the literature reported in detail a careful description of the manufacture process: mullet roes from Turkey (Celik et al., 2012) subdued a salting step lasting 2.5 to 6 hours, but no information is reported about the lasting of the drying step which was performed pressing the roes with wooden blocks (250 g); Lu et al. (1979) reported a salting step performed with brine (15% NaCl) overnight, and a drying step in which roes were kept under pressure for 1-2 days and dried for another 3-5 days. Greek mullet roes subdue a salting step lasting 2-3 h and a drying step of 3-4 days in which roes were kept into casts; Greek dried roes were then covered with melted beeswax (usually eight layers) (Kalogeropoulos et al., 2008).

Moreover, different analytical protocols were used in literature to determine the macro constituents of *Bottarga*: for moisture content, Celik et al. (2012) dried the samples at 105°C to constant weight; Barra et al. (2008) reported the drying of the samples at 60°C until constant weight was achieved; Lu et al. (1979) just reported the reference (AOAC, 1970); Kalogeropoulos et al. (2008) determined moisture gravimetrically after freeze drying the roes. For protein content, Barra et al. (2008), Celik et al. (2012), and Lu et al. (1979), used the Kjeldhal method converting the N content into protein content by multiplying for the factor 6.25 whereas Kalogeropoulos et al. (2008) used the Kjeldhal obtained N content multiplied by the factor 5.70. For fat determination, Celik et al. (2012) used the Bligh and Dyer method (extraction with chloroform/methanol 1:2) whereas Lu et al. (1979) and Kalogeropoulos et al. (2008) used the Folch method (extraction with chloroform/methanol 2:1); Barra et al. (2008) performed an extraction with a Soxhlet apparatus using diethyl ether/petroleum ether 1:1. Salt content was determined by Barra et al. (2008) and Lu et al. (1979) through the Volhard's method (Volhard, 1875).

These differences in the manufacturing process and on the analytical protocols could lead to variations in the gross composition of the final products, which could be more consistent than the variations due to the geographical origin of the fishes.

As can be seen in Table 1, the pH value for fresh roes was reported only by Celik et al. (2012) (5.79) whereas the pH values measured in dried roes ranged from 5.3 to 5.86. Dried roes of Sardinian origin were reported to have a pH value of 5.3 (Barra et al., 2008).

As what regards moisture, fresh roes of Turkish origin (Celik et al., 2012) were reported to have a mean value of 50.2%, whereas fresh roes of the USA origin (Lu et al., 1979) presented a mean value of 61.5%. The differences found in the unprocessed fresh products could be due to the different geographical origin of the fishes from which the roes came, but also by variables related to not described processing steps and by analytical bias. Widely variable is the moisture content of dried roes reported in literature: this variability may be due both to the different geographical origin of the fishes and most likely to the adopted manufacture process which involves a loss of moisture. Dried roes of Brazilian origin were reported to have a mean value of moisture of 22.4% (Barra et al., 2008) whereas dried roes of US origin reached a mean value of 30.5% (Lu et al., 1979). Greek mullet dried roes were reported to have a moisture content of 45.1% (Kalogeropoulos et al., 2008): the authors ascribed the high moisture content to the presence of the

beeswax applied on the already dried roes, which should prevent additional loss of water, but this high value was most probably due to differences in the manufacturing process. In fact, the combination of the salting and drying steps was limited in time (2-3 h and 3-4 days respectively) with respect to those used in other countries. Published moisture content in dried roes of Sardinian origin has a mean value of 29.5% (Barra et al., 2008).

As what regards the other macro-components of *Bottarga* reported in literature, a great variability in protein, fat and NaCl contents measured on the dried weight of edible part both in fresh and in dried roes was observed. In fact, protein contents (expressed on the dried weight) in fresh mullet roes from US and Turkey were reported to be 58.7% (Lu et al., 1979) and 51.6% (Celik et al., 2012) respectively; in dried mullet roes, protein content on the dried weight varied widely from 43.0% for roes of fishes caught in the FAO31 area (Western Atlantic Ocean) (Barra et al., 2008) to 67% (the reported value was converted by using the factor 6.25 instead of the reported 5.70) for roes of fishes caught in the Eastern Ionic Sea (Greece) (Kalogeropoulos et al., 2008). Dried mullet roes of Sardinian origin were reported to have an average protein content (on the dried weight) of 47.7% (Barra et al., 2008). Data reported in literature regarding the fat content (expressed on the dried weight) ranged from 26.3% in fresh roes of Turkish origin (Celik et al., 2012) to 35.6% in fresh roes of US origin (Lu et al., 1979); a great variation on the fat content in dried roes has also been reported in literature, with a minimum value on samples of Sardinian origin (13.3%) (Barra et al., 2008) and a maximum value of 37.0% in samples of US origin (Lu et al., 1979). Dried mullet roes of Sardinian origin were reported to have an average fat content (on the dried weight) of 13.3% (Barra et al., 2008). No published data is available regarding the NaCl content in fresh mullet roes, whereas dried roes were reported to have a NaCl value expressed on the dried weight, varying from 4.3% in dried roes of Sardinian origin (Barra et al., 2008) to 8.0% for dried roes from Mauritania (Barra et al., 2008).

The great variability reported in the literature for all the chemical parameters measured in mullet roes is somewhat unexpected, and this is quite puzzling for parameters measured for the fresh roes, evidently unaffected by differences in the technological process used to obtain Bottarga. It has to be reported that most of the data found in the literature are likely to be affected by a poor accuracy: in fact, as can be seen in Table 1, in most of the cases, the sum of all the chemical constituent values measured on the dried weight of the roes, reached a value that is too far from the hypothetical 100%. Both in the works of Barra et al. (2008) and Celik et al. (2012), this value reached about 78% in the best case. Celik et al. (2012) explained this underestimation by the presence of carbohydrates, which were not analytically measured, but were estimated by difference, whereas no explanation has been found in the work of Barra et al. (2008). Fat content was the most underestimated parameter: in fact the sum of the constituents values got lower when the fat content was lower. Moreover, all the values of the macro-constituents of mullet roes reported in literature, with the exception of those reported by Kalogeropoulos et al. 2008, did not present the measurement uncertainty, so they lacked of indication about the variability of the data.

Therefore, the only possible comparison between our data and data in the literature regarding the fresh roes is to be done with the values reported by Lu et al. (1979) whereas the comparison of data regarding the dried roes is to be done with the values reported by Kalogeropoulos et al. (2008) and Lu et al. (1979). Table 2 shows the physical-chemical characterization of fresh, salted and dried roes studied in this work.

Table 2 near here

The pH value obtained in the fresh roes decreased during the manufacture process, with a significant difference between fresh and dried roes, reaching a mean value of 5.46 in the final product. The greatest variability (CV = 2.8) was observed for the dried roes.

The mean value of the moisture content in the fresh roes was 53% of edible part, decreasing on average to 49% of edible part after the salting step, and reaching the value of 25% of edible part after the drying step (Table 2). The loss of water was such that the moisture differences between fresh, salted and dried roes were significant (P = 0.05). The greatest loss of water and the highest variability of this parameter (CV = 8) was observed during the drying phase of the roes. As what regards the other main constituents, the protein content on the dried weight in the fresh roes had an average value of 58.8%, which gradually decreased during the process to reach an average value of 56% in the final product. The values resulted to be statistically different for all the three steps of the manufacturing process. The average fat content (values on the dried weight) is 36% in the fresh roes, decreasing to 34% after the salting step (P < 0.05) and keeping constant after the drying step. NaCl content on the dried weight in the fresh roes was low (0.49%). After the salting step NaCl content obviously increased, reaching a value of 3.5% and 3.6% in salted and dried roes, respectively. NaCl values of fresh and salted roes were significantly different (P < 0.05).
Little differences respect to those values reported by Lu et al. 1979 and Kalogeropoulos et al. 2008 are present regarding pH and the fat and protein contents measured on the dried weight of the fresh roes. On the other hand, a great variation has been found on the moisture content, with a difference of 8.5% between the value we reported and the one reported by Lu et al., 1979. As what regards the composition of the dried roes, the differences are meaningful (with the exception of pH), and this could be related to the different technological process used to obtain *Bottarga*. In fact, the composition of the final products differed even if the raw material (fresh roes) had a similar composition (Lu et al., 1979). In our study, the protein and the fat contents (on the dried weight) got respectively lower of 3% and 2% after the manufacturing process, whereas the NaCl content increased by 3.1%. In the work of Lu et al. (1979), the protein content on the dried weight decreased by 7.6%, whereas the fat content increased by 1.4%. As reported by Lu et al. (1979), NaCl content was not measured in the fresh roes but, as it has to be assumed to have a low value, the variation of NaCl content due to the process was more significant than the one obtained in this study: in fact, the final product had a final content greatly higher than the one we determined in the dried roes (7.5% *vs* 3.6%). Therefore, the technological process reported by Lu et al. (1979) caused a greater loss of proteins and a greater absorption of NaCl than those obtained in the manufacturing process of *Bottarga* reported in this work. Greek dried roes presented a higher protein content and a similar fat content on the dried weight (Kalogeropoulos et al., 2008) respect to the ones we obtained. Since no information was reported in the study of Kalogeropoulos et al. (2008) about the chemical changes during the process, a comparison of the differences caused by the process is not possible.

3.2 Biometric evaluation

The weight of fresh, salted and dried roes, the weight losses consequent to the salting and the drying processes, and the results of the biometric analysis of the dried roes are reported in Table 3.

Table 3 near here

As programmed in the sampling plan, the weight of the fresh roes ranged between 251 and 354 g. The weight of the dried roes ranged between 148 and 217 g. The average loss of weight due to the salting process (14 g; 5%) was minimal; in addition the loss of weight due to water is partially compensated by the weight of the salt added during the salting step. Most of the loss of weight happened during the drying step with an average decrease of 94 g (34%).
As what regards the biometric evaluation of the dried roes, about half of the samples (55%) had a length (L) greater than 150 mm and more of the 70% had a thickness (T1) greater than 15 mm. Only 30% of the samples had a width (W2) larger than 85 mm.

In literature, dried mullet roes of Mauritanian origin (Beddih et al., 2005) were reported to have a length ranging from 140 and 190 mm (mean ± sd = 163 mm ± 20): the length decrease which followed the manufacture process consisted of 22%. The weight ranged from 97 to 200 g (mean ± sd = 135 g ± 44), and the average decrease related to the manufacture process was 38%, similar to the value 37% we obtained. Greek dried roes are reported to weight on average 269 g (without considering the beeswax which has an average weight of 47.5 g) (Kalogeropoulos et al., 2008). *Bottarga* analyzed in this study is therefore bigger than those reported in the literature with the exception of the Greek product.

3.3 Rheological analysis

The calculated results of the penetrometry test on all the dried roes are shown in Table 4.

Table 4 near here

The average value 11.2 N of the Fmax (corresponding to the hardness parameter) presented the lowest dispersion (CV = 22) of data: nonetheless, the force magnitude for breaking the envelope of *Bottarga* and for penetrating into the samples was different, ranging from 7.6 to 15.4 N. The large dispersion of data (CV = 31) around the mean value (111 N) of the positive area indicates that even the consistency of *Bottarga* was different for each sample. The greatest coefficients of variation were obtained for the negative area (CV = 42) and for the negative Fmax (CV = 40), both indices of the adhesiveness of the samples of *Bottarga* which therefore were the most variable among the rheological parameters. The negative Fmax was lower than the positive Fmax indicating that the force for coming out of the gonads was lower than the one needed for the penetration.

Fig. 2 shows the biplot of the Principal Component Analysis performed on the dried roes using both rheological and chemical results.

Figure 2 near here

As can be seen looking at the position of the variables in the biplot, some correlations between gross composition and rheological parameters were found: moisture was negatively correlated with hardness (r = -0.87) and consistency (r = -0.88) whereas fat content was negatively correlated with adhesiveness (r = -0.68). No correlations were found between the rheological parameters and the protein and NaCl values. Moreover, hardness and consistency presented a negative correlation with the biometric parameter thickness in the large section of the gonads (T2) (r = -0,674 and r = -0,743 respectively); this could be due to the fact that a more thickly gonad generally had a higher moisture value and consequently was less hard than a less consistent gonad.

3.4 Colorimetric analysis

Table 5 shows the colorimetric parameters measured on all the sampled roes (fresh, salted and dried) after thawing and Fig. 3 shows the biplot of the Principal Component Analysis obtained on the colorimetric parameters of all samples.

Table 5 near here

Figure 3 near here

As can be seen in the biplot, the dried roes are separated from the fresh and salted roes along the first component which is described by the L* (lightness) and the b* (yellowness) parameters which decreased

during the productive process of *Bottarga*. The a* (redness) parameter explains most of the variance of the second principal component, which in part separates the fresh by the salted roes. The L* parameter significantly decreased at the end of the drying step compared with the salting step, whereas the b* parameters significantly decreased in all the steps of the process (p=0.05); these decreases are related to the water loss happening during the manufacture stages, in fact a high correlation between L* and the moisture content (r=0.97) and b* and the moisture content (r=0.92) have been found: roes became therefore darker following the processing. Instead, the a* parameter significantly decreased only between fresh and salted roes, remaining basically unchanged in the drying step. No data in literature was found regarding the colorimetric evaluation of *Bottarga*.
Table 6 shows the colorimetric parameters L*, a*, b* measured on the fresh roes before freezing and after thawing.

Table 6 near here

A significant difference was obtained on the L* parameter which decreased with the process of freezing/thawing and on the a* parameters which instead increased; substantially the fresh roes became less light and more red after thawing. This trend can be seen in the biplot of the Principal Component Analysis in Fig. 4, in which a separation is clear between the two groups of samples, mainly due to the changes in the L* parameter.

Figure 4 near here

3.5 Building of linear discriminant model

The three colorimetric parameters (L*, a*, b*) measured on the fresh roes both before the freezing process and after the thawing were used to build a linear discriminant model able to discriminate the fresh samples about the presence of the process of freezing. The two classes ("not frozen roes" and "frozen roes") consisted in 18 samples each, therefore we used the Cross Validation method to assess the capability of the model in the discrimination of the samples. As can be seen in Table 6, the LDA model was able to correctly classify in Cross Validation 100% of the samples of fresh roes for both classes. This result showed that the colorimetric parameters combined with the LDA multivariate approach are an important tool in the identification of *Bottarga* produced from not frozen or frozen roes. This colorimetric/multivariate approach could be used to discriminate roes of local origin (Sardinian) from the imported ones; this can be possible because only the imported roes subdue a freezing process to avoid degradation during transport while roes of local origin are handled soon after the evisceration of the fishes which follows the catching of the animals.
To assess the capability of the discriminant model on unknown samples, the LDA model should be validated using a set of independent samples not used in the building of the model. The technique could be very useful and our future goal will be to calibrate and validate the method on dried roe samples, including local and imported roes, in order to be able to discriminate whole commercial samples regarding their origin. In the context of a certification of origin, such as PDO and PGI, this approach could be used as a fast, easy, non-destructive and cheap method, capable to evaluate commercial *Bottarga* samples for the real time assessing of their origin (local or imported).

4. Conclusions

The manufacture process for obtaining *Bottarga* produced in Sardinia caused in the roes a significant decrease of pH, moisture, fat, and protein and an increase of its NaCl content. Rheological parameters hardness and consistency were negatively correlated with moisture whereas adhesiveness was negatively correlated with the fat content. Protein and NaCl values were not correlated with any rheological parameter. All CIELab parameters decreased during the manufacturing of the roes which became darker after the process. Colorimetric parameters also changed when roes were subdued to a freezing process and these values were used to build a preliminary LDA model that could be used to discriminate if unknown fresh roes have or have not undergone a freezing process. Since a freezing process is used only on the imported roes, the importance of this model relies on its potential to be a valid, fast, cheap, non destructive and a real time technique to be used in the discrimination of samples of *Bottarga* regarding their origin (local or imported).

Acknowledgements

In our mind always will be remembered Fabrizio Selenu, President of “Cooperativa Pescatori Tortolì” deceased on December 2015, the first enthusiastic supporter of this study, who kindly supplied the biological samples and made available the plants and the operators of the “Cooperativa” for the manufacturing of *Bottarga*. This research was supported by Legge regionale no. 7, 7 Agosto 2007, “Promozione della ricerca scientifica e dell’innovazione tecnologica in Sardegna” (Delibera G.R. n. 32/8 26 luglio 2011; Determinazione Centro Regionale Programmazione n. 11362/1620, 28.12.2011).

Caption for figures

Fig. 1. Scheme of the biometric measurements carried out on the dried roes: W1 (width in the short part); W2 (width in the large part); W3 (intermediate width of a gonad); W4 (intermediate width of a gonad); T1 (thickness in the short section); T2 (thickness in the large section); T3 (intermediate thickness); L (length)

Fig. 2. Biplot of the PCA obtained on the dried roes using chemical and rheological parameters

Fig. 3. Biplot of the Principal Component Analysis obtained on the colorimetric parameters of all the samples

Fig. 4. Biplot of the PCA performed on the colorimetric data measured in fresh roes before freezing (raw) and after thawing (thawed)

Table 1. Comparison between the physical-chemical mean values obtained in this work and the literature data on fresh and dried *Mugil Cephalus* roes

Fresh roes							
	Origin	**pH**	**Moisture[1]**	**Proteins[2]**	**Fat[2]**	**NaCl[2]**	**Sum of the constituents on the dried weight**
Celik et al., 2012	Turkey	5.79	50.2	51.6	26.3	n.a.	77.9
Lu et al., 1979	USA	n.a.	61.5	58.7	35.6	n.a.	94.3
Salted and dried roes							
	Origin	**pH**	**Moisture[1]**	**Proteins[2]**	**Fat[2]**	**NaCl[2]**	**Sum of the constituents on the dried weight**
Celik et al., 2012	Turkey	5.86	26.3	56.7	18.1	n.a.	74.8
Lu et al., 1979	USA	n.a.	30.5	51.1	37.0	7.5	95.6
Barra et al., 2008	Brazil	5.6	22.4	48.9	14.2	6.8	69.9
Barra et al., 2008	Mauritania	5.6	28.1	49.6	20.8	8.0	78.4
Barra et al., 2008	USA	5.3	23.2	48.2	16.9	5.8	70.9
Barra et al., 2008	Sardinia	5.3	29.5	47.7	13.3	4.3	65.3
Barra et al., 2008	FAO 31 area	5.6	31.4	43.0	21.3	6.8	71.1
Kalogeropoulos et al., 2008	Greece	n.a.	45.1 (7.8)	67[3] (12)	32 (5)	n.a.	93

[1] Data expressed in g/100g of edible part; [2] Parameters expressed as percentage of the dried weight of edible part; [3] The literature protein value was divided by 5.70 and multiplied by 6.25 for data comparison; n.a. = not available.

Table 2. Effect of the manufacture process on pH and on gross composition of fresh, salted and dried roes. Results are expressed as mean (Coefficient of Variation, CV) and ranges

	Fresh roes		Salted roes		Dried roes		P value
	Mean (CV)	**Range**	**Mean (CV)**	**Range**	**Mean (CV)**	**Range**	
pH	5.60[a] (1.25)	5.45-5.75	5.53[ab] (1.4)	5.30-5.62	5.46[b] (2.8)	5.17-5.63	*
Moisture[1]	53[a] (2)	50.2-54.1	49[b] (2)	47.4-52.27	25[c] (8)	21.5-27.4	*
Proteins[2]	58.8[a] (2.6)	56-60.7	57[b] (2)	55-60.8	56[c] (2)	53.2-58.4	*
Fat[2]	36[a] (3)	34.9-39.9	34[b] (3)	30.65-36.5	34[b] (3)	32.6-37.0	*
NaCl[2]	0.49[b] (12)	0.392-0.58	3.5[a] (11)	2.70-4.2	3.6[a] (17)	2.46-4.37	*

[1] Data expressed in g/100g of edible part; [2] Parameters expressed as percentage of the dried weight of edible part. Values within rows not sharing a common superscript are significantly different (P<0,05).

Table 3. Weight of fresh, salted and dried roes, loss weight during salting and drying process, and biometric parameters of the dried roes. Results are expressed as mean (Coefficient of Variation, CV) and ranges

Weights and weight losses of the roes	**Mean (CV)**	**Range**
Weight of fresh roes (n=18), grams	291 (10)	251-354
Weight of salted roes (n=18), grams	277 (10)	239-332
Weight of dried roes (n=18), grams	183 (12)	148-217
Weight loss after the salting step, %	5 (40)	0.5-7
Weight loss after the drying step, %	34 (6)	30-42
Biometric parameters of the dried roes	**Mean (CV)**	**Range**
W1 (width in the short part), mm	46 (15)	38.8-66.6
W2 (width in the large part), mm	84 (7)	76.3-99.2
W3 (intermediate width of a gonad), mm	42 (7)	38.1-51.3
W4 (intermediate width of a gonad), mm	40 (7)	36.1-45.5
T1 (thickness in the short section), mm	14 (14)	10.0-17.2
T2 (thickness in the large section), mm	16 (12)	13.8-19.9
T3 (intermediate thickness), mm	16.8 (9)	13.9-19.8
L (length), mm	150 (6.7)	132.2-170.0

Table 4. Rheological parameters of the dried roes (n=18). Results are expressed as mean (Coefficient of Variation, CV) and ranges

Rheological parameters	Mean (CV)	Range
Fmax (N): Hardness	11.2 (22.3)	7.6-15.4
Positive Area (N mm): Consistency	111 (31)	74.5-182.3
Negative Fmax (N): Adhesive Force	2.1 (40)	0.7-4.1
Negative Area (N mm): Adhesiveness	26 (42)	6.8-48.4

Table 5. Colorimetric parameters measured on fresh, salted and dried roes after thawing. Results are expressed as mean (Coefficient of Variation, CV) and ranges

	Fresh roes (n=18)		Salted roes (n=18)		Dried roes (n=18)		
	Mean (CV)	**Range**	**Mean (CV)**	**range**	**Mean (CV)**	**range**	**P value**
L*	59.0^{a} (4.2)	55.3-64.6	58^{a} (7)	51.2-70.0	30^{b} (7)	27.8-33.1	*
a*	6^{a} (30)	2.7-10.9	4^{b} (50)	0.4-7.3	4^{b} (50)	0.9-6.6	*
b*	42^{a} (17)	29.8-53.1	36^{b} (20)	25.2-50.9	12^{c} (20)	7.4-16.4	*

Values within rows not sharing a common superscript are significantly different (P<0,05).

Table 6. Results obtained for the colorimetric evaluation of the fresh roes before freezing and after thawing (results are expressed as mean (Coefficient of Variation, CV) and ranges) and results of the Linear Discriminant Analysis performed on colorimetric parameters used to classify samples based on the presence of a freezing process.

Colorimetric Parameters						Linear Discriminant Analysis		
	Before freezing		After thawing			Confusion Matrix in Cross Validation		
Fresh	**Mean (CV)**	**Range**	**Mean (CV)**	**Range**	**P**			
L*	75[a] (3)	71.6-79.1	59.0[b] (4.2)	55.3-64.6	*		**Not Frozen**	**Frozen**
a*	5[b] (40)	2.2-8.0	6[a] (30)	2.7-10.9	*	**Not Frozen**	18	0
b*	46[a] (22)	28-61	42[a] (17)	29.8-53.1	ns	**Frozen roes**	0	18

Values within rows not sharing a common superscript are significantly different (P<0,05).

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Figure 1

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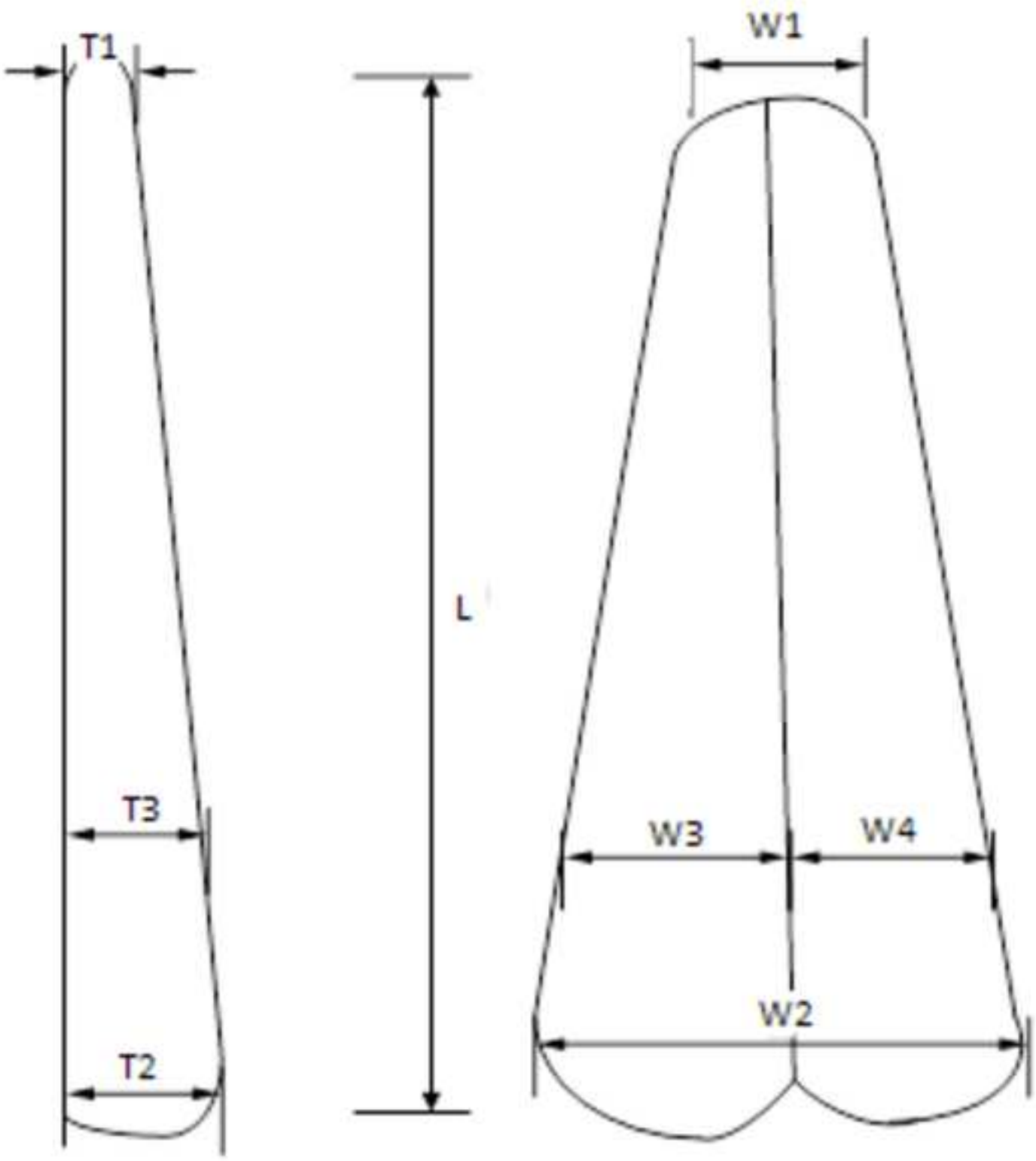


Figure 2

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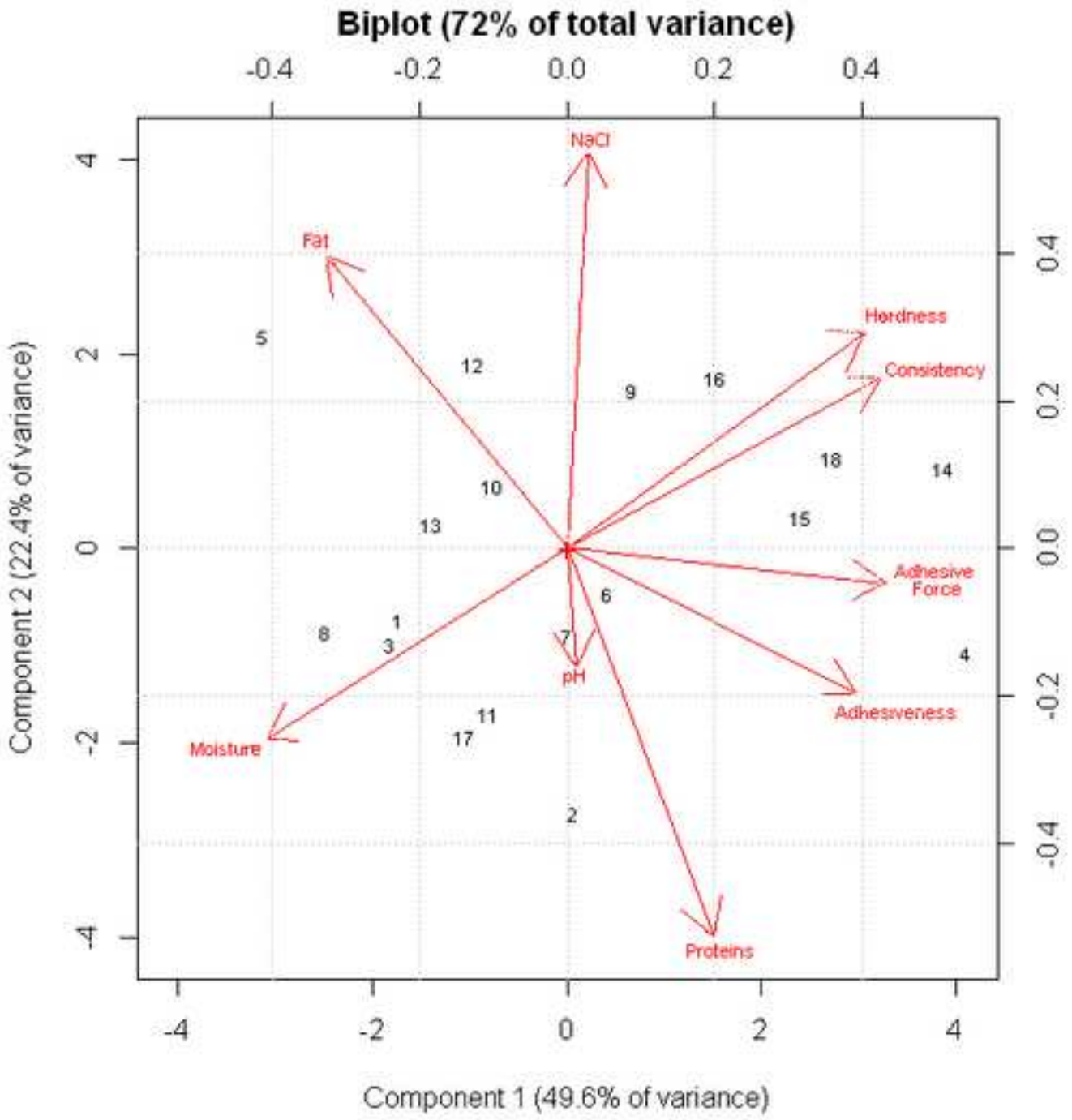


Figure 3

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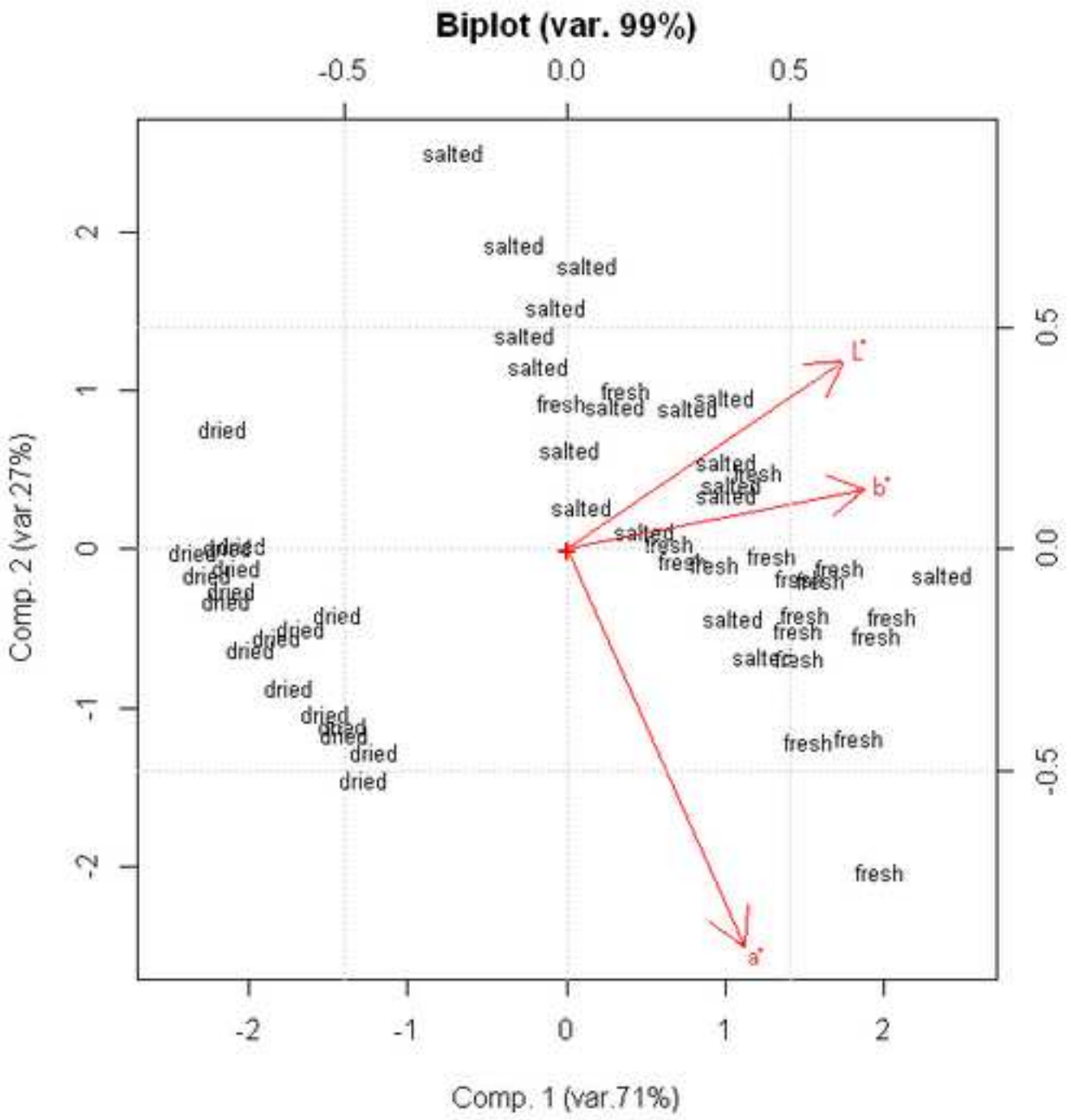
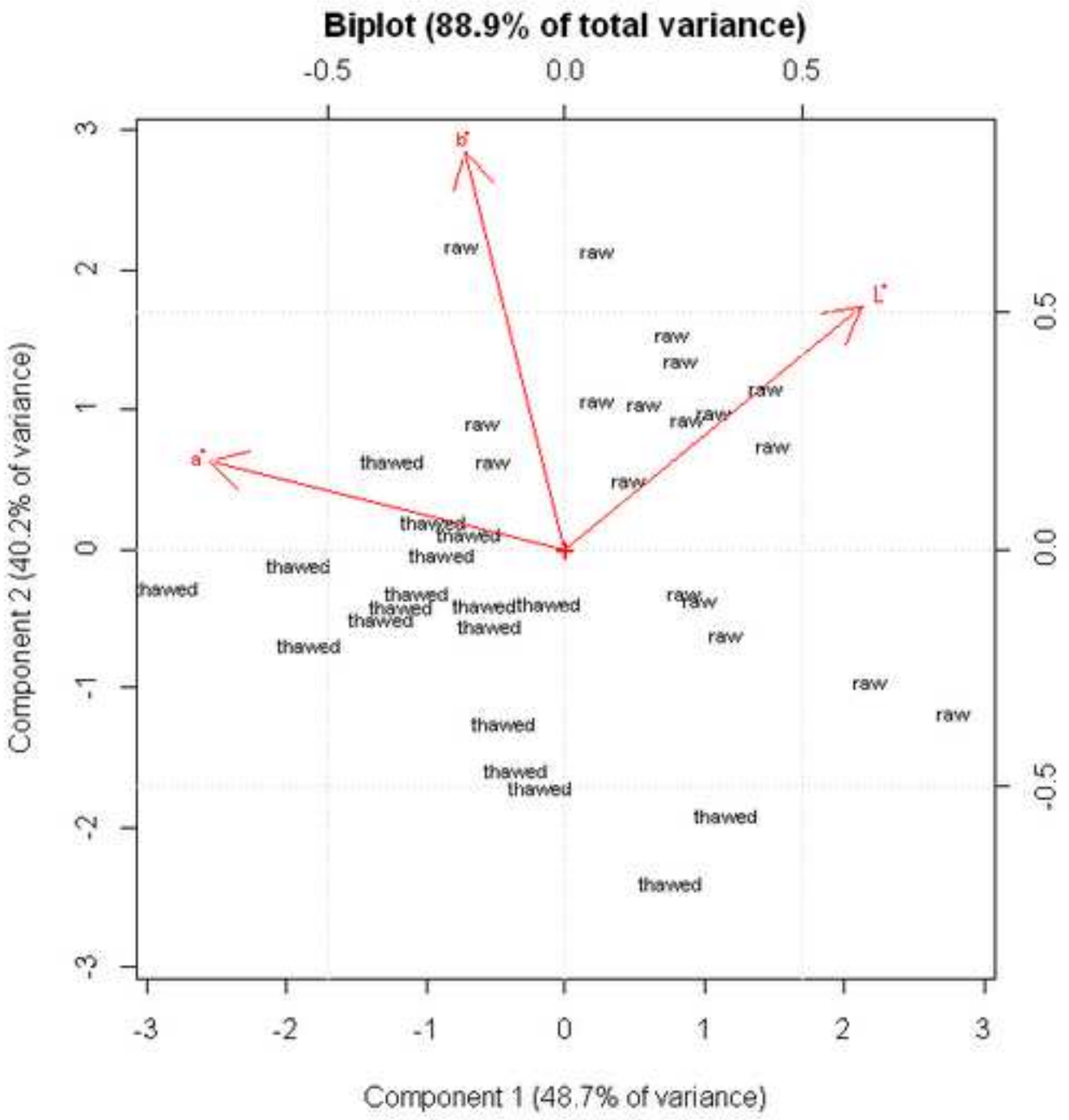


Figure 4

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