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Relation of quality and sensory perception with changes in free amino acids of thawed seabream (*Sparus aurata*).

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Abstract

This study aimed to investigate how the freshness before frozen storage affect the quality and sensory characteristics of seabream in different commercial presentations and to correlate the findings with free amino acids composition. The fish were slaughtered, allocated to three processing treatments (whole, gutted and filleted) and stored at refrigeration (0 +/-1°C) for different times (5, 9, 11 and 18 days) before one-month frozen storage (-30 °C). After this time, physicochemical (pH, TVB-N, TBARS and free amino acids), bacterial count and sensory evaluation (Torry Index & Quality Descriptive Analysis -QDA-) were studied. Significant differences were found among treatments over time for TVB-N, TBARS and bacterial growth. The quality index (Torry) exhibited a gradual decrease. QDA showed that fillets had the lowest assessment. Free amino acids contents varied significantly during frozen storage with a particular behavior that depended on the previous treatment applied and the fish freshness degree (elapsed days before frozen).

Keywords

Fish quality

Free-amino acids

Sensometrics

Seafood technology

PLS-DA

1. Introduction

Sea bream is one of the most cultivated species in the Mediterranean area being the leading producers Turkey, Greece and Spain, that covered 87.2 % of the European production in 2015 (EFAH, 2016). The demand for fresh sea bream has increased significantly over the past decade in Europe due to its desirable aroma and quality, and consequently, its high value has made the farming of the fish a profitable business (Alasalvar, Taylor, Oeksuez, Garthwaite, Alexis & Grigorakis, 2001). Total increase in seabass and seabream production in 2017 is expected to be some 8 to 12 % this year, with relatively higher growth projected for seabream. These figures have also been reflected in the export statistics of both Greece and Turkey, the two most significant producers, which showed substantial gains in 2017 (FAO, 2016).

Fresh seabream is stored in ice with a temperature close to 0˚C for commercial purposes, which has proven to be an effective strategy to delay fish spoilage. Fish freshness has always been acknowledged as one of the essential integrated quality attributes for assessing the quality of fish, either for direct consumption or as raw materials for the processing industry (Chen & Sun, 2014). Freshness can be described by a number of sensory, biochemical, microbial and physical parameters, and can, therefore, be defined as an objective attribute (Ólafsdóttir et al., 1997). The freshness degree is determined by the physical and chemical changes that appear in the fish once it has been slaughtered and is closely associated with the way of manipulation and storage after death (Rodriguez et al., 2006). Fish processing, namely that processes associated with fish between the time it is caught or harvested, and the time the

final product is delivered to the customer, represents a critical aspect for quality product. This term involves preliminary processing of raw material, in the case of whole fish, and the application of primary processing such as gutting or filleting of fish before it be refrigerated or frozen. Moreover, sensory characteristics of fish such as taste, smell and texture are expected to be critical determinants for fish consumption, and they are essential to evaluate freshness (Carlucci et al., 2015). Those changes have a direct effect upon consumer acceptance (Hernández, López, Álvarez, Ferrandini, García & Garrido, 2009). Unfortunately, even when stored in ice, fish will not keep in first-class condition for more than a week or so, and it is therefore condemnable after 14 to 16 days. To address this problem, freezing would be an alternative preservation method. Fresh fish adequately frozen and stored, will keep in good condition for a long time (Banks, 2001). Freezing by itself is not able to guarantee appropriate sensory characteristics of fish because it does not improve the previous quality of raw material. Final quality depends on the quality of the fish at the time of freezing as well as on other factors related to freezing technology, storage and distribution (Johnston, Nicholson, Roger & Stroud, 1994). Long-term frozen fish may suffer chemical changes and dehydration during storage with resulting toughening and flavor changes including the development of distinctive and undesirable frozen storage flavor, which finally affect consumer purchase and acceptance (Caballero et al., 2011; AGRIMER, 2010).

In salted-fermented fish, stored at room temperature, changes in the muscle and the free amino acid composition after processing and storage have been observed (Rabie, Simon-Sarkadi, Siliha, El-seedy & El Badawy, 2009). Free amino acids undergo considerable modification during processing and chilled

storage of fish, and therefore the different technologies used decisively influence the free amino acid profile (FAP) throughout storage (Ruiz-Capillas & Moral, 2001). Studies on variations in FAP in fish kept on ice are to be considered of great interest, since they reveal the proteolytic processes that happen after slaughtered in fish, and they could, therefore, be used as quality control indexes (Ruiz-Capillas & Moral, 2001; Sakaguchi, Murata & Kawaii 1982).

The relationship between free amino acid composition and sensory quality has not been widely studied in thawed seabream. As far as we know, this research is the first attempt to correlate sensory perception and quality with free amino acid composition and other physicochemical parameters. In this sense, it may be argued that the freshness degree before freezing and the way of early postmortem fish processing are the most crucial parameters for final quality in thawed seabream that could be related to free amino acid composition changes.

2. Materials and Methods

2.1 Sampling procedure

The sampling procedures developed in this research were the following: A batch composed of 405 gilthead seabream juveniles (IBW of 25 g) from the local fish farm (Castellón, Spain) were transported to the Fish Nutrition Laboratory of the Polytechnic University of Valencia. Once received in the laboratory, all the fish were randomly distributed to each tank (45 fish/tank) and subjected to a period of acclimatization. The trial lasted 12 weeks and 60 fish with medium size (400 g in average) from the initial stock were randomly

sacrificed by a lethal bath of clove oil (Guinama®, Valencia, Spain) containing 150 mgL−1. Fish were used to prepare the different treatments of the study and for analytical assays. Three treatments with 20 fish each one were designed: Whole (W), Gutted (G) and Filleted (F), like a traditional ways of selling in supermarket and fishmonger. All fish were kept in ice (0 +/- 1 °C) in polyspan boxes for 5, 9, 11 and 18 days. These sampling times were selected taking into account a previous sensory assessment of iced gilthead seabream that showed that reject threshold was at 15 day of storage. However, the earliest and most pronounced changes occurred in gills color at 5 days, while eye shape and gills odor did it in 11 days (Huidobro, Pastor y Tejada, 2000). The critical time for freshness is located between 5 and < 21 day of storage and therefore were selected those sampling time. After each ice storage time, fish of all treatments were frozen (-30 °C) for 30 days. Elapsed this time physico-chemical, microbiological and sensory analysis were carried out in three animal from each treatments.

2.2 Physico-chemical analyses

Physico-chemical and microbiological analyses were developed in upper mass muscle of the fish identified on the slice samples at different steps processing. All determination were done in triplicate. The pH in fish and fillets were measured using a digital pH-meter with puncture electrode (Crison, model PH25) and the results were expressed as the average. TVB-N determination was carried out in a Kjeltec unit by direct steam distillation over boric acid, following the protocol described in the EC 2074/2005, Chapter III, “Determination of the Concentration TVB-N in Fish and Fish Products”.

Potential of lipid oxidation was measured by the 2-thiobarbituric acid (TBA) method (Pfalzgraf, Frigg & Steinhart, 1995). Fish samples of 10 g were taken and homogenized with 10% trichloroacetic acid using an Ultra-Turrax T25 (Janke & Kunkel, Staufen, Germany). Samples were centrifuged at 4000 rpm for 30 min at 10 °C and the supernatants filtered through quantitative paper. Two milliliters of the filtrates were taken and mixed with 2 ml of TBA (20 mM), homogenized and incubated for 20 min in boiling water. Absorbance was measured at 532 nm. The TBA-reactive substances (TBARS) values were calculated from a standard curve of malondialdehyde (MA), and expressed as MA mg/kg sample.

2.3 Free amino acid determination

Samples from dialysate were homogenised in 0.1 N HCl (dilution 1:5) thorough Ultra-Turrax® T25 (IKA, Germany) for 4 min. Afterwards, samples were centrifuged at 10,000 g and 4 ˚C for 20 min. Supernatant was collected and then filtered. Alfa-amino butyric acid, 2.5 mM was added as internal standard solution and subsequently samples were deproteinised with acetonitrile. According to the method of Bidlingmeyer, (1987) samples were derivatised with phenylisothiocyanate. The derivatised amino acids AA were analysed by reverse-phase HPLC in a Nova Pak C18 column (3.9 x 300 mm) (Waters Corporation, MA, USA) dotted with a Nova Pack C18 pre-column (3.9 x 20 mm). The separation was achieved in 65 min at 52 ˚C with a flow of 1 ml/min, using a gradient between two solvents: 70 mM sodium acetate at pH 6.55 with 2.5% of acetonitrile (solvent A) and water-acetonitrile-methanol,

40:45:15 v/v (solvent B). The detection was monitored in an UV detector at 254 nm

2.4 Microbiological analyses

Psychrotrophic viable count (PST) was studied in samples of whole fish, gutted fish and fillets. A piece of fish muscle (10 g) was taken from the dorsal region of each fillet, transferred aseptically into a stomacher bag (Seward Medical, UK), mixed with 90 ml of 0.1% peptone water containing 1% NaCl and homogenized for 60 s using a Stomacher (Lab Blender 400, Barcelona, Spain). It was determined by pour plate methods in Plate Count Agar (Merck, Darmstadt, Germany) using conventional dilution procedures. Plates were incubated at 7 days at 10 °C (FTC 90i Refrigerated incubator, VELP Scientifica S.L., Italy) (ISO 4833-1:2013).

2.5 Sensory analyses

2.5.1 Assessor selection and training

A panel of twelve selected assessors with previous experience in sensory analysis of fish freshness was chosen from the staff of laboratory of *Universitat Politècnica de València* in order to carried two different sensory analyses. Assessment of quality index (QI) in cooked cultured seabream (Alasalvar, Taylor, Oeksuez, Garthwaite, Alexis & Grigorakis, 2001) and a quality descriptive analysis -QDA- (Lawless & Heymann, 2010) in thawed whole fish and fillets for different storage times. The assessors had demonstrated sensory sensitivity in preliminary tests, received a considerable training and they were able to make consistent and repeatable sensory assessments of various

samples fish. The panel received a prior training with respect to the use of Torry assessment scoresheets and intensity scales to evaluate different attributes in fish according to requirements of ISO standards (ISO 8586: 2012). Along this process, panelists became familiarized with the different descriptors and their intensity scales in order to assess the samples in a more accurate form (Braghieri et al., 2012).

2.5.2 Procedure

The samples presentation was made taking into account a complete block design, where each assessor evaluated all the samples to achieve a proper balance. Fish samples were blind-coded by using 3-digit numbers, randomly selected. In each treatment tested, a fish portion (15.0 +/- 2.0 g) from loin area were cooked in sample containers (suitable for microwave cooking) with screw caps partially closed and using a microwave oven (*Codex Alimentarius*, 1999). A moderate warming of samples took place (600 W) for 45 s then served to the assessors directly after 30 sec. Water and bread without salt (neutral) was provided to the assessors for cleansing the palate between the samples. After testing, they had to register their valuation in the respective forms provided for that purpose. Sensory analysis was performed in a test room designed according to ISO guidelines (ISO 8589:2007).

2.5.3 Assessment of fish samples

Sensory evaluation was developed in two different times. The first round was carried out to establish QI index in thawed and cooked seabream from all treatment assayed based on the average obtained for odor, flavor and texture

valuation using a QI scoresheet (Alasalvar et al, 2001). This scale goes from 8 (very fresh) to 1 (rotten) where ≤ 4 represented “No admitted” (spoilage) according to criteria established in the UE Freshness Rating for whitefish (Council Regulation 2406/96). In a second round, a QDA was carried out to characterize the fish spoilage. For this purpose, in a previous training session a vocabulary of the sensory attributes was developed with selected assessors based on sensory descriptors collected in the Torry scheme for cooked seabream (Alasalvar et al, 2001) and taking into account the definitions of terms used in sensory assessment of fish established by Seafish (2010). Two well differentiated attributes, first represents an ideal condition (freshness) and second shows a poor quality (dull & spoiled), were selected for odor (seaweedy & stale), flavor (meaty & bitterness) and texture (dryness & softness). The attribute intensities were rated on structured graphical intensity scales. The scales had 11 point with a mid- point and verbally anchored at each end, the left side of the scale corresponding to the lowest intensity (value 0) and the right side to the highest intensity (value 10) of the attribute. To validate the discriminant ability of the selected descriptors for seabream spoilage degree, the panel also practiced the assessment of attribute definitions and their intensities during two sessions of training. All the descriptors were discriminant among treatments and were consequently retained for sensory training. The panel performance was checked following guidelines established in the section 8 titled “Analysis of Results” of the ISO standard 8556:2012 (ISO 8586: 2012). For that, analyses of variances (ANOVA) were made, one-way type for established the discriminatory capacity of the panel and ANOVA of three factors (products, assessors & session) to evaluate the reproducibility. The evaluation

of individual performance was carried out by determination of the individual coefficients of variation (CV) for each assessor.

2.6 Statistical analysis

Results were analyzed using a XLSTAT Version 2016 (Addinsoft©). Normally and homogeneity of variance were tested. (Kolmogorov-Smirnoff and Levene test respectively) and statistical analysis of data was done by ANOVA -two ways- (Treatment / storage time) taking into account interaction, an artificial factor (not measured) which reflects the interaction between two measured factors ($p < 0.05$), and Duncan like *a posteriori* test was used to assess significant differences among means. For stablish a general profile of the relationship among free amino acids, physico-chemical variables, microbiological count and sensory analyses on the quality of thawed seabream, a Principal Components Analysis (PCA) was carried out (Cardinal et al., 2011). The Partial Least Square Discriminant Analysis (PLS-DA), a logistic regression, was used for explaining and predicting the membership of observations to several classes (treatment applied to fish) using quantitative (physico-chemical parameters including free amino acids composition) and qualitative (sensory assessment attributes) explanatory variables. This chemometric analysis type is recommended when the number of observations is low and the amount of explanatory variables is high. It was developed using an Unscrambler software (V.10.2, CAMO©, Norway) and taking into account the recommendations indicated by Viljanen, Heinio, Juvonen, Kosso & Puupponen-Pimia (2014) for a validation procedure.

2.7. Ethical statement

The experimental protocol was reviewed and approved by the Committee of Ethics and Animal Welfare of the Universitat Politècnica de València (UPV), following the Spanish Royal Decree 53/2013 on the protection of animals used for scientific purposes (BOE, 2013).

3. Results and discussion

3.1 Physico-chemical analyses and microbiological count

Results for physico-chemical and bacterial count are reported in Table 1. TVB-N and TBARS were significantly different ($p < 0.05$) among treatments; specifically treatment filleted was different from the rest and showed the highest values from day 5. It was also the only treatment to show a significant increase over time with highest values of total volatile basic nitrogen and lipid oxidation (86.2 mg/100g and 1.5 mg/kg Malonaldehyde respectively). Regarding to oxidation in seafood and fish, Boonsumrej, Chaiwanichsiri, Tantratian, Susuki & Takai (2007) found that TBA values of shrimp increased during freeze–thaw cycles indicating an increase in lipid oxidation. This could be due to the release of oxidative enzymes and pro-oxidants from various ruptured cellular organelles. Microbial growth was observed in all treatments and significant differences between treatments F (filleted) and W (whole) were found ($p < 0.05$) but this last was similar to G. Bacterial counts it increased significantly over time in both treatments F and G, which showed higher values at the end of the trials with the exception of whole fish where counts remained relatively stable from day 9 to final sampling time. These results agreed with the findings

reported by Lougovois, Kyrana & Kyranas (2003) that indicated values from 10^8 to 10^9 ufc/g after 16 days of storage in iced seabream, although in our experimental design.

Grigorakis Taylor & Alexis (2003) indicated that sea bream, according to the EU categorization, should rather be included in the group of fish for which the TVB-N limit for human consumption was set at 25 mg N_2 / 100 g. In this vein, IFST (1999) established a limit for human consumption of 7 log cfu/g for fish as the maximum recommended. In Fillet, both parameters exceeded these values at day 9 while the rest of treatments (C & G) did it after day 18. Considering the above, C and G treatments should be considered as suitable for human consumption until 11 days of storage on ice and 1 month of frozen.

3.2 Free amino acid composition

Fish is known to have an excellent essential amino acid composition, which is the basis for it is recommendation for a balanced healthy diet. A total of 26 amino acids and one dipeptide were considered in this study; nevertheless, asparragine, citruline and tryptophan were not detected, thus reducing the amount to 23. The free amino acid profile in fish changes considerably depending on species, harvesting, year season, processing and storage (Moutinho et al., 2017; Dordevic, Buchtóva & Borkovcova, 2016; Rabie et al., 2009). Significantly different ($p < 0.05$) quantities of free amino acids were detected in all treatments (Table 2). W and G treatments were similar and significantly distinct ($p < 0.05$) from F, which showed the highest values. At the end of storage (18 days), treatment F showed a strong spoilage and provided a

total value of 1392 mg/100 g free amino acids, around three times higher than the rest of the treatments.

The most important variation occurred in essential amino acids, which also increased differently ($p < 0.05$) in all treatments over time. G and F showed a gradual increase in each sampling time. For its part, W exhibited an increase of 80 % only between day 9 and 11. Histidine was in most cases, the most abundant essential free amino acid, followed by lysine. It should be noted that both of them, together with tyrosine, are key factors for food safety since they are the main precursors of biogenic amines (Rabie et al., 2009). Despite seabream is considered a histidine-poor fish, treatment F provided a 176.8 mg/100g of amino acids precursors of biogenic amines (hystidine, lysine & arginine) after 18 days that would pose a major risk for public health because of potential allergic reactions. Regarding to the above, Parente et al. (2001) established that histamine quantities higher than 100 mg might cause slight, intermediate and intensive poisoning. On the other hand, the increase of free essential amino acids could cause a loss in the nutritional quality of fish, due to their probable degradation to biogenic amines. The ratio E/N (Dordevic et al., 2016) for free amino acids is a good approximation to estimate the production of free essential amino acids in each treatment. A value of 1 represents an equal production of essential and non essential free amino acids (Table 2). There were gradual E/N ratio increases in C and G treatments, which showed values around 0.18 - 0.27 for all sampling times. However, this ratio triggered to 11 days until values of 0.63 and continued to raise until nearly to 1 (0.81) at the final time (18 days) in F treatment, demonstrating that a similar production occurred for essential and non essential free amino acids. This production might

be due to chemical and enzymatic reactions where they act as substrates leading to formation of secondary products (Ruiz et al., 1999).

Taurine is to be considered a major free amino acid in seabream (≥ 150 mg/100g from the beginning). It exhibited significant increases ($p < 0.01$) over time, particularly in F, which differed significantly ($p < 0.01$) from the W and G treatments. Among non-essential amino acids only glutamic acid, glutamine, hydroxyproline seemed to remain stable. The remaining amino acids showed significant differences ($p < 0.05$) among treatments, being F different from the rest. It is to be noted that distinct behaviors were established within each treatment over refrigerated storage time and after 1 month of frozen storage.

3.3 Sensory analyses

Quality Index (Table 1) showed some small changes in sensory descriptors (odor, flavor and texture) of cooked cultured sea bream through 18 days of storage in ice and 1 month of frozen storage. Significant differences ($p < 0.05$) were established between W and the other treatments (G & F), being the former the best valued in the first 9 days of experiment. The decreasing behavior of these appeared only as a non-significant tendency. This finding might be confirmed by previous studies (Alasalvar et al., 2001; Grigorakis et al., 2003), that reported a loss of intensity in most of the sensory descriptors studied in fresh seabream throughout time. In agreement with this, a research carried out by Lougovois et al. (2003) demonstrated that during the first half of the edible storage life there was a continuous loss of intrinsic fresh odors and flavors characteristic of the species, until the flesh became insipid, flavorless, by about 10–12 days. Because of this, insipid or neutral characteristics in odor and flavor

may have interfered with the perception of the sensory assessors because they may be determinant of quality indices.

Quality descriptive analysis showed that the sensory score for “stale odor” increased linearly over time in all treatments and exhibited a slight rise in the final time (day 18). These results agreed with those reported by Parlapani et al. (2014) who demonstrated that opaque and dull appearance and stale odor appeared in seabream after 10 days of refrigerated storage (0 °C). Conversely, significant differences ($p < 0.05$) were found among treatments at any sampling time for “seaweed odor”. In general, W (r^2= 0.96) and G (r^2= 0.89) showed low scores and decreased linearly through the refrigerated storage while F showed a rise in the first 9 days followed by a sudden fall. Regarding “meaty flavor”, it was significantly distinct ($p < 0.05$) among treatments and throughout storage time. W and G fall slightly in a linear way (r^2= 0.75 and r^2= 0.86, respectively) meanwhile F remained stable. Low values were found for “bitternes” (≤ score 4) from the beginning in all samples, which could be referred to the fact that freezing had a negative effect, and not significant differences were detected among treatments. However, significant differences ($p < 0.05$) were found for sampling times in each treatment. W decreased linearly (r^2= 0.86) over time, meanwhile G and F treatments did it in a polynomial way due to a sudden increase from day 9 to final time (18). These findings confirmed that slight sour and off-flavors were evident in fish with a high degree of spoilage while cooked flesh could be still palatable (Lougovois et al., 2003).

Regarding texture, “dryness” represents a good sensory attribute for seabream; it is associated with toughness and adhesiveness, which are usually well evaluated at the 11th day of storage in ice (Huidobro et al., 2000). Low

valuations, below the middle value of the scale were found although they showed significant differences ($p < 0.05$) among treatments. These low values may be referred to the negative effect of freezing and frozen storage. The texture properties of sea bream significantly deteriorated during storage in ice. In this sense, the cytoskeletal protein dystrophin decreased, causing a muscle structure degradation that contributed to the softening of seabream flesh (Caballero et al., 2009). Softness, another important texture property increased in a similar way for all treatments over time. For each condition, significant differences were found throughout the refrigerated storage. W ($r^2 = 0.84$) and F treatment ($r^2 = 0.89$) exhibited a linear increase; meanwhile, G showed a noticeable increase in the last sample point being then the highest of the experience. It must be taken into consideration that texture assessment of cooked fish fillet might be difficult because of its dependence on the state of myofibrillar proteins. If proteins denature and lose the ability to hold moisture the cooked products may taste dry and stringy; otherwise oil content and amount of gelatinized connective tissue contribute to the pleasant sensation while chewing (Bremner, 2012).

3.4 Relationship among free amino acids profiles and quality parameters

Both physico-chemical results and microbial results were related to sensory perception (QI & QDA). Statistical multivariate analysis (PCA) showed excellent correlations between sensory and quality parameters results. Fig.1 exhibits the plot for thawed whole fish (W) where the two first principal components explained an 86.75 % of variation. A spoilage shifts from right to left and a clear separation among days was clearly appreciated. At day 5, the most highlighted

aspects were seaweed smell and meaty taste, located near to the freshness index (QI). Following this order, on the 9th day the first free amino acids (hydroxyproline and β-alanine) were situated in the upper right quadrant in the figure. After day 11, the highest microbial counts were found, and valine, taurine and lysine, that showed significant differences ($p < 0.001$) through time, were very close within the upper left quadrant of the plot. In addition, anserine, glutamic acid, prolyne and γ-aminobutyric acid, the latter being the only one with significant change ($p < 0.01$) were located near to stale odor and bitterness. This finding coincided with that of Sforza et al. (2001), who related bitterness with a higher amount of lipophilic amino acids (valine and proline) in dry cured ham.

At final time (18), moderate values of TBARS and soft texture were appreciated, as well as the presence of free aromatic (phenylalanine) and aliphatic amino acids (leucine and isoleucine) that may show affinity for lipids. Due to the above, strong off flavor and off odor were detected with concomitant sensory rejection (completely opposite to QI).

The relationship among physico-chemical, microbial and sensory results in thawed gutted seabream (G) is shown in Fig.2. The two principal components explained an 88.92 % of variation. The spoilage shift occurred from left to right, opposite to that found in whole fish and showed, too, a clear separation by days. At day 5, fish had a meaty flavor and seaweedy odor, indicating a satisfactory freshness (QI) and valine was closed to them. This latter is a branched molecule with a high affinity for lipophilic compounds such as short chain fatty acids responsible for fresh odor and flavor (Olafsdottir et al., 1997; Ruiter, 1995). Glutamic acid was first observed between days 5 and 9, very

close to dryness. Proline, aspartic acid and β-alanine were found at day 11. Previous research with processed and cooked seafood showed that these free amino acids changed sensory properties (Ram, Chand, Forrest & Southgate, 2017). After 18 days, off-flavor and off-odor were strong, sensory rejection was evident and large amounts of free amino acids were detected.

At final time, quality parameters showed the lowest values , which matched with bacterial counts higher than 8 log ufc/g. Substances with unpleasant odor and flavor are originated by the action of decarboxylase enzymes upon free amino acids, especially sulfurous amino acids that produce hydrogen sulphide and metilmercaptane (Ruiter, 1995). As can be seen in the plot, most of the essential free amino acids grouped in the lower part of the plot next to TBARS and bacterial counts (PST).

The comparative for sensory profile and quality parameters in thawed fillet (F) is shown in Fig.3. The first two components explained a 94.05 % of variation. Fillet spoilage moved in the same way as the G treatment, from left to right, but with days 5, 9 and 11 practically overlapping. Seaweedy and QI exhibited an independent behavior; meanwhile arginine, glutamic acid and hydroxyproline were detected between days 5 and 11. The greatest bacterial counts were recorded on day 11, together with negative sensory attributes (bitterness, stale and softness), while (TBARS & TVB-N) were located at day 18 when a great amount of free amino acids were produced. These facts make difficult to identify relevant relationships among them. Regarding this, bitterness has been related with higher amounts of lipophilic amino acids in oysters stored in ice and refrigerated cured ham (Martuscelli et al., 2009; Murata & Sakagushi, 1986). The plot for thawed fillets showed a low discrimination among storage

days, and a clear polarization of sensory descriptors was found (satisfactory quality & poor quality); namely, fillets went from “acceptable” to “spoiled” very quickly and without midpoints.

Statistical multivariate analysis**,** PLS-DA, was performed in order to sharpen the separation among groups of observations, by hopefully rotating PCA components such that a maximum separation among classes be obtained, and to understand which variables carried the class separating information. This analysis was developed taking into account the first two principal components that explained 80% of the variance of the model (Fig.4). Three groups were clearly separated, each one corresponding to a specific treatment applied to fish. Whole and gutted were located in the left side of the plot, opposite to filet that set in the right side. Thawed whole seabream was related to hydroxyproline and quality index (QI). Periago et al. (2005) found that the muscle fibre density had a positive correlation with collagen and hydroxyproline contents, In Mediterranean fish (seabass), thus improving texture. Besides this, texture correlated very well with flesh pH. Our results agreed with those and pH was associated with whole treatment and a typical dry texture of fresh fish. Those authors claimed that most post-mortem degradation in the flesh occurred during the first hours of storage due to calpain activity. Related to gutted treatment, arginine (ARG) and glutamine (GLU) stands in the plot. This result agrees with previous research; Martuscelli et al., (2009) found that arginine was the most abundant essential amino acid in final food products with high value of protein (cured ham). Specially in seafood, arginine was the third most important free amino acid in escolar fish (*L. flavobrunneum*) packed in vacuum and stored under refrigeration during 9 days (Dordevic et al., 2016). Glutamine was also

detected by Rabie et al. (2009) in Egyptian salted-fermented fish after ripening and storage at room temperature for 40-60 days. Glutamine is known to be sensitive to heat and it is destroyed by cooking, but it seems to remain stable in a product without thermal treatment, like a gutted fish. According to our results, it was associated, too, with a bitter taste.

Fillet was the most discriminated treatment. A strong relation between glycine (GLY) and TBA was detected. Glycine is synthesized from the essential amino acid serine (SER), so both compounds were located close in the plot. Glycine is an amphoteric amino acid which could act as a hydrogen donor, thus contributing to lipid oxidation detected by TBA index. In a previous study performed by Ram et al. (2017), it was established that glycine was the most abundant protein-bound amino acid in sandfish (*H. scabra*). Another amino acid found in this treatment was ~~the~~ taurine (TAU), that showed a highly particular behavior. It is the most abundant free amino acid in animal tissues, accounting for 53% in muscle and many studies have demonstrated the essentiality of dietary taurine for many commercially relevant species, especially marine teleosts (Brosnan & Brosnan, 2006). As it was expected, storage and microbial growth were located very close and highly related with glutamic acid (GLU). In previous studies, alanine and glutamic acid were the most abundant free amino acids in fish and crustaceans and they were claimed to be responsible for flavor and taste. (Rabie et al., 2009; Sakaguchi et al., 1982). Ruíz-Capillas & Moral (2001) observed that composition in glutamic acid remained stable overtime but an increase in alanine quantities was detected in a study of hake packed in controlled atmosphere and refrigerated storage. In contrast with this view, our research found that glutamic acid was stable during the first eleven days after

thawing but increased suddenly at day 18 in all treatments, especially in fillets. Besides this, alanine increased steadily throughout the experiment. Finally, the presence in the fillets of big amounts of essential amino acids (lLE, PHE & MET) that overlap each other, allowed to affirm the existence of an advanced proteolysis stage that was confirmed by TVBN location. In this sense, aromatic and aliphatic amino acids such as methionine and alanine have been associated with off-flavor and bitter flavor (Martuscelli et al., 2009).

4. Conclusions

Most of the chemical (TVB-N, TBARS), microbial (PST) and sensory (QI) parameters demonstrated that filleted seabream (F) had a shelf life significantly lower than gutted (G) and whole (W) fish (< 11 and 11-18, respectively). This was probably due to the high microbial counts (7 log UFC/g) that fillets exhibited within 11 days of refrigerated storage. All free amino acids increased their quantities with spoilage, reaching higher concentrations in fillet treatment than in gutted and whole fish. PLS-DA discriminated treatments applied to seabream in a comprehensive way; whole and gutted fishes had similarities but were completely discriminated from fillet. In addition, PLS-DA allowed to relate each treatment with a specific free amino acid, so that hydroxyproline was associated with whole seabream, meanwhile arginine and glutamine were associated with gutted fish. For its part, fillet treatment was highly correlated with glycine, taurine and glutamic acid. All of them had the highest relationship with negative changes in sensory attributes. It is important to highlight that all free essential amino acids increased greatly throughout storage. Future research

should aim to deepen in the effects of these changes on the nutritional quality of seabream.

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Highlights

- All free amino acids increased their quantities with the spoilage
- Sea Bream freshness degree before frozen is related to free amino acids formation
- The technological processes modified the free amino acid composition in seabream
- The free essential amino acids were increased through the storage

TREATMENT											
(W) WHOLE (n=20)				**(G) GUTTED** (n=20)				**(F) FILLETED** (n=20)			
REFRIGERATION (0° C) DAY BEFORE FROZEN											
5	**9**	**11**	**18**	**5**	**9**	**11**	**18**	**5**	**9**	**11**	**18**
FROZEN STORAGE (-30 °C) -30 DAYS- (5 fish for each refrigeration storage time in all treatments)											

Analyses carried out:

Physicochemical analysis: TVB-N, TBARS, pH & Free amino acids free profile

Microbiological count: Psycrotrophics viable counts (PST)

Sensory analysis: Torry sensory quality index (QI), Descriptive sensory analysis (QDA)

	FILLETED				GUTTED				WHOLE			
DAYS ON ICE	**5**	**9**	**11**	**18**	**5**	**9**	**11**	**18**	**5**	**9**	**11**	**18**
PHYSICO-CHEMICAL & MICROBIOLOGICAL ANALYSES												
TVB-N*	24.5 Ba	32.4 Ba	40.9 Ba	86.2 Bb	21.0 A	24.4 A	21.5 A	36.5 A	22.8 A	24.1 A	24.9 A	32.0 A
pH	6.3	6.3	6.1	6.4	6.4	6.3	6.3	6.4	6.2	6.4	6.4	6.5
TBARS*	0.6 Aa	0.7 Aa	0.9 Aa	1.5 Ab	0.3 B	0.3 B	0.5 B	0.6 B	0.1 B	0.4 B	0.6 B	0.8 B
PST*	5.5 Ba	6.4 Bb	7.4 Bb	8.2 Bb	4.6 ABa	6.3 ABb	6.9 ABb	7.0 ABb	4.0 Aa	6.4 Ab	6.8 Ab	6.6 Ab
SENSORY ANALYSES												
Torry quality index in cooked fish												
QI*	7.5 Ab	6.4 Aa	6.6 Aa	6.7 Ba	7.8 Bb	7.5 Bb	7.5 Bb	4.0 Aa	8.0 Bb	7.6 Bb	6.7 Aa	6.6 Ba
Quality descritpive analysis (QDA)												
STALE*	3.4 A	3.7 A	4.6 A	4.6 A	3.0 A	3.1 A	3.3 A	3.7 A	3.4 A	3.5 A	4.5 A	5.0 A
SEAWEEDY*	1.4 Aa	5.3 Ac	4.8 Ab	2.6 Ab	2.0 Ac	1.8 Bc	2.4 Bb	1.3 Ba	4.6 Ba	2.7 Bb	2.5 Bb	1.5 ABc
MEATY*	4.4 A	4.7 A	4.8 B	4.0 B	4.5 Aa	3.9 Bb	3.8 Bb	3.3 Bc	4.8 Aa	4.4 Aa	4.7 Aa	3.4 Ab
BITTERNESS*	2.3 a	2.3 a	3.5 b	3.7 b	2.0 a	2.0 a	2.8 b	4.0 c	1.7 a	1.8 a	3.1 b	3.4 b
DRYNESS*	4.0 Ab	4.3 Ab	4.7 Ab	2.9 Aa	2.5 Ba	3.0 Ba	4.1 Ab	3.3 Ba	3.8 Ab	2.8 Ba	2.8 Ba	3.5 Ab
SOFTNESS*	3.5 a	4.0 a	4.3 a	6.5 b	3.3 a	2.8 a	4.8 b	5.6 c	3.3 a	3.4 a	3.8 a	6.0 b

TVB-N= Total volatile nitrogen (mg/100g). TBARS = Tiobarbituric Acid Index (mg/kg Malonaldedhyde). PST= Psychrotrophic viable count (log cfu/g). Odors: seaweedy & stale. Flavors: meaty & bitterness. Texture= dryness & softer and QI=Sensory quality index

Capital letters within columns show significant differences among treatments over time Lowercase letters within columns show significant differences in sampling times inside each treatment ($p < 0.05$).

		WHOLE				GUTTED				FILLETED			
	DAYS ON ICE	**5**	**9**	**11**	**18**	**5**	**9**	**11**	**18**	**5**	**9**	**11**	**18**
		ESSENTIAL AMINO ACIDS -E- (mg/ 100g)											
HIS	Histidine*	20,5b	8,3 a	20,6 b	14,3 b	14,4 b	11,6 a	16,3 b	19,5 b	12,6 a	16,8 a	20,8 a	39,0 b
ILE	Isoleucine**	2,2 Aa	3,7 Aa	4,7 Ab	6,2 Ab	2,8 Aa	5,2 Ab	4,6 Ab	6,8 Ab	2,0 Ba	6,4 Bb	25,9 Bc	62,4 Bd
LEU	Leucine**	4,9 Aa	8,4 Ab	10,5 Ac	13,2 Ad	5,9 Aa	11,6 Ab	11,8 Ab	15,8 Ac	5,4 Ba	13,5 Bb	50,0 Bc	125,0 Bd
LYS	Lysine**	10,8 Ab	8,8 Aa	15,5 Ac	16,1 Ac	8,0 Aa	11,5 Ab	12,6 Ac	16,3 Ad	3,6 Ba	14,7 Bb	57,6 Bc	101,4 Bd
MET	Methionine**	4,3 Aa	4,9 Aa	6,1 Ab	7,0 Ab	4,4 Aa	6,7 Aa	6,1 Aa	8,0 Ab	3,1 Ba	9,6 Bb	32,1 Bc	63,6 Bd
PHE	Phenylalanine**	1,7 Aa	5,7 Ab	5,2 Ab	6,8 Ac	3,1 Aa	6,5 Ab	5,9 Ab	8,3 Ac	2,0 Ba	6,8 Bb	22,3 Bc	63,9 Bd
THR	Threonine**	10,6 A	8,6 A	13,0 A	12,9 A	9,2 Aa	10,0 Aa	10,1 Aa	16,3 Ab	10,1 Ba	14,1 Ba	32,6 Bb	71,5 Bc
VAL	Valine***	8,8 Aa	7,9 Aa	18,2 Ab	10,6 Ab	13,4 A	12,7 A	12,2 A	11,9 A	8,1 Ba	17,0 Bb	42,2 Bc	74,9 Bd
	Total free E AA	68,8	65,4	104,9	105,1	66,1	84,9	90,6	120,8	51,8	107,8	294,6	619,9
		NON-ESSENTIAL AMINO ACIDS -N- (mg/ 100g)											
ALA	Alanine	29,4	26,4	31,9	31,9	23,4	25,2	26,2	40,9	29,5	30,7	29,2	75,3
ANS	Anserine	0,8	0,5	3,6	3,3	1,5	1,7	1,7	2,6	1,5	1,6	1,9	3,2
ARG	Arginine*	3,9 B	4,8 B	6,3 B	7,0 B	3,4 Ba	6,4 Bb	5,7 Bb	7,8 Bb	2,4 A	3,8 A	3,6 A	2,3 A
ASP	Aspartic acid.*	0,9 A	1,3 A	1,2 A	1,5 A	0,8 A	2,1 A	1,5 A	1,3 A	0,9 Ba	2,5 Bb	6,4 Bb	24,8 Bc
BAL	B-alanine *	0,2 A	0,2 A	0,2 A	0,1 A	0,2 A	0,2 A	0,2 A	0,2	0,2 Ba	0,1 Ba	0,6 Ba	1,8 Bb
CYS	Cysteine**	0,1 A	0,1 A	0,2 A	0,1 A	0,2 A	0,1 A	0,2 A	0,2 A	0,0 Ba	0,6 Ba	2,7 Bb	9,8 Bc
GABA	Γ-aminobutyric acid**	0,0 Aa	0,0 Aa	0,7 Ab	1,1 Ab	0,0 Aa	0,3 Aa	0,6 Aa	1,1 Ab	0,9 Ba	1,3 Ba	4,7 Bb	15,2 Bc
GLN	Glutamine	16,4	26,8	21,2	19,1	22,3	21,4	28,0	19,7	25,4	18,8	10,7	19,3
GLU	Glutamic acid.	21,0	27,8	38,9	32,6	22,2	28,4	31,4	41,6	20,7	23,2	23,2	67,7
GLY	Glycine *	67,9 AB	48,5 AB	70,2 AB	60,8 AB	58,3 A	54,2 A	53,6 A	64,7 A	63,9 Ba	58,5 Ba	71,3 Ba	107,8 Bb
HXP	Hydroxyproline	3,5	1,8	2,3	2,2	1,7	1,6	1,6	2,0	3,2	1,6	2,6	2,6
SER	Serine *	12,8 AB	11,0 AB	13,9 AB	13,1 AB	11,1A	11,2 A	9,9 A	16,2 A	12,6 B	10,2 B	9,7 B	28,1 B
ORN	Ornytine**	0,5 Aa	1,0 Ab	1,4 Ab	1,5 Ab	1,0 A	1,3 A	1,2 A	1,5 A	0,3 Ba	3,7 Bb	16,8 Bc	41,6 Bd
PRO	Proline*	4,3 A	4,3 A	6,2 A	6,8 A	7,7 AB	5,0 AB	4,7 AB	8,5 AB	3,7 B	4,2 B	7,3 B	29,6 B
TAU	Taurine**	163,5 Aa	153,0 Aa	194,9 Aa	199,0 Ab	205,2 Aa	206,6 Aa	196,5 Aa	224,6 Aa	228,7 Ba	222,2 Ba	250,7 Ba	306,7 Bb
TYR	Tirosine*	4,3 Aa	6,6 Aa	5,7Aa	7,7 Ab	3,9 Aa	7,1 Ab	6,1 Ab	9,3 Ab	2,6 Ba	6,8 Ba	25,3 Bb	36,4 Bc
	Total free N AA	328,6	313,6	395,1	384,3	361,1	371,0	367,5	439,7	395,0	388,3	464,8	769,1
		DIPEPTIDE -DP- (mg/100g)											
ANS	Anserine	0,8	0,5	3,6	3,3	1,5	1,7	1,7	2,6	1,5	1,6	1,9	3,2
	Total free AA (E+N)	397,3	379,0	500,0	489,4	427,3	455,9	458,1	560,4	446,8	496,1	759,4	1388,9
	Ratio Free AA (E/N)	0,21	0,21	0,27	0,27	0,18	0,23	0,25	0,27	0,13	0,28	0,63	0,81
	TOTAL FREE A.A.	398,1	379,5	503,5	492,7	428,7	457,6	459,8	563,0	448,3	497,7	761,4	1392,1

Means (n=3) Capital letters within columns show significant differences among treatment. Lowercase letters within columns show significant differences inside each treatment over time

** p < 0.050 ** p < 0.010 *** p < 0.001*