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Effect of Mechanical Separation Process on lipid oxidation in European aquacultured sea bass, gilthead sea bream, and rainbow trout products

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Abstract

Mechanical separation systems are a good option to create new fish products and open new market, however studies on the effect on quality of mechanical treatment on species of interest for European aquaculture, such as European sea bass, gilthead sea bream, and rainbow trout are scarce. Thus in this research, the effect on colour, nutritional quality, and lipid stability was considered immediately after separation process and after 90 days of frozen storage. Results revealed that mechanical separation technique significantly affected colour and lipid stability of the three studied species. Increases in L* and secondary oxidation products were observed, together with a decreased of antioxidant capacity. Nutritional value instead was unaffected by treatment. Thus, mechanical separation process could represent a new way to better exploit species of interest for European aquaculture and acquire new market niches, but oxidative processes during the treatment have to be limited and kept under control.

Keywords: mechanical separation; MSM; TBARS; fishburger; antioxidant capacity.

1. Introduction

Products development in aquaculture sector has been very limited during these last years (EC, 2013). In this sense, mechanical separation systems are a good option to create new fish products and open new market. In accordance with Regulation (EC) No 883/2004 (Regulation (EC), 2004) mechanically separated meat (MSM) is a product obtained by removing remaining meat from bones using mechanical means, where the normal structure of the muscle fibre is mostly lost or modified in such a way that it is not comparable with regular meat. During the last decades, MSM has grown in importance, especially in poultry and pig sectors, raising a production of 700 000 t in 2007 (EC, 2010). Concerning seafood industry, no specific restrictions about MSM utilized are presented in EU Regulation and mechanical separation treatment may represent a new technology in fish supply chain.

Recently, MSM obtained from Nile tilapia (*Oreochromis niloticus*) (Fogaça et al., 2015; Freitas et al., 2012; Kirschnik et al., 2013; Marengoni et al., 2009), and Brazilian catfish (*Brachyplatystoma vaillantii*) (Oliveira et al., 2015) by-products have been chemically and sensory characterized. However, the utilization of mechanical separation (MS) on the whole fish has to be investigated, as well as its effects on European seawater and freshwater species. Indeed, MS has typically been utilized for the recovery of fish by-products but it may be also a valid process to use on no directly marketable European farmed fish, such as the undersized or damaged ones. That will entail the utilization of whole fish, rich in fat and protein, and not only the frame derived from filleting process.

Lipid oxidation is a very important event leading the loss of nutritional values and food quality, especially for fish, due to the high presence of polyunsaturated fatty acids (PUFAs). However, the use of such mechanisms in new products, like MSM of fish is not yet well investigated. Thus, it seems reasonable to check the effect on lipid stability of MS technology applied to some sea and freshwater European farmed species, in order to understand its possible role for the creation of new products.

2. Materials and methods

2.1 Preparation of fish samples and storage conditions

Different species of sea and freshwater fish were utilised in the present trial. Six specimens of European sea bass (*Dicentrarchus labrax*) and 6 specimens of gilthead sea bream (*Sparus aurata*) were obtained from a fish farm located in Orbetello (Grosseto, Italy), while six specimens of pigmented rainbow trout (*Oncorhynchus mykiss*) were obtained from a farm located in the north west of Tuscany (Lucca, Italy). Fish were killed by percussion and, immediately after death, they were transferred into polystyrene boxes, covered by ice, and moved to the industry where the fish of each species, degutted and without head, were minced by the MSM machine Baader 60-1 (Lübeck, Germany). Then, the remained whole fish and the MSM were brought to DISPAA (Florence, Italy) where all the whole fish were filleted. Whereas six fillets (right) for each species were stored as whole fillet (WF samples), six fillets (left) for each species were grounded by using a New Style Chopper (Westmark Gmbh, Elspe, Germany) in order to obtain 6 fish-burger (FB samples), while six MSM-fish burger were obtained from MSM (MSM samples). Three samples for each treatment (whole fillet, burger from grounded fillet, burger from MSM) and each species were analysed at time 0 (T0), while the other samples were analysed after storage at – 20 °C for 90 days (T90). WF, FB, and MSM for the three species were analysed for: colour, pH, total lipids, fatty acid composition, primary (conjugated dienes) and secondary (thiobarbituric acid reactive substances, TBARS) oxidation products, and antioxidant capacity.

2.2 Colour and pH

A Dr Lange Spectro-colour® colorimeter (Keison International Ltd, UK) equipped with a Spectral qc 3.6 software was utilized for colorimetric measurement. Colour was measured in triplicate on the epaxial-cranial sites of fillet (WF) and in three points of the burgers (FB and MSM). Colour measurements were carried out according to the CIELab system (CIE, 1976).

Lightness (L*), redness index (a*), yellowness index (b*), Hue, and Chroma were recorded, whereas the numerical total color difference (ΔE) between samples was calculated by the formula

$$\Delta E_{(\beta-\alpha)}=[(L^*_\beta-L^*_\alpha)^2+(a^*_\beta-a^*_\alpha)^2+(b^*_\beta-b^*_\alpha)^2]^{0.5}$$

where β represents the values of colour parameters (L*, a*, and b*) measured at T90 and α represents the values of the same parameters measured at T0. A variation in colour (ΔE) equal to 2.3 units corresponds to a just-noticeable difference (JND) for the human eye; higher variation is considered discernible (Sharma, 2003). The pH values of the samples were measured by a Mettler Toledo pH-meter (Columbus, OH, USA) in three different points. Dorsal region of whole fillet and the burger's diameter were utilised for the measurements. Finally, ΔpH, as difference between the values measured at T90 and at T0, was also calculated.

2.3 Fatty acids

The total lipid content of the samples was determined according to Folch et al. (1957) method and fatty acids (FA) in lipid extract were determined after trans-esterification to methyl esters (FAME) using a base-catalyzed trans-esterification followed by a boron trifluoride catalyzed esterification (Morrison & Smith, 1964). The FA composition was determined by gas chromatography (GC) using a Varian GC 430 gas chromatograph (Varian Inc., Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a Supelco Omegawax™ 320 capillary column (30 m × 0.32 mm i.d., 0.25 μm film and polyethylene glycol bonded phase; Supelco, Bellefonte, PA, USA). The oven temperature was held at 100 °C for 2 min, increased to 160 °C over 4 min, then increased to 220 °C over 14 min and finally kept at 220 °C for 25 min. The injector and the detector temperatures were set at 220 °C and 300 °C, respectively. One μL of sample in hexane was injected into the column with helium as carrier gas kept at a constant flow of 1.5 mL/min. The split ratio was 1:20. Chromatograms were recorded with the Galaxie Chromatography Data System 1.9.302.952 (Varian Inc., Palo Alto, CA, USA) computing integrator software. Fatty acids were identified by comparing the FAME retention time with the standard Supelco 37

component FAME mix (Supelco). Fatty acids were quantified through calibration curves using tricosanoic acid (C23:0) (Supelco) as internal standard. This analysis was not carried out in FB samples, because the similarity of composition of these samples and WF samples.

2.4 Lipid oxidation products

Conjugated dienes (CD) content in the lipid extract were measured by the colorimetric method (Srinivasan et al., 1996) using hexane (Sigma Aldrich, St. Luis, MO, USA) as solvent. Conjugated dienes were quantified at 232 nm (50 Scan spectrophotometer Varian, equipped with a Cary Win UV Software; Palo Alto, CA, USA) and using a molar extinction coefficient of 29000 mL /mmol cm. The results are expressed as mmol hydroperoxides/kg lipid.

The 2-thiobarbituric acid reactive substances (TBARS) were measured at 532 nm, using the colorimetric method described by Vynke (1970). Briefly, TBARS were extracted in TCA (5%), then added with TBA 0.02mol/L. After 40 min of incubation at 97 °C, the oxidation products were quantified with reference to calibrations curves of TEP (1,1,3,3,-tetra-ethoxypropane) in 5% (w/v) TCA (0.2 to 3.1 μmol/L).

2.5 Antioxidant capacity

The antioxidant capacity was measured by the radical cation decolorization assay ($ABTS^{\bullet+}$), the radical scavenging activity ($DPPH^{\bullet}$), and the ferric reducing ability assay (FRAP). Samples (3 g) were extracted with 10 ml of ethanol. The antioxidant capacity was performed on ethanol extracted samples according the minor modifications reported in Mancini et al. (2015) to the methods of Re et al. (1999) for ABTS reducing activity assay (ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)), of Blois (1958) and Jung et al. (2010) for DPPH scavenging activity (DPPH, 2,2-diphenyl-1-picrylhydrazyl), and Descalzo et al. (2007) for FRAP assay method (ferric reducing ability).

2.6 Statistical analysis

The statistical analysis was performed using SPSS version 17.0 software (SPSS Inc. Illinois, USA). Normality of data distributions was tested by the Kolmogorov-Smirnov test. ΔE and fatty acids were subjected to one-way analysis of variance (ANOVA) with 'treatment' as a fixed effect, using the Bonferroni post-hoc test to check the significance of the differences among levels (WF, FB and MSM samples). The primary and secondary oxidation products and antioxidant capacity were subjected to two-way ANOVA with 'treatment' and 'storage' and their interaction as fixed effect, using Bonferroni post-hoc test to check again the significance of the differences among levels (WF, FB and MSM samples), and storage (T0 and T90). The same model (two-way ANOVA) was performed on the fatty acid profiles, but in this case only two treatment levels (WF and MSM) were considered. A repeated measures models, considering 'treatment' and 'storage' as main factors, was performed on pH and colour parameters (L*, a* and b*). Bonferroni post-hoc test was used as post-hoc test. Pearson correlation coefficients were calculated between the parameters evaluated for all the species.

3. Results and Discussion

Table 1 presents the results of ANOVA for pH and colour parameter values. Treatment significantly affected colour for European sea bass, gilthead sea bream, and partially for rainbow trout. The differences in colour parameters were similar for the seawater species, indeed the L*, a* and b* of WF resulted significantly lower than the minced (FB) and MSM burger. Trout fillet showed a significantly lower lightness (L*) and higher redness (a*) compared to MSM and minced fillet burger. No significant differences were observed for b* values in rainbow trout due to the time of storage. The fish fillet colour is linked with heme-based pigment, physical structure of muscle, and the amount of unbound water influences light scattering. Since sea bass and sea bream are white fish, it is reasonable to suppose that changes of pigments under high-pressure treatment are of

minor importance, so that these colour changes may be attributed to modifications of protein matrix as reported by Chéret, Chapleau, Delbarre-Ladrat, Verrez-Bagnis, and Lamballerie (2005). The redness in trout is due to astaxanthin added to the feed, and the significant interaction TxS (Treatment × Storage) could be related to the significantly higher a* value for WT samples at both the storage times. Storage time decreased lightness and redness in rainbow trout, as previously reported (Choubert & Baccaunaud, 2006). Evolution of colour during storage can be associated with enzymatic and non-enzymatic reactions resulting in degradation of myofibrillar proteins and disorganization of myofibrils (Cherét et al., 2005). These modifications were observed also in the white flesh fish. According to that, the storage of all treated fish led to a significant decrease in L* values after 90 days. However, the decrease of L* values was found to be higher in rainbow trout than in gilthead sea bream, and European sea bass. These results emphasised the importance of using white flesh fish (sea bass and sea bream, for example) to develop fish products, as supported by Bito (1965) who assessed that the colour of the white fish burgers was more stable than that of tuna stored at the same temperature, which lost its colour after 2 months. The pH values were not affected by treatment, indeed no significant differences were highlighted, as reported in Table 1. As expected the WF, FB and MSM resulted with the same pH likely because MSM was obtained by the whole fish and not from residues as reported by other authors (Oliveira et al., 2015). The storage of samples at negative temperature for 90 days resulted in significantly changing in terms of pH, showing an increase of pH during storage for gilthead sea bream and rainbow trout. The results are in agreement with those of several authors that reported the same pattern for sea bream (Kyrana, Lougovois, & Valsamis, 1997). Usually no significant differences were found in pH for trout during the storage in ice, even though the tendency is for increasing values (Chytiri, Chouliara, Savvaidis, & Kontominas, 2004). Our results showed a significant increase of pH ($p<0.05$) for trout samples stored for 90 days at negative temperature.

The variation in colour (ΔE) for each species showed no differences in terms of storage and treatments (data not reported), indicating that no noticeable difference (JND) for the human eye occurred, since only variation higher than 2.3 units is considered as discernible (Sharma, 2003).

The fatty acid (FA) composition of European sea bass, gilthead sea bream, and trout fillets and MSM immediately after treatment and after refrigerated storage is reported in Table 2. No statistical differences were found in the fatty acid profile between fillets and MSM for none of the considered seawater species. However, some slightly differences were found in fatty acid profile of rainbow trout. Indeed, a significantly higher amount of stearic acid (18:0) and lower amount of linolenic acid (18:2ω6) have been found in the whole fillet compared to MSM. As a consequence, the total amount of PUFAω6 was significantly higher in the MSM than in the whole fillets. Although a certain caution is required when the results are compared with previous studies, it seems that the raw material for MS process deeply influences fatty acid composition. Indeed, when MSM is obtained by filleting residues (Oliveira et al., 2015), lipid fraction is mainly constituted by SFA (around 54%), and MUFA (34%) whilst the most important PUFA applied for 10% of total fatty acids. On the contrary, when whole fish, degutted and without head, is utilised as in the present research, MSM fatty acid profile reflected that of the fillet. In conclusion, using no marketable fish instead of fish by-products may result in a high quality MSM chemical composition despite the species utilised.

The fatty acid composition of European sea bass, gilthead sea bream, and rainbow trout samples instead fell within previous data about farmed fish (Badiani et al., 2013; Grigorakis, 2007; Secci, Parisi, Dasilva, & Medina, 2016; Tibaldi et al., 2015).

The total amount of saturated fatty acids (SFA) in muscle was found to be around 20% in seawater species, whilst in rainbow trout stopped at around 15%. Regardless the quantitative difference, this fraction was found to be mainly composed of palmitic (16:0), stearic (18:0), and myristic (14:0) acids in all the species. In general, the dominance of these three fatty acids has been reported previously in farmed fish fed with different ratio of marine and plant feed ingredients

(Baron et al., 2013; Timm-Heinrich, Eymard, Baron, Nielsen, & Jacobsen, 2013). Among monounsaturated fatty acids (MUFA), the most abundant were oleic (C18:1ω9), and palmitoleic (C16:1ω7), with some species-specific differences. Particularly, gilthead sea bream resulted in the lowest oleic content, applying for 15% of total fatty acids, whereas rainbow trout contained almost the 10% more than the other. The opposite trend was found for palmitoleic acid, which resulted to be the highest in gilthead sea bream and the lowest in rainbow trout. In both cases, oleic and palmitoleic values for European sea bass were more similar to sea bream than to rainbow trout, confirmed the affinity of these two marine species (Grigorakis, 2007), often reared with similar techniques and feed. Oleic acid is often reported to be the most abundant MUFA in the lipids and it is one of those more affected by replacement of fish oil by plant oil in feeds (Baron et al., 2013).

As regards to muscle PUFA, their amount ranged from 49% (gilthead seabream) to 52.5% of trout but the main differences lean on its composition. Indeed, PUFA fraction of sea water species are mainly composed by ω3 (around 74%) and the ω6 represented about 20% of total polyunsaturated fatty acids. Freshwater species instead had 57% of ω6 and 42% of ω3. Even in that case, results confirmed the differences between marine and freshwater fish highlighted by Tocher (2003). However, the dominance of C18:2ω6 on ω6 fraction of PUFA has been reported both in marine (Badiani et al., 2013; Tibaldi et al., 2015) and in freshwater farmed species (Secci, Parisi, Dasilva, & Medina, 2016) though its percentage seemed to be strictly connected with the sources of feed ingredients (Baron et al., 2013). Specifically, present results revealed that C18:2ω6 applied for 70 % and 89 % of PUFAω6 in sea bass and trout, respectively, in agree with the 87% and 90% previously obtained for the same species (Badiani et al., 2013; Secci, Parisi, Dasilva, & Medina, 2016).

The major contributors to ω3 fraction were docosahexaenoic acid (DHA) for all the three considered species, followed by eicosapentaenoic acid (EPA) in European sea bass (around 20% of PUFAω3) and gilthead sea bream (28 % of PUFAω3).

Considering the storage effect, the three fish species showed different modification in fatty acid profile. However, a global trend can be discerned. Indeed a decrease of PUFA fraction and an increase of total saturated fatty acid (SFA) after 90 days of refrigerated storage was found for all fish. Specifically, a significant decrease of PUFA is reported for both the seawater species and it is lead by a significant PUFAω3 reduction (- 39 % and – 4 % in sea bass and sea bream, respectively). Furthermore, the decrease of that fraction is mainly due to the significant DHA reduction. Finally, such a trend is accompanied by a significant increase of SFA (+12 % and +8 % in sea bass and sea bream, respectively) and MUFA, even if a statistical difference was highlighted only for European sea bass. On the other hand, indeed no significant differences were found in PUFA fraction of trout fillets, thus confirming the stability of that species during storage (Secci, Parisi, Dasilva, & Medina, 2016). In trout, only an increase of SFA was reported which could be related to a small decrease found for the other classes of FAs. The present results confirm that storage significantly influences lipid composition of fish, especially of those rich in PUFAω3 which are reported to be highly susceptible to oxidation.

Concerning lipid stability due to treatment and during storage, such as high values of PUFAs ω3 fraction could be the main cause of lipid degradation of MSM as a consequence of the mechanical treatment. Indeed, results revealed that MS treatment significantly affected lipid oxidation of seawater fish whereas no effect emerged on rainbow trout (Table 3). Specifically, primary oxidation products, obtained by measuring conjugated dienes content, were affected nor by treatment or storage in sea bass and trout samples, whilst CD content of sea bream was found to be significantly affected by treatment and storage. Nonetheless, the extent of lipid oxidation was underlined by TBARS values. Globally, treatment significantly affected lipid oxidation of the three species. Mechanical separation process seemed to promote lipid oxidation in seawater species immediately after treatment, being TBARS more than two times higher in MSM than in WF. Burgers obtained from minced meat resulted in an intermediate level of oxidative status (Table 3). Moreover, gilthead sea bream appeared the most susceptible species to be oxidised by treatment

raising a value near the threshold of 8 mg MDA/ kg sample for the rancid perception, as proposed by Shormüller (1968). Lipid fraction of rainbow trout instead seemed not to be affected by mechanical separation process.

However, the present results are not in complete agreement with previous findings. Results by Fogaça et al. (2015) on the effect of MSM on tilapia (*Oreochromis niloticus*) showed a low oxidative value (1.03 mg MDA/ kg tissue) despite the high lipid content (around 7 %). Lowest value was obtained for no-washed MSM from tilapia by Kirschnik et al. (2013), who found a TBARS content around 0.5 mg MDA/ kg tissue which however, raised up to 0.7 mg MDA/kg tissue after 90 days at -18 °C. However, it has to be note that these studies were conducted on fish filleting waste which had a lowest PUFA percentage (Oliveira et al., 2015) than the values found in the species considered in the present research.

Concerning storage, it significantly affected TBARS values of all the studied species, in agreement with previous studies (Indergård, Tolstorebrov, Larsen, & Eikevik, 2014; Secci, Parisi, Dasilva, & Medina, 2016) and confirming the data obtained for fatty acid composition. At T0, trout was found in a lower oxidative status in comparison with the other two species, by being three times lower than the values obtained for sea bream and almost half the sea bass ones. Such as difference was in agreement with a previous study that showed the scarce susceptibility of rainbow trout to be oxidised, both for its low content of ω3 fraction and for carotenoid content (Secci, Parisi, Dasilva, & Medina, 2016). PUFA ω3 may be responsible instead for the three times higher TBARS contained in sea bream than in sea bass. Indeed, the 5 % of difference in ω3 amount of two species may increase sea bream lipid susceptibility to oxidation.

After 90 days of frozen storage, secondary lipid oxidation products doubled in sea bass and sea bream samples, whereas increased more than 4 times in rainbow trout. The rate obtained for sea bass was in agreement with that obtained by Simitzis et al. (2014) who found that TBARS in percussion killed fish doubled during 90 days at -20 °C. On the other hand, oxidation in trout highly increased confirming that astaxanthin seems to protect against the very early stages of lipid

oxidation but not during the long term frozen storage (Jensen, Birk, Jokumsen, Skibsted, & Bertelsen, 1998).

Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods. The ABTS, DPPH, and FRAP have been widely used to test the ability of compounds to act as free radical scavengers and thus to evaluate the antioxidant activity (Mancini et al., 2015). At the best of our knowledge, this is the first quantification of antioxidant capacity of European sea bass, gilthead sea bream and rainbow trout. All the treated fish, irrespective of the species, showed some reducing abilities, which probably could be attributed to the intrinsic antioxidant system of the muscle (Table 4). Particularly, the presence of astaxanthin in trout muscle may be responsible for the highest global antioxidant capacity, especially ABTS value, because it can improve scavenging and antioxidant activity. Nakajima, Yoshie-Stark, and Ogushi (2009) reported a lack of difference for DPPH value determination between methanolic extracts of Atlantic salmon (*Salmo salar*), Coho salmon (*Oncorhynchus kisutch*), Alaska pollack (*Theragra chalcogramma*), and southern blue whiting (*Micromesistius australis*). Treatment significantly reduced antioxidant capacity in all the studies species. When a statistical significance was found, WF showed the higher value of antioxidant capacity followed by FB with medium-higher values and by MSM with medium-lower values.

Ninety days of frozen storage (T90) significantly reduced antioxidant capacity of the three studied species. European sea bass showed a significance reduction for all the three evaluation methods, FRAP method reported a reduction of antioxidant capacity for gilthead sea bream and rainbow trout. This last species also showed a significant reduction of its DPPH value during the storage time. These trends were partially reported by Sanchez-Alonso, Jimenez-Escrig, Saura-Calixto, and Borderias (2007, 2008) for horse mackerel (*Trachurus trachurus*) whilst no information are available in literature for the species examined in this trial.

As reported by Pazos, González, Gallardo, Torres, and Medina (2005), under post mortem conditions, the endogenous antioxidants are consumed sequentially and the loss coincides with fish

muscle lipid oxidation development. Present results are in agreement with this pattern, because storage was found to significantly affect both antioxidant capacity and lipid oxidation. Specifically, storage significantly reduced the antioxidant assay whereas significantly increased TBARS content. Moreover, as suggested by Gómez-Estaca et al. (2011) higher oxidative stability should be expected from samples with higher reducing ability during refrigeration or under other oxidizing conditions.

A correlation pattern (Pearson test, $\alpha = 0.05$) was evaluated for fatty acid composition, oxidative parameters, antioxidant capacity, ΔE and ΔpH. Results are summarized in Table 5. CDs are positively correlated to SFAs ($p<0.01$) but no correlation has been found of SFAs with TBARS and oxidant capacity. On the other hand, MUFA resulted positively related to the ABTS ($p<0.05$), DPPH ($p<0.05$) and FRAP values ($p<0.01$). PUFAω6 fraction was strongly negatively correlated with primary and secondary oxidation products (CDs: $p<0.01$; TBARS: $p<0.05$), whereas ω3 FAs were strongly positively related to CDs ($p<0.01$) and TBARS ($p<0.01$). These correlations were in line with the correlation found by Secci et al. (2015). Indeed, ω3 fatty acids contribute to quality deterioration and decreases marketability of fish products leading to a major lipid oxidation.

As regard the antioxidant capacity, ABTS, DPPH and FRAP was significantly correlated to each other ($p<0.01$). Primary oxidation products were negatively correlated to ABTS ($p<0.05$), whereas TBARS were negatively related to ABTS, DPPH and FRAP ($p<0.05$, $p<0.01$, and $p<0.01$, respectively). Finally, it was also investigated the correlation among colour parameters and pH variations and the oxidation products and antioxidant capacity. An interesting positive relationship was found between ΔE and TBARS and ABTS values ($p<0.05$ and $p<0.01$, respectively). A negative correlation was reported between ΔE and CDs ($p<0.01$), and FRAP ($p<0.05$). A positive significant correlation was found for ΔpH with ABTS ($p<0.01$) but not with the other antioxidant capacity quantifications.

3. Conclusions

In conclusion, mechanically separation process significantly affected quality of the derived-products in terms of colour, antioxidant capacity and oxidative stability. Globally, MSM of the seawater species investigated resulted more damaged by mechanical treatment than that of rainbow trout, maybe because of the high content of ω3. However, using no marketable fish instead of fish waste may result in a high nutritional quality MSM despite the species utilised. Thus, mechanical separation process could represent a new way to better exploit species of interest for European aquaculture, but oxidative processes during the treatment have to be limited. Washing MSM, as proposed by other authors (Kirschnik et al., 2013) could be an option to wash out pro-oxidant molecules, such as heme, however further researches on the utilisation of antioxidant during the process or added to the MSM are suggested.

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Table 1. pH and colour parameter values of European sea bass, gilthead sea bream, and rainbow trout fillets (WF), minced (FB) and MSM burgers at T0 and after 90 days of frozen storage (T90).

Species	Parameter	Treatment (T)				Storage (S)			T×S
		WF	FB	MSM	SEM[1]	T0	T90	SEM	
European sea bass	pH	6.36	6.39	6.35	0.03	6.35	6.38	0.03	NS
	L*	42.65^{b}	47.41^{a}	49.04^{a}	1.07	47.55^{a}	45.18^{b}	0.46	NS
	a*	-1.40^{b}	0.59^{a}	0.15^{ab}	0.48	-0.29	-0,15	0.13	NS
	b*	1.35^{b}	5.19^{a}	5.57^{a}	0.43	2.26^{b}	5.81^{a}	0.23	NS
Gilthead sea bream	pH	6.16	6.18	6.23	0.06	6.15^{b}	6.22^{a}	0.01	NS
	L*	39.05^{b}	46.50^{a}	44.37^{a}	1.19	46.66^{a}	39.95^{b}	0.77	NS
	a*	-2.50^{b}	-0.64^{a}	-0.24^{a}	0.75	-0.12^{b}	-2.13^{a}	0.19	NS
	b*	0.77^{b}	5.36^{a}	6.57^{a}	1.57	2.16^{b}	6.31^{a}	0.73	NS
Rainbow trout	pH	6.46	6.48	6.49	0.02	6.42^{b}	6.53^{a}	0.01	0.01
	L*	28.81^{b}	39.68^{a}	38.79^{a}	1.98	42.70^{a}	28.82^{b}	1.21	NS
	a*	3.05^{a}	0.44^{b}	0.56^{b}	0.73	2.11^{a}	0.59^{b}	0.53	0.02
	b*	10.24	12.11	11.06	0.10	11.38	10.89	0.64	0.01

[1] SEM: Standard Error of the Mean

Within criterion, a, b: $p<0.05$;

NS, Not Significant ($p>0.05$).

Data were obtained from three replicates.

Table 2. Total lipids and fatty acids profile (g/100g of total fatty acids) of European sea bass, gilthead sea bream, and rainbow trout fillets (WF) and MSM burgers immediately after treatment (T0) and after frozen storage (T90).

	European sea bass						Gilthead sea bream						Rainbow trout					
	Treatment (T)		Storage (S)		SEM[1]	T×S	Treatment (T)		Storage (S)		SEM	T×S	Treatment (T)		Storage (S)		SEM	T×S
	WF	MSM	T0	T90			WF	MSM	T0	T90			WF	MSM	T0	T90		
Total lipid %	10.04	10.65	9.63	11.06	0.93	NS	8.82^{b}	12.12^{a}	7.72^{b}	10.77^{a}	0.46	0.008	6.55	6.76	7.35	5.96	0.37	0.04
C14:0	3.01	2.77	1.88^{b}	3.90^{a}	0.40	NS	4.38	4.37	4.00^{b}	4.75^{a}	0.14	NS	1.49	1.26	1.44	1.31	0.19	NS
C16:0	13.63	13.20	12.06^{b}	14.78^{a}	0.47	NS	12.91	13.42	12.78^{b}	13.55^{a}	0.16	NS	11.02	10.81	10.58	11.25	0.11	NS
C16:1ω7	3.83	3.47	2.50^{b}	4.80^{a}	0.53	NS	6.34	6.43	5.86^{b}	6.91^{a}	0.27	NS	2.43	2.45	2.42	2.46	0.06	NS
C18:0	3.64	3.55	4.63^{a}	2.56^{b}	0.57	NS	2.66	2.64	2.61	2.69	0.09	NS	3.27^{a}	3.06^{b}	3.11	3.23	0.04	NS
C18:1ω9	16.23	15.72	12.81^{b}	19.13^{a}	1.62	NS	15.87	15.84	15.74	15.97	0.56	NS	23.98	24.08	24.08	23.98	0.28	NS
C18:2ω6	6.21	6.19	3.65^{b}	8.76^{a}	1.26	NS	8.71	8.33	8.49	8.55	0.22	NS	25.58^{b}	26.43^{a}	26.03	25.98	0.24	NS
C20:1ω9	2.68	2.66	1.66^{b}	3.68^{a}	0.53	NS	1.81	1.85	2.09	1.57	0.32	NS	1.09	1.34	1.37	1.07	0.15	NS
C20:5ω3	8.47	8.17	7.28^{b}	9.36^{a}	0.63	NS	10.06	10.15	10.37	9.84	0.17	NS	3.07	3.09	3.11	3.05	0.09	NS
C22:1ω11	2.19	2.26	1.28^{b}	3.16^{a}	0.52	NS	1.61	1.59	1.83	1.37	0.28	NS	0.69	0.70	0.71	0.68	0.04	NS
C22:5ω3	3.30	3.26	4.18^{a}	2.38^{b}	0.35	NS	6.36	6.10	6.10	6.36	0.75	NS	1.59	1.49	1.73^{a}	1.34^{b}	0.06	NS
C22:6ω3	24.69	26.44	36.90^{a}	14.23^{b}	5.42	NS	15.06	15.33	16.25^{a}	14.14^{b}	0.35	0.02	11.63	11.16	11.47	11.32	0.46	NS
ΣSFA	21.29	20.58	19.73^{b}	22.14^{a}	0.28	0.05	20.98	21.45	20.37^{b}	22.07^{a}	0.30	NS	16.36	15.68	15.67^{b}	16.37^{a}	0.21	NS
ΣMUFA	28.71	27.89	21.52^{b}	35.08^{a}	3.41	NS	29.73	29.78	29.54	29.97	0.85	NS	31.16	31.38	31.32	31.22	0.36	NS
ΣPUFAω6	9.16	9.31	7.54^{b}	10.93^{a}	0.84	NS	11.04	10.68	10.91	10.80	0.21	NS	29.55^{b}	30.32^{a}	29.93	29.94	0.15	NS
ΣPUFAω3	39.79	41.22	50.52^{a}	30.49^{b}	4.62	NS	35.81	35.73	36.96^{a}	34.58^{b}	0.71	NS	22.17	21.83	22.30	21.71	0.50	NS
ΣPUFA	50.01	51.53	58.76^{a}	42.78^{b}	3.64	NS	49.29	48.77	50.08^{a}	47.97^{b}	0.70	NS	52.49	52.94	53.01	52.42	0.41	NS

The fatty acids C12:0. C13:0. C14:1ω5. C15:0. C15:1. C16:1ω9; C16:2ω4. C16:3ω4. C16:4ω1. C17:0. C17:1. C18:1ω7. C18:3ω6. C18:3ω4. C18:4ω1. C20:0. C20:1ω11. C20:1ω7. C20:2ω6. C20:3ω6. C20:3ω3. C20:4ω6. C20:4ω3. C21:0. C21:5ω3. C22:0. C22:1ω9. C22:1ω7. C22:2ω6. C22:4ω6. C22:5ω6. C24:0. and C24:1ω9 were also detected but not reported because in percentage <3%. They were utilised to calculate Σ.

[1] SEM: Standard Error of the Mean

NS. Not Significant (p>0.05).

Data were obtained from three replicates.

Table 3. Primary (CD. mmol Hp/kg sample) and secondary (TBARS. mg MDA/kg sample) oxidation products in European sea bass. gilthead sea bream. and rainbow trout fillets (WF). and minced (FB) and MSM burgers at T0 and after 90 days of frozen storage (T90).

Species	Parameter	Treatment (T)				Storage (S)			T×S
		WF	FB	MSM	SEM[1]	T0	T90	SEM	
European sea bass	CD	0.42	0.39	0.46	0.04	0.43	0.41	0.05	NS
	TBARS	1.10^{b}	1.37^{b}	2.34^{a}	0.28	1.11^{b}	2.09^{a}	0.23	NS
Gilthead sea bream	CD	0.44^{a}	0.37^{ab}	0.34^{b}	0.03	0.35^{b}	0.42^{a}	0.02	NS
	TBARS	2.72^{c}	5.40^{b}	7.26^{a}	0.57	3.72^{b}	6.53^{a}	0.47	NS
Rainbow trout	CD	0.22	0.25	0.21	0.02	0.21	0.24	0.01	NS
	TBARS	3.15	3.09	2.11	0.73	0.72^{b}	4.85^{a}	0.59	NS

[1] SEM: Standard Error of the Mean

Within criterion. a. b. c: $p<0.05$;

NS. Not Significant ($p>0.05$).

Data were obtained from three replicates.

Table 4. Antioxidant capacity. expressed as ABTS (mmol Trolox eq./kg sample). DPPH (mmol Trolox eq./kg sample). and FRAP (mmol Fe^{II} eq./kg sample). in European sea bass. gilthead sea bream. and rainbow trout fillets (WF). minced (FB) and MSM burgers at T0 and after 90 (T90) days of frozen storage.

Species	Parameter	Treatment (T)				Storage (S)			T×S
		WF	FB	MSM	SEM[1]	T0	T90	SEM	
European sea bass	ABTS	0.27[a]	0.21[b]	0.20[b]	0.01	0.28[a]	0.17[b]	0.01	0.01
	DPPH	0.12[a]	0.12[a]	0.05[b]	0.00	0.12[a]	0.07[b]	0.00	0.00
	FRAP	0.20	0.20	0.20	0.01	0.24[a]	0.17[b]	0.01	0.00
Gilthead sea bream	ABTS	0.14[a]	0.08[b]	0.09[b]	0.01	0.11	0.10	0.00	NS
	DPPH	0.04[a]	0.02[b]	0.02[c]	0.00	0.03	0.02	0.00	NS
	FRAP	0.13	0.14	0.11	0.01	0.16[a]	0.10[b]	0.01	NS
Rainbow trout	ABTS	0.46[a]	0.38[b]	0.13[c]	0.01	0.34	0.31	0.01	0.01
	DPPH	0.09[a]	0.08[a]	0.05[b]	0.00	0.08[a]	0.06[b]	0.00	0.00
	FRAP	0.21[a]	0.20[a]	0.13[b]	0.01	0.24[a]	0.12[b]	0.01	NS

[1] SEM: Standard Error of the Mean

Within criterion. a. b. c: $p<0.05$;

NS. Not Significant ($p>0.05$).

Data were obtained from three replicates.

Table 5. Correlation coefficients (*r*) of analysed parameters considering the three treatments (WF. FB and MSM). the two storage times (T0 and T90) and the three species (European sea bass. gilthead sea bream and rainbow trout).

	SFA	MUFA	PUFAω6	PUFAω3	PUFA	EPA	DHA	CDs	TBARS	ABTS	DPPH	FRAP	ΔE	ΔpH
SFA	1	-0.06	-0.98**	0.90**	-0.63**	0.97**	0.84**	0.90**	0.44	-0.34	-0.17	-0.15		
MUFA	-0.06	1	0.07	-0.39	-0.73**	-0.14	-0.21	0.29	-0.45	0.50*	0.52*	0.81**		
PUFAω6	-0.98**	0.07	1	-0.94**	0.61**	-0.99**	-0.92**	-0.88**	-0.49*	0.37	0.19	0.20		
PUFAω3	0.90**	-0.39	-0.94**	1	-0.31	0.96**	0.94**	0.70**	0.60**	-0.51*	-0.35	-0.46		
PUFA	-0.63**	-0.73**	0.61**	-0.31	1	-0.56*	-0.41	-0.84**	0.03	-0.16	-0.29	-0.52*		
EPA	0.97**	-0.14	-0.99**	0.96**	-0.56*	1	0.93**	0.86**	0.48*	-0.38	-0.18	-0.26		
DHA	0.84**	-0.21	-0.92**	0.94**	-0.41	0.93**	1	0.68**	0.56*	-0.44	-0.26	-0.30		
CDs	0.90**	0.29	-0.88**	0.70**	-0.84**	0.86**	0.68**	1	0.06	-0.29*	-0.03	-0.06	-0.56**	-0.32
TBARS	0.44	-0.45	-0.49*	0.60**	0.05	0.48*	0.56*	0.06	1	-0.32*	-0.64**	-0.59**	0.47*	0.28
ABTS	-0.34	0.50*	0.37	-0.51*	-0.16	-0.38	-0.44	-0.29*	-0.32*	1	0.62**	0.47**	0.50**	0.52**
DPPH	-0.17	0.52*	0.19	-0.35	-0.29	-0.18	-0.26	-0.03	-0.64**	0.62**	1	0.52**	-0.26	-0.10
FRAP	-0.15	0.81**	0.20	-0.46	-0.52*	-0.26	-0.30	-0.06	-0.59**	0.47**	0.52**	1	-0.39*	-0.01
ΔE								-0.56**	0.47*	0.50**	-0.26	-0.39*	1	0.31
ΔpH								-0.32	0.28	0.52**	-0.10	-0.01	0.31	1

* Significant Correlation at p< 0.05.

** Significant Correlation at p< 0.01.

Highlights

- Mechanical separation system were tested on sea bass, sea bream, and rainbow trout
- Mechanical separation system decreased antioxidant capacity
- Mechanical separation system increased oxidative processes
- Using no marketable fish instead of waste result in high nutritional quality MSM