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Mechanical Separation Process for the value enhancement of Atlantic horse mackerel (*Trachurus trachurus*), a discard fish

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Abstract

Mechanically separated meat (MSM) is the product obtained by removing meat from bones by pressure application. Whole fillets and fish burgers from minced muscle and from mechanical separation of Atlantic horse mackerel (*Trachurus trachurus*) were evaluated immediately after processing (T_0) and after 90 d of storage at -20°C for parameters related to quality loss. Firstly, mechanical separation inhibited water losses (2.67% against 4.57 and 5.57% in whole fillets and burgers from minced muscle, respectively), but the colour of MSM was duller and redder than the samples from other groups. Horse mackerel contained low fat (<1 g/100 g muscle), and high PUFAω3 content (around 57 g/100 g total fatty acids), especially EPA and DHA (around 52 g/100

g) regardless the treatment. However, the species was susceptible to oxidation, as revealed by the high TBARS content at T_0 (more than 8 mg MDA-eq/100g muscle). Nevertheless, Atlantic horse mackerel showed a high antioxidant capacity (ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid, DPPH, 2,2-diphenyl-1-picrylhydrazyl, and FRAP, ferric-reducing ability) at T_0 which may protect muscle against oxidative damages both during processing treatment and storage.

Keywords: Mechanical separation process, horse mackerel, discard, mineral, lipid oxidation, antioxidant.

1. Introduction

In 2012 global fish production amounted to 158 million tonnes, coming from fishery (58%) and aquaculture (42%) (FAO, 2014). According to FAO, almost 80% of fish stocks in the world is completely, or almost, overexploited, depleted or in a state of collapse. Moreover, only few species, are commercialized and appreciated by consumers thus resulting in the production of high volumes of discards (FAO, 2014). Considering the state of collapse of fish stocks and marine habitats and the increase in per capita consumption of fish, the interest of the companies should be oriented to the use of fish by-products or discards.

Atlantic horse mackerel (*Trachurus trachurus* L. 1758) is an edible fish that belongs to the Carangidae family and it is still underutilised. This species is captured in amounts larger than consumption levels and thus a large portion of the catch is underutilised and transformed into animal feed. In the Mediterranean, the catch by pelagic trawl, bottom trawl, and trammel fisheries is very common, producing medium and low discard levels (15-39% and <15% of the catch, respectively). Most of the discards is represented by inedible, undersized, damaged and no or very low commercial value fish, such as Atlantic horse mackerel (*Trachurus trachurus*) which account for more than 40% of discards (http://ec.europa.eu/fisheries/documentation/studies/discards/annex_en.pdf).

Nevertheless, Atlantic horse mackerel has a very high nutritional value due to its omega-3 polyunsaturated fatty acids (PUFAω3) levels. This species in effect is characterised by the presence of highly unsaturated fatty acid and quite low fat content, highly depending on the catching season (Orban et al., 2011). Unfortunately, its composition negatively influences horse mackerel commercial shelf-life which is relatively short, estimated as 7 days when stored on ice (Sanjuás-Rey, Barros-Velázquez, & Aubourg., 2011). As well, frozen preservation is even limited because of a rapid deterioration of lipid fraction (Sofi, Zofair, Surasani, Nissar, & Singh, 2014).

Recently, technologies such as mechanical separation process have been demonstrated to be successfully applied in fish sector. Mechanically separated meat (MSM) is the product obtained by removing remaining meat from bones using mechanical means by applying high ($>10^4$ kPa) or low ($<10^4$ kPa) pressures. The normal structure of the muscle fibre is mostly lost or modified during this operation. Good quality meat has been obtained by utilising fish by-products from Brazilian catfish (*Brachyplatystoma vaillantii*) (Oliveira, Lourenço, Sousa, Peixoto Joele, & Ribeiro, 2015), and Nile tilapia (*Oreochromis niloticus*) (Kirschnik, Trindade, Gomide, Moro, & Viegas, 2013). Moreover, the use of undersized and damaged fish from marine and freshwater aquacultured species has been successfully investigated in order to obtained fish products (Secci et al., 2016). Nevertheless, scarce information on its possible role in fish discard recovery is present in the literature. A deep knowledge of the physicochemical properties of fish species with a low commercial interest, such as Atlantic horse mackerel, represents a fundamental step toward a process of discard species valorisation.

Thus, the present study focused on the physicochemical changes, and antioxidant content as well as oxidative stability of horse mackerel subjected to mechanically separation process and stored up to three months at -20 °C.

2. Material and Methods

2.1 Preparation of fish samples and storage conditions

Thirty-six Atlantic horse mackerel (*Trachurus trachurus*) were fished in south region of Tyrrhenian Sea, Italy, on April 2015. Fish (weight 178.48 ± 24.90 g; size 25.5 ± 2.4 cm), killed by asphyxia in ice slurry, immediately after death were transferred into polystyrene boxes, covered by ice, and moved to the processing company where twelve fish were minced by the belt-drum MSM machine Baader 601 (Lübeck, Germany). Fish were manually inserted into the soft separator where a conveyer belt pressed the fish on the surface of a perforated drum (hole diameter 5 mm). Meat passes through the holes, while bones, skin and thicker layers of connective tissue remain on the outside of the drum and are ejected through a discharge chute. A one-step separation was conduced. MSM was not subdued to washing or centrifugation phases. Then, the remained fish and the MSM were brought to the laboratories of Agri-Food Production and Environmental Sciences Department (University of Florence, Florence, Italy) where all the whole fish were beheaded, gutted and finally filleted. Whereas twelve fillets (right) were stored as fillet (WF samples), twelve fillets (left) were grounded by using a New Style Chopper (Westmark Gmbh, Elspe, Germany) in order to obtain the meat to form six fish-burgers (FB samples), while six other fish burgers (MSM samples) were obtained from MSM. Both FB and MSM burgers were made with 100% horse mackerel meat. Samples for each treatment were analysed at time 0 (T_0), and after 90 days of frozen storage (– 20 °C) (T_{90}). Six replicates of WF, 3 FB, and 3 MSM were analysed at each time for the following parameters: colour, pH, weight loss, chemical and mineral composition, total lipids (TL), fatty acid composition (FAs), primary (conjugated dienes, CD) and secondary (thiobarbituric acid substances, TBARS) oxidation products, and antioxidant capacity (by ABTS-reducing activity assay, DPPH-scavenging activity and FRAP assay), according to the methods detailed below.

2.2 Physical analyses

WF, FB, MSM weight loss, pH and colour were measured at T_0 and after 90 days of frozen storage. pH was monitored using a pH meter (Columbus, OH, USA) in three different points located on the dorsal region of whole fillets (WF) and along the diameter for burger measurements (FB and

MSM). A Dr Lange Spectro-colour® colorimeter (Keison International Ltd, Chelmsford, Essex, UK) equipped with a Spectral qc 3.6 software was utilized for colorimetric measurement. Colour measurements were carried out according to the CIELab system (CIE, 1976), and ΔE_{00} was calculated with the formula retrieved from Mokrzycki and Tatol (2011):

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

where β represents the values of colour parameters (L*, a*, and b*) measured at T_{90} and α represents the values of the same parameters measured at T_0.

2.3 Chemical composition

Moisture, crude protein (Nx6.25), and ash contents were determined by using 950.46, 976.05, and 920.153 AOAC (2012) methods, respectively.

2.4 Mineral content

At T_0, three samples for each treatment were lyophilized by using a vacuum pump (Vacuum Pump Welch Director – Welch Vacuum Technology Inc., Skokie, Illinois, USA) and utilised for determination of mineral composition. Calcium (Ca), phosphorous (P), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), sodium (Na), potassium (K), selenium (Se), arsenic (As), cadmium (Cd), and lead (Pb) contents were determined. One-hundred g of lyophilized sample was dissolved in 10 mL of concentrated nitric acid (67% Suprapur®, Merck, Darmstadt, Germany) in teflon tubes. The tubes were mineralized in a microwave (Mod. Mars, CEM Corporation, North Carolina, USA) by applying the mineralization stages at 1600 watt: 200 °C (ramp time 20 m, hold time 15 m). After cooling, the volume was made up to 25 mL with bi-distilled water. Minerals were measured by inductively coupled plasma - optical emission spectrometry (ICP-OES) (Mod. IRIS Intrepid II ICP Spectrometer, Thermo Electron Corporation, Massachusetts, USA). Trace minerals were quantified on the basis of peak areas and comparison with a calibration curve obtained with

the corresponding standards, the samples were not analysed a T_{90}, presuming their mineral composition similar to that at T_0.

2.5 Fatty acids

The total lipid content of the samples was determined according to Folch, Lees, and Sloane Stanley (1957) method and fatty acids (FAs) in lipid extract were trans-esterified 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), purchased from Agilent (Palo Alto, CA, USA). Chromatograms were recorded with computing integrator software (Galaxie Chromatography Data System 1.9.302.952; Varian Inc., Palo Alto, CA, USA) and FAs were identified by comparing the FAME retention time with the standard Supelco 37 component FAME mix (Supelco). FAs were quantified through calibration curves, using tricosanoic acid (C23:0) (Supelco) as internal standard. This analysis was carried out on WF and MSM samples, but not on FB samples, because they were obtained from minced fillets and consequently considered with the same characteristics in term of FA composition.

2.6 Antioxidant capacity

Samples of fillets and burgers (3 g) were extracted with 10 ml of ethanol. The antioxidant capacity was analysed on the ethanol-extracted samples according to Mancini et al. (2015). ABTS-reducing activity assay (ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)), DPPH-scavenging activity (DPPH, 2,2-diphenyl-1-picrylhydrazyl) and FRAP assay (ferric-reducing ability) were analysed.

2.7 Lipid oxidation products

Conjugated diene (CD) content in the lipid extract was measured by the colorimetric method proposed by Srinivasan, Xiong, and Decker (1996). The molar extinction coefficient of 29000 mL /mmol cm was utilised for CD quantification. The results are expressed as mmol hydroperoxides/kg lipid.

The 2-thiobarbituric acid reactive substances (TBARS) were measured using the colorimetric method descripted by Vyncke (1970) at 532 nm. A calibration curve prepared with TEP (1,1,3,3,-tetra-ethoxypropane) in 5% (w/v) TCA (0.2 to 3.1 μmol/L) was necessary for TBARS quantification.

2.8 Statistical analysis

The statistical analysis of data was performed using the SPSS version 17.0 software (SPSS Inc. Illinois, USA). Normality of data distributions was tested by the Kolmogorov-Smirnov test. A repeated measure model, considering 'treatment' (T) and 'storage' (S) as main factors, was performed on weight loss, pH, and colour parameters (L*, a* and b*). Bonferroni post-hoc test was used as post-hoc test. Chemical composition, primary and secondary oxidation products and antioxidant capacity were subjected to a two-way ANOVA with T and S and their interaction (TxS) as fixed effects, using Bonferroni post-hoc test to check again the significance of the differences among the treatment (WF, FB and MSM samples) and the storage (T_0 and T_{90}) levels. The same model (two-way ANOVA) was performed on the fatty acid profile data, but in this case only two treatment levels (WF and MSM) were considered. Mineral composition was subjected to one-way analysis of variance (ANOVA) with T as fixed effect, using the Bonferroni post-hoc test to check the significance of the differences among the levels (WF, FB and MSM samples).

3. Results and Discussion

3.1 Physical analyses

Burgers from MSM and minced meat (FB), and the whole fillets (WF) were analysed at the beginning and at the end of 90 days of frozen storage. Table 1 shows that weight loss was unaffected by treatment though a pattern may be discerned. Specifically, MSM showed the lowest losses, followed by WF and FB. Concerning storage, it did not significantly influence the weight loss.

No significant differences among treatments have been found for pH value (Table 1). The observed pH levels are in agreement with a previous study carried out on Mediterranean horse mackerel (Tzikas, Amvrosiadis, Soultos, & Georgakis, 2007). As a consequence of enzymatic and microorganism activities (Simeonidou, Govaris, & Vareltzis, 1997), pH value increased during storage at the same manner in all the considered treatments. Indeed, no significant effect of the interaction T x S emerged.

On the contrary, colour was significantly affected by MS process (Table 1). MSM samples resulted in the dullest meat, with significantly higher redness and yellowness indexes in comparison with WF samples. Such a pattern may be due to the presence of red muscle, myoglobin, mitochondria, glycogen, and cytochromes (Chaijan, Benjakul, Visessanguan, & Faustman, 2005) in horse mackerel muscle which may result more concentrated in handled meat and may influence the values of a* and b* chromaticity indexes. Furthermore, colour changes may be synthesised in ΔE_{00}. The ΔE_{00} values calculated at T_0 for horse mackerel subdued to different treatments were the follows: $\Delta E_{WF\text{-}FB}$ 9.08, $\Delta E_{WF\text{-}MSM}$ 9.66, and $\Delta E_{FB\text{-}MSM}$ 3.19. Mokrzycki and Tatol (2011) suggested that ΔE_{00} values higher than 5 represent samples which give the impression that the observer is looking at two different colours. Thus, every sample seems to be perceived as noticeably different from the samples of the other treatments, especially when MSM is compared to WF. Globally, results indicated that the colour of the samples was deeply affected by the handling/processing performed.

Even though no significant differences emerged, the pattern of colour alteration during frozen storage was in agreement with previous study (Chaijan et al., 2005). L* values tended to increase during frozen storage, in agreement with Torres, Saraiva, Guerra-Rodríguez, Aubourg, and Vázquez (2014), whilst the a* index decrease during storage regardless the treatment considered may be caused by the formation of meta-myoglobin, as suggested by Chaijan et al. (2005). At the end of the storage, the yellowness index (b*) slight decreased reaching a value comparable to that proposed by Torres et al. (2014). In summary, present results indicated a trend to a shift in the colour towards brown after the storage of three months at low negative temperature.

The colour variations emerged within the treatments at T_0 and T_{90} resulted evident by the calculated ΔE_{00} values that were 9.25, 8.14, and 3.64 (respectively for WF, FB and MSM), which means that WF, and FB at T_0 and T_{90} were perceived as different whilst only clear difference in colour was noticeable when MSM samples were compared at T_0 and at T_{90} ($3.5<\Delta E_{00}<5$, as reported by Mokrzycki and Tatol, 2011). Interestingly, MSM showed the lowest ΔE which signifies that mechanical separation process visibly altered the colour of the raw material even though such as parameter tended to remain stable during the storage at low negative temperature (-20 °C).

3.2 Chemical and mineral composition

Data of chemical composition of samples are reported in Table 2. Interestingly, MSM resulted the treatment with less ash content. A value for this parameter around 1-2% is generally accepted as an index of good separation process (Sen, 2005) for MSM fish. Indeed, such as value could be influenced by the presence of frame fragments, so lower the ash content better the quality of the process. Thus, the low ash content quantified in the present trial suggests that the mechanical separation process has been correctly applied and no exceeding fragments have been extracted during the mechanical process.

Lipid content (Table 2) did not go beyond 1% in WF, FB, and MSM. Data agree with the value found by Orban et al. (2011). Concerning moisture and crude protein, the same table shows that

MSM samples contained more water and less protein than both WF and FB samples. Cellular disruption should be responsible for differences in the sample analysed, especially considering the possible release of water soluble protein, like the sarcoplasmatic ones, but the MSM samples of this trial strangely resulted the richest in moisture and subjected to a reduced loss of weight during the trial, besides storage did not significantly influence proximate composition of samples except for protein content which increased, as a consequence of the decrease of water level.

Macronutrients (Ca, K, Mg, Na, P), essential trace elements (Cu, Fe, Se, Zn), as well as potentially toxic elements (As, Cd, Cr, Pb) were searched in the samples analysed, notwithstanding the content of As, Cd, Cr, Cu, Pb, and Se was below the instrument detection limit (1 mg/kg wet weight). Even though the macronutrients are present at varying concentrations, they are not often reported in literature for fish, for which heavy metals are considered more interesting for the concerns due their overexposure. The inclusion of these elements is in any case an important facet of the nutrient profile. Mineral composition of WF, FB, and MSM samples is reported in Table 3. In accordance to their categories, high levels of the K, P, and Na macronutrient were found together to low amounts of Zn and Fe. Wide concentration ranges were proposed by Özden (2010) in horse mackerel analysed at different catching months. Specifically, ranges of 297.12-550.16, 28.18-63.87, and 71.614-168.29 mg/100 g wet weight were found for K, Mg, and Na, respectively; thus the values found for these macronutrients in the present trial are comprised in that range that can be considered as reference, due the scarce literature related to the species considered. Zn concentration is just below the fifth percentile, equivalent to 6.417 mg/kg wet weight, proposed by Olmedo et al. (2013) in a study of the concentration of essential metals in fish and shellfish bought at the central market of Granada (Spain). On the other hand, iron concentration in the samples analysed (1.59-1.88 mg/100 g wet weight) was lower than those found by other authors. Particularly, range values from around 4 (Yilmaz, 2003) to 21 mg/100 g (Özden, 2010) were proposed for *Trachurus mediterraneus* and *Trachurus trachurus*. Such as high difference however may be related to the wide variability in fish composition. In detail, the mineral fraction is influenced by diet and water

where fish live or are reared (Alasalvar, Taylor, Zubcov, Shahidi, & Alexis, 2002) as well as by other factors, intrinsic (fish size, age, gender) and extrinsic (salinity, temperature, handling processes; Yilmaz, 2003; Erkan & Özden, 2007). Interestingly, treatment seemed to affect K and Mg contents by reducing their values whereas no effect on Ca and P content was found, confirming the efficiency of the mechanical separation process in separating the muscular part by the skeletal part of the fish carcass. It was concluded that MSM from horse mackerel can slightly contribute to mineral and trace element nutrition, anyway not being a significant contributor to dietary exposure of toxic elements.

3.3 Fatty acid composition and lipid oxidative stability

In Table 4, the fatty acid composition of WF and MSM samples is reported. SFA fraction was mainly composed by palmitic (C16:0) and stearic (C18:0) acids, while in the MUFA fraction, oleic acid (C18:1ω9) was the most abundant FA. Horse mackerel have remarkably high values of PUFA which accounted for 65%, of which around 89% was represented by PUFAω3 and only 9% by PUFAω6. Interestingly, almost 53% of the total fatty acids was represented by EPA and DHA. Plenty ranking is in agreement with Orban et al. (2011) results, even though the fish of the present trial had almost two times more the content of DHA in comparison with the values found in the paper previously cited. Mechanical separation process did not significantly alter the fatty acid composition. Thus, the large prevalence of PUFAω3 on PUFAω6 that characterises the lipid fraction of samples is an element of nutritional relevance and an index of the lipid quality of this species of fish. As a result, the high proportion of PUFAω3, especially of EPA and DHA, and the low fat content make horse mackerel a good source of PUFAω3 when this species is included in the human diet. A slight but significant increase of SFA content together to a not significant decrease of PUFAω3 content and a small but significant decrease in C22:5ω3 percentage can be attributed at the storage effect.

Lipid oxidation is a very important event leading the loss of nutritional quality and it is mainly due to degradation of PUFAω3. A previous study (Secci et al., 2016) performed on MSM from sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) showed that mechanical separation process significantly promoted lipid oxidation in seawater species, being TBARS more than two times higher in MSM (2.34 and 7.76 mg MDA-eq/kg muscle in sea bass and sea bream, respectively) than in WF (1.10 and 2.72 mg MDA-eq/kg muscle in sea bass and sea bream, respectively). Interestingly, in the present study mechanical separation process seems not to reduce lipid stability, indeed no significant differences between MSM and WF were found for CDs nor for TBARS levels (Table 5). Nevertheless, horse mackerel oxidative status was deeply compromised from the beginning (T_0), as revealed by the high TBARS values which exceeded the threshold of 8 mg MDA-eq/kg for the rancid odour perception (Shormüller, 1968). Such as damaged condition can be explained by considering that stress during fishing and killing have been shown to negatively affect the oxidative stability, thus resulting in an increase of lipid oxidation (Secci & Parisi, 2016). Specifically, asphyxia in air is a practice considered poor both for animal welfare and fish quality. In addition, the prevalence of red muscle in horse mackerel, rich in a pro-oxidant molecule like myoglobin, may be another cause of such high TBARS value (Tokur & Korkmaz, 2007; Maqsood, Benjakul, & Kamal-Eldin, 2012).

Interestingly, storage did not significantly affect lipid oxidation as demonstrated by TBARS values which are maintained at the same high levels found in the T_0 samples. Markedly low values of MDA-eq content were proposed by Aubourg, Piñeiro, and González (2004) for horse mackerel stored 90 days at -20 °C. Indeed, fish raised from 0.17 ± 0.03 to 1.04 ± 0.11 mg MDA-eq/kg muscle. Nevertheless, the increasing rate during the storage at similar condition of the present trial proposed by Aubourg et al. (2004) was higher than that of the present research, 6 times more after the storage period against 1.4 times more, respectively. Such as difference between the two studies may be attributed to the higher PUFA content of the samples analysed in the present trial than in the previous study (Aubourg et al., 2004).

In Table 5, the results obtained for antioxidant capacity assessment are also shown. The antioxidants naturally contained in muscle can act as radical scavenger against ABTS and DPPH or by reducing iron. Globally, MSM samples resulted significantly damaged by the treatment in comparison to both fillets and the burgers from minced muscle. As well, storage significantly reduced antioxidant content, in agree with previous findings (Sánchez-Alonso, Jiménez-Escrig, Saura-Calixto, & Borderías, 2007; Secci et al., 2016). The information about antioxidant content in fish are scarce. Horse mackerel contains tocopherol, but different levels of such antioxidant have been reported during last decades (Eymard, Baron, & Jacobsen, 2009; Farvin, Grejsen, & Jacobsen, 2012). Even though no tocopherol determination was conducted in the present study, data in Table 5 reveal a high potential antioxidant activity. This capacity may be attributed to the superoxide dismutase or catalase, two enzymes contained in red cells of pelagic fish, like horse mackerel, which act as antioxidant chemical species (Trenzado et al., 2006).

Furthermore, the statistical analysis underlined the presence of a significant interaction between treatment and storage for ABTS and DPPH. This fact can be explained by considering the different ongoing of the considered parameters. Specifically, ABTS capacity is highly compromised both immediately after treatment and at the end of the storage (Table 6). Indeed, WF showed a higher ABTS than FB, and MSM at T_0 thus suggesting that an exploitation of antioxidant capacity during processing might due to cellular disruption. Besides, a decrease of ABTS was especially evident in WF where it has undergone a 74% of reduction, and in MSM, with 41% of reduction. On the contrary, in FB ABTS significantly increased and raised the highest value at T_{90}. Concerning DPPH, data at T_0 confirm that both mincing and separation processes altered this parameter, like for ABTS. Again, all the species are subjected to a decrease during storage, in the measure of 24, 32, and 47% in WF, FB, and MSM, respectively. Finally, a high reduction of the antioxidant activity during storage is shown in each sample group, however, it seemed that the major part of the antioxidant was consumed at T_0 for preventing oxidative damages caused by the mincing and separation treatments.

High value of both oxidation and antioxidant parameters may be related to the post-mortem unbalance between the fish defence processes and the production of pro-oxidant species (Gülçin, 2006). In the case of an inadequate defence, a large amount of reactive oxygen species (ROS) can be produced and can promote lipid oxidation (Pazos, González, Gallardo, Torres, & Medina, 2005).

4. Conclusions

A deep knowledge of the physicochemical properties of fish species with a low commercial interest, such as horse mackerel, represents a fundamental step toward a process of valorisation of discard species. Some advantages and disadvantages emerge from the present study. Horse mackerel has low fat content, high PUFAω3 percentage, especially EPA and DHA, and good balance in mineral composition, thus resulting in a very interesting nutritional source. However, polyunsaturated fraction increases its susceptibility to oxidation and, in this sense, the traditional catching activities seem to deeply compromise lipid oxidation. Horse mackerel has however an high antioxidant power which may protect muscle against oxidative damages during both treatment and storage. Thanks to this content indeed, mechanical separation process seems to be applied without many detrimental effects on horse mackerel quality during frozen storage. Moreover, MSM resulted the treatment with the less weight loss and ash content but which deeply modify the colour of the burgers. In conclusion, mechanically separated meat from horse mackerel could be a high quality ingredient to add at meat from other fish species in order to obtain new fish products as burgers, nuggets, sticks, or even sauces which may represent a way for the valorisation of discard species and for enlarging the fish potential market.

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Table 1. Physical properties (weight loss %, pH and colour) of whole fillets (WF), minced burgers (FB), and MSM burgers (MSM) stored for 90 days at – 20 °C.

	Treatment (T)				Storage (S)			T x S
	WF	**FB**	**MSM**	**SEM**[1]	**T_0**	**T_{90}**	**SEM**	
Weight loss	4.57	5.57	2.67	1.64	0	4.27	1.43	NS
pH	6.79	6.80	6.66	0.12	6.61	6.65	0.12	NS
L*	26.09a	23.74a	14.74b	2.23	20.10	22.95	1.82	NS
a*	2.18b	10.01ab	15.95a	2.07	10.88	7.88	1.69	NS
b*	3.45b	12.82a	11.45a	0.67	9.36	9.12	0.55	NS

[1] Standard Error of the Mean.
Within criterion a, b: $p<0.05$.
NS: Not Significant ($p>0.05$).
Six replicates for WF and three replicates for FB and MSM have been utilised for each determination.

Table 2. Proximate composition (g/100 g muscle) of whole fillets (WF), minced burgers (FB), and MSM burgers (MSM) stored for 90 days at – 20 °C.

	Treatment (T)				Storage (S)			T x S
	WF	**FB**	**MSM**	**SEM**[1]	**T_0**	**T_{90}**	**SEM**	
Moisture	77.58b	76.95b	80.50a	0.35	78.77	77.91	0.29	NS
Total Lipid	0.82	0.66	0.42	0.13	0.63	0.64	0.10	NS
Crude Protein	19.90a	20.56a	17.37b	0.22	18.84b	19.71a	0.18	NS
Ash	1.44a	1.48a	1.26b	0.02	1.37	1.42	0.02	NS

[1] Standard Error of the Mean.
Within criterion a, b: $p<0.05$.
NS: Not Significant ($p>0.05$).
Six replicates for WF and three replicates for FB and MSM have been utilised for each determination.

Table 3. Mineral composition (mg/100g wet weight) of whole fillets (WF), minced burgers (FB), and MSM burgers (MSM) at T_0.

	Treatment			SEM[1]
	WF	**FB**	**MSM**	
Ca	39.79	53.37	34.88	15.97
Fe	1.59	1.88	1.59	0.22
K	326.78ab	328.81a	288.19b	8.34
Mg	41.54a	39.62a	32.79b	1.21
Na	121.43	109.63	99.58	8.39
P	198.15	205.90	175.47	7.44
Zn	0.67	0.57	0.56	0.04

As, Cd, Cr, Cu, Pb, and Se were also researched but the contents were below the detection limit (1mg/kg).

[1] Standard Error of the Mean.
Within criterion a, b: $p<0.05$.
NS: Not Significant ($p>0.05$).
Six replicates for WF and three replicates for FB and MSM have been utilised for each determination.

Table 4. Fatty acid composition (g fatty acid/100 g total fatty acid) of whole fillets (WF), and MSM burgers (MSM) stored for 90 days at – 20 °C.

	Treatment (T)		Storage (S)			T x S
	WF	**MSM**	**T_0**	**T_{90}**	**SEM[1]**	
C16:0	12.26	11.95	11.08b	13.13a	0.31	NS
C18:0	6.24a	5.75b	5.58b	6.41a	0.13	NS
C18:1ω9	10.24	8.41	9.02	9.63	0.83	NS
C20:5ω3	5.57	6.41	6.24	5.74	0.32	NS
C22:5ω3	4.28	4.32	4.75a	3.84b	0.12	NS
C22:6ω3	46.68	48.49	48.80	46.37	1.21	NS
ΣSFA	20.82	19.83	18.95b	21.70a	0.49	NS
ΣMUFA	15.37	13.44	13.97	14.83	1.10	NS
ΣPUFAω6	5.91	6.00	5.90	6.01	0.20	NS
ΣPUFAω3	57.46	60.31	60.80	56.98	1.39	NS
ΣPUFA	63.81	66.73	67.07	63.46	1.56	NS

C12:0, C13:0, C14, C14:1ω5, C15:0, C15:1, C16:1ω7, C16:1ω9, C16:2ω4, C16:3ω4, C16:4ω1, C17:0, C17:1, C18:1ω7, C18:2ω6, C18:3ω3, C18:3ω6, C18:3ω4, C18:4ω1, C20:0, C20:1ω9, C20:1ω11, C20:1ω7, C20:2ω6, C20:3ω6, C20:3ω3, C20:4ω6, C20:4ω3, C21:0, C21:5ω3, C22:0, C22:1ω11, C22:1ω9, C22:1ω7, C22:2ω6, C22:4ω6, C22:5ω6, C24:0, C24:1ω9 were detected but not reported in the table because < 3%. All the mentioned fatty acids have been utilised for calculating the lipid fractions.

[1] Standard Error of the Mean.

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

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

Six replicates for WF and three replicates for FB and MSM have been utilised for each determination.

Table 5. Lipid oxidation (CD, mmolHp/100g fat; TBARS, mg MDA-eq/100g muscle) extent and antioxidant capacity (ABTS$^{\bullet}$, mmol Trolox/kg muscle; DPPH, mmol Trolox/kg muscle; FRAP, mmol Fe^{2+}/kg sample) of whole fillets (WF), minced burgers (FB), and MSM burgers (MSM) stored for 90 days at – 20 °C.

	Treatment (T)				Storage (S)			T x S
	WF	**FB**	**MSM**	**SEM[1]**	**T_0**	**T_{90}**	**SEM**	
CD	0.15ab	0.20a	0.14b	0.01	0.17	0.15	0.01	NS
TBARS	8.14	11.59	8.07	1.82	7.70	10.84	1.49	NS
ABTS	0.73a	0.46b	0.36c	0.02	0.67a	0.36b	0.02	0.00
DPPH	0.18a	0.16b	0.13c	0.00	0.19a	0.13b	0.00	0.02
FRAP	0.32a	0.26b	0.16c	0.02	0.33a	0.16b	0.01	NS

[1] Standard Error of the Mean.
Within criterion a, b, c: $p<0.05$.
NS: Not Significant ($p>0.05$).
Six replicates for WF and three replicates for FB and MSM have been utilised for each determination.

Table 6. Interaction (TxS) data for ABTS$^{\bullet}$ **(**mmol Trolox/kg muscle) and DPPH (mmol Trolox/kg muscle) of whole fillets (WF), minced burgers (FB) and MSM burgers (MSM) at T_0 and T_{90}.

	ABTS			DPPH		
	T0	**T90**	**SEM[1]**	**T_0**	**T_{90}**	**SEM[1]**
WF	1.16ax	0.30by	0.00	0.21ax	0.16bx	0.02
FB	0.40by	0.52ax	0.00	0.19ay	0.13by	0.02
MSM	0.45ay	0.26bz	0.00	0.17az	0.09bz	0.02

[1] Standard Error of the Mean.
Within storage: a, b, c: $p<0.05$.
Within treatment: x, y, z: $p<0.05$.

Industrial relevance

The mechanical separation process described in the article has been largely utilized for terrestrial animal products. However, it is seldom adopted by fish industry, especially for recovering discard fish species. Horse mackerel is an underutilized species, normally transformed into animal feed despite its high levels of omega-3 polyunsaturated fatty acids. Therefore this study was conducted in order to determine the effect of a mechanical separation technique on the physicochemical properties of horse mackerel. Our study showed that, although this species is susceptible to oxidative changes, mechanically separate meat can be a high-quality ingredient in burgers, nuggets, sticks, or even sauces which may represent a way for the valorisation of discard species.

- Mechanical separation technology was applied on horse mackerel
- The benefit and limitations of the mechanical separation technique were described
- Mineral and fatty acid composition, antioxidant capacity and oxidative status were evaluated
- Mechanically separated meat from discard fish could be a high quality ingredient