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Application of the Mechanical Separation Process in Different Fish Species for the Development of a New Product Based on Fish

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Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor substantially overlapping with material submitted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment is made in the text.

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AKNOWLEDGEMENTS

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PART 1

Abstract

This PhD thesis is based on three main trials. The overall aim was to investigate the feasibility of using discard fish by adopting mechanical separation process to different fish species for creating new fish products (fish burgers). Different formulae of fish burgers were evaluated physically, chemically, nutritionally, and sensorily.

The first research was conducted in order to investigate the impact of exploiting fish waste by mechanical separation process (MS) in order to produce ready-to-eat/cook foods based on fish. The aim was to evaluate the effect of MS process on physical and chemical characteristics, and on the nutritional value of the three farmed species: the European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), and rainbow trout (*Oncorhynchus mykiss*). Specifically, mechanically separated meat (MSM) burgers were compared with manual minced-burgers and whole fillets by evaluating yield, colour, pH, dienes, proximate composition, fatty acid profiles, and mineral composition. Results revealed that rainbow trout showed the highest yield for both manually and mechanically separated meat (53 and 50 g/100 g, respectively). The yield of MS process of sea bass and sea bream was higher than the manual operation yield (42 and 45 g/100 g, respectively against 39 and 40 g/100 g). The proximate composition, pH, colour, and mineral compositions are differently affected by MS process in the different fish species. However, MS slightly increased water content in sea bream and trout (71.12, and 70.65 g/100 g, respectively against 68.05, and 68.11 g/100 g of fillets) and decreased minerals, especially in trout, which showed loss of Ca, Mg, Na, and P. Interestingly, the fatty acid profiles of whole fillet, MS burger, and minced burger did not change. In conclusion, the MS process enabled manufacturing products with good characteristics in terms of yield and maintained nutritional value.

In the second research, non-directly marketable specimens of European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) were used in order to produce healthy clean label products, and to examine the instrumental, chemical and sensory properties of raw and cooked fish burger recipes obtained from MSM characterized by differences in the recipe composition. Consumer attention towards healthy and more natural foods and producer attempts to reduce food loss have become more popular nowadays. For this reason, shear stress, proximate composition, fatty acid composition, and sensory characteristics of four formulations of fish burgers were examined. The four formulae differed in the ratios of European sea bass to rainbow trout (50:50 and 30:70) and the ratios of fish to potato flakes (dry matter ratio, DMR: 2.5:1 and 1.5:1). Results showed that the sensory attributes were affected mostly by the potato content of fish burger, whereas the effect of sea bass to trout ratio was negligible. The recipes with higher DMR were related to sandy, crusty, and dry

features, salty taste and the flavour of raw fish, while the lower DMR recipes were distinguished by soft texture, and a starchy flavour and a flavour of fish cooked in the oven. Moreover, shear stress was unaffected by the different ratios of fish or potato flakes in raw and cooked burgers. However, raw burgers with lower DMR had higher moisture and ash, and lower protein content, while cooked burgers with lower DMR had higher moisture and lower protein content. Interestingly, the fatty acid profiles of the four cooked burger recipes were not significantly different, and a quantity of 100 g of burger provided more than the recommended daily intake of the essential fatty acids. In brief, development of ready-to-cook products based on under-utilized fish through four clean label recipes of high nutritional value and good sensory attributes was attained, irrespective of prevalence of rainbow trout over the more expensive sea bass, or using higher ratio of potato flakes.

In the third study instead, two mechanically separated meat from two fish species, the European sea bass (*Dicentrarchus labrax*), and rainbow trout (*Oncorhynchus mykiss*), were used for obtaining fish burgers submitted to a frozen storage. Recently ready-to-cook fish products, which are generally marketed as frozen and need some culinary preparation, stimulated the fish consumption. Therefore, the target of this research was to study the effect of two recipes, distinguished by the ratios of European sea bass to rainbow trout (50:50 and 30:70), and storage duration at sub-zero temperatures for obtaining convenient, easy-to-prepare, and good quality products. Particularly, the physical, chemical, and nutritional properties of raw and cooked fish burger of different formulations were assessed during storage. Results revealed that raw recipes with more trout have higher moisture, shear stress, yellowness, and intense colour. Conversely, they have lower values of primary (conjugated dienes) and secondary (TBARS) oxidation products. On the other hand, cooked recipes with more trout have more moisture, but lower protein content, and higher water holding capacity, yellowness, and intense colour. Furthermore, storage was found to significantly affect the shear stress, water-holding capacity and colour in raw and cooked fish burgers, causing their values to decline at the end of the storage. Excitingly, the nutritional value of raw and cooked fish burgers was decent and was not altered by the different formulae and storage durations. The highest oxidative stability was obtained in fish burger containing a high proportion of rainbow trout, which could be a matter of importance for the seafood industry because to the lower economical value of this species than sea bass.

1. Introduction

1.1 The fish sector

1.1.1 The importance of fish as a source of nutrients

Fish has always been one of the essential elements for the nourishment of humankind. From the beginning of human history, humans worked to provide themselves and their families with this precious food, acquiring the techniques and skills for fishing from the water along the rivers as in the open sea. Coastal civilizations had always a direct access to fish capture, enjoying seasonality and natural availability. However, peoples who lived in the hinterland could not benefit by the same scale. Only with the birth of trades, fish became a food of greater enjoyment for everyone, and is still one of the most traded food products worldwide (Allison, 2011).

Nowadays, hunger and malnutrition are the world's most devastating problems, and inextricably linked to poverty. About 795 million people are globally undernourished (FAO, IFAD & WFP, 2015). Fish and other aquatic animals make an indispensable contribution to food and nutrition security in many Asian and African countries and the aquaculture sector, especially the capture fisheries that provide a vast majority of livelihood opportunities, highly nutritious fresh and processed fish of the poor (Belton & Thilsted, 2014). However, the global population is increasing and, in order to maintain at least the current level of per-capita consumption of aquatic foods, which was equal to 20.3 kg in 2016 (FAO, 2018), the world will require an additional 23 million tonnes thereof by 2020.

Fish is acknowledged as an integral component of a well-balanced diet, providing a healthy source of energy, high-quality proteins, vitamins and a wide range of other important nutrients as it is shown in Figure 1. Fish protein accounts to about 17% of the protein in the diet at the global level but exceeding 50% in many least-developed countries. In addition, fish is rich of long-chain polyunsaturated fatty acids, particularly omega-3 fatty acids, and in particular of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) which have shown beneficial effects for optimal neurodevelopment in children and for improving cardiovascular health (FAO, 2016; FAO, 2018).

Moreover, fish consumption is accompanied with many health outcomes: reducing the risk of death from coronary heart disease; improving neurodevelopment in infants and young children, when mothers consume fish before and during pregnancy and during breastfeeding; and playing a major role in correcting unbalanced diets (FAO, 2014). Fish not only provide macronutrients, but also micronutrients that are not widely available from other sources in the diets of the poor people. Micronutrient deficiencies affect hundreds of million people, especially women and children in the developing world. More than 250 million children worldwide are at risk of vitamin A deficiency, 200

million people have goitre, and 20 million are mentally retarded, as a result of the iodine deficiency. Almost 2 billion people (nearly 30% of the world's population) are iron deficient, and 800 000-child deaths per year are attributable to zinc deficiency (FAO, 2014).

Fish products are considered among the main sources of vitamins and minerals. Small-sized fish species are consumed as a whole, with heads and bones, and can be an excellent source of many essential minerals such as iodine, selenium, zinc, iron, calcium, phosphorus, potassium, and vitamins such as vitamins A, D and B (Roos et al., 2007). The levels of these nutrients are also high in larger fish, but the highest contents are in the parts that are usually not eaten, such as heads, bones and viscera. Fatty fish can also be an important and unique source of vitamin D, which is essential for bone health. In areas lacking sun in winter where the skin is not exposed to sunshine, vitamin D deficiency is increasingly acknowledged as a serious health problem, but it can potentially be corrected by increased consumption of fatty fish.

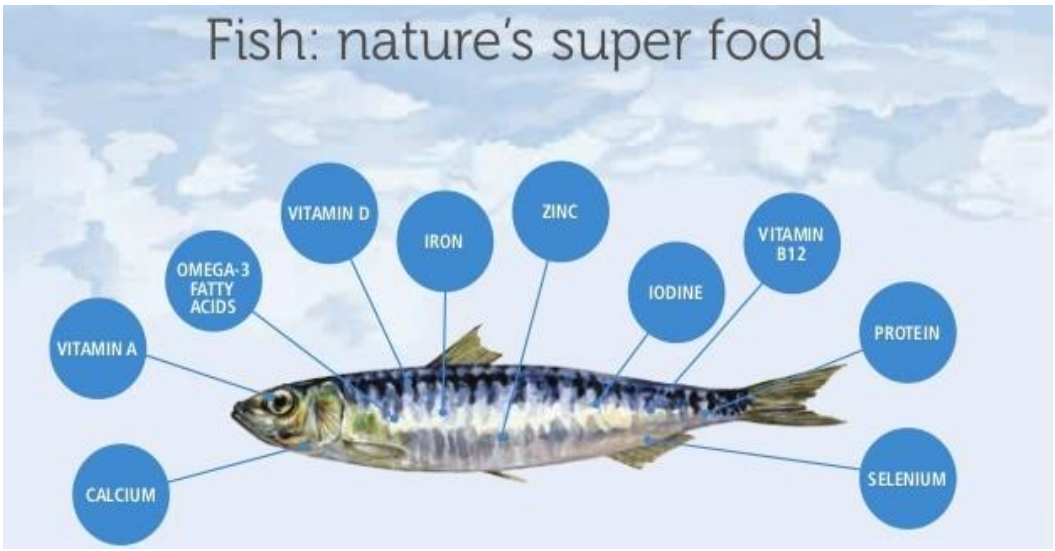

Figure 1. Fish as a source of nutrients (FAO, 2017).

1.1.2 Fish production: a global overview

As mentioned above, fisheries and aquaculture are important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world, thanks to its contribution to eliminate hunger and poverty, promote health, and create employment. Recently, fish production is not only based largely on fish capture, but also on aquaculture production that is steadily growing in many parts of the world. Figure 2 shows that within the last four decades, the aquaculture production has

substantially increased, almost by four times from less than 20 million tons in the 1990s to almost 80 million tons in 2016, and it is projected to further go up and to exceed 100 million tons by 2025. In fact, its contribution to the total world production of aquatic animals has recently (in 2014) exceeded that of capture destined for human consumption (OECD/FAO, 2017).

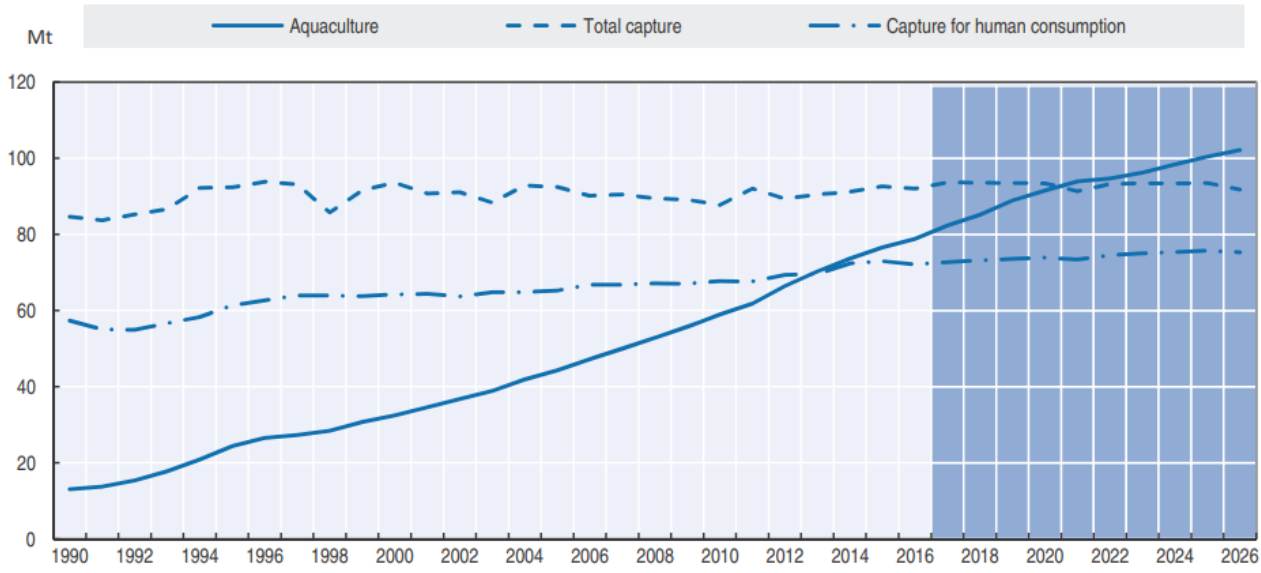

Figure 2. Comparison of world capture and aquaculture in the total production of aquatic animals (excluding algae) (OECD/FAO, 2017).

World production from fishing in 2014 was 93.4 million tons, from which 81.5 million tons came from marine waters, while the remaining 11.9 million tons from inland waters. The main groups of species produced from inland aquaculture and marine aquaculture differ across continents. They include 362 species of finfish (including hybrids), 104 of molluscs, 62 of crustaceans, 6 of frogs and reptiles, 9 of aquatic invertebrates, and 37 of aquatic plants. Figure 3 shows the aquaculture production by continent and for the ten top producing countries. All continents display a general trend of an increasing share of aquaculture, with Asia having the highest percentage of fish production (89%), while Oceania's share has declined in the last three years (FAO, 2016).

Worldwide, 35 countries produced more farmed than wild-caught fish in 2014. These countries have a population of 3.3 billion (45% of the world's population), including the five major producers namely: China, India, Viet Nam, Bangladesh, and Egypt (FAO, 2016). In terms of marine fishery capture, the top four producers in the world are China (14,811,390 tons), Indonesia (6,016,525 tons), the United States of America (4,954,467 tons) and the Russian Federation (4,000,702 tons) (FAO, 2016; FAO, 2018).

Production of aquatic animals from aquaculture in 2016 was 80 million tons, with an estimated sales value of 231.6 million USD dollars (FAO, 2018). In 2014, China produced more than 60% of world aquaculture production, amounting to 45.5 million tons. Other important producing countries were India (4,884 tons), Vietnam (3,411.4 tons), Bangladesh (1,956.9 tons) and Egypt (1,137.1 tons) (FAO, 2016).

Fish populations are highly exploited by biologically unsustainable capture systems leading to excessive fishing and risking the depletion of this precious natural resource. According to FAO (2016), there have been recent declines in the captured fish quantities due to several factors, from which the most important may be the restrictions imposed on illegal and over-fishing in order to safeguard fish resources and enhance their sustainability, which may have also encouraged the adoption of biologically sustainable fishing systems, leading to further decline in fishing. However, these recent declines were not translated into significant improvements in fish marine stocks, despite the progress achieved in some geographical areas in monitoring and controlling the capture systems (FAO, 2016).

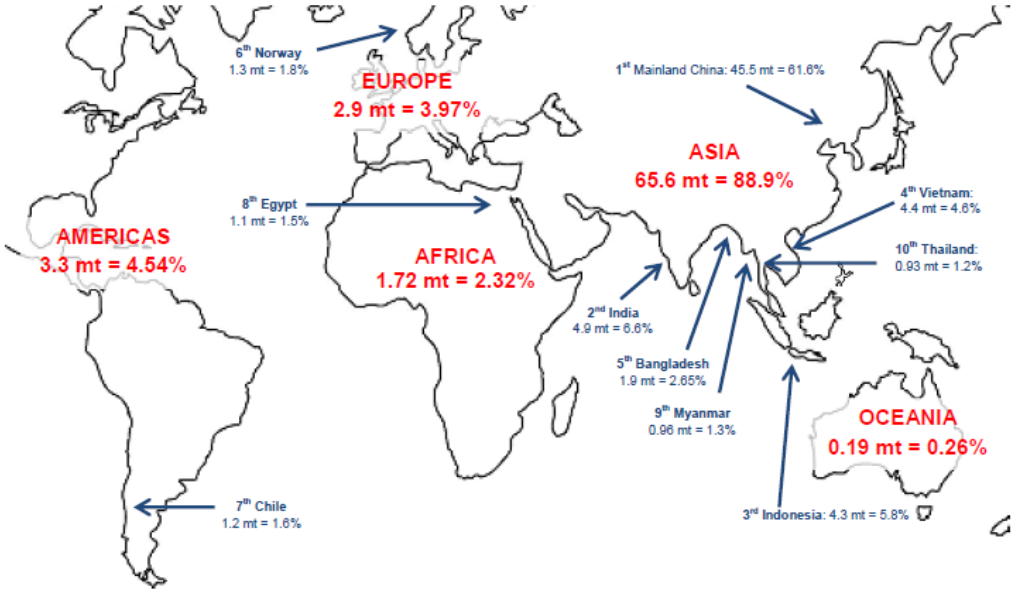

Figure 3. Farmed aquatic animal production in 2014: regional production and top 5 producers (FAO, 2017).

The proportion of world fish production used for direct human consumption has increased considerably in the last few decades, from 67% in the 60s of the last century to 87% in 2014. In that year, more than 146 million tons were destined for direct human consumption, while the remaining 21 million were destined for non-food consumption, of which 76% was in the form of fish meal and

fish oil. Almost half of the fish (46%) used for direct human consumption was in the form of live, fresh or chilled fish (equal to 67 million tons), which are the most popular forms and have higher prices. The other half was in processed forms with about 12% (17 million tons) processed through drying, salting, and smoking, 13% (19 million tons) processed in other preserved forms, and 30% (44 million tons) frozen (FAO, 2016).

1.1.3 Fish production in the EU

In 2015, the volume of aquaculture production in the EU was estimated at 1.3 million tons, equating to one fifth of total EU fisheries production (Figure 4). Globally, the EU aquaculture sector was classified ninth, with a 1.2% share in volume. The value of aquaculture production accounted for 4 billion €. This equates to 1% of the output value of agricultural production. However, the EU is the world's largest importer of fisheries and aquaculture products (Eurostat, 2018a).

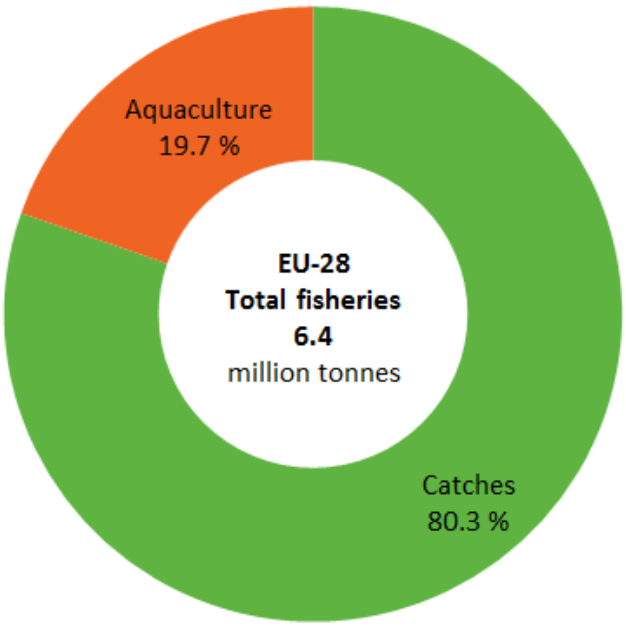

Figure 4. Aquaculture production and fish catches in EU-28, in 2015 (Eurostat, 2018).

Five members Countries (Spain, the United Kingdom, France, Italy and Greece) form three quarters of both production value and production volume at the EU level, where the sector shows a high diversity. In 2015, within the EU-28, 137 different species were farmed in aquaculture. However,

only 10 species make up 90% of the EU production in volume, in 2015. The most produced species is the Mediterranean mussel that accounted for nearly one fourth (24.7%), followed by Atlantic salmon (14.8%), rainbow trout (13.8%) and blue mussels (10.2%). They are followed by gilthead seabream, Pacific cupped oyster, common carp and European seabass, with each of them having an individual share of around 6% (Figure 5). At the country level, there is a tendency for specialization in only a few species (Eurostat, 2018b).

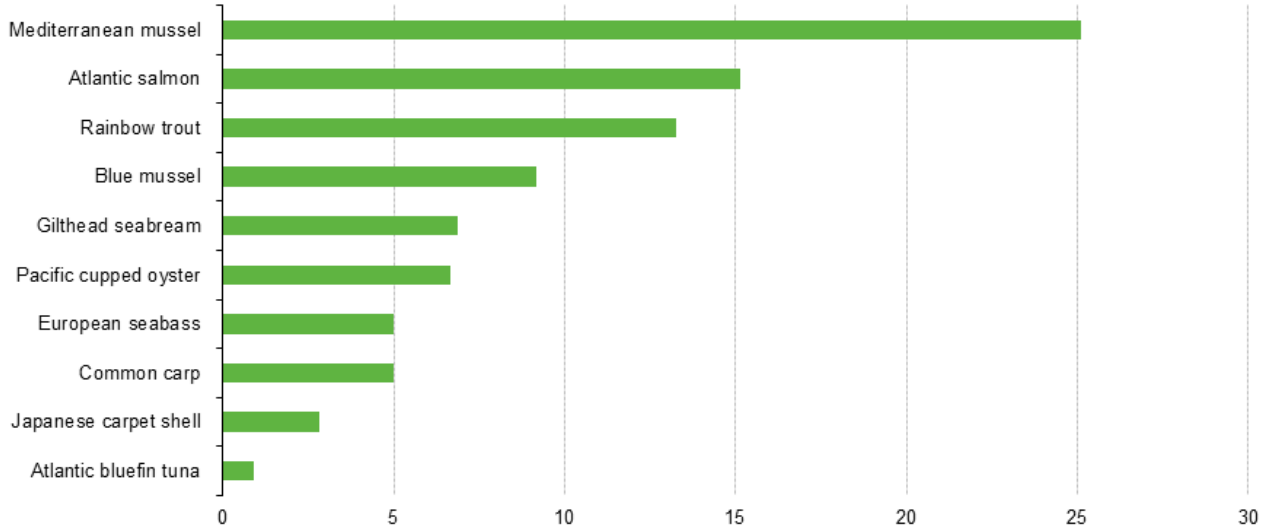

Figure 5. Ten major species of the aquaculture production in EU-28, in 2014 (in % of the total aquaculture production, expressed in tons live weight; Eurostat, 2018b).

The main producing countries in EU's aquaculture production in 2015 were Spain, the United Kingdom and France, producing together more than half of the EU's production volume (with shares of 23.3%, 16.8 % and 13.0%, respectively). In addition, Italy and Greece were considered major producers with shares of 11.8% and 8.4%, respectively. From the aspect of economic value, the United Kingdom had the highest share (24.1%), followed by France (15.0%), Spain (12.4%), Greece (11.2%) and Italy (10.6%). Therefore, only five EU countries were responsible for almost three quarters of the aquaculture production volume and value (Eurostat, 2018a).

Norway in 2015 was by far the biggest aquaculture producer in Europe, exceeding the EU in volume and value. This country produced 1.4 million tons, worth 5.2 billion €, making it the world's eighth largest producer in farmed fisheries, with a 1.3% share. Furthermore, Norway in 2013 was classified as the world's largest producer of marine finfish, according to Food and Agriculture Organisation of the United Nations, due to its salmon production (Eurostat, 2018a).

In general, aquaculture plays a major role in the countries around the Mediterranean and the Black Sea: Slovenia, Malta, Cyprus, Romania, Greece, Bulgaria and Italy. Those Member States tend to catch fish mostly along their coasts, using small-scale vessels with an average capacity lower than the EU average (equal to 18.9 gross tons, in 2015). As a counterbalance, their aquaculture activity plays a major role, representing 81.6% (Malta), 78.7% (Cyprus), 69.5% (Romania), 62.2% (Greece), 54.9% (Bulgaria) and 43.6% (Italy) of their respective total fisheries production.

In 2014, finfish and molluscs formed 98.2% of all aquaculture production in the EU. Whilst, the production of crustaceans, algae and other organisms was small. More than 130 species were farmed in the EU in 2014, however the most three common species were the Mediterranean mussel (*Mytilus galloprovincialis*), Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), which accounted for over half of all production (53.5%) and two-fifths (42.4%) of the value. Figure 5 illustrates the most10 major species in terms of aquaculture production.

These three common species formed about 90% of production and 87% of value. The Atlantic salmon was the leading species in the European aquaculture, although it ranked second for volume (15.1%). However, rainbow trout was the most widespread: it was grown in 24 European countries, both in inland freshwaters (84.2%) or in the saltwater of the North East Atlantic (15.8%), where this species is reared mainly in tanks (64.9%). Three countries together accounted for more than half of the total weight: Italy (17.9%), Denmark (17.3%) and France (16.8%).

1.1.4 Fish production in Italy

As mentioned above, Italy is one of the five major aquaculture producers in both production value and production volume at the EU level. Aquaculture sector contributes approximately 48% to total national fish production, amounting to about 162,600 tons, of which 38,800 tons derive from freshwater aquaculture (24%) and 123,800 tons from marine and brackish aquaculture (76%) (FAO, 2015). Nevertheless, fisheries and aquaculture sectors not occupy a gross position in the Italian economy.

In 2010, these sectors accounted for less than 0.1% of the Italian Gross Domestic Product (GDP) and 5.7% of the Agricultural Added Value. The total production of the fishing sector was 340,000 tons in 2013. The control policy of the European Union (EC Reg. 1967/2006) led to a significant reduction in the Italian fleet capacity with a consequent reduction in catches of about 44% between 2006 and 2013. During this period, the budget deficit was aggravated not only owing to reduction in exports and great increase in imports, but also to lower domestic production. The estimated commercial value

of fish product imports was 5.8 billion US dollars in 2013, while exports were of only 760 million US $ (FAO, 2015).

The aquaculture sector in Italy includes both marine and freshwater farming with about forty species of fish, shellfish and crustaceans. However, 97% of the production is based on five species: rainbow trout in freshwater and European seabass, gilthead seabream, Mediterranean mussel, and Japanese carpet shell in marine waters (FAO, 2015). In 2013, there were 820 Italian aquaculture companies, mainly situated in the north (64%), while the remaining was located in the south and the Islands (26%) and in the centre (10%). Since 2002, the companies' numbers increased by 22%; however, in the recent years this number decreased due to the reform of enterprises (especially shellfish) and the temporary or permanent closure of some mariculture farms (FAO, 2015).

Presently, the aquaculture sector encounters several problems including the intense price competition in the seabass and seabream markets coming from some Mediterranean countries such as Turkey and Greece, while there has been an increasing production cost for the Italian fish farmers due to the elevation of energy price and to the running costs especially for fish meals, as important ingredient for aquafeeds. The lack of a close connection with the public research sector, which can play an important role in creating innovative solutions to reduce the costs and enhance the sector competitiveness, is another constraint.

1.1.5 Fish consumption: an overview

Global fish per capita consumption has dramatically grown from 9 kg in the 1961 to 20.2 kg in 2015 at an average rate of about 1.5% per year, and the initiatory estimates for 2016 and 2017 referred to continuous growth to about 20.3 and 20.5 kg respectively. Various factors played a role in this increased consumption, including: production increases, reduced wastage, better utilization, improved distribution channels, and growing demand interlinked with population growth, rising incomes, and urbanization (FAO, 2018). The increases in fish consumption, in terms of quantity and variety consumed per head, are distributed differently among and within countries and regions. For example, the fish per capita consumption decreased in some countries of sub-Sahara Africa, while increased in Japan. In addition, it is noted that the consumption is usually higher in the coastal, riverine and inland water areas. Moreover, fish consumption is also unequal between the developed and developing countries. There was an evident increase in the annual per capita consumption of fishery products in developing countries (from 5.2 kg in 1960 to 18.8 kg in 2013) and in countries with low income and food deficit (from 5.2 kg to 7.6 kg). However, the previous values are still markedly lower than the consumption recorded in the developed countries (23 kg/ per capita of fish in 2013) and in industrialized countries (26.8 kg / per capita) (FAO, 2016).

In 2014, the EU annual fish per capita consumption reached to 25.5 kg, with an increase equal to 1 kg compared the figure in 2013. The growth was mainly for farmed products (19.05 kg of per capita consumption) rather than for fisheries products (6.48 kg of per capita consumption) (EUMOFA, 2016). Croatia, France, Greece, Italy, Slovenia, Spain and Portugal (European Mediterranean) are among the world's top consumers of seafood in the Mediterranean (Eurostat, 2014), but Italy, Spain, and France amounted for more than half of the EU consumption, despite having only around one third of the EU's population (Eurostat, 2014).

Italy was characterized by an apparent per capita fish consumption of 28.9 kg in 2014, slightly above the EU average (EUMOFA, 2016). It is worth mentioning that, in 2014, 3.6 kg/year per capita consumption of seafood in Italy were in the form of packaged/processed fish and seafood products (Inside Italy, 2014). Italy is the third-largest market for fish and seafood suppliers among the European Union countries, just after Spain and France, and the sixth-largest in the world. Italy imported US$5.6 billion worth of seafood products in 2015. Spain, the Netherlands, and Denmark were the Italy's top three suppliers, and the top imported products were prepared or preserved tuna, skipjack or bonito (Inside Italy, 2016).

In 2016, Italy ranked second in volume of fresh fish consumed by EU households, with 330,000 tonnes, and ranked third in its value of 2.8 billion €, after Spain and the UK. In volume, mussel was the most important species consumed in Italy, whereas the most valued species consumed were seabream, squid, octopus, cod and European seabass, which covered 30% of the total consumption (EUMOFA, 2017a). The most used purchasing channel for fish products was the supermarkets with an incidence of approximately 35.4% of the total volumes consumed by households, in 2015. However, this percentage mainly includes the purchases of processed products such as frozen foods, canned, salted, and smoked products. The presence of the fresh fish counters in the retail outlets such as fishmongers is considered a very effective and competitive element for specialized shops. Yet in 2015, specialized stores with 33% of total sales in volume of the fresh fish products have a share close to that covered by supermarket sales (ISMEA data, 2015).

1.1.6 Main cultured fish species in Italy

Three species for European aquaculture are considered in this study, which are rainbow trout (*Oncorhynchus mykiss*), European sea bass (*Dicentrarchus labrax*), and gilthead sea bream (*Sparus aurata*).

In the following section, the major characteristics of these three species are presented, with European sea bass and gilthead sea bream being presented together because they are both carnivorous marine finfish that have very similar biology and life history.

Rainbow trout (*Oncorhynchus mykiss*)

Rainbow trout is cultured on every continent, except Antarctica, and it is the most farmed fish species within the European Union. Historically, the major EU producers were Denmark, France, Germany, Italy, and Spain. According to FAO data, world production of rainbow trout has grown exponentially since the 1950s, especially in Europe and more recently in Chile and Iran as well as in Turkey, whose production exceeded that of all European countries, as shown in Figure 6. The annual world production (2014-2016) is estimated at about 800 000-820 000 tons. In 2015, the EU production of farmed rainbow trout was estimated at 240 000 tons (FEAP, 2015).

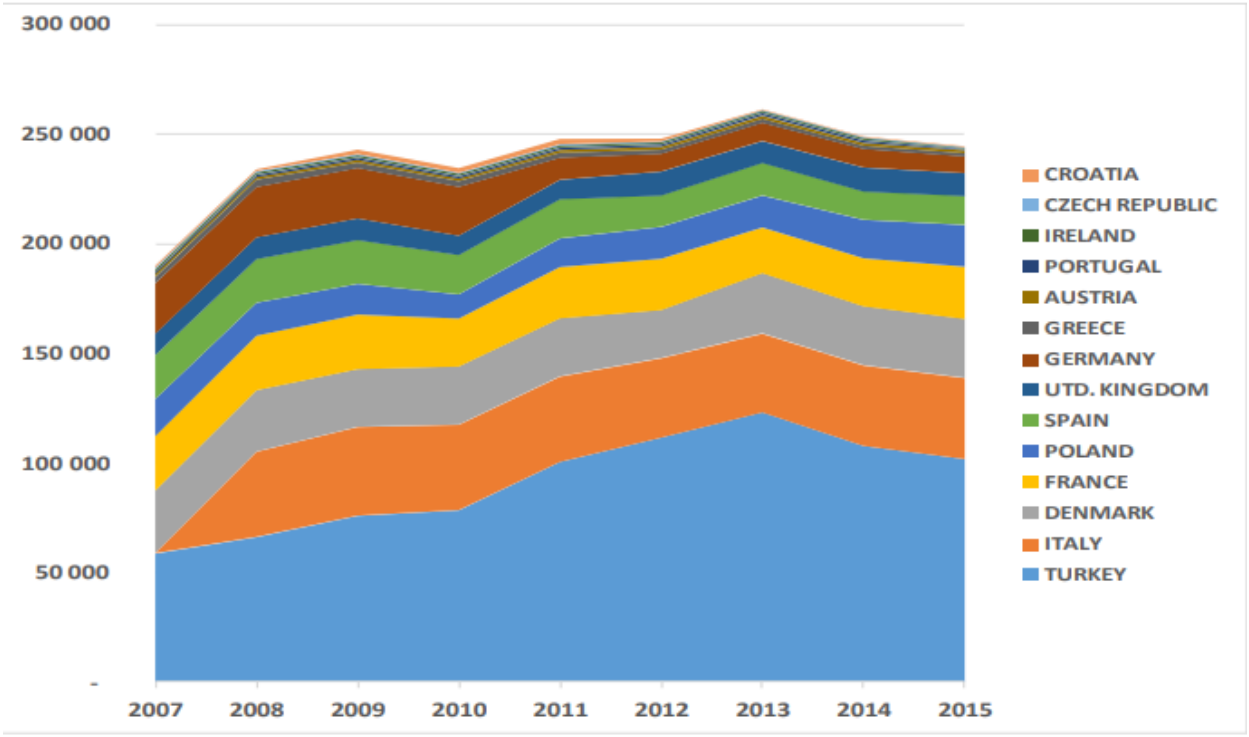

Figure 6. Production of portion-size trout (tonnes) in Europe and Turkey (FEAP, 2015).

The name of rainbow trout (*Oncorhynchus mykiss*) refers to the rainbow-coloured line on its skin. Rainbow trout is salmonid, which has a fusiform body shape with 60-66 vertebrae, 3-4 dorsal spines, 10-12 dorsal soft rays, 3-4 anal spines, 8-12 anal soft rays, and 19 caudal rays. The body size is generally 5 times greater than the height with blue to olive green and a pink colouration along the lateral line (Figure 7). The head has a conical shape and the mouth is slightly sloping, with teeth arranged in one or two series.

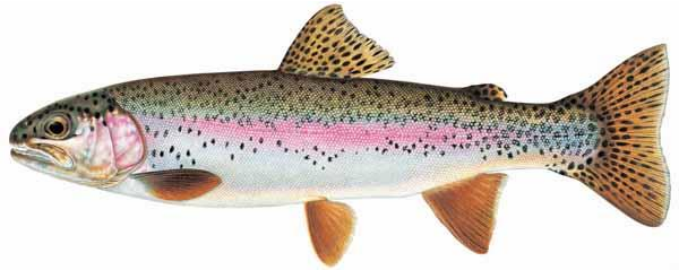

Figure 7. Rainbow trout (*Oncorhynchus mykiss*) (http://www.featheredhook.com).

Fish colours in freshwater are dark green, yellow-green or brown with dark spots on the body, the dorsal fin and the tail. In salt or brackish water, rainbow trout is silvery with the top half of the fish body being darker and having dark spots above the lateral line. Traditionally, rainbow trout is farmed in freshwater systems for production of portion size fish (300 g), while larger fish (3-5 kg) can be produced when fish are kept in seawater for the major grow-out period (Dhamotharan et al., 2018).

The rainbow trout is highly adaptable and possesses desirable characteristics that contribute to their culture and their worldwide distribution. The fish spawns easily with the fry being large compared to most other aquaculture species. They readily accept prepared feeds from their first feeding on. They grow rapidly, a little over 2.54 cm per month at the ideal water temperature of 15 °C and reach market size (400 to 650 g) from 10 to 13 months of age (Gary, 2002). In addition, rainbow trout has the capability of living in different type of habitats, extending from permanently inhabiting lakes to an anadromous life cycle. This means that some strains, such as steelhead, are migratory, spending most of their life in seawater and returning to its original freshwater only to breed. The anadromous strain is characterized by its rapid growth, achieving 7-10 kg within 3 years, whereas the freshwater strain can only reach 4.5 kg in the same time span (FAO-Cultured Aquatic Species Information Programme- *Oncorhynchus mykiss*).

Moreover, rainbow trout is tolerant to a wide range of temperatures and environmental variables as it can withstand vast ranges of temperature variation (0-27 °C). However, spawning and growth occurs in a narrower range (9-14 °C), and the optimum water temperature for rainbow trout culture is below 21 °C (FAO-Cultured Aquatic Species Information Programme - *Oncorhynchus mykiss*). Therefore, growth rate depends on temperature and food availability, causing variation in maturity age, which is usually attained at one year for males and two years for females (Skelton, 2001). One more important thing is that rainbow trout needs well-oxygenated water to survive. The dissolved oxygen level needed for survive is as low as 3 mg/l, but it is recommended to keep minimum levels above 5 mg/l, and ideally above 7 mg/l (Gary, 2002).

Rainbow trout is a predatory species, feeds on terrestrial and aquatic invertebrates, other small fish, and fish eggs. In the wild, the fish can eat also freshwater shrimps, which contain the carotenoid responsible for the fish's pink flesh. In aquaculture, this pink colour is produced artificially by feeding the fish with aquafeed including synthetic or natural pigments (FAO-Cultured Aquatic Species Information Programme - *Oncorhynchus mykiss*). This pink pigment is the most important for the appeal of consumers and is recognized as an important characteristic and selection criterion for food choice by consumers (Koteng, 1992).

European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*)

European sea bass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) are both predatory marine finfish that are often cultured together, with similar production systems at the same farming site. They also have similar biology and life histories as they both have been historically cultured in coastal lagoons and saltwater ponds because they are euthermic (5-28 °C) and euryhaline (from 3‰ to full strength seawater). The culture of sea bass and seabream is an important production sector in the Mediterranean Sea area, and these two species combined represent the largest volume of aquaculture production in that region. In 2015, a total of 181,442 tons of gilthead seabream was produced. Greece is the main producer, followed by Turkey, Egypt, Tunisia, Italy, and Spain. In the same year, 176,970 tons of European sea bass were produced, mainly in Turkey, Greece, and Spain within the Mediterranean Sea (FAO, 2016).

European sea bass (Dicentrarchus labrax)

Sea bass was the first marine non-salmonid species to be commercially cultured in Europe and presently is one of the most important commercial cultured fish species in the Mediterranean areas. It is a worldwide species that grows all over the Mediterranean, the Black Sea and the North Eastern Atlantic, from Norway to Senegal. In the late 1960s, reliable mass-production techniques of juvenile seabass were developed in France and Italy. By the late 1970s, these techniques were well developed in most Mediterranean countries to provide hundreds of thousands of larvae. Greece, Turkey, Italy, Spain, Croatia and Egypt are the main producers worldwide (FAO-Cultured Aquatic Species Information Programme - *Dicentrarchus labrax*).

A picture of European sea bass is represented in Figure 8. The species has rather oblong body with a well-developed caudal peduncle. The head is quite long, and the mouth is terminal, wide, and provided with thin pointed teeth on both jaws, on the palate, and on the tongue. The jaw is slightly prominent and further small teeth are present on the vomer. The sea bass has two separate dorsal fins. The first one has 8-10 hard spines, while the second has one spine and 12-13 soft rays. In addition, it

has a slightly concave caudal fin and the anal fin with 3 spines and 10-12 soft rays. Colour of dorsal portion is silvery grey to bluish, the sides are silvery, and the belly is whitish. Some dark spots on body of juveniles are distinct, while adults are never spotted (FAO-Cultured Aquatic Species Information Programme - *Dicentrarchus labrax*).

Figure 8. European sea bass (*Dicentrarchus labrax*) (https://ec.europa.eu/fisheries/marine_species/farmed_fish_and_shellfish/seabass_en).

European sea bass is eurythermic (tolerant to a wide range of temperature, 5-28 °C), and euryhaline (tolerant to the water salinity of 3‰). Thus, it can live in very different environments such as coastal inshore waters and estuaries and brackish water lagoons. Sometimes, it moves upstream into freshwater. Sea bass enters the sea from brackish environments and from the estuary areas of the rivers, adapting itself to waters characterized by very low salinity (FAO-Cultured Aquatic Species Information Programme - *Dicentrarchus labrax*).

The European sea bass is an opportunistic predator and is known to attack the prey species quite violently. Sea bass utilize as food mainly small pelagic fish such as sardines, sprats, and sand smelts. They also feed on sand-eels and other bottom-living species, crustaceans, and squids. Young fish tend to eat more invertebrates than do the older fish (Pickett & Pawson, 1994).

In Europe and in particular in the Mediterranean basin (except Italy), the sea bass is reared totally by intensive methods, in ground tanks or in sea cages. In Italy, both methods are applied, the intensive method and the semi-extensive ones in certain areas such as Veneto valleys, some lagoons of central Italy, some Sardinian ponds, and in the "cold" pools of the saltpans of Puglia and Western Sicily (FAO-Cultured Aquatic Species Information Programme- *Dicentrarchus labrax*).

Gilthead sea bream (Sparus aurata)

The sea bream, whose latin name refers to a golden band on its head (Figure 9), has compressed oval body with a thin caudal peduncle. Head profile is regularly curved, and in the middle of the small eyes, it has a black band and another golden one. The mouth is low, the buccal apparatus has 4 to 6 frontal teeth similar to the canines, followed posteriorly by less sharp teeth up to be molar-like. Colour of the sea bream's back is silvery grey, and the sides have silver colour, covered with greyish longitudinal lines. The gill operculum has a reddish margin, while the dorsal fin has bluish shades and the caudal has grey-greenish one.

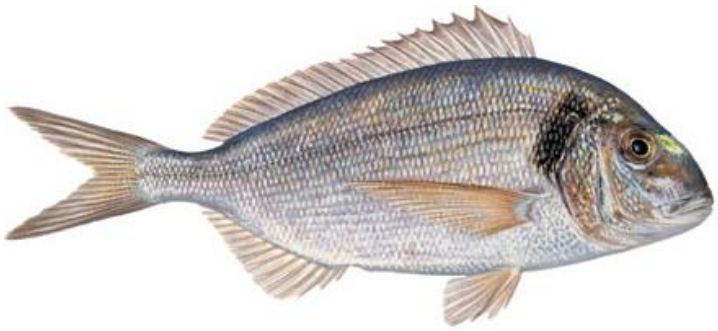

Figure 9. Gilthead sea bream (*Sparus aurata*) (https://ec.europa.eu/fisheries/marine_species/farmed_fish_and_shellfish/seabream_en).

As the sea bass, sea bream is spread along the Atlantic coasts, from Senegal to England, in all Mediterranean coasts but, differently from sea bass, it rarely exists in the Black Sea. Sea bream is a species that can live in waters with different temperature up 4 °C. In addition, it is tolerant to a wide range of salinity, although less than the sea bass. Therefore, its habitat ranges from the marine environment to the coastal lagoons, where it enters during the summer season. It feeds mainly on molluscs and benthic organisms.

In the past, this species was cultured only extensively in the coastal lagoons and salt-water basins. In the 80s of the last century, the first forms of intensive farming were developed and become today the most used production technology in most the Mediterranean areas, including Italy. This was mostly achieved by using cages in sea, and the tanks in the ground. The Italian production is lower than the Greek one, which is the largest European producer, but in the recent years, Italy's production has increased, the regions of Tuscany, Puglia and Sicily showing the highest production of sea bream in the country (FAO-Cultured Aquatic Species Information Program - *Sparus aurata*).

1.1.7 Discard fish- species of low commercial value

Discards fish are considered the species that have no commercial value (even rare/endangered/protected species) as well as the species with low market value (Tsagarakis et al., 2008). Low commercial value appears to be the main reason for the discarding of commercial species in small-scale fisheries (Tzanatos et al., 2007) as well as in trawl fisheries (Machias et al., 2001).

The FAO reports that 35% of global catches are wasted. About a quarter of these wastes are by-catch or discards, mostly from trawlers where unwanted fish are thrown back dead because they are too small or an unwanted species (FAO, 2018).

Fish are wasted or discarded for a number of reasons (Clucas, 1997), including:

- wrong fish species (not of the particular operator's target species);
- wrong size or wrong sex (where sex is important from the processing and marketing point of view);
- damaged fish or deformed fish (caused by gear or predation in nets or mishandling, etc.);
- fish with no local market value and not desirable by the consumers.

Fish discards come also from the considerable amount of leftover raw materials generated by the filleting process of the fish, including viscera, heads, fins, skins, scales, and bones (Rustad et al., 2011). The discards from the processing plants account for 20 million tons, which is equivalent to 25% of the world's total production from marine capture fisheries (AMEC, 2003). These wastes can be used to produce fishmeal, fish oils and enzymes (such as pepsin and chymotrypsin) as well as other value-added products.

Many studies showed that the nutritional value of the low commercial fish is very close to that of the more valuable ones. The chemical composition of discarded species presented in trawling discards demonstrates how the protein content of these minor species is similar to that of the more valuable ones and with the same nutritional value (Ferro, 2000). The oily discard fish also have considerable nutritional benefits due to the content of ω3 and a high content of phosphorus, vitamins, minerals and iron. In addition, the discarded species are small, with low risk of the mercury accumulation that can be found in oily fish species of higher commercial value (Simeone & Scarpato, 2014).

Fish mince is produced by mechanically or chemically recovering flesh, either from the filleting waste process or from the whole fish, where the eviscerated and beheaded fish or fish waste pass through a machine that separates the meat from the bones (Oliveira et al., 2010).

Using the residuals from the fish filleting raised the profitability in the industry and encouraged the producers to exploit them (Tomczak-Wandzel et al., 2015). In addition, fish waste uses in terms of noncommercial- sized fish could be profitable for the aquaculture farmers and the industry. The fish farmers could gain profit through selling trash fish at good price to fish meal-processing plants (Heng

& Lai Kim, 2008). Fish industry profits could be raised through presenting innovative products for the human consumption, thereby decreasing the environmental impact, adding value by using fish wastes, providing easy-to-prepare and nutritious foods for consumers, and increasing industrial profits (Palmeira et al., 2016).

1.2. Mechanical separation of the meat (MSM)

1.2.1 Historical background of mechanically separated meat

Recovery of meat from the bones of filleted fish was the first application of mechanical flesh separation, which started in Japan at the end of the ‘40s of the last century, with the aim of increasing the amount of filleted fish produced and thus increasing the yield of the process. In fact, the effectiveness of the separation process was demonstrated by the increase in yields of 10-20% for fish and 40% for carcasses of crustaceans such as lobster, crab and shrimp. Hence, even the less requested species such as small fish or fish with a significant incidence of bones could be considered preciously recoverable and could find a commercial outlet (Paulsen & Nagy, 2014).

At the end of the ‘50s of the last century, this technique spread to the poultry to obtain further meat that can be used for human nutrition from the neck, back and other bones of the animal. While, it was applied on the carcasses of pigs and red meat species only at the end of the 70s for the purpose of obtaining meat from the vertebral column and other bones after boiling (Field, 2004).

Over years, the technology of mechanical separation of meat has been applied for multiple purposes. The first one was the increasing yields through exploiting the specimens that do not comply with the standards, and so to make them thus suitable to be allocated for the market. Secondly, it aimed to reduce the rate of repetitive strain injury of workers caused by short cyclic boning work in cutting rooms of meat operations. The use of a press was developed for this purpose, and this technology was quite successful and spread all over Europe and the USA within a reasonably short period (CEN, 2010).

The term “Mechanically Separated Meat” was adopted at the 10th Session of the Codex Committee on Processed Meat and Poultry Products, held in Copenhagen in 1978 (Field, 1988). Nevertheless, other terms remain in common usage for such products including “Mechanically Recovered Meat”, “Mechanically Deboned Meat”, and “Mechanically Deboned Poultry”. Furthermore, this technology has taken various names according to meat type on which it is applied. Thus, when it is applied to obtain meat from fish, it can be called "minced" mechanically separated fish or mechanically recovered fish. While, mechanically deboned chicken or mechanically deboned turkey are some of

common names that are used to designate mechanically separated meat from poultry. The multiple names of this technology can lead to confusion among consumers. Hence, the Food Safety and Inspection Service of the US Department of Agriculture (USDA) in 1995 modified the regulations aiming to clarify and standardize the identity and the composition of products obtained from carcasses and parts of chickens recovered by mechanical separation process (Field, 2004).

1.2.2. Definition and legislative requirements of mechanically separated meat

Mechanically Separated Meat (MSM), and more rarely Mechanically Deboned Meat (MDM), is thus a technology used to obtain meat products by removing the remaining meat from the skeleton and the carcasses of animals of various kinds, and it is considered as important raw material of animal origin for the manufacture and preparation of food products. The exploitation of this type of industrial process turns out to be a valid tool to increase the economic advantages related to food sector (European Commission, 2010). Instead, “Mechanically Recovered Meat” (MRM) is meat obtained by mechanical means from flesh bearing bones apart from the bones of the head, the extremities of the limbs below the carpal and tarsal joints and, in the case of swine, the coccygeal vertebrae. It is intended for establishments approved in accordance with the Article 6 of the European Directive 77/99/EEC. European Directive 64/433/EEC was applicable only to ruminant animals (cattle, sheep and goats), pigs and horses. Council Directive 71/118/EEC on fresh poultry meat did not provide a definition of MRM although a requirement was added to that Directive by Council Directive 94/65/EC, in December 1994. This requirement stipulated that mechanically recovered poultry meat could be traded only if it had previously undergone heat treatment, in accordance with the European Directive 77/99/EEC on meat products in the establishment of origin or any other establishment designated by the competent authority.

The current EU legislation (Annex I, point 1.14 to Regulation (EC) No. 853/2004 and Article 3 (Point 1) to Regulation (EC) No. 999/2001) defines MSM as follows: Mechanically separated meat or MSM is a product obtained by removing meat from flesh-bearing bones after boning or from poultry carcasses using mechanical means resulting in the loss or modification of the muscle fiber structure. Moreover (Annex I, Point 3.4), the same legislation defines the concept of "mechanically separated fishery product" as a product obtained by removing meat from fishery products using mechanical means leading to the loss or modification of the meat structure. Since the mechanically separated meat is a technique that destroys or modifies the muscle-fibrous structure and consequently contains parts of the bones and of the periosteum (fibrous layer that covers the bones), this type of meat is not comparable to the meat normally obtained. Therefore, it is appropriate to review their use for human consumption (Regulation (EC) No. 1923/2006).

The main topics of the legislative procedure are the characteristics of the production system, the requirements that producers must have, and the chemical, physical and microbiological parameters of the finished product (European Union, 2015). The Regulation (EC) No 853/2004 (Annex III, Section V) describes the specific legal requirements for MSM production as follows.

Requirements for raw materials and production establishments

The raw materials used to produce mechanically separated meat submitted to requirements defined by the Annex 3, Section V, Chapter II, must meet the following requirements:

- it must meet the requirements for fresh meat derived from skeletal muscles, including adherent fat tissues
- poultry used must not come from the feet, neck skin and the head, whereas for other animals they must not come from the bones of the head, feet, tails, femur, tibia, fibula, humerus, radius and ulna
- fishery products could be used only in the case of whole gutted washed fish and fish carcasses obtained from the filleting operation.

Hygiene requirements during and after production

The Community Regulation, according to the used production techniques, identified two MSM subtypes: low pressure MSM (obtained at <100 bar) and high pressure MSM (obtained at 100-400 bar). Low pressure MSM process does not alter the structure of the muscle. Thus, the product obtained from low pressures does not differ much in terms of structure from the minced meat, as seen in Figure 11. In addition, the calcium content in low pressure MSM is often not significantly higher than that of minced meat. In particular, the calcium content for MSM, as referred to in Regulation (EC) No 2074/2005, shall not exceed 100 mg/100 g (=0.1% or 1000 ppm) of fresh product as it was determined by a standardized international method. Conversely, the high pressure MSM process alters the structure of the muscle fibers, giving a pasty aspect to the product that lost almost its structure, as can be seen in the Figure 10.

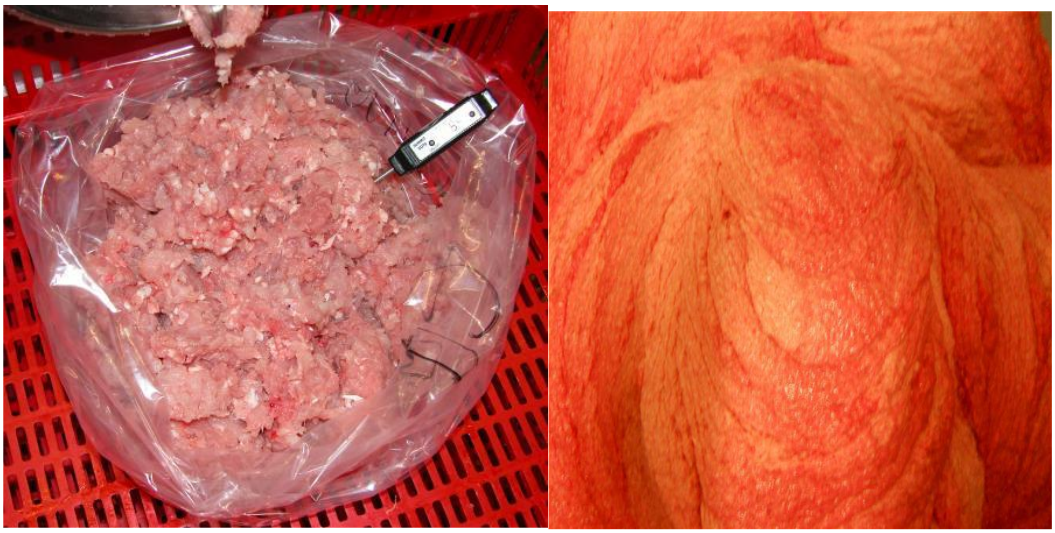

Figure 10. Low pressure (left) and high-pressure (right) obtained MSM (EFSA, 2013).

Hygiene requirements of MSM during and after production were defined in the Annex 3, section V, Chapter III. The legislator also defined the requirements to be met for each of the two types of mechanically separated meat, i.e. low pressure and high pressure MSM (Annex 3, Section V, Chapter 3, Points 3 and 4) as follows:

- for low-pressures MSM, the raw materials derived from on-site slaughterhouse must not have more than seven days of storage, except of poultry carcasses that must not be stored for more than three days. Other raw materials from other site must not be stored for more than five days. The MSM has to be done after de-boning, otherwise it must be packaged and refrigerated at a temperature not exceeding 2 °C, or frozen at a temperature not exceeding -18 °C. These temperatures must be maintained during the storage and transport. Then, the use of MSM depends on the microbiological criteria for the minced meat demonstrated by the analyses. When MSM complies with microbiological criteria, according to the Regulation (EC) no. 853/2004, it can be used in meat preparations, which are clearly not intended to be consumed without first undergoing heat treatment. Otherwise, it can be used only in heat-treated meat products before consumption. In all cases, the obtained meat must have a calcium content, as reported in Reg. EC 2074/2005, not more than 100 mg/100 g (1000 ppm) of fresh product.
- For high-pressures MSM: the raw materials from on-site slaughterhouse must not have more than 7 days of storage. Otherwise, they must not have more than 5 days of storage. However, poultry carcasses must not have more than 3 days of storage. The MSM is not necessarily to be separated after deboning, but in this case must be stored and transported at refrigerated

temperature (not higher than 2 °C) or frozen temperature (not higher than -18 °C). When the mechanical separation is done, it must be packaged and refrigerated at 2 °C if processed within 1-24 hours. Otherwise, it must be frozen within 12 hours after production, reaching an internal temperature of -18 °C within six hours. The maximum time of frozen storage is three months and can only be used for heat-treated meat products before consumption, in approved establishments. The calcium content in high pressure MSM is not defined.

Particularly MSM fishery products must comply with the following requirements (Annex 3, Section VIII, Chapter 3, Part c, Points 1 and 2):

- mechanical separation must be carried out directly after threading and, if the whole fish are used, they must first be gutted and washed
- when mechanical separation is completed, fishery products must be frozen to kill the live pests: at -20 °C for at least 24 hours or at -35 °C for at least 15 hours. Otherwise, fishery products must be incorporated into products undergoing heat treatment before consumption. Frozen fishery products must be kept at a temperature of no more than -18 °C; however, the whole fish initially frozen in brine and used for the manufacture of preserves may be kept at a temperature of no more than -9 °C (Annex 3, Section VIII, Chapter 7, Point 2).

1.2.3. Methods for meat recovery

In the beginning, primitive presses derived from other types of industries were used to separate the meat from the bones, using high pressures of up to 200 bar. High pressure MSM presents 77% of the European production (European Commission, 2010), and yields a fine textured meat paste suitable for use only in cooked sausages. After time, technological improvements were introduced, and flesh-bearing bones were pressed at much lower pressure (up to 20 bar). Low pressure MSM represents 23% of the European production (European Commission, 2010) and produces a coarse texture higher quality meat that could no longer be distinguished from traditional minced meat (so called 3 mm or Baader meat) (EFSA, 2013).

Generally, there are two types of separators: press type and sieve type, with different names. The basic separators in the market are: 1) drum & belt system, 2) endless screw system, 3) hydraulically powered presses.

The pressure used may vary with the machine type and the specific settings used. Most machines can operate at low or high pressure, but some types of machine are more normally used at low pressure than other types. For example, drum & belt separators normally operate at low pressure, but endless screw separator can operate at both low and high pressures, while hydraulically powered presses typically operate at high pressures but can be used at low pressures as well.

Therefore, most of the machines used for MSM production may produce both MSM (low pressure) and MSM (high pressure) by adjusting the pressure settings (EFSA, 2013).

<u>Drum & belt system</u> (Baader type)

This method was firstly developed for fish (Figure 11) but it is also used for poultry and red meat species. The conveyer rubber belt transfers the product and presses the carcass on the surface of the perforated steel drum (holes diameter from 1 to 10 mm). The softer material passes through the holes, while the hard parts such as connective tissues, bones, skin, nerves, tendons and thicker layers remain outside the drum and are ejected through a discharge chute (Barbut, 2002).

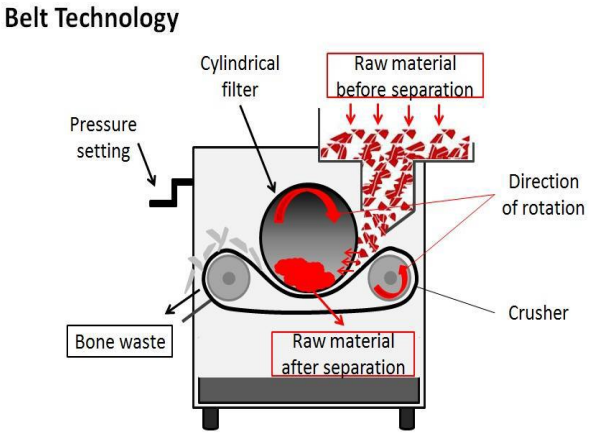

Figure 11. Scheme of belt-drum system (EFSA, 2013).

Pressure on the belts can be adjusted, and sometimes pressure rollers are used to ensure an even distribution of the tissue on the belt. The derived mince after deboning may be refined by passing it through a filter (hole diameter from 1 to 2 mm) that removes most particles and small pieces of belly lining. The mince can range from a coarse texture to a fine paste depending on source material, machine type and setting, and processing method (EFSA, 2013). Drum & belt system can operate at low pressure to produce the low pressure MSM or Baader meat or 3 mm or "desinewed meat", according to different terminologies used in the meat sector (EFSA, 2013). This meat has the appearance of traditional minced meat (EFSA, 2013), and the quality of minced meat is better than the endless screw style, because the product is exposed to less mechanical rubbing; however, the yield is lower.

<u>Endless screw system</u> (Beehive type)

Endless screw system (Figure 12) is used for fish, poultry and red meat species. This separator uses a rotating auger inside a perforated cylinder to force the meat through holes in the perforated cylinder

(Field, 1988; Barbut, 2002; EFSA, 2013), similar to the action of a standard meat mincer. The perforated cylinder acts like a sieve with the meat passing through the holes while the bone remaining in the cylinder and being pushed out at the end by the auger. The size of the holes can be adjusted and are usually around 0.5 mm in diameter (Barbut, 2002). The extracted yields are very high and are correlated to the pressure values that can be reached, but the quality is not the best. Meat recovered by auger separators set at high pressure falls within the definition of mechanically separated meat (MSM) given in Section V, Annex III of Regulation (EC) No. 853/2004, because of the high pressure used that causes bone disruption and loss or extensive modification of the muscle fiber structure.

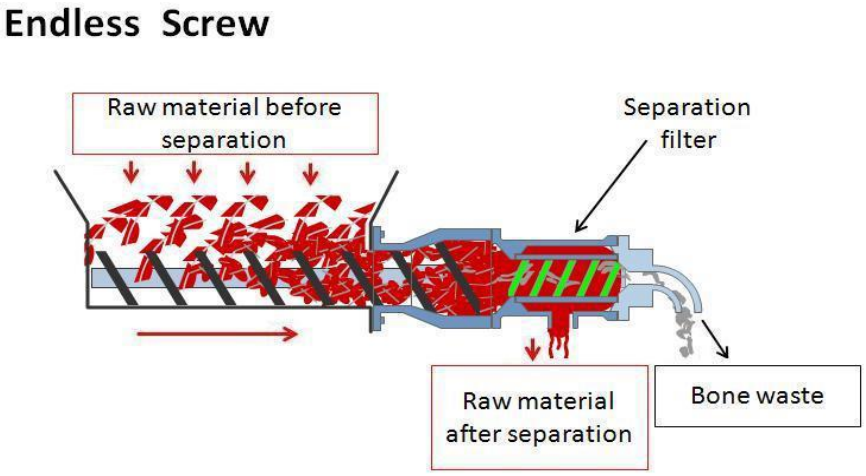

Figure 12. Scheme of endless screw technology (EFSA, 2013).

Hydraulically pressed batch (Protecon type)

Press separators system (Figure 13) is used mainly for red meat species, and also for fish and poultry. It uses a hydraulic piston to force flesh-bearing bones under low or high pressure to the separation chamber while crushing them and squeezing the meat puree through thin slits between the concentric rings. Recovered meat is transferred to a desinewing step where it passes between a belt and a drum with holes 1.0-1.3 mm in diameter (Barbut, 2002; EFSA, 2013). Sinews, cartilage and bone particles are removed at this stage and the product is ready for use (Field, 2004; EFSA, 2013).

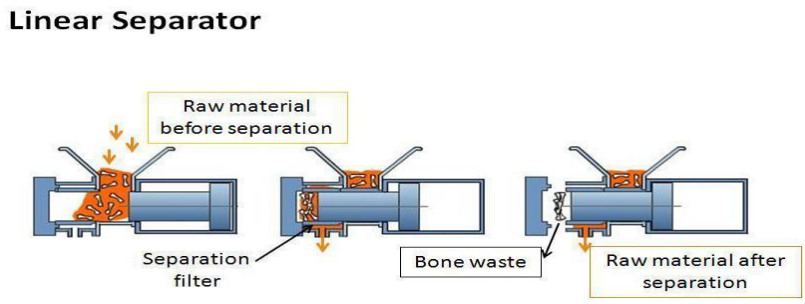

Figure 13. Scheme of hydraulically pressed batch (EFSA, 2013).

Meat recovered by hydraulically powered press separators, as the auger separators, fall within the definition of high pressure mechanically separated meat (MSM) given in Section V, Annex III of Regulation (EC) No. 853/2004, because of the high pressure often used that causes bone disruption and loss or extensive modification of the muscle fiber structure.

The MSM production process is conducted in a two-phase technology, by joining press or endless screw technology followed by belt-drum separation. In the first phase, the meat is extracted from the crushed bones by pressure; then the belt-drum system refines the material by eliminating cartilages residues and thick connective tissue layers.

Machine settings and parameters needed for MSM production include discharge plate hole diameter, drum perforation diameter, machine speed, machine tension, pressure in various modules, pressure time yield, and meat cut fed.

1.2.4 Meat recovery through other than mechanical methods

Recovery of meat from bones could be accomplished by other technologies, such as biochemical, chemical, and physical methods, apart from mechanical methods. Newman (1981) reported a brief description of each, and why most of them are not preferable to the mechanical methods as follow.

Biochemical methods: Various proteolytic, collagenolytic and elastolytic enzymes are used in meat separation from bones, but control of the process is difficult because the enzyme needs to be inactivated in the final product. This could also modify the properties of the derived product. Further, the enzymes presently available are not optimal for this use.

Chemical methods: Alkalis and dilute acids are effective in flesh elimination, but the process leads to breakdown of proteins as well as thawing of bones, especially the acid treatments. The resulting products are suitable only for use in the manufacture of sausages and similar formulated products.

Physical methods: Can be classified as follow, according to the technique utilized:

Thermal technique: Cooked meat is separated from bones through pressure generated by paddles forcing it against a perforated grid. The disadvantage is that the material has been cooked and has lost its binding capacity; however, it still finds uses in the processed food industry.

Ultrasonic technique: This involves ultrasonic vibration of ground meat-bone homogenates in the presence of an extraction solvent. The product has the consistency of thin honey and has been successfully incorporated into frankfurter emulsions.

Cryogenic techniques: The meat is frozen to temperatures of -70 °C to -110 °C and comminuted under known impact loadings. The different structural and mechanical properties of the meat and the bone result in a differential fragmentation and a selective comminution of the mixture. Electrostatic forces are then used as a method of separation.

Cutting techniques: Numerous patents have been granted for utilizing fine liquid or gas jets to cut meat from bones. It is claimed that this assures the complete deboning of whole joints such as legs, whilst maintaining the meat almost intact and not denatured totally.

1.2.5. Composition and characteristics of mechanically separated meat and fish

The composition of mechanically separated meat can present several variations due to the type of the mechanical separator equipment used, bone location, temperature, animal species, and amount of lean meat (Field, 1988). Therefore, a clear distinction of the different types of MSM based on objective and measurable parameters of the final product is a difficult task owing to the high variability of these products in their chemical and physical properties (EFSA, 2013).

During the production process of MSM, particularly in the case of the high pressure MSM, bones are crushed and then higher quantity of bone particles, which contain high level of calcium, are found in such meat. While, the drum-belt system provides lower calcium content than the endless screw system, since it is used under low pressure (Josefowitz, 2008). Therefore, the bone content and consequently the calcium content in MSM are generally higher than the content found in the minced meat (Mayer et al., 2007). The calcium content is frequently used as one of the criteria to identify this

MSM. In some countries, the calcium content of meat is controlled; in Europe, the maximum calcium content allowed for low pressure MSM is 100 mg/100 g of MSM (1000 ppm). In addition, the size of bone particles is also important because the larger the fragments are, the more they can cause a sandy texture and potential dental problems for consumers. The presence of the skeleton in the mechanical separation operations affects the calcium content and the presence of bones fragments in the finished product. The sizes of these residues vary and depend not only on the part of the skeleton involved but also on holes diameter of separator drum and on the applied pressure. The elements found in these products may be longer than 6 mm, which can be reduced by controlling the holes diameter of the drum and the pressure of the belt. However, these operating modifications in the separating machine affect the texture and the degree of fragmentation in the finished product. Bone residues from terrestrial animals of a size equal or less than 2 mm are considered not dangerous to the consumer health (Paulsen & Nagy, 2014).

The iron content increases in the meat obtained by mechanical separation, especially if high pressures are applied. Iron content is twice as high as of the manually deboned meat due to bone marrow incorporation (Komrska et al., 2011). This increase is due to the presence of hemoglobin (EFSA, 2013; Froning, 1981). The hem content varies considerably depending on the bone-meat ratio, the setting of the mechanical separator, the skin content and the age of the animal (Froning, 1981). Furthermore, as reported by Lee & Toledo (1977), separator steel components contain metal particles, which could merge with the MSM meat. Iron, like other metal ions, acts as a catalyst for lipid oxidation, so the extent of its presence can compromise the quality of MSM meat during the storage. The phosphate content is not considered a health matter for the food safety by the European Food Safety Authority (EFSA) and only subject to quantitative limits in the finished meat products, so no limits are set in particularly for MSM. The phosphorus content of MSM depends on animal species, age of the slaughtered animals, cuts of meat, presence of cartilage, bone type (necks, wings, bones, back), previous treatment of the bones (trimming, freezing, etc.), and the machine type and operating conditions used in the recovery process (Froning, 1981; Michalski, 2009).

The moisture content of the MSM varies according to the raw material subjected to mechanical separation but, in general, the MSM has a lower moisture than the hand-deboned meat because of the higher lipid content. However, water activity is in a range allowing growth of all microorganisms in all types of such products, if unfrozen (EFSA, 2013).

The lipid component of MSM has an important role in the organoleptic characteristics and the stability of the product, determining its future shelf life and the modulation of its sensory features (Robb et al., 2002). The composition of MSM has higher lipid content than that of the manually deboned meats. These extra lipids originate mainly from bone marrow and bone tissue, but they may also come from

fat under the skin, the skin or abdominal fat, which depends on the animal species and method used (Trindade et al., 2004). The fatty acid content in MSM varies, ranging from 7% to 48% in relation to the animal species and the type of bone. The MSM fat is rich of polyunsaturated fatty acids thanks to the presence of phospholipids from the fraction of bone and accompanying the spinal marrow (Viuda-Martos et al., 2012). Mott et al. (1982) compared the MSM of whole hens with the MSM of skinned hens and found higher concentrations of unsaturated fatty acids in the first. On the other hand, according to Moerck & Ball (1974), the composition of fatty acids in the marrow and in the MSM of chicken was similar to that of the breast, thigh and skin.

Cholesterol content of mechanically separated pork and poultry meat is usually higher than that of hand-boned meat. Cholesterol is an indicator of the obtained matrix characteristics and an important element in the definition of the nutritional value of the product. The cholesterol can be found in some tissues at concentrations of 40 times greater than in the lean meat of the same animal (Paulsen & Nagy, 2014). Cholesterol's increase is basically related to the increase in fat and the released marrow from the bones resulted from mechanical deboning. Serdaroglu and Yildiz Turp (2005) compared the composition of mechanical deboned and hand deboned turkey and beef meat. They found that mechanical deboned beef and mechanical deboned turkey had higher fat content (31.8% and 14.0%, respectively) than the hand-deboned beef and hand-deboned turkey (19.6% and 4.8%, respectively). In addition, the moisture contents of hand deboned beef and hand deboned turkey were 63.4% and 74.4%, which were significantly higher than those of the mechanical deboned beef and mechanical deboned turkey (54.9% and 69.2%).

The process of mechanical separation not only affects the lipid composition, but also makes the meat very liable to lipid oxidation since this technique induces cell damage and subsequently the release of oxidative enzymes. In addition, during the process, the surfaces are widely exposed to the air, accompanied by the inevitable production of heat, so the extraction of hemoglobin and lipids from bone marrow can make the meat more susceptible to rancidity (Froning, 1981; Field, 1988; Ostovar et al., 1971). Moreover, MSM fat is rich of polyunsaturated fatty acids (PUFA) and phospholipids, which make the meat more susceptible to oxidation. The application of adequate refrigeration to maintain the temperature at values below 10 °C limits the microbial development and oxidation of lipids and hemoglobin (Ostovar et al., 1971).

Protein content of MSM is related to the type of mechanical separation technique applied and correlated with the lipid content of raw material because often fatty raw materials are used, such as skin and subcutaneous fat that reduce the protein content in favor of lipids content in separated meat (EFSA, 2013; Trindade et al., 2004). MSM protein quality depends on the material used, which is usually rich in connective tissues and the collagen. High contents of collagen in MSM is negatively

correlated with technological and nutritional characteristics. Al-Najdawi & Abdullah (2002) assessed the collagen contents in manually and mechanically deboned meats of whole and skinned hens and observed higher contents of collagen in the MSM (3.45% for whole carcass and 3.0% for skinned carcasses) in comparison to the meat of manually deboned hens (1.60% for whole carcass and 0.85% for skinned carcasses). Mello et al. (2010) estimated the moisture value and the protein content of 80.69% and 16.5%, respectively, for the minced tilapia, whereas Melo et al. (2011) obtained a moisture value of 72.75% and a protein content of 14.29% for MSM tilapia.

The content of lipids and proteins also varies greatly in the same individual fish (dorsal and abdominal area, nearness to the fins, etc.). Therefore, fillet composition will be different from MSM fish composition, since it could be obtained not only from the fillets, but also from whole fish (Bordignon et al., 2010; Ogawa & Maia, 1999; Oliveira et al., 2015). In addition, the content of lipids and proteins in fish also varies according to species, age, reproductive cycle and diet (Oliveira et al., 2015). The mechanically separated meat is characterized by a low quantity and quality of proteins because they have more collagen and less myofibrillar proteins than the minced meat. This, in turn, decreases the ability to retain water during processing and storage, and reduce the capability to emulsify lipids and to form stable gels during cooking (EFSA, 2013).

The modification on the structure of the muscle fibers results from the mechanical separation process as it is assured by the Regulation (EC) No 853/2004, and this is considered a criterion to distinguish the MSM meat from other types of meat. The mechanical separation process includes crushing of the bones and destruction of muscle fibers, leading to changes in the sensorial characteristics of the product such the loss of the fresh taste, mainly due to preservation at low negative temperatures, which cause the dryness of the matrix. In addition, the presence of blood causes colour changes from white-pink to red that tends to brown colour with cooking. Furthermore, the sensation of a "metallic" taste due to the excessive presence of iron is a common consequence of the mechanical separation implemented on the fish matrix (Paulsen & Nagy, 2014). The final product is also more vulnerable to the chemical, physical, microbiological, and functional properties changes (Abdullah & Al-Najdawi, 2005). These changes include the development of undesirable aromas (rancidity), loss of its characteristic red colour because of lipid and pigments oxidation, loss or modification of the muscle fiber structure, reduced stability during storage as well as the functionality and processing ability (Mielnik et al., 2002; Bodner & Sieg, 2009).

Quality loss can result from heavily contaminated MSM with microorganisms coming from the carcass and raw material, which, in turn, are contaminated when bacteria primarily on the feathers and in the gastrointestinal tract or on the skin are transferred to the carcasses during slaughter. Storage and transport of the raw material to processing plants are considered as a critical point for microbial

contamination and microbial growth. The degree of muscle fiber destruction is more extensive, especially in the case of the high pressure MSM than in the minced fresh meat. Such damage releases intracellular fluids rich in nutrients and of low acidity that supports bacterial growth (Field, 1988; Froning, 1981). In addition, cross-contamination and redistribution of contamination mainly because of poor hygienic measures (environment, handlers, and equipment) can contribute to the quality loss. Although MSM products may be stored in frozen conditions and/or heat treated, MSM is considered more perishable than fresh and minced meat (Viuda-Martos et al., 2012). Thus, minimizing the microbial risks associated with MSM depends on the operation of effective HACCP plan and a supporting prerequisite programme of Good Manufacturing Practices/Good Hygienic Practices (GMP/GHP) in the slaughterhouse and boning hall, and the efficient chilling of low pressure MSM and frozen storage of high pressure MSM. As required by Regulation, MSM must be used exclusively to produce products that must be consumed after cooking (EFSA, 2013).

Lipid oxidation in the washed MSM fish had lower value of TBARS compared to the non-washed MSM fish (close to 0.2 mg/kg and close to 0.5 mg/kg of malondialdehyde, respectively). After the fish mechanical separation, the sequence processes involve washing the minced flesh fish with chilled water (5-10 °C). The washing technique used is an important key in determining the quality of the product because it results in removing most of the primary and secondary lipid oxidation products and helps to reduce them during the storage (Kirschnik et al., 2013). Therefore, washing process for fish MSM is an used technique that aims to remove blood, pigments, soluble components, lipids and other impurities that can catalyze protein degradation and lipid oxidation. Washing process is useful for the production of surimi and similar products leading to higher stability, better quality, and conserved flavour characteristics of the obtained meat (Jesus et al., 2001; Oliveira Filho et al., 2012; Tenuta-Filho & Jesus, 2003). However, this process also leads to the loss of proteins and fluids and other soluble nutrients (Kirschnik et al., 2013).

1.2.6. Oxidative instability of mechanical separated fish and the supporting mechanism

Fish lipid content is considered an important constituent in the human diet. This fact is mainly due to the high levels of PUFAs, low levels of linoleic acid (C18:2ω6) and linolenic acid (C18:3ω3) and high levels of the long-chain ω3 PUFAs, especially EPA (C20:5ω3) and DHA (C22:6ω3). These latter fatty acids have a positive influence on human health, including prevention of the coronary heart disease, improving retina and brain development, and having anti-inflammatory and anti-carcinogenic effects (Huang et al., 2012; Linseisen et al., 2011; Lund et al., 2011; Stevanato et al., 2010; Tacon & Metian, 2013). However, these unsaturated fatty acids are very susceptible to be

oxidized especially during post-mortem, handling and storage (Chaijan, 2008; Mapiye et al., 2012). Oxidation has negative consequences on both food properties and on human health such as off-flavour development, rancid odour, loss of pigments, loss of vitamins, change of meat colour, loss of nutritional value, decrease in shelf life and formation of toxic compounds (Contini et al., 2014; Farvin et al., 2012; Secci & Parisi, 2016; Palmieri & Sblendorio, 2007). Therefore, the control of oxidation is greatly important in order to prevent the possible waste of nutrients, especially in the phases of production, handling and conservation of MSM, which is particularly relevant in the case of fish MSM.

Autoxidation is the major mechanism that causes lipid oxidation in meat products, and it starts by reactive oxygen species (ROS) (Gray & Monhan, 1992; Kanner, 1994; Min & Ahn, 2005). Light, oxygen, metal, and high temperature are common free radical initiators for this process, and only trace amounts of initiators or catalysts are enough to start the lipid oxidation process. Autoxidation involves the reaction of unsaturated fatty acids with the oxygen resulted in production of free radicals, and it consists of three steps: initiation, propagation and termination (Chaijan, 2008; Mapiye et al., 2012). The initiation step requires low activation energies and it starts by reactive oxygen species that remove hydrogen atoms from the fatty acid and form a fatty acid (alkyl) radical (R•), where the energy source can be heat, light or high-energy radiation or metal ions (e.g., copper ions) (Pegg & Shahidi, 2012). This lipid free radical will react rapidly with oxygen to form peroxy radical (ROO•) and this is the final product of the initiation reaction. The peroxy radical (ROO•) eliminates hydrogen atom from another hydrocarbon chain (unsaturated fatty acids) and yields hydroperoxides (ROOH), which characterizes the propagation step (Chaijan, 2008; Enser, 1987; Mapiye et al., 2012; Pearson et al., 1977). In the final step, i.e. the termination step, free radicals such as lipid peroxyl radicals (ROO•) interact each other to produce non-radical products. Whereas, hydroperoxide will be decomposed at high temperature or in the presence of metal ions (Choe & Min, 2006; Halliwell & Gutteridge, 1990). The decomposition of hydroperoxide results in secondary compounds such as aldehydes, alkanes and conjugated dienes, which lead to unwanted effects on the sensory properties of the foods (due the production of volatile aromatic compounds), and others resulting in injurious health effects (e.g., the malondialdehyde production) (Chaijan, 2008; Zaki et al., 2014).

Another kind of lipid oxidation is the enzymatic oxidation. In this situation, muscle lipoxygenases (peroxidases) is the main endogenous enzyme associated with fatty acids oxidation, which remains active at low temperature (-20 °C) (Abreu et al., 2010).

Many factors along the chain of fish play a fundamental role in maintaining the quality of muscle. At the beginning of the production chain, the enriched aquafeed with antioxidants such as vitamin E or astaxanthin can help preventing lipid oxidation (Secci & Parisi, 2016). The protection against lipid

oxidation and attaining long-term stability of any material could be achieved through several strategies including the elimination, or at least the inhibition, of the initial alkyl radical production in lipids (Schaich, 2005). The antioxidant defense mechanisms are essential to keep the balance between the generation of ROS and their inactivation by the antioxidant body systems (Gülçin, 2006). ROS are produced normally during the physiological processes of the animal and include free radicals such as superoxide anion radicals ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$) and non-free radical species such as H_2O_2 and singlet oxygen ($^1O_2$), which are various forms of activated oxygen (Gülçin, 2006). The inequity between antioxidant and ROS defense mechanisms causes oxidative modification in the cellular membrane or intracellular molecules (Buyukokuroglu et al., 2001; Duh et al., 1999). The degree of lipid oxidation in fresh and processed meat products relies on different internal factors such as fat content, fatty acid composition, and antioxidant content (Addis, 1986; Choe & Min, 2006; Du et al., 2000; Min et al., 2008).

In recent years, antioxidants are added in aquafeeds, and in the meat during processing or at packaging time (Iglesias et al., 2009; Medina et al., 2007; Sánchez-Alonso et al., 2007) in purpose of inhibiting the lipid oxidation, retarding development of off-flavours, and improving the stability of frozen meat products. Abdel-Aal (2001) found that 0.5% ascorbic acid and 0.1% Na_2EDTA were effective antioxidants in retarding the MSM oxidation in African catfish (*Claries lazera*) stored at -18 °C for 6 months. Also, Hussein & Hayam (2012) reported that the addition of essential oils of marjoram and rosemary at level of 200 mg/kg reduced significantly the TBARS and increased the sensory scores of beef patties, formulated by incorporating 200 g/kg of mechanically deboned poultry meat, during frozen storage period at -18 $^{\circ}$C.

Sodium erythromate, which is an ascorbic acid isomer, has a strong antioxidant effect and has the capability of preventing the development of oxidation when it applied to concentrations above 100 mg/L (Trindade et al., 2008). Polyphosphates, such as sodium tripolyphosphate (STPP), are additives used especially in seafood industry. The most important advantages of seafood phosphate treatments are the increase of the water holding capacity of protein in fish and the reduction of the drip loss during freeze/thaw that occurs during processing and storage. Consequently, STPP contributes to save nutrient loss and reduces deterioration of the quality during storage (Turan et al., 2003). Crapo & Crawford (1991) studied the influence of polyphosphate soak and cooking procedures on the quality and yield of Dungeness crab. They found that soaking the crab meat in a 10% of STTP solution for more than 60 minutes resulted in optimum meat yield, quality, and frozen storage stability.

Polyphosphates also have sequestration function of some ions (Ca^{+2}, Mg^{+2}, Fe^{+2}), which lower the WHC (water holding capacity) by reduction the electrostatic repulsion between negative groups, causing contraction and thickening of the protein structure. In addition, phosphate compounds rise

the pH further away from the isoelectric point of the muscle proteins, enhancing the water binding capacity of protein because larger electrostatic repulsive forces create large gaps between actin and myosin with subsequent bounding larger amount of water in the muscle fibers (Puolanne et al., 2001; Young et al., 2005).

Using non-fresh raw material leads to poor quality MSM (Keay, 1979), characterized by short shelf life, and compromises the organoleptic profile (EFSA, 2013). Organoleptic characteristics of MSM can be very different when compared to those of whole carcass or fillets from the same species of fish. Firstly, the product consistency changes making it doughier and more elastic, while the colour tends to the red because of the pigment incorporation, resulting from the inclusion of rich hem tissues (e.g. kidney). The taste alters because of the breaking of the muscle structure and the consequent diffusion of substances in the mixture. The factor that mostly affects the aroma is the lipid fraction of the animal's tissues. The variation of the lipid content and the modification of the proportions between the different elements can lead to a relevant alteration of the flavour.

1.2.7. Health and safety characteristics of the MSM and productive limitations

The amount of MSM produced in the EU Member States is around 70,0000 tons a year (2006/2007), of which 77% is obtained by MSM high pressures, while the remaining 23% is obtained with MSM low pressures. The most used species for MSM production is chicken which represents about 88% of the total product, followed by the swine by about 11%, while the MSM production from other species is negligible. Financially, the total value of MSM production is estimated at between 400 and 900 million € a year (www.eur-lex.europa.eu). Only 20% of the total production is exported, and the annexed revenue is negligible. In 2008, MSM production (mainly by high pressure) was 150,000 tons (higher of 32% compared to the previous year), oriented for export to the East Europe and Russia, with a value of around 80 million € (Eur-lex.europa.eu/legal-content/EN/TXT/?=CELEX%3A52010DC0704).

MSM is a food matrix that is very different from meat, as it is normally understood by consumers. The common "minced meat", i.e. "boned meat which are subjected to a fragment grinding process" (Regulation (EC). No. 853/2004) is very different from MSM. Although the most modern technologies obtain MSM very similar in appearance to the common ground meat (at macroscopic level), it differs greatly at the microscopic (histological) characteristic level. One example is the more valuable Desinewed Meat (DSM), which is deboned meat and tendons obtained from process carried out at very low pressures. Initially this meat is equated to real meat, but depth studies carried out by the European Commission in 2012 recognized it as a separate ingredient, belonging to MSM type

(EFSA, 2013). The structure of the fibers, the concentration of dissolved and mineral substances, and the lipid content are some aspects that make the difference.

For clearer and transparent communication for the consumer, labeling represents an important element of redefinition, especially with the recent entry into force of Regulation (EC). No. 1169/2011, relating to provide food information to consumers. According to the referee of the Regulations, the MSM must be labeled following specific instructions, since they have been legally recognized as different from the regular/normal meat. When MSM is present in the food, it must be mentioned in the label with the name of the derived animal species (possibly with relative proportions) and the quantity of incorporated MSM on the finished product (Regulation (EC). No. 1169/2011).

Healthy and security features of the whole fish and the MSM fish is the same, i.e. the mechanical separation of a fresh gutted and beheaded fish produces MSM fish with the same hygiene and safety properties of the animal from which the meat is derived. Differently from fish, the MSM meat derived from the terrestrial animals does not have the same microbiological and healthy characteristics of the original animal. The evisceration and removal of all parts of the animal are responsible for great contamination (Paulsen & Nagy, 2014). Nevertheless, in the case of fish, the negative part is, in greater importance, the presence of the skeleton fragments that can be found in the mass obtained by mechanical separation. The fish skeletal structure is less consistent and more delicate and distinct; therefore, the discovery of physical residues is a common incident. These residues are considered as major problem in the case of fillets or slices products obtained from manual operations, rather than the MSM, which is statistically proven to contain a lower quantity (Paulsen & Nagy, 2014). Since cooking treatments are mostly able to make these fragments harmless, the presence of danger residues to the consumer can be judged to be reduced. However, the fragments evaluated as dangerous must always be removed from the mass as they leave a space when pressed axially between the fingers (Patashnik et al., 1974).

Considering the various types of meat intended for human consumption, the meat deriving from some species is subjected to restrictions relating to their use for mechanical separation. After the diagnosis of the first case of Bovine Spongiform Encephalopathy (BSE) in 1986 in the United Kingdom and 10 years later of the first transmission of this pathology to humans, the world attention is increased a lot to Transmissible Spongiform Encephalopathies (TSE). Since the consideration that the consumption of contaminated meat is the source of contagion, very restrictive measures are put to safeguard consumers health (EFSA, 2016). These measures were initially prepared for controlling and eradicating the diseases. After that, the objective was to minimize the risk of new outbreaks. According to article 9 paragraph 2 of the Regulation (EC) n. 1923/2006, that modified the EC Regulation n. 999/2001, "the bones of bovine, sheep and goat animals coming from countries or

regions, which have a controlled or undetermined risk of BSE, must not be used for the production of mechanically separated meat" (Regulation EC. 1923/2006). Therefore, this instruction prohibited the use of ruminant bones and carcasses for the production of MSM since 2001.

1.3. Ready to Eat products

1.3.1 Consumer attitude towards fish products

Recently, consumers' trend in choosing food is becoming more complex than in the past. The idea about the choice of food is more dynamic and varied as consumers' interest in safe food is growing and affecting food acceptance and choice. Cultural differences can also influence food preferences since food tastes usually differ across countries. Therefore, people perceptions of healthy food, convenience, versatility, cost, quality and quantity may substantially differ. In some countries, for example, people may pay a little attention to products' quality, while in other countries the consumers consider food quality for human health. Furthermore, other consumers are interested in the technological innovation in the food field, which can be considered as a rational approach to the food choice (Conte et al., 2014; Grunert et al., 2001).

Fish and seafood have always been considered as an important part of a balanced and healthy human diet (Trondsen et al., 2003), having significant health benefits including lower instances of cardiovascular disease (Sidhu, 2003; Verbeke & Vackier, 2005). Consumers expect good quality products to be derived from animals raised in a healthy environment, so they are natural, fresh tasting and nutritious (Kennedy et al., 2004). Particularly, a critical driver of consumer food preferences is better taste and nutrition while production processes may have a minor impact on that (Cardello et al., 2007). However, new production processes can be regarded as a potential that can be taken into consideration for creating new markets and developing new products (Grunert, 2006). In the seafood market, consumers use experiences combined with a reliance on retailers' reputation in order to obtain information on the credibility of purchased food products (Anderson, 1995). They seem to pay more attention to fish general state, the visual aspects of the product, the fish origin, the prices, the product form, and the freshness (Brécard et al., 2009).

According to EUMOFA (2017b), who conducted survey and analyses of the EU consumer attitudes and habits, the main factors for consuming fish and fish products can be categorized into two main groups: (1) personal preferences and (2) external factors.

The main personal factors derived from Eurobarometer survey include "*wellness and health*", which combines three elements: "healthy", "contain little fat", and "easy to digest"; "*hedonism*", which

combines the elements: “good taste”, “products for special occasions” and “look good looking on the table”; and “*convenience and ease of preparation*”, which combines the elements: “easy to prepare” and “quick to prepare”.

Health consciousness has been found to be positively related to seafood consumption (Olsen, 2003; Ragaert et al., 2004, Trondsen et al., 2004a; 2004b). Likewise, health perception appears to influence satisfaction and pleasure associated with eating seafood (Brunsø et al., 2009). Taste has also consistently been found to be a driver of seafood consumption (Bredahl & Grunert, 1997; Olsen, 2004). In addition, taste is an important factor in explaining food consumption because “food is a matter of pleasure, and few people eat things if they do not like their tastes” (Brunsø et al., 2009). The convenience has also been found to influence seafood consumption, in particular for younger consumers (Kittler & Sucher, 2004; Ryan et al., 2004). The convenience is becoming more and more important as consumers are more and more seeking for time savings, as well as, reduce effort and mental exertion for meal acquisition process. The meal acquisition process involves planning the meal, sourcing and purchasing ingredients, storing and preparing food, consuming the meal, cleaning up and disposing of, or storing any leftover food (Beck, 2007; Brunsø et al., 2009; Olsen et al., 2007).

The personal preferences differ highly on the EU countries and sub-regions. For example, the consumption motivations for the Central EU countries rely more on the factor *wellness and health*, while the factor *hedonism* is more important for the Eastern EU countries and Northern EU countries. At socio-demographic category level, elderly people are more sensitive to health aspects and their fish consumption is linked to the positive effects on their personal well-being, whereas the highest socio-professional classes are more sensitive to elements of *hedonism*, especially during special occasions.

The main external factors are those not linked to personal preferences but still affecting (in a positive or negative way) the purchasing behavior, and they mostly regard young consumers, including *price levels*, *products assortment*, and *promotional strategies*.

Perceptions of high prices have been found to be key barriers to seafood consumption in many European studies, as about 68% of EU consumers would increase their consumption if their price level were lower (Brunsø et al., 2009; Myrland et al., 2000; Olsen, 2004; Trondsen et al., 2003; Verbeke & Vackier, 2005). Therefore, price represents a factor slowing the consumption growth and, consequently, promotional strategies could encourage the consumption. Moreover, 51% of consumers would increase their consumption if they could choose within a wider products assortment. Rortveit & Olsen (2009) revealed that the variety or the number of considered dinner alternatives have a significant positive effect on consumption frequency. Variety can also be considered in terms of whether people can purchase seafood in the form they want, in the desired portion size, and whether

pre-packaged seafood products are available. Therefore, the diversification of the supply, independently from the price level and in connection with promotional strategies, would encourage the consumption.

The Italian Institute of Services for Agricultural and Food Market (ISMEA, 2014) has conducted a study to analyze the behaviour of habitual and non-habitual consumers of fish, showing the various aspects concerning the barriers and the motivations to purchase. Besides, the study clarified the features that affect the tendency to purchase and the ways to stimulate the purchase of fish products that can be summarized as follows:

- the lack of confidence in the shopkeeper, mainly relative to freshness (for example, the consumer is not sure if the fish is really fresh and not defrosted). In some cases, the uncertainties about freshness is linked to possible negative health consequences
- absence of complete trust in the labels and in the available written or oral information. The fact that fish is a highly perishable product makes the consumers fear (at least at emotional level) of negative health effects to a greater extent than in the case of other foods. These health fears are aggravated in the case of mollusks and raw fish
- the difficulties in preparation and cooking the product ("it is difficult to do it well"), and the worries of cleaning it (a problem that could be resolved, at least partially, by buying already cleaned fish). In addition, there is the intensive smell when cooking fish
- the family habits and local traditions. In some cases, fish is consumed only on Fridays. Besides, many inland areas have a poor culture of consuming fish, being away from the coastal areas where fish is produced and can be purchased fresh and less costly. In such cases, the recipes are limited to the simplest and the most common ones.
- the low acceptance of fish taste by some members of the family (children's tastes are often a hindrance to purchase). This is accompanied by the presence of thorns (for some, non-negligible source of fear) and therefore the inconvenience for consumption. Children under ten age, and the adolescents are consumers showing a great dislike of fish products consumption. Hence, they are one of the main barriers to consumption on the families (Altintzoglou et al., 2010; Birch & Lawley, 2012; Grieger et al., 2012; Myrland et al., 2000; Neale et al., 2012; Olsen , 2001; Scholderer & Trondsen, 2008; Trondsen et al., 2003; Verbeke & Vackier, 2005). Nevertheless, Myreland et al. (2000) and Trondsen et al. (2004b) thought that families with young children, under twelve age, have a high consumption of processed fish products compared to families with teenagers or without children. Myreland et al. (2000) explained this by the fact that the fish within these families is breaded or processed in another way, rather than fresh fish that has characteristics children may not like. Olsen et al. (2008)

and Story et al. (2002) affirm that parents show a significant motivation and a greater ability to consume a new fish product compared to their children.

Food labeling is an effective instrument for consumers' information. An increased consumers' awareness and trust towards fish, together with information on the label, can have a noticeable impact on food choice. In all countries, labeling is considered an essential guarantee for safe fish, and quality marks are considered of consumers' interest. For fish products, consumers correlate information to product safety and quality mark, and they put their trust in information when the mark is supported by plausible controls and by guarantees deriving from a good traceability system (Pieniak & Verbeke, 2008; Verbeke et al., 2007).

Regulation (EU) No 1379/2013 of the European Parliament and of the Council on the common organization of the markets in fishery and aquaculture products stated (Article 35, point b) that fish must be labeled according the production method, in particular by following words (caught or farmed). This compulsory rule can help those consumers wishing to avoid intensively farmed fish (European Commission, 2000). Labeling and traceability can be considered two of the most important means to safeguard consumers' safety, and these attributes will help people to differentiate and choose food products.

Eco-labeling or environmental label product, which is a logo, symbol, text, or seal of approval given to the products, inform consumers about environmental production attributes or the impact of the product on the environment (Bjerner et al., 2006; Bonilla et al., 2008). Thus, it conveys to consumer unobservable information about the environmental attribute of fishery goods and consequently may influence consumer-purchasing decision towards environmentally friendly products (Roheim et al., 2011; Salladarré et al., 2010).

Briefly, the main factors for promoting fish products according to Focus Group proposed by ISMEA (2011) are:

- lowering sales price;
- improving the availability of products by increasing the distribution of fishmongers or street sealer, or by promoting openings of more specialized shops;
- expanding the consumption target to include the children and young adults (18-35) categories through the development of new distribution and selling systems (street food and fast food), new cooked products (ready-to-eat) or ready to be cooked (ready-to-cook) of high ease of use;
- promoting strategies of ready meals in order to reduce consumption barriers, through television programs, fishmongers and fish section in supermarket, and packaging.

The trend is to make the fish-based products rely on economically sustainable food. The consumer point of view for the label is to be simple and clean, free of complicated ingredients, and not cooked

in a way that could weaken the nutritional value. In fact, it is underlined that complex products, which are made of many ingredients, such as panata products, induce distrust in consumers who consider them artificial (ISMEA, 2011).

In short, the market moves towards creating range of fashionable products oriented towards the youngest. This can be an encouraging factor for children to consume fish in general due to both the absence of thorns, and the less perceptible taste of the fish.

1.3.2 Ready-to-eat seafood products

Currently the consumers, particularly the urban ones, show more and more interest in food products that are available in ready-to-eat or ready-to-cook forms such as fish fillets, fingers, cutlets, patties, burgers, sausages and fish balls (Kolekar & Pagarkar, 2013). Ready-to-eat (RTE) products are a group of food products that are pre-cleaned, precooked, mostly packaged and ready for consumption without further washing, cooking, or additional preparation, except for a possible thawing. According to FDA (2009), RTE foods should be in an edible form without an additional preparation step to achieve food safety. While, the ready-to-bake products are called ready-to-cook (RTC).

RTE products enable utilizing by-products fish or unfavorite fish species among consumers due to some of the factors like small/unconventional size, ugly shape, too much spiny body, and unfavorable flavour/taste (Datta, 2015). This, in turn, enhances their acceptability, their nutritious value, their sensory characteristics, and their shelf life and convenience (Pagarkar et al., 2011).

The development of new eating habits (snacks, fast food, etc.) versus the traditional meal is pushed by the loss of culinary expertise due to the reduced time spent in the kitchen, the change in family structures with an increasing number of singles. The new status of women today, having a higher education level and enjoying a higher employment rate with less time to spend in culinary preparations stimulated the demand for RTE foods. Thus, pre-packed fixed weight portions with no waste and pre-prepared items such as fish portions with some culinary content (sauce, bread-crumb, pre-cooked) are increasingly appreciated (FAO, 2010).

As previously mentioned, fish sector is increasingly focused on proposing and promoting RTE products. These products are derived either from fish itself including beheaded, gutted, and filleted products, or from processed fish such as fish burger, meatballs, nuggets, fish sticks, and different formulations of breaded fish. Presently, the RTE products on the market are obtained mainly from codfish (*Gadus morhua*) or from pollock (*Gadus pollachius*) captured from the North Sea (Oetterer, 2002). From the health prospect, consumers differently perceive the diverse types of RTE products. Fillet is considered the healthiest product, followed by fish burgers and fish nuggets. This is because

the fish burgers and nuggets are considered as processed products and are composed of the same basic formulation of fish but differently prepared. Fish nuggets are breaded, pre-fried, and frozen, but need to be fried before consuming, while fish burgers are shaped in round form, and consumed after grilling (Mitterer-Daltoé et al., 2014). Although burgers are considered healthier than nuggets since they are not fried, they still have a lower penetrating capacity in the market (Olsen et al., 2008). The different presentation of fish products leads to different responses by consumers, and the healthy characteristics of a product is expected to be only one of the decisive factors for choosing a food product.

As demonstrated by the ISMEA (2011) surveys, the time required for the preparation of fresh fish products may discourage some consumers from buying them and consequently they prefer RTE fish products (Brunsø et al., 2009). For example, consumers prefer fish patty and fish croquettes made of carp rather than the traditional preparations of the fresh carp (Sehgal & Sehgal, 2002). However, this cannot be generalized because the perceptions about food products can vary considerably depending on geographical origin (Olsen et al., 2008). For example, in Spain there is a strong culinary tradition, and it is a part of the Spanish culture to spend a lot of time cooking and eating, so the concept of convenience and solutions for saving time are not important and not appreciated. In Belgium, on the contrary, the consumers are familiar with these concepts and consider them important (Brunsø et al., 2009).

The RTE products are expensive and this could be a great barrier to consumption. However, it is proved that the current consumers are willing to pay more for this type of product, thus new business opportunities could be suggested for the fish industry (Cosmina et al., 2012).

1.3.3 Fishburger derived from fish MSM and their acceptability

The mechanical separation of meat (MSM) is considered an alternative way for the diversification of new products derived from fish meat and also as a solution for the use of residuals from the fish filleting industry (Freitas et al., 2012; Secci et al., 2016a; Secci et al., 2017). The filleting residues from the process represent, in some cases, more than 60% of the total weight of production. Therefore, the use of edible leftovers from traditional filleting or slicing becomes highly important. The importance could be at the three different levels: i) economically for the industry, through reducing the cost and gaining additional income; ii) environmentally through minimizing the pollution matters associated with the disposal of processing by-products (Jaczynski, 2005); and iii) nutritionally through presenting nutritional value equivalent to that of the entire muscle (Boscolo, 2001; Lima et al., 2015). In addition, the use of this technique in the aquaculture sector would optimize the production costs through exploitation of undersized and damaged specimens, which cannot be commercialized directly as whole fish. Even the species that are considered to be waste of the catch,

such as horse mackerel (*Trachurus trachurus*), could find a fair but interesting economical value (Secci et al., 2017).

Moreover, MSM technique would offer to the market different types of products with high capacity for use (Secci et al., 2016a). Various types of products could be got by using processed mechanical separated fish, including fishburgers, nuggets and breaded steaks (Marengoni et al., 2009). The products obtained from MSM system can be considered new, so it is recommended to evaluate their acceptance by potential consumers (Freitas et al., 2012). Several researches studied the fishburger obtained from mechanical separation process (Bochi et al., 2008; Di Monaco et al., 2009; Fogaça et al., 2015; Marengoni et al., 2009), evaluating their acceptability from a part of consumers and concluding that this type of product has, in general, a good consumer acceptance.

1.3.4 Characteristics of ready-to-eat (RTE) and ready-to-cook (RTC) fish

In this paragraph, we will compare the characteristics of RTE and ready-to-cook (RTC) fish products, which could be divided in different categories: sticks, crisps and flowers, fillets, fishburgers and nuggets.

Fish sticks (fish fingers)

Fish sticks are fish processed products characterized by a rectangular form, breaded, pre-fried and frozen. Many products, present in the market, contain several codfish species: Pacific cod (*Merluccius gayi* and *Merluccius productus*), Atlantic cod (*Merluccius hubbsi*), South African cod (*Merluccius capensis* and *Merluccius paradoxus*), New Zealand cod (*Macruronus novaezelandiae*), and Alaska pollock (*Theragra chalcogramma*). Fish sticks may be prepared from a single species of fish or from a mixture of species with similar sensory properties. The percentage of codfish in the products is around 60% and the ingredients used for the breading include mainly wheat flour, starch, vegetable oil of sunflower or rapeseeds, water, yeast, salt and spices including turmeric and paprika. For free-gluten-products, intended for people intolerant to gluten, wheat flour is replaced with other types of flour such as rice flour, corn flour, and chickpeas flour. There were two methods of cooking these products: baking or cooking in a pan. The first way requires the use of a preheated oven at around 200 °C and cooking for 13-15 minutes, while the second way requires frying the products in butter or oil, with around 5 minutes of cooking time.

Crock and hearts of fillet

The crock and heart fillets are produced directly from fish fillets, cut into smaller sizes. These products could be in natural form or in a breaded form (pre-fractions). Crock and heart of fillets derive particularly from the hearts of the fillets. The main fish species used in the market for these products are, as in the previous products, species of codfish: Pacific cod (*Merluccius gayi* and *Merluccius*

productus), Atlantic cod (*Merluccius hubbsi*), South African cod (*Merluccius capensis* and *Merluccius paradoxus*), New Zealand cod (*Macruronus novaezelandiae*), and pollock of Alaska (*Theragra chalcogramma*), beside species of pink salmon (*Oncorhynchus gorbuscha*), Atlantic salmon (*Salmo salar*), Keta salmon (*Oncorhynchus keta*), and yellowfin tuna (*Thunnus albacares*). Breaded products contain generally the following ingredients: wheat and maize flour, sunflower or canola oil, wheat and potato starch, water, salt, yeast, dextrose and spices such as mustard, paprika and turmeric. Products in natural form could be cooked in different ways, including cooking in preheated oven at around 220 °C (about 25 minutes), in a pan with a little quantity of oil (for about 15 minutes), and in a pot with boiling water (for about 6 minutes). Other cooking methods are grilling and steaming, particularly utilized for salmon. In some commercial products, the cooking ways are not specified, advising to cook the product as if it was fresh. However, for breaded products, the culinary methods are either cooking in preheated oven at around 220 °C for about 15-25 minutes or cooking in the pan for 8-10 minutes. Some cooking guidelines propose frying the product instead of using a pan. Other cooking guidelines, especially with a salmon, propose grilling in the oven.

Fillets

Frozen fish fillets, present in the market, mainly include fillets form the following species: European sea bass (*Dicentrarchus labrax*), Alaska cod (*Merluccius hubbsi*), Atlantic cod (*Merluccius hubbsi*), hake (*Merluccius capensis*), gilthead sea bream (*Sparus aurata*), plaice (*Pleuronectes platessa*), pink salmon (*Oncorhynchus gorbuscha*) and yellow fin tuna (*Thunnus albacares*). Fish fillets are available in different shapes, such as natural breaded gratinated, and as a ready dish. The natural fillets are sold either in packages containing a couple of fillets, or in bags containing a larger quantity of product.

These products are recommended to be defrosted before cooking, and then the culinary methods are either cooking in preheated oven at 200-220 °C for 10-15 minutes or cooking in a pan with a small amount of oil for 6 minutes. White paper is usually left to the consumer to treat the product as if it were a fresh product. Breaded fillets are products that are breaded and pre-fried. The ingredients used for breading are: wheat and corn flour, sunflower or canola oil, wheat and potato starch, water, salt, yeast, dextrose and spices such as mustard, paprika and turmeric. The culinary ways include cooking in preheated oven at around 220 °C for 15-17 minutes, and cooking in a pan for 6-8 minutes.

Gratin fillets are products that have a breading only on one side of the fish fillet. The ingredients of the breading are similar to those of breaded products. However, they have more breadcrumbs to give specific crispness to these products, more spices such as parsley, basil and white pepper. These products could be cooked in a preheated oven at 200 °C for 30-40 minutes. The products as ready meals are composed of fish fillet, and often accompanied by a side dish of vegetables and various seasonings including extra virgin olive oil, lemon juice, and different spices. The products can be

cooked in preheated oven at 170 °C for 22-25 minutes, in the pan for 12 minutes, and in microwave at 750 W for 6 minutes.

Fish burgers

Fish burgers are products characterized by a circular form and could be breaded and pre-fried or they can be without breading. The fish species used in the market for these products are the following: European sea bass (*Dicentrarchus labrax*), codfish (*Gadus morhua*), Alaska cod (*Merluccius hubbsi*), Atlantic salmon (*Salmo salar*) and yellowfin tuna (*Thunnus albacares*). These kinds of products are mainly obtained from fish fillets or from fish pulp. The breaded products are coated with crumbs, which consist of wheat flour, sunflower or canola oil, wheat starch, lemon juice, water, yeast, salt, dextrose, mustard and turmeric. The culinary methods are in a preheated oven at 220 °C for 20 minutes or in a pan with plenty of oil for 7-10 minutes. Often, the products without breading are made from the pulp of two fish species, and codfish (*Gadus morhua*) is often one of them. The ingredients that accompany the products are sunflower oil, wheat flour, potato flakes, egg white, salt, lemon juice and natural flavors. The culinary styles for unbreaded products are cooking in a preheated oven at 250 °C for 10-12 minutes, in a non-stick pan and opened flame plate for 1.5 minutes for each part, and in electric toaster at the highest temperature for 7 minutes.

Nuggets

The nuggets are processed products based on fish and are breaded, pre-fried and deep-frozen. The products found on the market contain codfish (*Gadus morhua*), Alaska cod (*Merluccius hubbsi*) and surimi fish. The based fish in these products are mainly made up of fish fillets or fish pulp. The used ingredients for breading include mainly wheat flour, starch and potato starch, vegetable oil from sunflower or canola seeds, water, yeast, salt and spices including turmeric and paprika. Cooking can be done in preheated oven at around 200 °C for 13-15 minutes or in a pan with oil for 4-8 minutes.

From the comparison of all the types of RTE products on the market, it is possible to observe the positive and negative characteristics of this type of products that are summarized in Table 1.

Table 1. Positive and negative characteristics of fish ready-to-eat products in the market.

Positive	Negative
Nutritional value	Breaded and pre-fried
Convenience of use	Presence of alien species
Conservation	High cost

2. Aims of the study

As revealed from the former chapters, the world and Italian per capita fish consumption increased. Currently, the Italian fish and fish products market encountered high competition from other European and Mediterranean countries, and the consumers' wants and needs changed according to the socio-economic situation and lifestyle items.

Application of the mechanical separation technique enabled transformation of undersized fish or /and fish waste in partially processed foods with added value for human consumption (Ferraro et al., 2010; Monteiro et al., 2012; Monteiro et al., 2014). Using the discards fish could be beneficial for both the aquaculture farmers through increasing industry profit, and for the fish consumers through creating innovative product, ready to eat and ready to cook. The former products are characterized by convenience, easiness to prepare, and nutritious value, and can contribute to keep competitiveness on the market and meet the new needs of consumers.

Fish and fish products are highly perishable. Therefore, the general aim of this study was to evaluate the physical, compositional and sensory properties of fish-burgers obtained from fish coming from the aquaculture farms of Tuscany and from the wild in order to create new fish products (fish burgers) by using mechanical separation system, and to open new market for the aquaculture products.

The specific goals were to study:

• Advantages of mechanical separation treatment technologically and nutritionally, when applied on European sea bass, gilthead sea bream, and rainbow trout. Specifically, assessing the physical and chemical properties and nutritional quality of mechanical separation and manual mincing techniques applied for the interest species, and comparing the results between them. (Research I).

•Utilization of the nonmarketable specimens from European sea bass and rainbow trout by studying the physical, nutritional, and sensory properties of 'clean label' fish burgers. Specially, the evaluation of four clean label recipes of fish burgers obtained from mechanically separated meat, that differed in the ratios of sea bass to rainbow trout (50:50 and 30:70), and in the ratios of fish to potato flakes (2.5:1 and 1.5:1). (Research II).

•Effects of cooking and frozen storage on nutritional and physico-chemical characteristics of fish burger formulation based on mechanically separated meat. In particular, studying two recipes of fish burger prepared from different ratios of European sea bass to rainbow trout (50:50 and 30:70), and enhanced with lemon, salt, water, and potato flake. (Research III).

3. Materials and Methods

Fish were obtained from different fish farms. The two species of sea water, European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) were bought from a fish farm located in Orbetello (Grosseto, Italy). Whilst, the fresh water rainbow trout (Oncorhynchus mykiss) specimens were acquired from a farm located in the north west of Tuscany (Lucca, Italy). All the considered fish species were killed by percussion method. Immediately after death, fish were transferred to the company for processing, in polystyrene boxes and covered by ice. After being washed, the fish specimens were eviscerated and decapitated, then minced by the soft belt-drum separator (BAADER Mod. 601; Baader, Lübeck, Germany).

Generally, the following analyses were conducted: physical analyses concluding texture, colour, pH, water holding capacity, and chemical analyses comprising proximate composition, fatty acid (FA) profiles, lipid oxidation product (conjugated dienes, thiobarbituric acid reactive substances, TBARS), antioxidant capacity, and mineral composition. In the different research activities, some of the previously listed analyses were carried out. An overview of these assessments is given in the Table 2 and described in depth in the following Sections of this Chapter.

Finally, experimental set-up for each research is described in the Part II, which collects the papers that have been originated from the three researches performed during the PhD period, that are summarised in Table 3.

Table 2. List of the analyses conducted in each research.

		Research I	Research II	Research III	Method
CHEMICAL ANALYSES	Proximate composition	●	●	●	AOAC (2012)
	Fatty acids composition	●	●	●	Morrison & Smith (1964), by GC
	Lipid oxidation products (conjugated dienes, TBARS)			●	Srinivasan et al. (1996); Vyncke (1970) by Spectrophotometer
	Antioxidant capacity (ABTS, DPPH, FRAP)			●	Re et al. (1999); Blois (1958) mod. by Jung et al. (2010); Descalzo et al. (2007) by spectrophotometer
	Mineral composition	●			by ICP-AES spectrophotometer
	Yield	●			g of deboned meat /100g whole fish
PHYSICAL ANALYSES	Texture		●	●	Texturometer
	pH	●		●	pH-meter
	Colour	●		●	Colorimeter
	Water Holding Capacity			●	Centrifuge system
SENSORY ANALYSES			●		Panellists

Table 3. List of papers derived from the PhD research activities.

Study Type	Obtained	Publication Type	Status	Journal/Congress
Research I	Paper I	Article	Published	LWT-Food Science and Technology
Research II	Paper II	Article	Published	International Journal of Food Science and Technology
Research III	Paper III	Article	Submitted	Journal of Food Processing and Preservation
	Annex I	Oral communication	Published	22nd Congress of Animal Science and Production Association, 2017
	Annex II	Poster	Published	78th West European Fish Technologist'Association, 2018

3.1 Physical analyses

3.1.1 Texture

The texture can be defined as a group of characteristics that arise from the structural elements of food and are related to deformation, disintegration and flow of the food under a force (Bourne, 2002). In this experimentation, the measure of the texture of raw and cooked fishburger was carried out by using a texturometer (Mod. 109 texturometer Zwich Roell, Germany) equipped with a 200 N load cell and the Text Expert® II software. The cutting and compression stress tests were carried out on cube-shaped sub-samples (3.5 cm on each side), which are taken from the centre of each raw and cooked fishburger. The appropriate Warner Bratzler blade was used for these tests (Figure 14).

Figure 14. Texturometer with the appropriate blade used.

3.1.2 pH

The pH is an important index to determine meat quality. The pH value in fish varies according to the biochemical muscular processes and the bacterial and enzymatic activity during the storage, which have an effect on the concentration of free hydrogens and promote the decomposition of molecules (Ogawa & Maia, 1999; Okeyo et al., 2009). The pH measurement was carried out on the fillets and on raw fishburgers using a pH-meter (Columbus, OH, USA) equipped with an inserted probe. The pH value was examined in three different points of the epaxial region of the whole fillet and of the burger's diameter.

3.1.3 Colour

A Dr Lange Spectro-color® colorimeter (Keison International Ltd, UK) equipped with a Spectral qc 3.6 software was utilised for colorimetric measurement. Colour was measured in triplicate on the epaxial-cranial fillet position and on three surface points placed along the diameter of the raw and cooked fishburgers. Colour measurements were carried out according to the CIELab system (CIE, 1976). CIELab is the second of two systems adopted by CIE (Commission Internationale de l'Éclairage) in 1976 as models that better showed uniform colour spacing in their values. CIELab is an opponent colour system based on the earlier (1942) system of Richard Hunter called L*, a*, b*. Colour opposition correlates with discoveries in the mid-1960s that somewhere between the optical nerve and the brain, retinal colour stimuli are translated into distinctions between light and dark, red and green, and blue and yellow. CIELAB indicates values of L*, a*, b*, chroma, and hue. The colour is the result of three components; hue, lightness, and saturation.

Hue describes a primary colour such as red, green, or blue.

Lightness or luminosity describes the brightness of the colour.

Chroma or saturation describes how vivid or dull the colour is.

Figure15 illustrates the relationship among the hue, lightness and saturation presented in a three-dimensional space. In the CIElab space, the hue and chroma values are obtained by using the following formulas:

Hue = arctg2 (b*, a*)

Saturation = $(a^2+b^2)^{(1/2)}$

The CIE system is one of the most popular system currently used by the meat industry and is known as the CIE L*, a*, and b*, colour space system as it is shown in Figure 16. The vertical axis represents the lightness of the surface (L*), whose values are in the range from 0 (black) to 100 (white). The other two axis values range from positive to negative. The redness index (a*) spans from -60 (green) to +60 (red), while the yellowness index (b*) spans from -60 (blue) to +60 (yellow). The zero represents neutral grey.

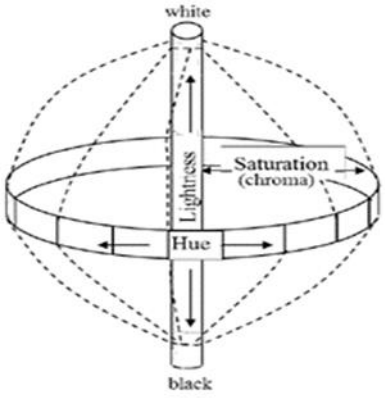

Figure 15. The relationship among hue, lightness and chroma.

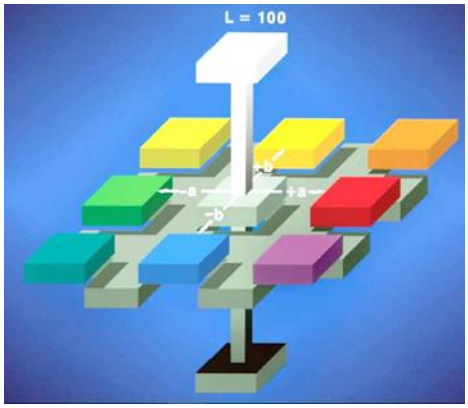

Figure 16. Colour distribution on CIELab scale.

3.1.4 Water Holding Capacity (WHC)

The capacity of water retention is the ability of meat to retain the water even though external pressure (e.g. gravity, heating) are applied to it and is related to the amount of free water. The WHC of the samples was determined by gravimetric method, in which the liquid is lost from a sample by application of centrifugal force. Approximately 2 g of sample were weighed on a special filter (Figure 17) and then inserted in a centrifuge glass tube. The samples were centrifuged (Mod. Sorvall Superspreed RC 2-B Automatic Refrigerated Centrifuge, Walthan, MA, USA) at 510 × g for 5 minutes. Next, the difference between the two weights (before and after centrifugation) is calculated to determine the amount of water lost. The water content of the sample was determined by weighing 2 g of the sample and putting it in the oven at 105 °C overnight; after that, the sample was re-weighed after cooling in the dryer (Figure 14). The water retention capacity was then calculated by comparing the amount of water lost to the amount of water initially presented in the sample and expressed as a percentage. The higher the percentage is, the greater the water retention capacity of the sample.

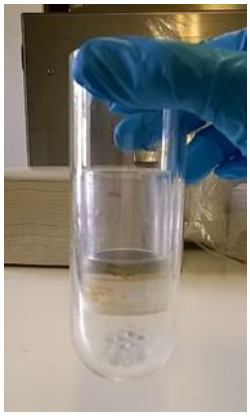

Figure 17. Filter inserted in the centrifuge tube (left). Weighed samples to put in the oven (on the right).

3.2 Chemical analyses

3.2.1 Proximate composition

The proximate analysis was conducted on the whole fillet and both raw and cooked burgers to determine the moisture, crude proteins, crude fat, and ash contents by using the official methods 950.46, 976.05, 991.36 and 920.153, respectively, of AOAC (2012).

The AOAC oven drying method was used for moisture analysis.

Approximately 4 g of lyophilized samples were weighed in a special porcelain capsules (previously calibrated and weighed), then dried for 4-5 hours at 105 °C in drying oven. The moisture percentage (M, %) formula was: M (%) = [(wet weight – dry weight) / wet weight] × 100.

The ash content was obtained by combustion of the samples, which are used in the analysis of the moisture content, on the heating plate at 300 °C for 10 minutes. After that, the samples are placed in the muffle for about 5 hours at a temperature of 500 °C. The ash percentage formula used was Ash (%) = (weight of crucible and ash – weight of crucible)/ sample weight) × 100.

Crude protein content, expressed as nitrogen content, was determined by the Kjeldahl method, which consists of three main steps: sample digestion, distillation, and titration to get ammonia determination. The Kjeldahl method uses 98% sulfuric acid and a variety of catalysts and salts to convert the organically bound nitrogen of the samples to ammonium with its subsequent measurement. The percentage of protein was calculated by multiplying the total nitrogen percentage by a factor of 6.25.

3.2.2 Total lipids and Fatty acid composition

Total lipids were extracted using the Folch method (Folch et al., 1957).

Approximately 2 g of sample were homogenized in 2:1 chloroform to methanol solution. The homogenate was then filtered through filter paper. A quantity of 10 ml of 0.88% KCl was added to the filtered sample, and the sample was placed in a refrigerator for at least 2 hours. As a result, the filtrate is separated into two phases, the lower phase was then taken and dissolved in 5 ml of chloroform and placed in sealed amber bottles, which are treated with nitrogen to prolong the stability of the lipids against the risk of oxidation, and then stored in the freezer at -20 °C. About 0.5 ml of lipid extract was put into a pre-weighed aluminium crucible and was then placed in an oven at 105 °C for 5 minutes. The crucible was weighed again, and the total quantity of lipids are calculated by applying the following formula:

Fat (%) = (crucible and lipid weight - crucible weight /5 ml × ml weighed)/sample weight] × 100.

The FA composition was determined according to Morrison & Smith (1964), depending on the trans-esterification methods by using a Varian GC 430 gas chromatograph (Agilent, 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 at the rate of 12 °C/min, and then increased to 220 °C over 14 min at the rate of 3 °C/min and 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 the sample in hexane was injected into the column with the carrier gas (helium) kept at a constant flow of 1.5 mL/min. The split ratio was 1:20. The chromatograms were recorded with the Galaxie Chromatography Data System 1.9.302.952 (Agilent) computing integrator software.

3.2.3 TBARS

The 2-thiobarbituric acid reactive substances (TBARS) were measured according to Vyncke (1970) method. About 2 g of sample were homogenised with 10 mL of 5% trichloroacetic acid (TCA) solution for 60 sec using an Ultraturrax® (Mod. Ultra-Turrax T25, IKA®-Werke GmbH and CO, KG, Germany). Samples were stored at -30 °C for 10 min in order to precipitate the protein fraction. Then the samples were centrifuged and filtered. Five mL of the extracts were added with 2 mL of 0.02 M thiobarbituric acid (TBA) and incubated at 93 °C for 40 min. The absorbance was read at 532 nm with a spectrophotometer (Mod. Lambda EZ 150 UV/VIS Spectrometer, Perkin Elmer, Norwalk, USA). The results were expressed as mg of malonaldehyde/kg sample using a calibration curve determined with eight standard solutions of TEP (1,1,3,3,-tetra-ethoxypropane) at concentration ranging from 0.2 to 3.1 µM.

3.2.4 Conjugated dienes

Conjugated dienes (CD), considered the primary lipid oxidation products, were measured according to Srinivasan et al. (1996). Briefly, 2 g of sample were homogenate in 6 mL water, then 0.5 mL of that extract were added to 5 mL hexane:isopropanol (3:2, v/v). Before reading the absorbance at 233 nm, the samples were centrifuged 5 min at 2000 × g. The concentration of conjugated dienes was obtained by using the molar extinction coefficient of 25200 mL/ ($mmol^{-1}$ cm^{-1}). The results were expressed as mol hydroperoxides/kg muscle.

3.2.5 Antioxidant power

The samples were analyzed to determine their antioxidant power, by determining the reducing activity of 2,2-azino-bis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS), the radical scavenging activity 1,1-diphenyl-2-picrilhydrazyl (DPPH) and iron-reducing ability (FRAP). A quantity of 3 g of sample was homogenized with 7 mL of ethanol using an Ultraturrax. Subsequently, the homogenized samples were centrifuged, at 6500 × g for 10 minutes to separate the solid material; then the extract obtained was filtered.

- ABTS

7 mM of ABTS molecule reacted with 2.45 mM potassium persulphate in distilled water in order to obtain the stable radical cation ABTS•+ (Re et al., 1999). This solution was incubated in the dark, at room temperature, for 12 to 16 hours before use.
The solution of ABTS•+ was diluted with ethanol to obtain a solution with absorbance equal to 0.70 ± 0.02 at 734 nm. Then, the sample, the control, and the blank were prepared by adding 3 mL of ABTS•+ to 30 µL of sample extract, 3 mL of ABTS•+ to 30 µL of ethanol, and the ethanol respectively. The readings were taken after 6 minutes at the spectrophotometer (at 734 nm) for each of the sample, the control, and the blank. The reaction resulted in elimination the colour from intense blue to an almost transparent blue, and termination of ABTS• + radical by the possible antioxidants of the sample. The reduction of ABTS•+ is calculated as a percentage of removing colour according to the following formula: [(control Abs - sample Abs) / control Abs] × 100. The final result was expressed as mmol of trolox/kg of sample.

- DPPH

According to Blois (1958) method, later modified by Jung et al. (2010), a solution of 0.2 mM of DPPH in ethanol was prepared. Then, 1 mL of the prior solution was added to 0.5 mL of distilled

water and 1 mL of sample extract. Readings were taken after 30 minutes at the spectrophotometer (at 517 nm) for each of the sample, the control (1 mL of DPPH, 0.5 mL of distilled water and 1 mL of ethanol), and the blank (ethanol). The reaction resulted in changing the colour from purple to yellow and reacting between DPPH radicals molecules with the antioxidant molecules presented in the sample. The reduction of the DPPH is calculated as a percentage of colour elimination. The final result was expressed as mmol of trolox/kg of sample.

- FRAP

The solution of FRAP containing TPTZ (2,4,6-tris (2-pyridyl) -striazine 10 mM in 40 mM of HCl) was prepared according to the method of Descalzo et al. (2007). The previous solution reacts with Fe^{3+} giving a yellow to orange colour. 2.5 mL of FRAP were added to 83 µL of sample extract, and then readings were taken after 4 minutes at the spectrophotometer (593 nm) of each of the samples, the control (2.5 mL of FRAP and 83 µL of ethanol) and the blank (ethanol). The reaction resulted in reduction of Fe^{3+} to Fe^{2+} by the possible antioxidants presented in the sample, and in a change of colour tending to intense blue/violet. The final result was expressed in mmol Fe^{2+}/ kg of sample.

3.2.6 Mineral composition

The analysis of the mineral contents (Al, As, Ca, Cd, Cr, Cu, Fe, K, Mg, Na, P, Pb, Se, Zn) was carried out exclusively on the lyophilized samples of the whole fillet and burgers analysed at time t = 0 (immediately after their obtention). For this purpose, 100 mg of sample were weighed and transferred to special Teflon tubes. A quantity of 10 ml of 67% nitric acid (HNO_3) was added and the tubes were placed in a microwave oven (Mod. Mars - CEM Corp., North Carolina, USA). The samples were subjected to a mineralization program at 200 °C for 15 minutes, at 1600 W. After cooling, the tubes contents were dissolved in 25 ml of bi-distilled water, then the samples were analyzed at the ICP-AES spectrophotometer (Mod. IRIS Intrepid II ICP Spectrometer, Thermo Electron Corporation-Massachusetts, USA). The light radiation emitted from each element was measured for three consecutive times.

3.3 Sensory analysis

In the Research III, the Sensory analysis was perfomed by the Descriptive Analysis.
In details, 10 subjects (5 males and 5 females, mean age 31 years) were recruited as panellists. They were regular fish consumers, had no history of disorders of oral perception and were paid to take part in the study. Written informed consent was obtained from each after the experiment had been described to them.

The burgers, each consisting of a 25 g portion served at 50 °C, were used for training and evaluation sessions. Panellists participated in three training sessions of about 60 minutes each. The subjects developed a vocabulary describing differences and similarities between experimental samples in two different sessions, according to a simplified version of the repertory grid method. A main list of 15 attributes was developed which described aroma (ortho-nasal odour), texture, taste and flavour (retro-nasal odour) of burgers. A nine-point scale (1-9 from extremely weak to extremely strong, respectively) was used for intensity ratings. Assessors and panel performance were validated by evaluating two repetitions of a subset of three samples.

The evaluation of samples of each recipe was replicated three times in two sessions. In each session, each panellist evaluated 6 samples identified by a three-digit code. Samples were presented singly, and presentation order was randomized between subjects and sessions. The order of attributes was randomized between subjects for each sensory mode, the attributes "overall aroma" and "overall flavour" were always at the end of the corresponding list.

Subjects were asked to evaluate aroma, then to take a first bite to evaluate taste and flavour and a second bite for texture evaluation. After evaluation of each sample, subjects rinsed their mouths with water for 30 s, ate plain crackers for 30 s and rinsed their mouths a second time with water for a further 30 s. They took a 15 min break after every three samples. Data was collected with the software Fizz (ver. 2.47.B, Biosystemes, Couternon, France).

3.4 Statistics

Data obtained from the different researches were statistically analysed.

Briefly, results of Research I were obtained by using SPSS version 17.0 software. A one-way ANOVA tested the treatment as fixed effect on pH, colour parameters, proximate composition, micro-elements, and fatty acid composition. The Bonferroni post-hoc test was applied to check the significance of the differences among treatments. The PLS-DA model defining a dummy variable for each type of flesh treatment.

In the Research II, data related to sensory attributes of four burger recipes were analysed by multi-block PCA (Tucker-1) and by p*MSE plot (Panel Check software, ver. 1.4.0, Nofima, Norway). Intensity ratings were analysed independently by a two-way ANOVA mixed model (sample as fixed and assessors as random factors), followed by a Fisher LSD post hoc test.

The result of texture and chemical characteristics were submitted to ANOVA (two-way) by the PROC GLM of the SAS (SAS, 2004), were the sea bass to rainbow trout ratio (MSMR, 50:50 and 30:70), the fish to potato ratio (DMR, 2.5:1 and 1.5:1), and the interaction MSMR × DMR were included in the model as fixed effects. Multiple comparisons among means were performed using

the Tukey's test and were considered significant for p values <0.05. Finally, sensory, physical and chemical data set (sensory aroma&flavour, sensory texture, saturated fatty acids, polyunsaturated fatty acids, laboratory determined texture) were subjected to multiple factor analysis (Escofier & Pagès, 1994).

The statistical analysis of data collected during Research III was performed using the General Linear Model procedures of the Statistical Analysis Software SAS (2004) for Windows. A two-ways ANOVA, where the recipes with different ratio of European sea bass to rainbow trout (R: 50:50, R1; 30:70, R2), storage time (S: T0, T30, T60, T90), and the Recipes × Storage time (R × S) interaction were included in the model as fixed effects.

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Technological and nutritional advantages of mechanical separation process applied to three European aquacultured species

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ABSTRACT

Recently, mechanical separation (MS) process has been applied on fish sector, however, its impact on fish quality is scarcely investigated. Aim of the present study was to compare the impact of mechanical separation with manual mincing applied on European sea bass, gilthead sea bream, and rainbow trout by evaluating physico-chemical properties and nutritional quality. MS process yield was found higher than the manual one when applied to sea bass, and sea bream (42, and 45 g/100 g, respectively against 39, and 40 g/100 g). Rainbow trout had the highest processing yield even if the high presence of residual on the drum (5 g/100 g) lead a lower MS yield than the manual processing. MS seemed to slightly increase water content in sea bream and trout (71.12, and 70.65 g/100 g, respectively against 68.05, and 68.11 g/100 g of fillets) and decrease minerals, especially in trout, which showed loss of Ca, Mg, Na, and P. Hopefully, lipid fraction of the three species remained unaltered, indeed no significant differences were found in the fatty acid composition of the products, and consequently for the calculated atherogenicity and thrombogenicity indexes. In sum, manufacturing of products by exploiting fish without altering the nutritional value of whole fish is a goal reached adopting mechanically separation process.

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Technological and nutritional advantages of mechanical separation process applied to three European aquacultured species

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Abstract

Recently, mechanical separation (MS) process has been applied on fish sector, however, its impact on fish quality is scarcely investigated. Aim of the present study was to compare the impact of mechanical separation with manual mincing applied on European sea bass, gilthead sea bream, and rainbow trout by evaluating physico-chemical properties and nutritional quality. MS process yield was found higher than the manual one when applied to sea bass, and sea bream (42, and 45g/100g, respectively against 39, and 40g/100g). Rainbow trout had the highest processing yield even if the high presence of residual on the drum (5g/100g) lead a lower MS yield than the manual processing. MS seemed to slightly increase water content in sea bream and trout (71.12, and 70.65g/100g, respectively against 68.05, and 68.11g/100g of fillets) and decrease minerals, especially in trout, which showed loss of Ca, Mg, Na, and P. Hopefully, lipid fraction of the three species remained unaltered, indeed no significant differences were found in the fatty acid composition of the products, and consequently for the calculated atherogenicity and thrombogenicity indexes. In sum, manufacturing of products by exploiting fish without altering the nutritional value of whole fish is a goal reached adopting mechanically separation process.

Keywords: Mechanical Separated Meat; Process Yield; Mineral; Fatty Acids; Color.

Introduction

Fish represent a source of high-quality protein, essential fatty acids, and a range of macro- and micronutrients that have shown beneficial effects on human health. Fatty acids of ω3-series, especially eicosapentaenoic acid (C20:5ω3, EPA) and docosahexaenoic acid (C22:6ω3, DHA), have been proved to be involved in anti-inflammatory responses, anti-carcinogenic, and anti-thrombogenic effects (Maskrey, Megson, Rossi, & Whitfield, 2013). Furthermore, fish muscle includes a large variety of mineral (Alasalvar, Taylor, Zubcov, Shahidi, & Alexis, 2002; Asghari, Zeynali, & Sahari, 2013), from K to Se. The main functions of essential minerals include skeletal structure, maintenance of colloidal system, and regulation of acid–base equilibrium. Minerals also compose hormones, enzymes, and enzyme activators (Belitz, Grosch, & Schieberle, 2001). Despite the high nutritive value of fish, the time for their preparation and the price can discourage some consumers from purchasing, leading to a preference for ready to cook or ready to eat products (Palmeira et al., 2016). The edible proportion of fish represents approximately 45% of total fish weight (depending on fish species); thus, 55% is composed by head, fins, guts, bones, frame, and meat adhered to bones and skin which are considered as fish waste from processing (Arvanotoyannis & Tserkezou, 2014). Numerous technological strategies have been adopted in the recent years in order to trade on wastes, such as the production of functional ingredients from bones and skin, semi-ready or ready to cook/eat products obtained by meat adhered to bones and skin. The latter is commonly named mechanically separated meat (MSM) and it derives from the removal of the remaining meat from bones applying low (<104 kPa) or high pressure (>104 kPa). Fish burgers (Marengoni et al., 2009), surimi (Fogaça, Otani, Portella, dos Santos Filho, & Sant'Ana, 2015), and flour (Oliveira, Lourenço, Sousa, Joele, & Amaral Ribeiro, 2015) have been developed using MSM from Nile tilapia (*Oreochromis niloticus*), and Brazilian catfish (*Brachyplatystoma vaillantii*) wastes (Freitas, Resende, Furtado, Tashima, & Bechara, 2012; Kirschnik, Trindade, Gomide, Moro, & Viegas, 2013; Marengoni et al., 2009; Fogaça et al., 2015; Oliveira et al., 2015). On the other hand, not only residual from commercial fish processing, but also damaged and noncommercial sized fish are considered discards even if they might be shifted to human consumption.

Seafood represents an important component of the food supply for the Italian population. However, the loss of high quality food image, the saturation of internal market, and the strong foreign competition have been responsible for the seafood industry stagnation, from which new marketing approaches, such as products diversification, might help to be turned out

(http://www.fao.org/fishery/countrysector/naso_italy/en). Therefore, current innovations could be directed towards the manufacturing of new products, also by exploiting fish wastes, without altering the nutritional value of whole fish.

For this reason, this study aimed to compare the impact of mechanical separation process and manual minced technology applied on three aquacultured species of interest for European aquaculture. Physico-chemical properties and nutritional quality of European sea bass, gilthead sea bream, and rainbow trout derived products were evaluated immediately after the fish treatments.

Materials and methods

Preparation of fish samples and storage conditions

Thirty-three European sea bass (*Dicentrarchus labrax*) and 33 gilthead sea bream (*Sparus aurata*) were purchased from a fish farm located in Orbetello (Grosseto, Italy) whereas 33 pigmented rainbow trout (*Oncorhynchus mykiss*) were purchased from a farm located in the north west of Tuscany (Lucca, Italy). All the fish were killed by percussion. Immediately after death, fish were transferred in polystyrene boxes, covered by ice, and moved to the industry where 18 fish for each species were minced by the soft belt-drum separator (BAADER Mod. 601; Baader, Lübeck, Germany) after being washed, eviscerated and decapitated, and washed again in order to eventually remove blood and gut residuals. In details, fish were manually inserted into the MSM machine, previously sanitized, where a conveyer belt pressed the carcass on the surface of a perforated drum (hole diameter: 5 mm). Bones, skin and thicker layers of connective tissue remained outside from the drum and were ejected through a discharge chute, while meat (MSM) passed through the holes and conveyed in a plastic box. A one-step separation was conducted without any washing or centrifugation additional phases. The remained whole fish and the MSM were immediately brought, in refrigerated condition, to the Agri-Food and Environmental Science Department laboratories (University of Florence, Firenze, Italy) where all the whole fish were filleted. Fifteen fillets (right) for each species were analysed as such (control, C samples) while fifteen fillets (left) for each species were grounded by using a manual mincer (Mod. Tritacarne New Style; Westmark Gmbh, Elspe, Germany) and the minced meat was shaped in 15 flat round cakes, similar to burger (FB samples) made with 100% of fish (referred as fish burger in this paper) that were manually formed with a plastic stamp. Fifteen MSM-fish burgers were directly obtained by forming MSM with the same plastic stamp (MSM samples). Three replicates of C, FB, and MSM were analyzed for pH, color, proximate composition, fatty acid profile, and mineral composition.

Processing yield

The whole, headed and gutted weights of each fish, as well as the minced meat weight were recorded. Similarly, the fillet yield of manually filleted fish was calculated considering the whole fish, headed and gutted weights and the weight of the two fillets from each fish. Yield was calculated as g/100g of deboned meat weight relative to whole fish weight (Booman, Márquez, Parin, & Zugarramurdi, 2010).

Physical analyses

pH and color were measured on C, FB, and MSM samples. The pH value was monitored in three different points of the epaxial region of the whole fillets and of the burger's (FB and MSM) diameter by using a pH-meter (Columbus, OH, USA).

Lightness (L*), redness index (a*) and yellowness index (b*) were measured according to the CIELab color space system (CIE, 1976) by a Spectro-color® colorimeter (Keison International Ltd, Chelmsford, Essex, UK) and data were recorded by the software Spectral qc 3.6.

Proximate composition

Moisture, crude protein (N×6.25), crude fat, and ash contents were determined by using 950.46, 976.05, 991.36, and 920.153 A.O.A.C. (2012) methods, respectively. For total lipid analysis, approximately 2 g of sample were ground and extracted using chloroform and methanol according to Folch, Lees, & Sloane Stanley (1957) method. Total lipids were measured gravimetrically.

Fatty acid profile

Fatty acids (FAs) were determined in the lipid extract after trans-esterification to methyl esters (FAME) using the method proposed by Morrison and Smith, 1964. The FA composition was determined by gas chromatography (GC) using a Varian GC 430 gas chromatograph (Varian Inc., 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, PA, USA) set as previously described in Secci et al. (2016). Fatty acids were quantified through calibration curves using tricosanoic acid (C23:0) (Supelco, PA, USA) as internal standard. This analysis was carried out on C and MSM samples, but not on FB samples, because they were obtained from minced (left) fillets of the same fish from which the C samples were obtained and consequently they were considered with the same characteristics of C samples in term of FA composition. Atherogenicity index (AI), according to the formula [C12:0 + (4 × C14:0) + C16:0] / (ΣPUFAω3 + ΣPUFAω6 + ΣMUFA), and thrombogenicity index (TI), according to the formula [C14:0 + C16:0 +

C18:0] / [0.5 × ΣMUFA) + (0.5 × ΣPUFAω6) + (3 × ΣPUFAω3) + (ΣPUFAω3/ΣPUFAω6)] were calculated as suggested by Ulbricht and Southgate (1991); hypocholesterolaemic/hypercholesterolaemic FA ratio (h/H), as (C18:1ω9 + C18:2ω6 + C20:4ω6 + C18:3ω3 + C20:5ω3 + C22:5ω3 + C22:6ω3) / (C14:0 + C16:0) was also calculated (Santos-Silva, Bessa, & Santos-Silva, 2002).

Mineral composition

Three samples for each treatment were lyophilized (Vacuum Pump Welch Directorr; Welch Vacuum Technology Inc., Skokie, IL, USA) and utilized for determination of mineral composition. The contents in 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) were determined. One-hundred mg of lyophilized samples 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, NC, USA) by applying the mineralization stages at 1600 Watt: 200 °C (ramp time 20 min, hold time 15 min). 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, MA, USA). Trace minerals were quantified on the basis of peak areas and comparison with a calibration curve obtained with the corresponding standards. The elements were read in triplicate for each sample.

Statistical analysis

The statistical analysis was performed using SPSS version 17.0 software (SPSS Inc., IL, USA). Normality of data distributions was tested by the Kolmogorov-Smirnov test. The analysis was performed independently for each evaluated species. Firstly, one-way analysis of variance (ANOVA) with 'treatment' (C, FB, MSM) as a fixed effect was performed on pH, color parameters (L*, a* and b*), proximate composition, micro-elements, and fatty acid composition data. The Bonferroni post-hoc test was applied to check the significance of the differences among treatments.

The PLS-DA model was built between the proximate composition, color variables and micro-elements (X-matrix) and the C, FB and MSM variable (Y) that was made by defining a dummy variable for each type of flesh treatment. The species of fish was not taken into account. Classification performance was assessed in terms of sensitivity, specificity and total accuracy (Kjeldahl & Bro, 2010).

Results and Discussion

Yield of separation process compared to the manual operation

From Figure 1 emerged that flesh removal yield seemed to be species-specific. Generally, rainbow trout showed the highest flesh removal yield by raising 53 g/100 g (on whole weight), and 50 g/100 g when manually or mechanically separated, respectively. Interestingly, despite an overall lower processing yield obtained for seawater species (around 40 g/100 g) than for trout, MS operation increased the yield by raised from 39 to 42 g/100 g in sea bass and from 40 to 45 g/100 g in sea bream, in agreement with Setiady, Lin, Younce, and Rasco (2007). However, as previously underlined by Souza, Melo, Moreira, and Souza (2015) numerous factors, such as species, size, gender, age and condition of fish might affect operation yields thus explaining the difference between sea water species and rainbow trout. Indeed, looking at the composition of MS output reported in Figure 2, emerged that the residual meat on the drum was sensibly higher in rainbow trout than in sea bass and sea bream thus suggesting that trout muscle is less prone to be MS processed maybe because of its thickness and bones structure. Such high residual quantity on the drum found for trout moreover may be responsible for the lower processing yield than the manual filleting process.

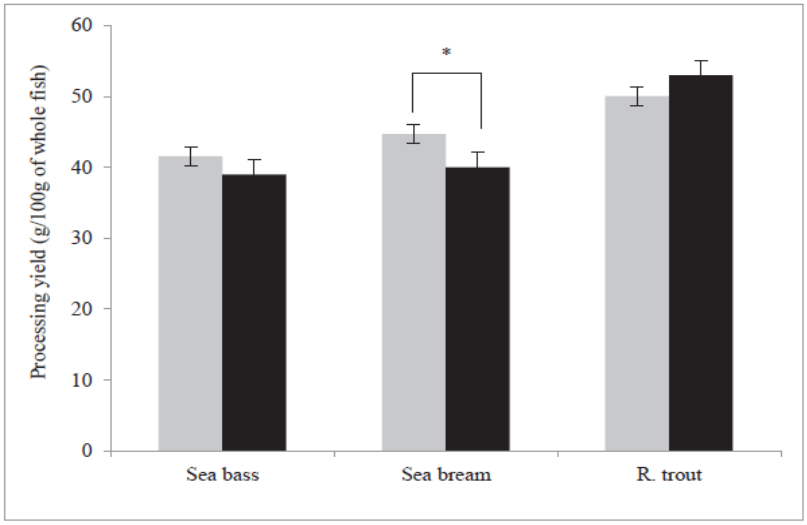

Figure 1. Graph bars of mechanical (grey bar) and manual (dark bar) yield (g/100 g whole fish) for each studied species (European sea bass, gilthead sea bream and rainbow trout). *$p < 0.05$.

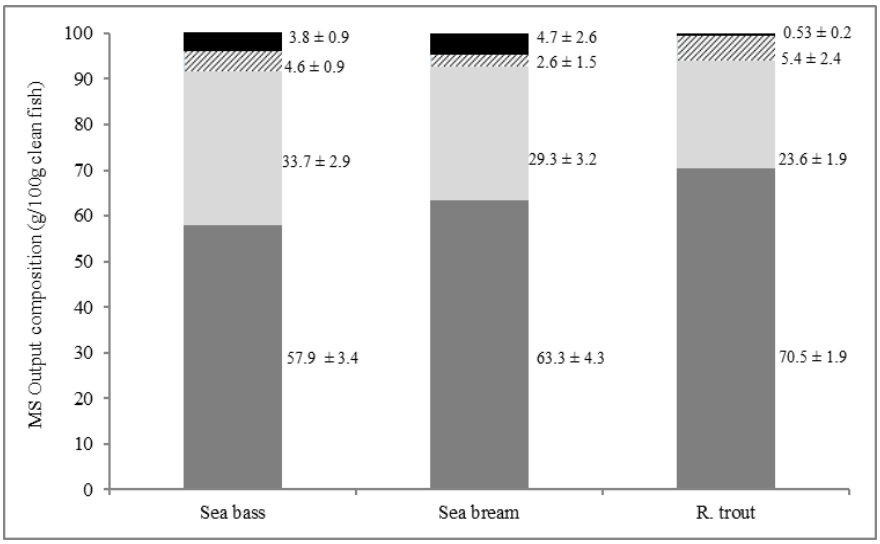

Figure 2. Graph bars of MS output composition (mechanical separated meat: dark grey bar; bones and skin: grey bar; drum residual: bar; process error: dark bar), expressed as g/100 g clean fish, for each studied species (European sea bass, gilthead sea bream and rainbow trout). Mean ± standard deviation is reported near to the corresponding bars.

Qualitative differences among treatments

The basic compositional traits and physical parameters results of products obtained is reported in Table 1. The treatments differently affected the products from the three species of fish. Sea bass whole fillet (C) and burger (FB) showed a lower fat and protein content ($p>0.05$), with higher amount of moisture, and significantly higher ash values compared to MSM. Proximate composition of sea bass was in line with previous papers where cultured sea bass composition of fillets resulted in 68-70 g/100 g of moisture, 20-22 g/100 g of protein, 8-10 g/100 g of lipids and 1.3-1.5 g/100 g of ash (Alasalvar et al., 2002; Badiani et al., 2013). In sea bream, the MSM samples were significantly different ($p<0.05$) from C and FB samples. Indeed, the moisture resulted higher in MSM with a significantly lower content of protein and fat. The basic compositional traits of sea bream MSM were well within the range presented by Grigorakis (2007) in his review encompassing fifteen years of research on nutritional quality of farmed specimens, but the results obtained for C and FB resulted with a lower moisture contents. Cellular disruption could be responsible for the increase in water content of MSM samples, especially considering the possible release of water soluble protein, and this trend confirms previous data obtained on MSM from Brazilian catfish (Olivera et al., 2015). However, the species-specific effect of the mechanical process is underlined by the presence of significant differences in water content differently distributed between treatments. Hence, sea bream and trout appeared to be the most susceptible to be damaged by the mechanical separator thus suggesting the fragility of the matrix.

The mean contents of moisture, protein, lipids, and ash in the analyzed trout fillets were in the ranges 70-72, 19-21, 6-7.5 and 1.45-1.55 g/100 g of product, respectively. The proximate composition of the rainbow trout reported in literature (Gokoglu, Yerlikaya, & Cengiz, 2004) showed some differences in fat content, mostly dependent on the animal diets (González-Fandos, García-Linares, Villarino-Rodríguez, García-Arias, & García-Fernández, 2004).

The pH value is an important index for determining the fish quality as bacterial and enzymatic activity affects pH and is affected by pH in fish fillets like in other meats. The pH values were not affected by treatment in sea bass, indeed no significant differences were highlighted, as reported in Table 1. As expected, C, FB and MSM products resulted with the same pH, because of the employment of whole fish in MSM production instead of residues as reported by other authors (Oliveira et al., 2015) and all the products were in the same freshness condition of the matrix. However, differences in pH were found in both sea bream and rainbow trout. The differences in pH could be easily explained by the moisture content, indeed the higher the moisture, the higher the pH as reported in previous papers (Ruiz-Ramírez, Arnau, Serra, & Gou, 2005; Rebouças, Rodrigues, Castro, & Vieira, 2012).

The results of one-way ANOVA for color parameters were reported in Table 1, that highlights that for the three species the color was significantly affected by treatment. In the case of the seawater species, the differences were similar, indeed L*, a* and b* of C samples resulted significantly lower than in FB and MSM burgers. In the case of rainbow trout, the fillets presented a significantly lower lightness (L*) and higher redness (a*) compared to MSM and minced fillet burgers.

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 samples, notwithstanding the content of As, Cd, Cr, Cu, Pb, and Se was below the instrument detection limit (1 mg/kg wet weight). The mineral composition of C, FB, and MSM samples is reported in Table 2. The inclusion of macronutrients is an important facet of the nutrient profile. In accordance to their categories, high levels of K and P were found together with low amounts of Fe and Zn in all the considered species. Similar values were proposed for European sea bass (Alasalvar et al., 2002), gilthead sea bream (Erkan & Özden, 2007) and rainbow trout (Asghari et al., 2013). Specifically, K ranged from 1212 mg/kg in trout to 4240 mg/kg in sea bream, whilst P levels were found around 3000 mg/kg in sea bass, sea bream, and trout. Nevertheless, the huge variation in mineral composition is confirmed by the fact that frequently authors expressed the results as 5th and 95th percentile instead of the mean (Olmedo et al., 2013).

Table 1. Proximate composition (g/100 g product), pH and color of European sea bass, gilthead sea bream, and rainbow trout fillets (C), minced (FB) and MSM, analysed immediately after treatment.

Species	Parameter	C	FB	MSM	RMSE1
Sea bass	Moisture	70.00	71.18	68.79	1.00
	Lipid	9.59	7.67	10.49	1.30
	Protein	18.86	19.68	19.17	0.22
	Ash	1.25b	1.38a	1.23b	0.03
	pH	6.30	6.34	6.33	0.02
	L*	42.93c	49.54b	51.19a	0.44
	a*	-1.20b	0.17a	-0.31a	0.16
	b*	-0.69b	3.77a	4.06a	0.18
Sea bream	Moisture	68.05b	70.21a	71.12a	1.03
	Lipid	9.87a	7.15b	7.76b	0.59
	Protein	20.34a	20.82a	19.35b	0.20
	Ash	1.43a	1.45a	1.28b	0.02
	pH	6.15ab	6.12b	6.18a	0.01
	L*	42.39b	48.05a	47.75a	0.33
	a*	-1.25b	0.33a	0.87a	0.22
	b*	-0.42c	3.36b	4.41a	0.23
Rainbow trout	Moisture	68.11b	67.71b	70.65a	0.44
	Lipid	8.06	7.89	6.65	0.59
	Protein	22.01	22.31	21.00	0.34
	Ash	1.52	1.53	1.50	0.02
	pH	6.37b	6.37b	6.48a	0.01
	L*	31.91b	46.87a	48.12a	1.05
	a*	5.13a	0.95b	1.28b	0.84
	b*	9.54b	11.27a	12.51a	0.47

1RMSE: Root Mean Square Error.
Within criterion, a, b, c: $p<0.05$.
Data were obtained from three replicates.

Such as high difference however may be attributed to the diet, to origin (wild or farmed) (Alasalvar et al., 2002), as well as age, gender, salinity, handling processes (Yilmaz, 2003; Erkan & Özden, 2007), and type of muscle, i.e. white or red (Erkan & Özden, 2007). Interestingly, treatments differently affected the mineral content of the analyzed species. Sea bass was almost unaffected by manual and mechanical separation processes (Table 2) being only Na to significantly decrease in MSM samples. The macronutrient P was significantly reduced by MS treatment when applied to sea bream. Finally, even Ca, and Mg were highly affected by the treatment. In all cases, separation process induced a reduction of mineral concentration in samples. Two aspects have to be underlined. The first one is the unexpected reduction of Ca, confirming the efficiency of the mechanical separation process

in muscle separation from the skeletal part of the fish, which is the main source of Ca. The second aspect is that, despite the tendency to decrease mineral fraction, mechanical operations did not severely compromise it, that is an important result from the nutritional point of view. Indeed look at the recommended daily intake (Tolonen, 1990) it is possible to find out that 100 g of MSM burger of all the considered species supplied for 100% K, Mg, and P, and it might represent a good source of Fe, Zn, and Na.

The fatty acid (FA) composition of the fillets and MSM samples, taken immediately after treatment, is reported in Table 3. No statistical differences were found in the fatty acid profile between fillets and MSM nor for seawater or freshwater species. All the considered fish had more than 8 g of fat for 100 g of product, but their fatty acid composition varied as follow. Linoleic (C18:2ω6), oleic (C18:1ω9) and docosahexaenoic (DHA, C22:6ω3) acids were the majority of FA for trout, whereas oleic, DHA, and palmitic (C16:0) acids were the main constituents of sea bass and sea bream lipid fraction. These major three fatty acids represented around 47% of the total fatty acids in trout, and sea bass whilst 43% in sea bream. As expected, in what concerns PUFAs, the level of ω3 was higher than that of ω6 in seawater species whilst rainbow trout contained two times the level of PUFAω6 and lower PUFAω3 content than sea bass and sea bream. These results are in agreement with previous findings about the differences between marine and freshwater fish species (Tocher, 2003; Li, Sinclair, & Li, 2011).

Despite the high percentage of fat contained in 100 g of product, the characteristics of intramuscular fat are very interesting for human nutrition as a consequence of the predominance of PUFA fraction, particularly ω3, on both MUFA and SFA ones especially in seawater species. Moreover, also the values of other health indexes as ω3/ω6 PUFA ratio, AI, TI, and h/H confirmed the optimal nutritional characteristics of fat. In addition, these characteristics seemed to be conveniently preserved during the mechanical separation process, as revealed by the absence of statistical differences between MSM and C samples.

Particularly, the ω3/ω6 ratio was found to be around 3 in sea bass and sea bream, in agreement with previous findings for intensive farmed sea bass (Orban et al., 2002), and sea bream (Orban et al., 2003b). Trout instead had the lowest ratio by staying under 1. In this case, the calculated value was inferior at what previously reported for pigmented rainbow trout (Orban et al., 2003a) because of the widest presence of ω6, certainly due to the feeding composition. This difference is not particularly surprising, since the dietary fish meal and fish oil inclusion in aquafeeds is dramatically decreased in the last decades, mainly in feed for salmonids (Tacon & Metian, 2008). The use of more sustainable diets has produced relevant changes in fatty acids profile, seriously affecting ω3/ω6 PUFA ratio.

Table 2. Mineral composition of European sea bass, gilthead sea bream, and rainbow trout fillets (C), minced (FB) and MSM samples immediately after treatment (mg/100 g).

		C	FB	MSM	RMSE1
Sea bass	Ca	26.59	27.67	85.47	36.78
	Fe	0.81	0.91	0.46	0.35
	K	346.09	358.71	358.65	7.50
	Mg	36.27	36.84	36.51	1.52
	Na	38.35a	36.07a	27.45b	1.25
	P	230.02	239.49	248.18	14.75
	Zn	0.54	0.48	0.53	0.08
Sea bream	Ca	79.41	22.92	29.00	19.19
	Fe	0.50	0.33	0.59	0.12
	K	385.06ab	406.47a	363.43b	9.07
	Mg	37.49	37.08	33.30	0.98
	Na	32.15	30.50	29.59	2.21
	P	275.12a	258.52ab	224.39b	9.38
	Zn	0.57	0.47	0.53	0.06
Rainbow trout	Ca	44.29a	15.99b	16.72b	5.74
	Fe	0.49	0.75	0.67	0.12
	K	438.61	459.98	442.91	7.52
	Mg	36.07a	35.42a	32.41b	0.57
	Na	47.98a	46.10a	32.88b	1.34
	P	238.75a	228.48b	217.75c	1.94
	Zn	0.56	0.50	0.43	0.05

[1]RMSE: Root Mean Square Error.
Within criterion, a, b, c: $p<0.05$.
Data were obtained from three replicates.

Recently, the attention toward EPA+DHA content has been increased. An intake of 250 mg per day has been suggested as the labeling reference intake value for EPA plus DHA, which is in agreement with the most recent evidence on the relationship between the intake of these fatty acids and cardiovascular health in healthy populations (EFSA, 2010). However, the intake of fish flesh with similar EPA+DHA content but with different ω3/ω6 ratio was shown to have varied effects on lipid quality in a group of healthy subjects. Specifically, a significant decrease of total cholesterol, LDL, and triglycerides as well as an improve of inflammatory variables such as interleukins 6 and 8 were found in subject weekly fed for 10 weeks with 12 g of EPA+DHA, and 2.4 ω3/ω6 PUFA ratio (Sofi et al., 2013). The present findings revealed that a portion (100 g) of MSM and C of all the considered species may provide more than the suggested quantity of EPA+DHA together with a high ω3/ω6

ratio, except for rainbow trout. Again, MSM might represent an optimum way to propose fish to people traditionally not well available for fish consumption, such as children or elderly people.

Finally, AI, TI, and h/H values are presented in Table 3. As reported by Valfrè (2008), AI and TI represent factors of promotion and protection against coronary disease, and their low values suggest a high cardio-protective effect. Very low values and in agreement with those proposed by Orban et al. (2003a; 2003b) were calculated for MSM and C samples. In conclusion, considering both the fatty acid profile and the calculated indexes, MSM seemed to be an optimum lipid sources, especially if obtained by seawater species

However, the higher ω3/ω6 PUFA ratio of the seawater species, regardless the different treatments tested in this trial, is an element of nutritional relevance as an index of the lipid quality.

Table 3. Total lipid (g/100 g of product), and fatty acids profile (g/100 g of total fatty acids) of European sea bass, gilthead sea bream, and rainbow trout fillets (C) and MSM samples immediately after treatment.

	Sea bass				Sea bream				Rainbow trout			
	C	MSM	Sign.	RMSE[1]	C	MSM	Sign.	RMSE	C	MSM	Sign.	RMSE
Total lipids	9.59	10.49	NS	1.83	9.87	7.76	NS	0.64	8.06	6.65	NS	0.84
Fatty acids												
C14:0	3.56	3.43	NS	0.04	4.24	4.10	NS	0.14	1.45	1.44	NS	0.01
C16:0	13.42	13.72	NS	0.30	12.58	12.59	NS	0.44	10.75	10.40	NS	0.26
C16:1ω7	4.80	4.43	NS	0.04	6.39	6.36	NS	0.16	2.47	2.37	NS	0.11
C18:0	2.31	2.46	NS	0.03	2.83	2.61	NS	0.057	3.24	2.98	NS	0.09
C18:1ω9	17.98	18.94	NS	0.72	14.94	14.83	NS	0.41	24.39	23.77	NS	0.31
C18:2ω6	9.05	8.67	NS	0.13	8.17	8.02	NS	0.32	25.82	26.24	NS	0.33
C18:3ω3	1.86	1.91	NS	0.02	1.29	1.31	NS	0.09	4.12	4.23	NS	0.09
C20:1ω9	3.49	3.73	NS	0.09	1.42	1.53	NS	0.06	1.38	1.36	NS	0.12
C20:5ω3	10.12	9.52	NS	0.16	10.41	10.32	NS	0.31	3.08	3.13	NS	0.14
C22:1ω11	3.03	3.38	NS	0.10	1.25	1.41	NS	0.09	0.71	0.70	NS	0.07
C22:5ω3	3.05	2.85	NS	0.13	7.66	7.37	NS	0.22	1.81	1.64	NS	0.05
C22:6ω3	15.91	15.75	NS	0.81	15.83	16.90	NS	0.52	11.00	11.95	NS	0.36
ΣSFA	20.17	20.47	NS	0.30	20.72	20.32	NS	0.63	16.00	15.35	NS	0.34
ΣMUFA	33.43	34.68	NS	0.88	27.99	28.07	NS	0.41	31.70	30.94	NS	0.56
ΣPUFAω6	11.40	10.89	NS	0.17	10.59	10.44	NS	0.31	29.68	30.17	NS	0.24
ΣPUFAω3	33.53	32.67	NS	1.02	38.06	38.71	NS	0.15	21.83	22.76	NS	0.38
ΣPUFA	46.39	44.85	NS	1.18	51.29	51.60	NS	0.25	52.30	53.71	NS	0.33
AI	0.35	0.35	NS	0.00	0.39	0.38	NS	0.01	0.20	0.17	NS	0.01
TI	0.15	0.16	NS	0.01	0.14	0.14	NS	0.00	0.16	0.15	NS	0.01
h/H	3.48	3.42	NS	0.02	3.54	3.60	NS	0.10	5.84	6.08	NS	0.15
ω3/ω6	2.94	3.00	NS	0.04	3.59	3.71	NS	0.10	0.74	0.75	NS	0.02

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 <3%.
They were utilized to calculate Σ.
[1]RMSE: Root Mean Square Error.
Within criterion, a, b, c $p<0.05$. NS, Not Significant ($p> 0.05$). Data were obtained from three replicates.

Discriminant analysis

To identify differences among C, FB and MSM, the discriminant analysis was performed and the results for the PLS-DA classification model (leverage validation method) were satisfactory. Considering all fish treatments, the coefficients of prediction were over 0.98 and over 0.75 for calibration (R2 C) and for validation (R2 V), respectively; calibration root mean square errors were under 0.06 and the validation root mean square errors were under 0.23, after six latent variables for all treatments (i.e. C, FB and MSM). In the validation phase, all samples deriving from the three different treatments were correctly predicted (sensitivity, or true positive rate, i.e. number samples correctly detected by the model divided by the total number of C, FB or MSM samples = 1.00), were correctly classified in the related group (specificity = 1.00), with an overall correct classification (accuracy) of 100%. The main variables that contributed to fish treatment classification resulted the proximate composition, L* and b*, and minerals (with exception of Fe content), as shown in Figure 3. The main differences between samples were accounted along the first component where C resulted well discriminated from FB and MSM, especially for moisture. Few differences appeared between the two minced meats, indeed MSM and FB were separated on the second component characterised by ash, protein, K and Na contents.

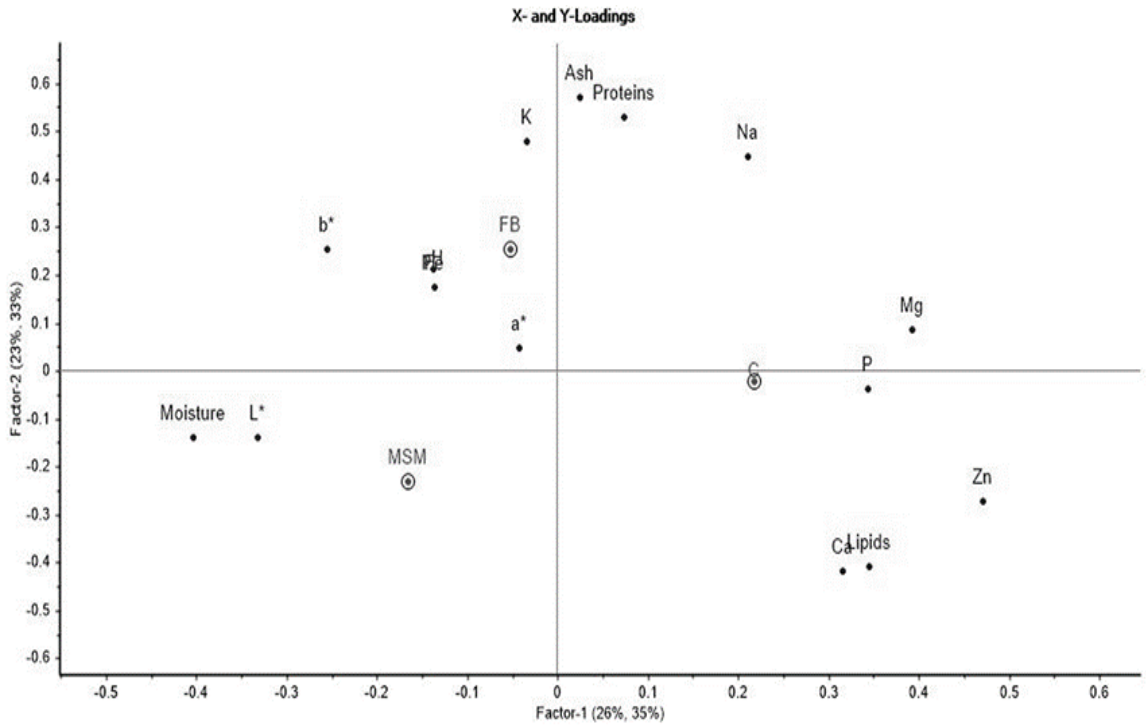

Figure 3. Correlation loadings of PLS e DA performed using proximate composition, mineral content and color parameter at T0 in the X matrix and treatment of fish in Y dummy variable.

Conclusion

The manufacturing of new products by exploiting unmarketable fish without altering the nutritional value of whole fish is a goal well reached adopting the mechanically separated process. This process allowed having a good performance in terms of yield without deeply altering the micro- and macro-nutrient contents. However, some limitations have to be taken into account. The species-specific characteristics, such as the muscle composition and bones structure, seemed to lead to different results in terms of yield and nutritional values. In this sense, sea bream and trout appeared to be the most susceptible to be damaged by the mechanical separator thus suggesting the fragility of these matrixes. The whole fillet of all the considered species slightly differs from the minced meat, in both manual or mechanical separation, however the advantages related to the utilization of no directly marketable specimens, so limiting the waste production, and to the "ready to cook" product by using the mechanical separation process was highlighted in this study. Moreover, further studies could focus on sensory properties of MSM in order to understand if the modification in nutritional value will impact the sensory profile or the acceptability of the transformed fish. Microbiological aspects, such as the microbial growth during the shelf life at different storage conditions might be investigated.

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Original article

Enhanced utilisation of nonmarketable fish: physical, nutritional and sensory properties of 'clean label' fish burgers

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Summary Four 'clean label' formulations for fish burgers made with mechanically separated fish meat were characterised in sensory, textural and chemical terms. The formulations differed in the ratios of European sea bass to rainbow trout (50:50 and 30:70) and the ratios of fish to potato flakes (dry matter ratio, DMR: 2.5:1 and 1.5:1). The sensory profile was mainly influenced by DMR. Recipes with the higher DMR were positively correlated with sandy, dry and crusty attributes, salty taste and overall flavour. Soft texture was perceived for recipes with the lower DMR, although no differences in texture were detected by a texturometer. Lowering DMR increased ash and water content and decreased protein content, as expected. The results indicated that 100 g of burger provided more essential fatty acids than the recommended daily intake, irrespective of formulation. In conclusion, multiple factor analysis indicated that the main changes detected and perceived were due to DMR.

Keywords Descriptive analysis, essential fatty acids, European sea bass, rainbow trout, texture.

Introduction

Awareness of the food we eat and sustainable consumption are topical subjects. The food industry and consumers are increasingly concerned about sustainable production, health and wellness. The industry, for example, has been trying to reduce food wastage, a significant contributor to unsustainability, while consumers are demanding healthy minimally processed food. As underlined by Balasubramaniam *et al.* (2016), consumers often read food labels to check whether the ingredients on the label can be found in their own kitchens. If so, they are prepared to purchase the product. The movement away from artificial chemical ingredients is known in the industry as 'clean label' (Saltmarsh, 2014).

Parallel to this movement, health claims are gaining importance in Western countries. They refer to a food's ability to prevent, manage or treat illness (Martirosyan & Singh, 2015) through known or unknown functional molecules that modulate one or more metabolic processes or pathways in the body. For example, the long-chain omega-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (C20:5n3, EPA) and docosahexaenoic acid (C22:6n3, DHA), can reduce the risk of cardiovascular disease, improve mental and visual functions and are involved in inflammatory responses (Hong *et al.*, 2005; Merched *et al.*, 2008; Abuajah *et al.*, 2014). They may also theoretically lead to a decrease in body fat over time and reduce obesity risk (Wildman, 2016).

Fish are an important source of EPA and DHA and can therefore be regarded as natural functional food. Fish also contain high-quality protein and essential micronutrients for humans. However, much precious food is lost in the production chain due to processing-related fish waste (up to 55% of the fish body is typically inedible) and the fact that damaged and noncommercial sized specimens are discarded.

The literature indicates that such fish waste can be viable, sustainable, easy-to-prepare and nutritious food (Palmeira *et al.*, 2016). Mechanical separation process has been successfully applied to the major aquaculture species on the European market in the case of specimens that cannot be marketed directly due to damage

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Enhanced utilisation of non marketable fish: physical, nutritional and sensory properties of 'clean label' fish burgers

Running title: Clean label fish burger characterisation

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Abstract

Four "clean label" formulations for fish burgers made with mechanically separated fish meat were characterised in sensory, textural and chemical terms. The formulations differed in the ratios of European sea bass to rainbow trout (50:50 and 30:70) and the ratios of fish to potato flakes (dry matter ratio, DMR: 2.5:1 and 1.5:1). The sensory profile was mainly influenced by DMR. Recipes with the higher DMR were positively correlated with sandy, dry and crusty attributes, salty taste and overall flavour. Soft texture was perceived for recipes with the lower DMR, although no differences in texture were detected by a texturometer. Lowering DMR increased ash and water content and decreased protein content, as expected. The results indicated that 100 g of burger provided more essential fatty acids than the recommended daily intake, irrespective of formulation. In conclusion, multiple factor analysis indicated that the main changes detected and perceived were due to DMR.

Keywords: European sea bass; rainbow trout; descriptive analysis; texture; essential fatty acids.

Introduction

Awareness of the food we eat and sustainable consumption are topical subjects. The food industry and consumers are increasingly concerned about sustainable production, health and wellness. The industry, for example, has been trying to reduce food wastage, a significant contributor to unsustainability, while consumers are demanding healthy minimally processed food. As underlined by Balasubramaniam et al. (2016), consumers often read food labels to check whether the ingredients on the label can be found in their own kitchens. If so, they are prepared to purchase the product. The movement away from artificial chemical ingredients is known in the industry as 'clean label' (Saltmarsh, 2014).

Parallel to this movement, health claims are gaining importance in western countries. They refer to a food's ability to prevent, manage or treat illness (Martirosyan & Singh, 2015) through known or unknown functional molecules that modulate one or more metabolic processes or pathways in the body. For example, the long-chain omega-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (C20:5ω3, EPA) and docosahexaenoic acid (C22:6ω3, DHA), can reduce the risk of cardiovascular disease, improve mental and visual functions, and are involved in inflammatory responses (Abuajah et al., 2014; Hong et al., 2005; Merched et al., 2008). They may also theoretically lead to a decrease in body fat over time and reduce obesity risk (Wildman, 2016).

Fish are an important source of EPA and DHA and can therefore be regarded as natural functional food. Fish also contain high-quality protein and essential micronutrients for humans. However, much precious food is lost in the production chain due to processing-related fish waste (up to 55% of the fish body is typically inedible) and the fact that damaged and non-commercial sized specimens are discarded.

The literature indicates that such fish waste can be viable, sustainable, easy-to-prepare and nutritious food (Palmeira et al., 2016). Mechanical separation process has been successfully applied to the major aquaculture species on the European market in the case of specimens of that cannot be marketed directly due to damage or size (Borgogno et al., 2017b; Secci et al., 2016). The authors found that due to its fatty acid composition, mechanically separated meat (MSM) of European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss*) was a suitable raw material for high quality fish-based products.

The present study was inspired by consumer demand for healthy clean label products, the need to enhance the value of discarded fish and the desire to provide new opportunities for the fishery/aquaculture sector. We focused on testing four "clean label" formulations for fish burgers made using mechanically separated fish. The sensory, textural and chemical properties of the four formulations were assessed.

Material and Methods

Fish burgers

Product recipe development

Fish burgers were developed according to three main ideas that emerged from a preliminary Focus Group conducted with Italian consumers (Secci et al., 2017): fish burgers should be based on a mixture of fresh mechanically separated fish, having a texture resembling minced meat, not meat emulsion, with very few other natural ingredients. We therefore prepared fish burgers from the following materials chosen for their functional properties:

- fresh mechanically separated fish of European sea bass (*Dicentrarchus labrax*) and pigmented rainbow trout (*Oncorhynchus mykiss*) as protein and lipid sources;
- rehydrated potato flakes as starch providing texture;
- clear lemon juice as natural source of the antioxidants ascorbic and citric acid;
- sodium chloride as flavour enhancer.

Moisture content (MC), fish to potato ratio (dry matter ratio, DMR) and mechanically separated fish ratio (MSMR) between pigmented rainbow trout and sea bass were chosen as operating parameters to set up the recipes. Moisture content was set at 72 g 100 g-1 to make the mixture workable. The DMR was decided on the basis of burger taste: the higher the DMR, the greater the fish taste and the less the potato taste and vice versa. Pigmented rainbow trout and sea bass were chosen because they are species of interest in European aquaculture, good sources of PUFAω3 and PUFAω6, and less vulnerable to mechanical separation than other previously tested species (Secci et al., 2016).

Four fish burger recipes (R1, R2, R3, R4) were set up with constant moisture, sodium chloride and lemon juice contents, two values of DMR (fish:potato, 2.5:1 and 1.5:1), and two values of MSMR (sea bass:trout, 50:50 and 30:70). These ratios were chosen after preliminary optimisation based on the workability and appearance of the mixture. The complete composition of the four recipes is reported in the supplementary material (Table S1). A mass balance was used to weigh the amounts of the ingredients according to an original spread sheet (shown as Supplementary material Table S2) which can solve the problem of the usual moisture variations of fish and potato flakes (and any other ingredient chosen by the industry) and should make the experiment more reproducible.

Mechanically separated fish and burger preparation

A total quantity of 18.5 kg of rainbow trout (average individual weight 450 ± 50 g) was purchased from a farm in northern Tuscany (Lucca, Italy) and 15.5 kg of sea bass (*Dicentrarchus labrax*, average individual weight 550 ± 30 g) was purchased from a local farm near Orbetello (Grosseto, Italy). The fish were processed on the premises of a fish-processing company (Grosseto, Italy) where they were

gutted and headed before being fed manually into a soft separator (Baader 601, Lübeck, Germany). In a one-step process, the fish carcasses were pressed (level 2.5 machine setting) by the conveyer belt onto the surface of the perforated drum (5 mm hole diameter). Mechanically separated meat passed through the holes, while bones, skin and thicker layers of connective tissue remained on the outside of the drum and were ejected through a discharge chute. Without washing or centrifuging, the MSM was used for burger preparation.

Potato flakes were puréed with cold water in a semi-automatic kitchen mixer for 10 s, then MSM of the two species was added and mixed for other 20 s. Five seconds before the end of the mixing time, lemon juice and salt were added without stopping the mixer. The mixture was divided into 100 g portions which were pressed manually in a burger-press (La Pressella Rigamonti, Lecco, Italy). Each burger was wrapped individually in cellophane. A total of 120 burgers were produced and frozen at -80°C until sensory, physical and chemical analysis on raw and cooked (oven-baked at 180°C for 35 min, core temperature 80°C) burgers. Three replicates per recipe (i.e. 12 burgers) were set aside for physical and chemical analysis of raw and cooked samples. The other 96 burgers were used for sensory evaluation.

Sensory evaluation

Descriptive Analysis

Ten subjects (5 males and 5 females, mean age 31 years) were recruited as panellists. They were regular fish consumers, had no history of disorders of oral perception and were paid to take part in the study. Written informed consent was obtained from each after the experiment had been described to them.

The burgers, each consisting of a 25 g portion served at 50 °C, were used for training and evaluation sessions. Panellists participated in three training sessions of about 60 minutes each. The subjects developed a vocabulary describing differences and similarities between experimental samples in two different sessions, according to a simplified version of the repertory grid method. A main list of 15 attributes was developed (Table 1) which described aroma (ortho-nasal odour), texture, taste and flavour (retro-nasal odour) of burgers. A nine-point scale (1-9 from extremely weak to extremely strong, respectively) was used for intensity ratings. Assessors and panel performance were validated by evaluating two repetitions of a subset of three samples. Data was analysed by multiblock PCA (Tucker-1) and P/MSE plot to assess panel calibration and assessor performance, respectively, using Panel Check software (ver. 1.4.0, Nofima, Trømso, Norway).

Table 1. List of the attributes describing fish burger sensory properties and their relative significance

Attribute	*P*
Texture (TxS)	
Crusty	< 0.05
Soft	< 0.05
Doughy	NS
Dry	< 0.05
Sandy	< 0.05
Fatty	< 0.05
Taste	
Salty	< 0.05
Umami	NS
Aroma/Flavour (A&F)	
Fish	< 0.05
Backed fish	< 0.05
Fatty	< 0.05
Starch	< 0.05
Vegetable broth	NS
Overall aroma	NS
Overall flavour	< 0.05

The evaluation of samples of each recipe was replicated three times in two sessions. In each session, each panellist evaluated six samples identified by a three-digit code. Samples were presented singly and presentation order was randomized between subjects and sessions. The order of attributes was randomized between subjects for each sensory mode, the attributes "overall aroma" and "overall flavour" were always at the end of the corresponding list.

Subjects were asked to evaluate aroma, then to take a first bite to evaluate taste and flavour and a second bite for texture evaluation. After evaluation of each sample, subjects rinsed their mouths with water for 30 s, ate plain crackers for 30 s and rinsed their mouths a second time with water for a further 30 s. They took a 15 min break after every three samples. Data was collected with the software Fizz (ver. 2.47.B, Biosystemes, Couternon, France).

Laboratory analysis of texture and of proximate and fatty acid compositions

Texture measurements were performed using a Zwick Roell® texturometer model KAF-TC 0901279 (Zwick GmbH & Co. KG, Ulm, Germany) equipped with a 1 KN load cell and a blade probe. Shear

force was determined on the middle part of the burger. Data was collected and analysed by Test-Xpert2 of the Zwick Roell® software version 3.0.

Moisture, crude protein and ash contents were determined (AOAC, 2012) in raw and cooked burgers. The total lipid content of the samples was determined according to Folch et al. (1957) and fatty acid composition was determined by gas chromatography (GC) using a Varian GC 430 instrument (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), as described in Secci et al. (2016). Tricosanoic acid (C23:0) (Supelco, Bellefonte, PA, USA) was used as internal standard for fatty acid (FA) quantification through calibration curves (standard Supelco 37 component FAME mix; Supelco, Bellefonte, PA, USA). Proximate composition and fatty acid profiles of MSM of sea bass and rainbow trout are published in Borgogno et al. (2017b).

Statistical analysis

Intensity data from the trained panel was analysed by multi-block PCA (Tucker-1) and by P*MSE plot (Panel Check software, ver. 1.4.0, Nofima, Norway) to assess panel calibration and assessor performance, respectively. The Tucker-1 plots did not detect any cases of disagreement between panellists for any attribute. P*MSE plots indicated that all subjects were reliable in terms of product differentiation ability and consistency across replicates. All panellists were therefore retained for data analysis.

Intensity ratings were analysed independently by a two-way ANOVA mixed model (sample as fixed and assessors as random factors), followed by a Fisher LSD post hoc test (significant for $P \leq 0.05$). PCA was computed on panel averages of each significant attribute arising from the ANOVA models using The UnscramblerX 10.3 software (Norway). Full cross validation was computed to validate interpretation of the first two components.

Data related to texture and chemical characteristics was processed by two-way ANOVA using PROC GLM of SAS statistical software (SAS, 2004), where the effects considered were the species of fish ratio (MSMR, two levels: 50:50 and 30:70 sea bass to rainbow trout, respectively) and fish to potato ratio (DMR, two levels: 2.5:1 and 1.5:1 fish to potato, respectively). The interaction MSMR × DMR was also included in the statistical model. Comparison of means was performed by Tukey's test (SAS, 2004) at $P < 0.05$.

Multiple Factor Analysis (Escofier & Pagès, 1994) was applied to the sensory and laboratory data set (five data blocks: sensory aroma&flavour, sensory texture, saturated fatty acids, polyunsaturated fatty acids, laboratory determined texture). Product spaces and correlation plots were constructed to

visualise sample differences and/or similarities according to sensory, chemical and physical characteristics.

Results and Discussion

Sensory Evaluation

Overall, sensory assessment indicated that potato content can significantly modify the perceived profile of fish burgers, while the sea bass to trout ratio only marginally affected sensory properties. Indeed, the PCA bi-plot (Figure 1) showed that samples were mainly discriminated along the first component (PC1: 73% explained variance) in relation to DMR. Recipes with the highest DMR (R1 and R3) clustered on the right side of the map, while those with the lowest DMR (R2 and R4) clustered on the left. Texture varied in relation to DMR: R1 and R3 were positively correlated with sandy, dry and crusty attributes, while soft texture characterized R2 and R4 samples related to their higher potato flake content. Potato flakes are composed of starch, frequently used as a binding agent in meat preparations (Totosaus, 2009). It is widely accepted that sensory profile has a key role in consumer acceptance and favour. In this sense, recent studies have shown that texture attributes are clear hedonic drivers of fresh fish and fish-derived products (Alexi et al., 2018; de Quadros et al., 2015). Specifically, juiciness and crumbliness/crunchiness seem to increase consumer hedonic response to different fish species (Alexi et al., 2018) and Serra Spanish Mackerel burgers (Scomberomorus brasiliensis; de Quadros et al., 2015). On the other hand, attributes like firmness and tenderness do not generate a single response among consumers (Alexi et al., 2018; de Quadros et al., 2015), probably due to specific attitudes of consumers towards different products, including familiarity.

The high proportion of fish in the R1 and R3 recipes was positively associated with salty taste, higher overall flavour and “fish” notes (typical aroma and flavour of fresh/raw fish). On the contrary, “baked fish” (typical aroma and flavour of fish cooked in the oven) and “starch” (typical aroma and flavour of boiled starchy foods such as rice and potatoes) were the main descriptors of R4 sample flavour. As found for texture, salty taste and fish flavour are other proven drivers of liking for fresh fish and ready to cook fish products (Alexi et al., 2018; de Quadros et al., 2015). However, when considering tilapia burgers, Ali et al. (2017) showed that the overall acceptability increased significantly ($P < 0.05$) up to 20% of mashed potato. Considering the present results, it is possible to say that not only can the four recipes be divided into two groups on the basis of fish to potato ratio, but they are also characterized by sensory attributes which are mainly linked to hedonic response. Hence, R1 and R3 as well as R2 and R4 could be baselines for the development of products directed at different consumer target groups.

Finally, the variation in sea bass to trout ratio only marginally influenced burger sensory properties along the second component (15% of explained variance). This fact could be of economic and other interest for the fish industry: firstly, a high ratio of potato flakes did not induce negative attributes, so recipes can be developed starting with 1.5:1 DMR; secondly, the prevalence (70%) of rainbow trout over sea bass (30%) only weakly affected burger sensory profile. Higher proportions of relatively economical ingredients as potato and trout (cheaper than sea bass) are therefore feasible.

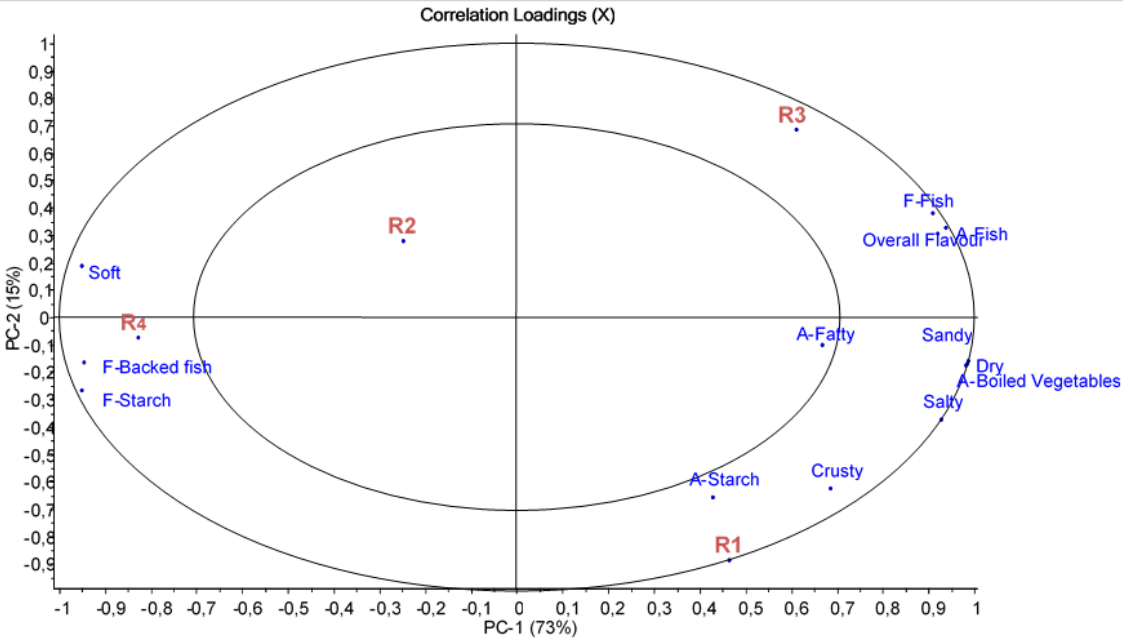

Figure 1. Principal Component Analysis of attributes significantly discriminating the four recipes (R1: sea bass:rainbow trout 50:50, fish:potato 2.5:1; R2; sea bass:rainbow trout 50:50, fish:potato 1.5:1; R3: sea bass:rainbow trout 30:70, fish:potato 2.5:1; R4: sea bass:rainbow trout 30:70, fish:potato 1.5:1) (correlation loading plot). F: flavour; A: aroma.

Laboratory analysis

Shear stress was not significantly affected by different percentages of the two fish species or of potato flakes in raw (mean value 8.09 ± 0.05 N) and cooked fish burgers (mean value 9.73 ± 0.32 N). Potato starch is commonly used as an emulsifier in meat products because it boosts the gel strength of protein gel matrix structure (Aktaş & Gençcelep, 2006) determining an increase in shear force (Bushway et al., 1982), although its percentage seems to have different effects on the texture of the mixture to which it is added. Many authors report that starch is more effective in increasing matrix gel strength when its concentration is less than 3% (Yoon et al. 1997; Zhang et al., 2013). In addition, Ali et al. (2017) showed that tilapia burgers with 10% or 15% (w/w) of potato starch did not differ in hardness. The present results are in line with those of Ali et al. (2017) since dry potato flakes in recipes with DMRs of 1.5:1 and 2.5:1 were 11.6% and 8.3% (w/w), respectively, and therefore similar to those tested by these authors.

Table 2 shows the results of proximate analysis of raw and cooked burger samples. Among the various constituents, significant differences were found for crude protein and ash content, which were only affected by DMR. Recipes with more potato and less fish (DMR 1.5:1) had significantly lower values of crude protein and ash than those with a higher proportion of fish. In line with this, Ali et al. (2017) found that addition of carbohydrate-rich ingredients obviously decreased the protein content of tilapia burgers. Total lipid content did not differ significantly with changes in fish species ratio or DMR. Addition of 10, 15 or 20% mashed potato did not significantly modify the crude fat content of tilapia burgers (Ali et al., 2017).

Concerning cooked burger composition (Table 2), water content was significantly higher in the recipes with DMR 1.5:1 than in those with DMR 2.5:1. This may be attributed to the high capacity of potato starch to retain water, in comparison with other types of flour used in cooked meatballs from mechanically deboned quail meat (Ikhlas et al., 2011). The authors found that potato starch had the second highest moisture retention capacity at 64.67%, exceeded only by cassava flour (64.99%). Crude protein was significantly higher in DMR 2.5:1 burgers than in DMR 1.5:1, due to the paucity of potato protein. Moreover, ash and total lipid content did not show any significant differences among the recipes, as illustrated in Table 2.

Figure 2 is a biplot of texture and proximate analysis results for cooked burgers.

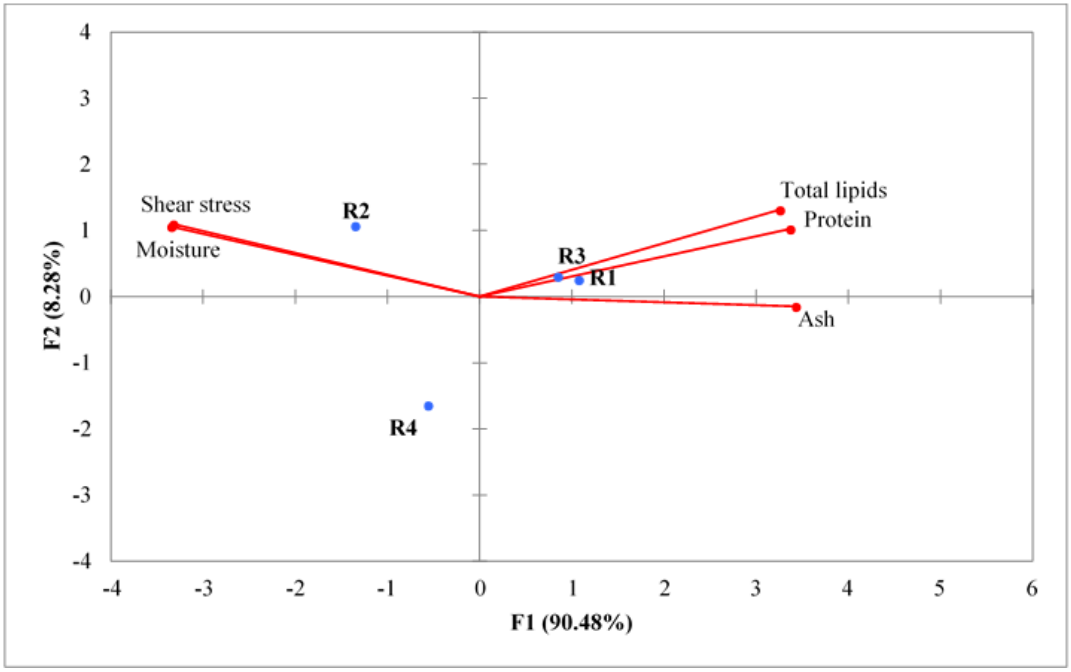

Figure 2. Biplot (F1 and F2 axes: 98.76%) of laboratory analyses conducted on cooked burgers made with the four recipes (R1: sea bass:rainbow trout 50:50, fish:potato 2.5:1; R2: sea bass:rainbow trout 50:50, fish:potato 1.5:1; R3: sea bass:rainbow trout 30:70, fish:potato 2.5:1; R4: sea bass:rainbow trout 30:70, fish:potato 1.5:1).

As found for the sensory data, the four recipes were separated along the first component according to DMR. Samples with the higher fish content (R1 and R3) associated positively with total lipids, protein and ash content, whereas R2 and R4 clustered on the opposite side of the map, showing a positive association with moisture content. Interestingly, the positions of R2 and R4 indicated a positive association of potato flake content with shear stress due to the composite reinforcing effect of starch in the meat gels, whereby absorption of water embedded in the protein gel matrix by starch granules tends to compress the matrix as the starch swells during cooking, thus resulting in a more compact product (Tee & Siow, 2017).

Table 2 shows the fatty acid composition (g 100 g^{-1} of total fatty acids) of differently formulated raw fish burgers.

Only a few differences emerge, despite the different fish species ratios and DMRs. Specifically, the recipes containing more trout (MSMR 30:70) were significantly poorer in certain MUFA (C16:1ω7, C20:1ω9) which however did not reduce the overall MUFA content.

No differences emerged in the fatty acid composition of cooked burgers (Table 2), apart from C18:3ω3, which was lower in 30:70 than in 50:50 burgers. The overall fatty acid profile of these products was good: SFA did not exceed 16 g 100 g^{-1} of total fatty acid and the MUFA fraction was mainly composed of oleic acid (C18:1ω9), the nutritional importance of which has been highlighted in relation to its role in controlling the processes responsible for development of colorectal cancer (Llor et al., 2003). Furthermore, the present results revealed that a portion (100 g) of all four recipes contained around 0.400 g of EPA+DHA. According to FAO/WHO (2008), the recommended daily intake of EPA+DHA for adult males and non-pregnant/non-lactating adult females is 0.250 g/day, which rises to 0.300 g/day for pregnant and lactating women. One burger (100 g) obtained with any of the MSM-based recipes tested therefore provides more than the suggested daily intake of EPA+DHA.

Table 2. Moisture, crude protein, ash, total lipid (g 100 g^{-1} of burger) and fatty acid composition (g 100 g^{-1} total fatty acid) of raw and cooked fish burgers distinguished by the ratios of the two fish species used (sea bass:rainbow trout 50:50 and 30:70) and fish to potato ratios (2.5:1 and 1.5:1).

	Sea bass:R.trout (MSMR)		Fish:Potato (DMR)		MSMR	DMR	RMSE[1]
	50:50	30:70	2.5:1	1.5:1	Significance		
Raw							
Moisture	73.17	72.72	72.46	73.44	NS	NS	0.75
Crude protein	12.54	12.65	13.62	11.57	NS	< 0.001	0.27
Ash	1.83	1.88	1.93	1.79	NS	< 0.01	0.07
Total lipids	4.58	4.25	4.07	4.13	NS	NS	1.21
C14:0	1.53	1.44	1.47	1.50	NS	NS	0.064
C16:0	10.27	9.99	10.06	10.20	NS	NS	0.168
C16:1ω7	2.42	2.29	2.34	2.37	< 0.05	NS	0.064
C18:0	2.92	2.95	2.92	2.95	NS	NS	0.087
C18:1ω9	30.10	30.08	30.23	29.94	NS	NS	0.334
C18:1ω7	2.30	2.28	2.30	2.29	NS	NS	0.022
C18:2ω6	15.44	15.52	15.49	15.47	NS	NS	0.196
C18:3ω3	4.14	4.08	4.09	4.13	NS	NS	0.053
C20:1ω9	2.28	2.16	2.20	2.23	< 0.01	NS	0.044
C20:5ω3 (EPA)	4.70	4.43	4.49	4.64	NS	NS	0.235
C22:5ω3	2.46	2.35	2.38	2.44	NS	NS	0.065
C22:6ω3 (DHA)	12.87	13.90	13.49	13.28	< 0.01	NS	0.374
ΣSFA	15.60	15.23	15.31	15.53	NS	NS	0.234
ΣMUFA	39.43	38.86	39.29	38.99	NS	NS	0.335
ΣPUFAω3	25.80	26.40	26.08	26.12	NS	NS	0.458
ΣPUFAω6	18.40	18.68	18.59	18.48	NS	NS	0.266
Cooked							
Moisture	67.76	67.26	66.95	68.08	NS	< 0.01	0.49
Crude protein	15.15	14.98	16.40	13.73	NS	< 0.001	0.48
Ash	2.40	2.41	2.52	2.29	NS	NS	0.19
Total lipids	4.37	4.34	4.68	4.02	NS	NS	0.85
C14:0	1.58	1.45	1.58	1.45	NS	NS	0.133
C16:0	10.36	10.09	10.38	10.07	NS	NS	0.479
C16:1ω7	2.43	2.27	2.39	2.31	NS	NS	0.103
C18:0	2.91	3.03	2.97	2.97	NS	NS	0.089
C18:1ω9	30.26	30.62	30.63	30.26	NS	NS	0.282
C18:1ω7	2.30	2.28	2.30	2.28	NS	NS	0.026
C18:2ω6	15.59	15.92	15.76	15.75	NS	NS	0.239
C18:3ω3	4.17	4.08	4.10	4.15	< 0.01	NS	0.042
C20:1ω9	2.22	2.05	2.11	2.17	NS	NS	0.112
C20:5ω3 (EPA)	4.55	3.98	4.16	4.38	NS	NS	0.300
C22:5ω3	2.39	2.15	2.18	2.36	NS	NS	0.165
C22:6ω3 (DHA)	12.72	13.55	12.96	13.32	NS	NS	0.434
ΣSFA	15.73	15.43	15.80	15.36	NS	NS	0.685
ΣMUFA	39.49	39.33	39.59	39.22	NS	NS	0.246
ΣPUFAω3	25.46	25.38	25.00	25.85	NS	NS	0.814
ΣPUFAω6	18.56	19.17	18.88	18.85	NS	NS	0.292

The percentages of fatty acids C12:0, C13:0, C14:1, C15:0, C16:2ω4, C17:0, C16:3ω4, C17:1, C16:4ω1, C16:1ω9, C18:2ω4, C18:3ω6, C18:3ω4, C18:4ω3, C18:4ω1, C20:0, C20:1ω11, C20:1ω7, C20:2ω6, C20:3ω6, C20:3ω3, C20:4ω3, C20:4ω6, C22:0, C22:1ω11, C22:1ω9, C22:1ω7, C22:2ω6, C21:5ω3, C24:4ω6, C22:5ω6, C24:0, present at a level lower than 1.5 g 100 g-1 of total FAs were utilized to calculate Σ of the lipid fractions, but were not reported in the table. [1] RMSE: Root Mean Square Error. NS: Not Significant.

Differences according to sensory and laboratory characterization

Multiple factor analysis provides useful information on the main associations between the different groups of variables and was successfully used to explore the contribution of the sensory and laboratory data sets for food sample characterization (Morvan et al., 2003; Valente et al., 2011). It was carried out on the combined data, namely intensity of sensory attributes describing aroma and flavour, intensity of sensory descriptors of texture, PUFA and SFA concentrations and laboratory descriptors of texture. Figure 3 summarizes differences and similarities across samples. The four recipes were distributed along the first component (F1) in relation to DMR. RV coefficients, reported in Table 3, indicated a shared sample configuration across sensory data (aroma&flavour, A&F, and texture, TxS), and fatty acid categories. Borgogno et al. (2017a) recently showed that SFAs positively correlated with metallic aroma/flavour, overall flavour and tenderness of boiled rainbow trout (Oncorhynchus mykiss) and that MUFAs correlated positively with boiled fish flavour and overall aroma, thus confirming the relations found in the present trial. In addition, sample discrimination according to sensory texture (TxS) had a low RV coefficient with respect to laboratory determined texture (TxI) which means that the two parameters did not discriminate samples in the same way. This fact agreed with Ali et al. (2017) who found a discrepancy between measured textural parameters, such as hardness, and the texture sensory descriptors in tilapia burgers containing different percentages of mashed potato (10% and 15%). Specifically, laboratory analysis did not reveal any difference in hardness of burgers while panellists were able to discriminate samples on the basis of texture sensory properties.

Table 3. RV coefficients obtained from Multifactorial Analysis of sensory and instrumental datasets.

	A&F	**TxS**	**TxI**	**SFA**	**PUFA**	**MUFA**
A&F	1.000	0.814	0.655	0.768	0.320	0.853
TxS	0.814	1.000	0.571	0.913	0.322	0.870
TxI	0.655	0.571	1.000	0.635	0.481	0.801
SFA	0.768	0.913	0.635	1.000	0.662	0.959
PUFA	0.320	0.322	0.481	0.662	1.000	0.675
MUFA	0.853	0.870	0.801	0.959	0.675	1.000

A&F: Aroma and Flavour; TxS: Texture from Sensory analysis; TxI: Texture from Instrumental analysis; SFA: Saturated Fatty Acid; PUFA: Polyunsaturated Fatty Acid; MUFA: Monounsaturated Fatty Acid.

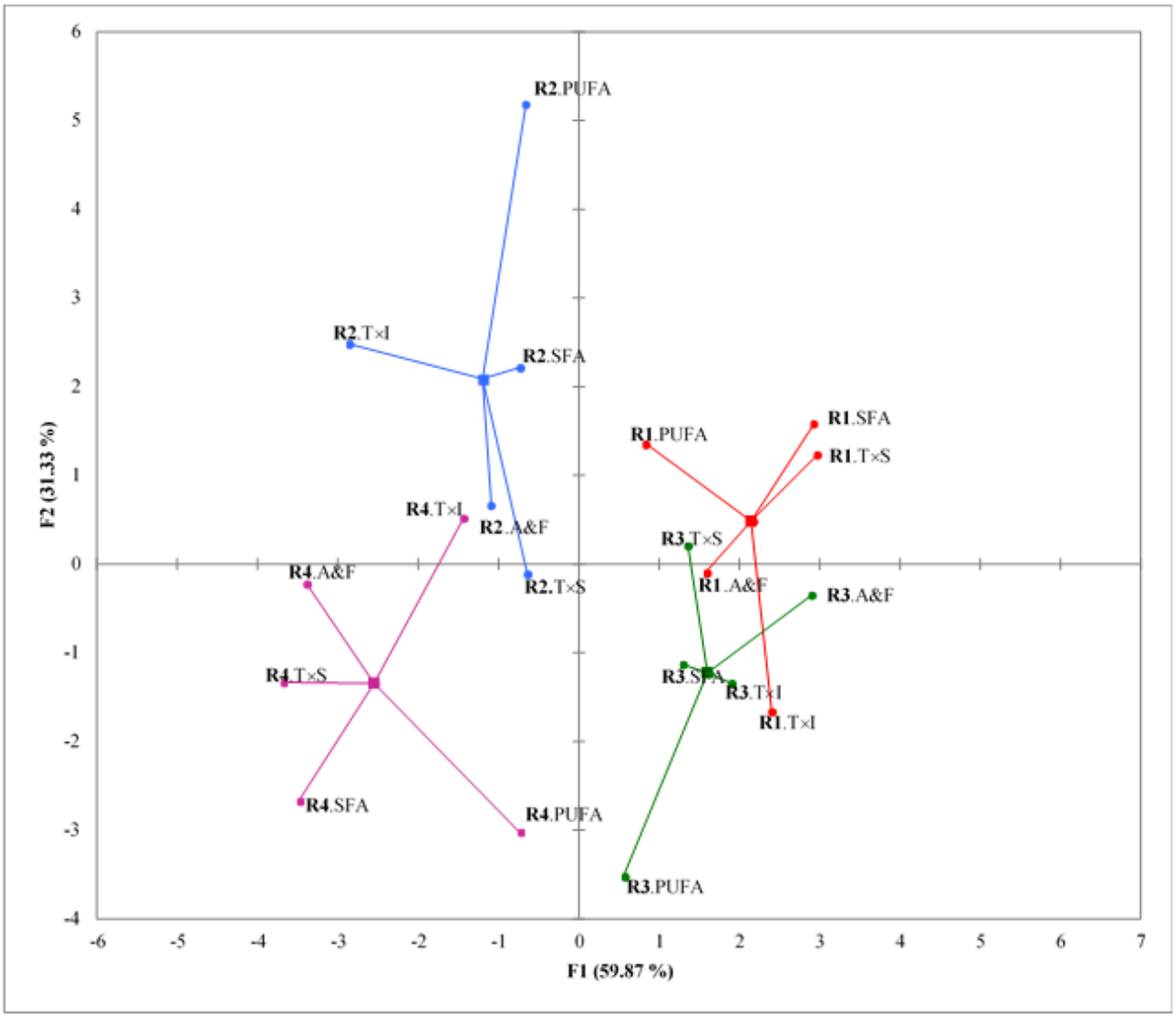

Figure 3. Multiple factor analysis (F1 and F2 axes: 91.20%) of sensory and laboratory data obtained for cooked burgers made with the four recipes (R1: sea bass:rainbow trout 50:50, fish:potato 2.5:1; R2: sea bass:rainbow trout 50:50, fish:potato 1.5:1; R3: sea bass:rainbow trout 30:70, fish:potato 2.5:1; R4: sea bass:rainbow trout 30:70, fish:potato 1.5:1).

Conclusion

Enhanced use of normally discarded fish through the development of fish burgers based on four clean label formulations of high nutritional value was achieved in this study with a mixture of fresh mechanically separated fish meat. The ready-to-cook products, thus achieved, prevent waste of not directly marketable specimens through recipes containing only simple natural ingredients. The four formulations used gave rise to products with different characteristics in terms of sensory properties and protein content, while high nutritional value was guaranteed by their fatty acid composition. The main changes detected and perceived seemed to be due to the fish to potato ratio, as underlined by

multiple factor analysis, while the prevalence of rainbow trout over the more expensive sea bass did not substantially modify burger characteristics. This finding is certainly of interest to the fish industry as it reduces production costs. In addition, these results could help optimize fish burger formulations to match the tastes and expectations of different consumer groups. Indeed, consumers less familiar with fish (such as children) would prefer the formulation higher in potato content, with its softer texture and more delicate flavour. On the other hand, consumers who like fish would prefer the formulation higher in fish content and characterized by fresh/raw fish olfactory notes. Further research on shelf life and consumers studies are suggested.

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SUPPLEMENTARY MATERIAL

Table S1. Fish burger recipes; *MC* = moisture content, *DMR* = dry matter ratio between fish and potato, *MSMR* = mechanical separated meat ratio between pigmented rainbow trout and European sea bass.

Fish burger code	***MC*** **(g 100 g^{-1} fish burger)**	***DMR*** **(g dry matter from fish g^{-1} dry matter from potato)**	***MSMR*** **(% of pigmented rainbow trout/ % of European sea bass)**	**Total fresh fish meat (g 100 g^{-1} fish burger)**	**Potato flakes (g 100 g^{-1} fish burger)**	**Added water for potato flakes rehydration (g 100 g^{-1} fish burger)**	**Sodium chloride (g 100 g^{-1} fish burger)**	**Lemon juice (g 100 g^{-1} fish burger)**
R1	72	2.5	50:50	71.75	8.31	18.11	0.83	1.00
R2	72	1.5	50:50	60.27	11.64	26.26	0.83	1.00
R3	72	2.5	70:30	75.25	8.31	14.60	0.83	1.00
R4	72	1.5	70:30	63.21	11.64	23.32	0.83	1.00

SUPPLEMENTARY MATERIAL

Table S2. Mass balance spreadsheet of fish burger recipes.

An original Excel spreadsheet was set up to determine the amount of fish burger ingredients by a mass balance. The following system of equations in three variables was written (assuming that the lemon juice and the sodium chloride were only taken into consideration as water and dry matter amounts, respectively):

$$F + P + W + L + S = B \quad [1]$$

$$F \cdot x_{wF} + P \cdot x_{wP} + W + L = B \cdot x_{wB} \quad [2]$$

$$\frac{F \cdot x_{dmF}}{P \cdot x_{dmP}} = DMR \quad [3]$$

where:

F = fish mixture mass (g)

P = potato flake mass (g)

W = water mass to rehydrate potato flakes (g)

L = lemon juice mass (g)

S = sodium chloride mass (g)

B = fish burger mass (g)

x_w = water mass fraction of F or P

MASS BALANCE TO DETERMINE FISH BURGER RECIPES

DATA IN

	Fish moisture (%)	*MSMR*
Trout	76.1	70
Sea bass	69.8	30

Fish burger mass (g)	Fish mixture moisture (%)	Potato flakes moisture (%)	*MC*	*DMR*	Fish mass fraction	Potato flakes mass fraction	Sodium chloride		Lemon juice	
350	74.21	6.64	72	1.5	0.258	0.934	0.83	g/100 g	1	g/100 g
							2.905	g	3.5	g

DATA OUT

Potato flakes mass (g)	40.7					
Fish mixture mass (g)	221.2	*of which:*	Trout mass (g)	154.9	Sea bass mass (g)	66.4
Water for potato flakes rehydratation (g)	81.6					

MASS BALANCE VALIDATION

Fish burger mass (g)	350.0
Fish burger moisture (%)	72.0

Paper 3

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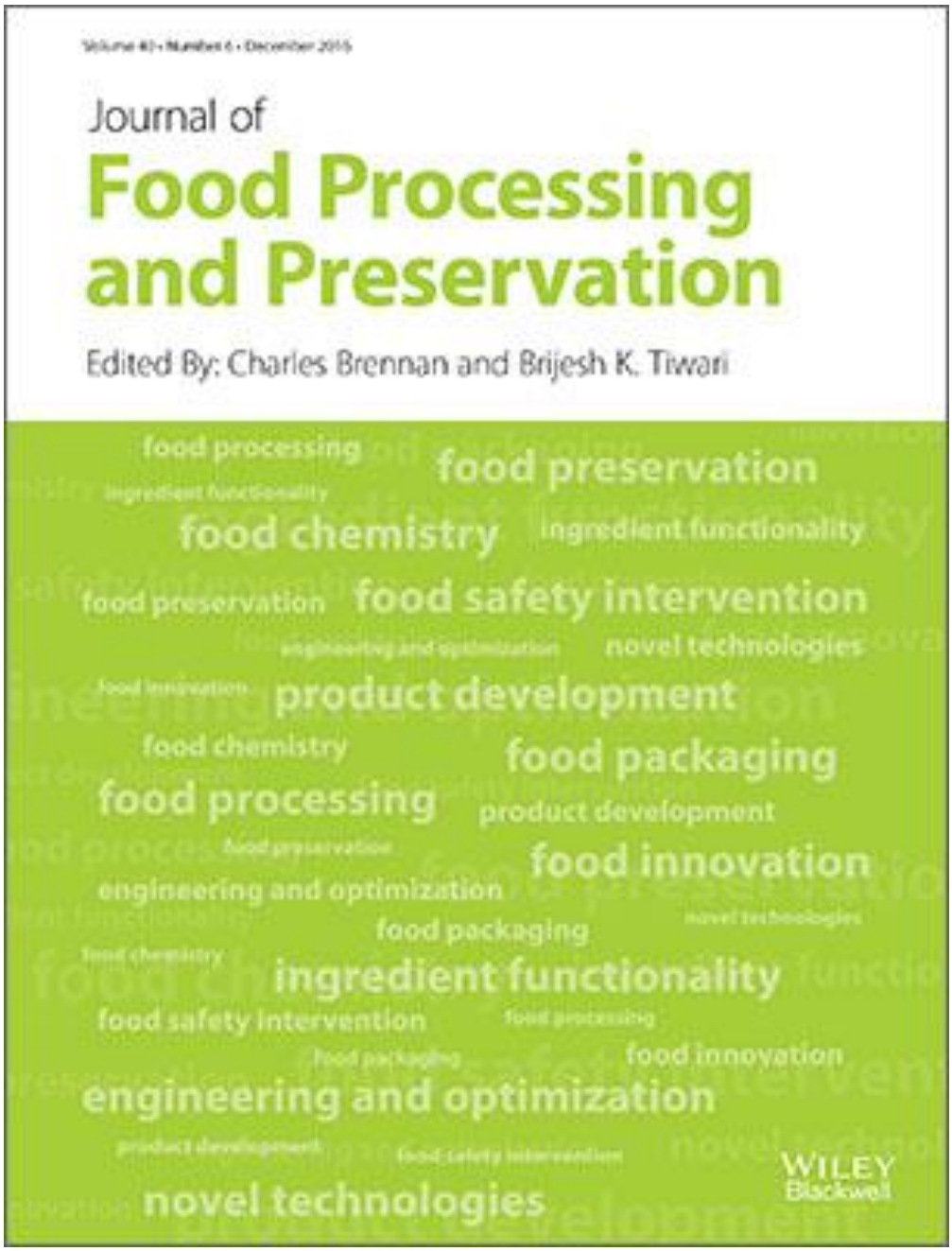
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Physical, chemical and oxidative stability of clean label burger based on mechanically separated fish as affected by formulation and frozen storage

RUNNING TITLE: Frozen clean label fish burger quality

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Abstract

Two clean label burgers were formulated based on different ratios of mechanically separated fish (European sea bass to rainbow trout: 50:50 and 30:70, R1 and R2), and on few simple ingredients. Their physical (shear force, pH, color and water holding capacity), chemical (proximate and fatty acid composition) and oxidative stability was assessed during 90 days of frozen storage (-18^{o}C) on raw and cooked samples. The shear force, b* and Chroma of raw R2 were higher than R1 ones (P < 0.05), while a* index, found -1.49 and -2.03 in raw R1 and R2, respectively. Cooked samples were similarly affected by the formulation. The proximate and fatty acid compositions of both raw and

cooked burgers were slight affected by the recipe. The R2 showed a greater ($P < 0.05$) oxidative stability of R1 at the end of the storage. Overall, the recipe R2, with more trout, better preserved its physic-chemical and oxidative status than R1.

Keywords: European sea bass, rainbow trout, baking, PUFA, antioxidant capacity.

Practical application

The study showed the feasibility to produce clean label fish burger starting from not directly marketable species through the process of mechanical separation. Few and simple ingredients such as lemon, salt and potato flakes were utilized for the preparation of high nutritional quality fish burger which were here demonstrated stable during frozen storage. The fact that the highest stability was obtained in fish burger containing a high proportion of rainbow trout could be of interest for seafood industry due to the lower economical value of this species than sea bass.

Introduction

Population growth and the growing concerns over healthy food habits in developed countries have induced an increase in the fish demand (Food and Agriculture Organization, FAO, 2016) which has made the world fish production grow in the last five decades. Fishery products accounted for one percent of all global merchandise trade in value terms, representing more than nine percent of total agricultural exports. Worldwide exports amounted to USD 148 billion in 2014, up from USD 8 billion in 1976. Developing countries were the source of USD 80 billion of fishery exports, providing higher net trade revenues than meat, tobacco, rice and sugar combined (FAO, 2016).

Consumer health awareness has boosted growing demand for fortified and rich foods in recent decades such as fruit juice that is fortified with vitamin C, eggs enriched with omega-3 fatty acids, and yogurt milk enriched with probiotic culture (Vicentini, Liberatore, & Mastrocola, 2016). Fish and

fishery products naturally represent very valuable sources of protein and essential micronutrients for balanced nutrition and good health. Regarding lipid fraction, fish represents a significant source of polyunsaturated fatty acids (PUFAs), especially the eicosapentaenoic (EPA, C20:5ω3) and docosahexaenoic (DHA, C22:6ω3) acids. These two fatty acids are supplied solely by the diet (i.e., they cannot be synthesized by the human metabolism) and seem to be involved in the reduction of the risk factors associated with cardiovascular disease, hypertension, general inflammation, asthma, arthritis, psoriasis and various types of cancer (Calder & Yaqoob, 2009; Hooper et al., 2006).

Strategic initiatives to promote fish consumption, mainly among the young people, are represented by the ready-to-cook fish products, such as burgers, patties and fillets (Altieri, Speranza, Del Nobile, & Sinigaglia, 2005; Boskou & Debevere, 2000; Corbo et al., 2008; Gildberg, 2001; Mahmoud, Kawai, Yamazaki, Miyashita, & Suzuki, 2007; Poli, Messini, Parisi, Scappini, & Vigiani, 2006). These products are generally pre-fried and commercialized as frozen meeting the consumers need for food easy to store and prepared. Pre-fried fish products anyhow are characterized by a high level of fat and, as fried foods, they are not very easily digestible (Oke et al., 2018). The surface can be carbonized or burnt very easily, if the temperature is too high. Besides, the high temperature of the cooking may radically affect the characteristics and composition of food by enhancing the oxidation of polyunsaturated fraction of lipids, consequently causing a depletion of the fish nutritional value (Secci & Parisi, 2016). On the other hand, frozen fish storage products are commonly used because of their consistent, reliable quality, ease of transportation and the fact that they are very close to fresh equivalents (Bavitha, Dhanapal, Madhavan, Vidyasagar Reddy, & Sravani, 2016). However, the labile PUFA fraction of fish can be also altered by the processing and storage conditions (Taheri, Motallebi, Fazlara, Aghababyan, & Aftabsavar, 2012). Indeed, oxidative rancidity is a critical factor that limits the fish shelf life during storage (Dellarosa, Laghi, Martinsdóttir, Jónsdóttir, & Sveinsdóttir, 2015; Karlsdottir et al., 2014).

In this regard, Secci et al. (2016) have shown that despite mechanically separated meat (MSM) from fish could represent a good source of PUFAω3, the mechanical process might enhance lipid oxidation

both immediately after the process and during frozen storage. This fact must be considered while utilizing MSM as ingredient for new fish derived products as in those recently proposed by Husein et al. (2018). Thus, the objective of this study was to investigate how the nutritional quality of raw and oven-baked fish burger containing MSM from European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) could be affected by the formulation of burger recipe and the frozen storage.

Materials and methods

Fish burgers preparation

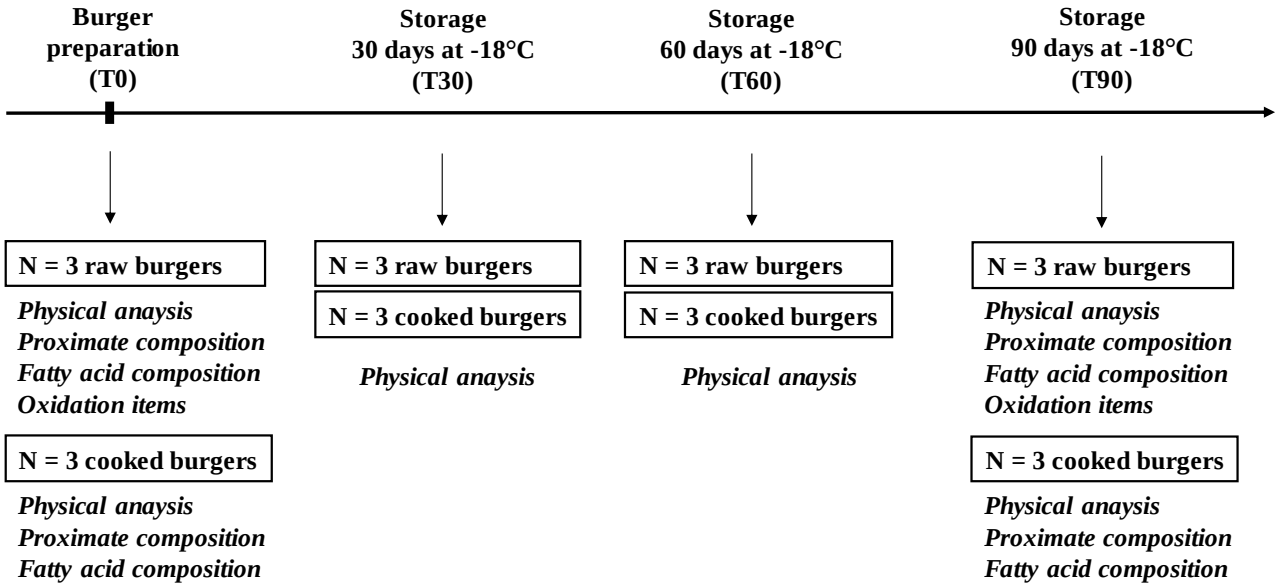
Two clean label recipes of fish burgers made with mechanically separated fish meat were formulated. The recipes differed in the ratio of European sea bass to rainbow trout (50:50, R1; 30:70, R2) and they were added with lemon, salt, water, and potato flakes to obtain products containing 72% of moisture (Table 1). Specimens of European sea bass were obtained from a fish farm located in Orbetello (Grosseto, Italy), while the pigmented rainbow trout were obtained from a farm located in the north west of Tuscany (Lucca, Italy). Fish were killed by percussion and immediately moved to the industry, where the fish were beheaded, degutted, and minced by the MSM machine Baader 60-1 (Lübeck, Germany). More details about the MS process and the formulation of the recipes can be retrieved in Husein et al. (2018). Overall, 5 and 3.5 kg of MSM from rainbow trout and sea bass were obtained. The ingredients were mixed by a kitchen mixer in the following order: potato flakes, water, MSM sea bass, MSM trout, salt, and lemon. Once ready, they were formed in burger pieces of 100.4 ± 0.5 g.

Table 1. Ingredients (g) of the two formulated recipes, R1 (ratio sea bass: rainbow trout 50:50) and R2 (ratio sea bass: rainbow trout 30:70).

Ingredients (g)	**R1**	**R2**
MSM sea bass	35.875	52.675
MSM trout	35.875	22.575
Potato flakes	8.31	8.31
Water	18.11	14.61
Lemon	1	1
Salt	0.83	0.83
Total	100.00	100.00

For each recipe, 24 replicates were prepared and subdued to the experimental design showed in Fig. 1. Six burgers for each treatment were immediately analyzed (three as raw and three as cooked). The cooking was performed in an oven at 180 °C for 35 min (core temperature: 72 °C) without fat or oil addition nor on the burgers surface or on their bottom. The other 18 samples were vacuum-packaged in plastic bags (Vacuum Pump S.p.A, Como, Italy), put overnight at -80 °C to obtain a fast freezing, and then stored at -18 °C for 90 days. Every 30 days (T30, T60 and T90), six samples (three as raw and three as cooked) for each recipe were subdued to the physical analyses (shear force, pH, color, water holding capacity-WHC). Proximate composition (moisture, crude protein, ash, and total lipids) and fatty acid composition were evaluated in six samples for each recipe (three as raw and three as cooked) immediately after burger's preparation and at the end of the storage time (T90). Oxidation items (primary and secondary oxidation products, and antioxidant capacity) were evaluated only in raw R1 and R2 burgers.

Figure 1. The experimental design of the trial.



Physical and chemical characterizations

Shear force, pH, color, WHC

A Zwick Roell® texturometer model KAF-TC 0901279 (Zwick GmbH & Co. KG, Ulm, Germany) equipped with a 1 kN load cell was utilized for shear force (N) determination by using a blade. Textural attribute was determined on the middle part of fish burger. Data were collected by the Test-Xpert2 by Zwick Roell® software version 3.0.

The pH values of the samples were measured by a Mettler Toledo pH-meter (Columbus, OH, USA) in three different points of the burger's diameter.

A Dr Lange Spectro-color® colorimeter (Keison Product, Chelmsford, UK) equipped with a Spectral qc 3.6 software was utilized for colorimetric measurement. The color values were measured in three points of the burgers and reported in the CIELab scale (CIE, 1976) as lightness (L*), redness and yellowness indexes (a* and b*, respectively), Chroma (C*) and color hue (H°).

The Water Holding Capacity (WHC), performed only on the raw burger, was determined according to Iaconisi, Bonelli, Pupino, Gai, and Parisi (2018). The WHC was calculated as the percentage of water loss after centrifugation (5 min at 210 × g) in relation to the water content of the sample. This

last value was obtained gravimetrically on 2 g of each minced sample by weighing the samples before and after 24 h at 105 °C. Two measurements for each sample were performed.

Proximate and fatty acid composition

Water content was determined using 2 g of sample by heating at 105 °C until constant weight (AOAC, 2012). Total nitrogen was determined by the Kjeldahl procedure (Kjeltec, 1035 Analyzer, Foss Tecator, Denmark) and converted to crude protein by multiplying by 6.25 (AOAC, 2012). Ash was determined as the remnant weight after calcination of a 5 g sample at 550 °C during 3 h (AOAC, 2012). The results were expressed as g/100 g product.

The total lipid content of the samples was determined according to Folch, Lees, and Sloane Stanley (1957) method and fatty acids (FA) were determined in the lipid extract after trans-esterification to methyl esters (FAME) using a base-catalyzed trans-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 (Supelco, Bellefonte, PA, USA). The condition of analysis (oven, injector, and detector temperatures as well as the carrier gas and the split ratio utilized) was set as mentioned in Secci et al. (2016). Chromatograms were recorded with the Galaxie Chromatography Data System 1.9.302.952 (Varian Inc., Palo Alto, CA, USA). Fatty acids were identified by comparing the FAME retention time with the ones of the standard Supelco 37 component FAME mix (Supelco, Bellefonte, PA, USA). Fatty acids were quantified through calibration curves, using tricosanoic acid (C23:0) (Supelco, Bellefonte, PA, USA) as internal standard.

Oxidative status

Primary and secondary oxidative products were quantified in raw burgers as conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS), respectively. CD content was measured by the

colorimetric method (Srinivasan, Xiong, & Decker, 1996) using hexane as solvent. Conjugated dienes were quantified in 0.5 µL of lipid extract dissolved in 3 mL of pure hexane. The absorbance at 232 nm (50 Scan spectrophotometer Varian, equipped with a Cary Win UV Software; Palo Alto, CA, USA) was determined and the mmol hydroperoxides/kg sample were calculated by using a molar extinction coefficient of 29,000 mL/mmol × cm.

The TBARS content was measured at 532 nm, by the colorimetric method described by Secci et al. (2016), using trichloroacetic acid (5%) as solvent and then added with TBA 0.02 mol/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 (from 0.2 to 3.1 mmol/L).

The antioxidant capacity was measured by the radical cation decolorization assay (ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)), the radical scavenging activity (DPPH, 2,2-diphenyl-1-picrylhydrazyl), and the ferric reducing ability assay (FRAP). The antioxidant capacity was performed on ethanol extracted samples (3 g of sample in 10 mL of ethanol), according to Mancini et al. (2015).

Statistical analysis

The statistical analysis was performed using the General Linear Model procedures of the Statistical Analysis Software SAS (2004) for Windows. A two-way ANOVA, where the recipes with different ratio of European sea bass to rainbow trout (R: 50:50, R1; 30:70, R2), storage time (S: T0, T30, T60, T90), and the Recipes × Storage time (R × S) interaction were included in the model as fixed effects.

Result and Discussion

Physical characteristics

Shear force values of raw and cooked fish burgers are reported in Table 2. Raw samples were significantly affected by the recipes and by the storage time. The R2 showed a higher shear force than

R1, coherently with the fact that texture of fish muscle is affected by the species (Dunajski, 1980). Indeed, fish species are different in the muscle fibers in terms of contractile and metabolic types, size and number, the content, composition and distribution of the connective tissue, and the content and lipid composition of intramuscular fat, which play a relevant role in the texture profile (Listrat et al., 2016). The shear force decreased dramatically during the storage, being the first 30 days critical for this parameter that from that moment forward remained unaltered until 90th day. The same results were found by Ocaño-Higuera et al. (2011) who measured the textural properties of ray fish (*Dasyatis brevis*) and recorded a significant decrease in texture with prolonging the storage time. Also, the ready-to-eat pineapple chicken curry showed a decrease in shear force values during frozen storage at -18^{o}C for 6 months (Sunooj & Radhakrishna, 2013).

The shear force of cooked samples did not show any effect because of recipes. However, the effect of storage was even clear in cooking samples, causing a significant decrease of the shear force after 30 days of frozen storage. The interpretation could be attributed to the relationship between texture and heat-induced denaturation of the meat proteins (Bertola, Bevilacqua, & Zaritzky, 1994). The texture of cooked meat is generally affected by heat-induced changes in connective tissue, soluble and myofibrillar proteins. The cross-linkage between the collagen molecules within the connective tissue is associated with collagen solubility (Zayas & Naewbanij, 1986). Eilert and Mandigo (1993) found that the changes in collagen solubility with heating temperature could affect the textural and water-binding properties in the ground chicken breast patties.

Table 2 presents the results of ANOVA even for pH and color parameter values. For the raw samples, the recipes did not affect the pH values. However, the samples stored at negative temperature for 90 days exhibited slight but significant changes in terms of pH. Specifically, the values of pH increased significantly during storage time between 0 and 30 days, remained unvaried until the 60th day, then a significant reduction was evident at the end of the storage. The increase in the pH value during the first 60 days of storage could be explained by the decomposition of nitrogenous compounds due to endogenous enzymes causing an increase in volatile bases, thus increasing the pH value

(Chomnawang, Nantachai, Yongsawatdigul, Thawornchinsombut, & Tungkawachara, 2007; Özyurt, Polat, & Tokur, 2007). Meanwhile, the decrease in pH at the 90th day of storage might be due to the presence of carbon dioxide, which solubility increases at low temperatures and produces a drop in pH value (Adams & Moss, 2000). CO_2 is the product of the consumption of the residual oxygen provoked by natural muscle respiration, which occurs in meat even at negative temperatures (Osman & Zidan, 2014). In agreement with the present results, Osman and Zidan (2014) reported an increase in the pH values during the first two month of frozen storage of fish burger prepared from silver carp (*Hypophthalmichthys molitrix*), followed by decreasing pH value during the storage at -18 ^{o}C.

Color is one of the most important quality criteria for consumers, which determines the acceptability and marketability of many fish minced products (Sachindra & Mahendrakar, 2010). The color parameters differed significantly due to fish ratio effect, except for the lightness (L*), and hue values. Indeed, the b* index and the color intensity (Chroma) of R2 burgers resulted significantly higher than those of R1 ones. The opposite was for the redness index, since R1 samples had higher a* value than the R2 burgers. Storage time considerably affected the color parameters. The lightness and redness values decreased, as previously also stated by Choubert and Baccaunaud (2006), while the yellowness, Chroma, and hue values increased significantly after 30 days of storage, and then remained unchanged. The color regression during storage time might be related to enzymatic and non-enzymatic reactions resulting in degradation of myofibrillar proteins and disorganization of myofibrils (Cheret, Chapleau, Delbarre-Ladrat, Verrez-Bagnis, & Lamballerie, 2005), in agreement with the texture evolution during the storage time.

Table 2 also shows the results of color of cooked fish burgers. Like the raw samples, the lightness and hue values did not change significantly in the two recipes. Nevertheless, the redness value of R1 burgers was significantly higher than those of R2 ones, meanwhile the yellowness and chroma values were significantly higher in R2 comparing to R1 samples. In addition, the effect of the storage was evident in the color parameters, except for the redness value. A significant decrease in L* value at 60 and 90 days was observed, while b* and C* values increased significantly in the second part of the

storage period. The development of color in cooked frozen food are related to the reactions that occurred during heating including Maillard reaction, protein denaturation, fat and water exudation (Fennema, 1996). The same results were found by HassabaAlla, Mohamed, Ibrahim, and AbdElMageed (2009) who showed change of color parameters for the frozen cooked catfish burgers stored at -18 ^{o}C for four months, especially the lightness value, which decreased at the end of the storage. Consumers liking and acceptability of the color of raw and cooked burgers deserve further investigation.

Water holding capacity is another quality attribute of meat, especially for the ready and processed meat products. Low WHC can represent significant loss of weight from carcasses and cuts and may affect the yield and quality of processed meat (Aaslyng, 2002; Woelfel, Owens, Hirschler, Martinez-Dawson, & Sams, 2002). In addition, low WHC can negatively affect the appearance of meat, and this can influence consumer willingness to purchase the product. Table 2 illustrates the water holding capacity of the two recipes and its changes during storage time. The WHC of raw fish burger did not show significant differences either due to the influence of recipes, nor due to storage time even though the numerical changes from the 30th day onwards were relevant. Differently, the WHC of the cooked burgers was significantly affected by the recipes and storage time. Firstly, WHC of R2 had higher value than the WHC of R1. This could be because water holding capacity differs according to species (Adam & Abugroun, 2010). Secondly, the WHC of cooked samples decreased significantly after 30 days of storage because of the storage effect on muscle fiber integrity and the muscle protein denaturation during cooking, leading to a decrease in their WHC and to shrinkage of the protein network. The shrinking network applies a mechanical force on the water between fibers, resulting in expelling the water to the surface of the meat (Van der Sman, 2007).

Table 2. Physical properties of raw and cooked fish burger as affected by the formulation and the storage time (T0, T30, T60 and T90). Data are expressed as mean values (n=3) and the Root Mean Square Error (RMSE) is reported for each item.

	Recipes (R)		Storage (S, days)				R	S	R × S	RMSE
RAW	**R1**	**R2**	**0**	**30**	**60**	**90**				
Shear force, N	4.09	4.99	7.62^{a}	4.55^{b}	2.70^{b}	3.30^{b}	**	***	NS	0.625
pH	6.09	6.10	5.97^{b}	6.18^{a}	6.21^{a}	6.03^{b}	NS	***	NS	0.058
Color										
L*	56.93	56.78	59.08^{b}	63.42^{a}	53.46^{c}	51.47^{c}	NS	***	*	1.181
a*	-1.49	-2.03	-0.88^{a}	-1.78^{b}	-2.00^{b}	-2.37^{b}	**	***	NS	0.399
b*	12.99	14.21	11.61^{b}	12.22^{b}	15.21^{a}	15.37^{a}	**	***	NS	0.871
C*	13.12	14.38	11.69^{b}	12.35^{b}	15.36^{a}	15.56^{a}	**	***	NS	0.869
H°	96.39	97.95	94.24^{b}	98.27^{a}	97.52^{a}	98.66^{a}	NS	**	NS	1.809
WHC, %	73.54	70.72		75.24	70.35	70.79	NS	NS	NS	6.008
COOKED										
Shear force, N	7.54	7.64	9.11^{a}	8.85^{a}	6.74^{b}	5.66^{b}	NS	***	NS	0.951
Color										
L*	60.75	61.51	64.94^{a}	65.17^{a}	55.56^{c}	58.83^{b}	NS	***	**	1.266
a*	-0.46	-0.71	-0.75^{a}	-0.44^{a}	-0.44^{a}	-0.70^{a}	*	NS	NS	0.268
b*	18.44	19.98	15.45^{c}	18.28^{c}	22.40^{a}	20.73^{b}	***	***	***	0.845
C*	18.45	20.00	15.47^{c}	18.29^{c}	22.41^{a}	20.74^{b}	***	***	***	0.841
H°	91.55	92.09	92.91^{a}	91.27^{b}	91.15^{b}	91.99^{a}	NS	**	NS	0.876
WHC, %	83.23	87.02		88.36^{a}	83.93^{b}	83.09^{b}	**	**	***	2.808

Within criterion, a, b, c are significant different means.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, Not Significant ($P > 0.05$).

Proximate and fatty acid composition

Results of proximate composition analyses of raw and cooked fish burgers were shown in Table 3. The considered constituents (moisture content, crude protein, ash, and total lipids) did not show any significant differences due to the recipes or due to the storage time. After cooking, water content in the cooked samples was significantly higher in the recipe R2, containing more trout, than in the R1 one. This might be due to the difference in water content of the mechanical separated meat from the species of fish that compose the recipes, since the water content in the MSM from trout was higher than the water content in the MSM from seabass (70.65% against 68.79%), as highlighted by Borgogno, Husein, Secci, Masi, and Parisi (2017). In addition, the moisture content in the cooked fish burgers increased significantly from T0 to T90 of the storage. The increase in water content of

frozen burgers is probably due to the decrease in water holding capacity, which might be due to the hydrolysis of muscle proteins or aggregation of myofibrillar proteins during frozen storage. This increase was in line with Kirschnik, Viegas, Valenti, and de Oliveira (2006) who found that the moisture content in iced stored samples of tail meat of the giant river prawn (Macrobrachium rosenbergii) increased approximately of 6% comparing to the samples at zero time. An increase in the protein content was observed in the cooked R1 compared to R2 probably because of the lower moisture in the R1 comparing to the R2. Nevertheless, the storage time did not have any significant effect on the protein values. Moreover, the content of ash did not show any significant difference between the two recipes, but the effect of storage was evident in this parameter since it caused a significant decrease at T90. These results are consistent with those obtained by Okeyo, Lokuruka, and Matofari (2009), who observed that the ash content of the frozen raw Nile perch (*Lates niloticus*) decreases with the storage time. Moreover, the content of total lipids in raw and cooked sample did not show any significant differences due to the recipes or the storage period.

Table 3. The proximate composition (g/100 g product), of raw and cooked fish burger recipes at T0 and after 90 days of frozen storage (90). Data are expressed as mean values (n=3) and the Root Mean Square Error (RMSE) is reported for each item.

	Recipes (R)		**Storage (S, days)**		**R**	**S**	**R X S**	**RMSE**
	R1	**R2**	**0**	**90**				
RAW								
Moisture	72.17	72.55	72.45	72.27	NS	NS	NS	0.27
Protein	13.77	13.74	13.62	13.89	NS	NS	NS	0.39
Ash	1.85	1.93	1.92	1.86	NS	NS	NS	0.17
Total lipids	5.56	4.52	4.70	5.38	NS	NS	NS	0.31
COOKED								
Moisture	66.87	67.86	66.94	67.78	*	*	*	0.53
Protein	16.73	15.79	16.40	16.13	***	NS	**	0.32
Ash	2.44	2.34	2.52	2.25	NS	*	NS	0.18
Total lipids	5.01	4.91	4.68	5.24	NS	NS	NS	0.63

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, Not Significant ($P > 0.05$).

Table 4 shows the fatty acid composition (g/100 g of total fatty acids) of the products and highlights that few differences can be noted as affected by the recipe. Indeed, the recipe containing more European sea bass (R1) resulted significantly richer in saturated acid (C14:0 and C16:0), EPA, linolenic acid (C18:3ω3) and some monounsaturated fatty acid (C16:1ω7, C18:1ω7, C20:1ω9, C22:1ω11). Meanwhile, the recipe with more rainbow trout (R2) was significantly richer in DHA, PUFA and especially PUFAω6. These differences reflect the differences found in the MSM from both sea bass and trout, as previously published by Borgogno et al. (2017) and Secci et al. (2016). Concerning the storage, the amounts of the fatty acids and their sum into the corresponding fractions were PUFA > MUFA > SFA, this assuring that the storage time had a neglectable effect on the nutritional quality of the raw fish burger.

Table 4 illustrates also the fatty acid composition of the cooked burger as affected by the recipes and the frozen storage. Overall, the same observations and differences in the monounsaturated fatty acid composition of raw samples were also maintained in the cooked samples. However, it appeared also that the cooked recipes R2 were richer in oleic acid (C18:1ω9), beside in DHA and PUFAn6. However, no significant differences between the two recipes in terms of PUFA, MUFA, and SFA emerged. Considering the storage time, a significant decrease of oleic acid and MUFA fraction can be discerned.

The predominance of the PUFA fraction, which accounted for 45 g/100 g of total fatty acids, on both MUFA and SFA fractions was discerned in cooked burger as affected by the recipes and storage. Moreover, PUFA fraction was mainly composed of n3 fatty acids, with the n3/n6 ratio being around 1.4. There is agreement regarding the need to increase the n3/n6 ratio in the food consumed by the western modern consumers and, according to some authors, the ideal ratio may be 1:1 (Granados, Quiles, Gil, & Ramírez-Tortosa, 2006).

Table 4. The fatty acid composition (g/100 g of total fatty acids) content of raw and cooked fish burger recipes after 90 days of frozen storage (T90). Data are expressed as mean values (n=3) and the Root Mean Square Error (RMSE) is reported for each item.

	Recipes(R)		Storage (S, days)		R	S	RMSE
RAW	**R1**	**R2**	**0**	**90**			
C14:0	1.53	1.37	1.47	1.44	***	NS	0.030
C16:0	10.25	9.85	10.06	10.03	**	NS	0.141
C16:1ω7	2.41	2.21	2.34	2.29	***	NS	0.038
C18:0	2.90	2.96	2.92	2.94	NS	NS	0.065
C18:1ω9	30.24	30.28	30.23	30.29	NS	NS	0.327
C18:1ω7	2.31	2.27	2.29	2.29	*	NS	0.020
C18:2ω6	15.38	15.62	15.48	15.51	*	NS	0.116
C18:3ω3	4,15	4.04	4.09	4.11	*	NS	0.057
C20:1ω9	2.30	2.10	2.20	2.19	***	NS	0.039
C20:4ω6	0.94	1.04	0.99	0.98	**	NS	0.036
C20:5ω3 (EPA)	4.69	4.19	4.49	4.39	***	NS	0.137
C22:1ω11	1.08	0.94	1.02	1.01	***	NS	0.016
C22:5ω3	2.45	2.27	2.38	2.34	***	NS	0.051
C22:6ω3 (DHA)	12.79	14.30	13.49	13.60	***	NS	0.440
Σω3	25.71	26.45	26.08	26.08	*	NS	0.489
Σω6	18.36	18.86	18.59	18.63	***	NS	0.116
ΣSFA	15.56	15.02	15.31	15.28	**	NS	0.194
ΣMUFA	39.60	38.98	39.30	39.29	*	NS	0.365
ΣPUFA	44.83	46.00	45.40	45.43	**	NS	0.499
EPA+DHA	17.49	18.49	17.98	17.99	**	NS	0.463
COOKED							
C14:0	1.58	1.46	1.58	1.47	NS	NS	0.125
C16:0	10.36	10.06	10.38	10.04	NS	NS	0.447
C16:1ω7	2.45	2.28	2.39	2.33	*	NS	0.101
C18:0	2.92	2.96	2.97	2.91	NS	NS	0.073
C18:1ω9	30.23	30.64	30.63	30.25	**	*	0.231
C18:1ω7	2.31	2.28	2.30	2.29	**	NS	0.013
C18:2ω6	15.60	15.77	15.75	15.61	NS	NS	0.239
C18:3ω3	4,17	4.06	4.10	4.13	**	NS	0.043
C20:1ω9	2.22	2.07	2.11	2.19	*	NS	0.106
C20:4ω6	0.92	1.00	0.96	0.96	***	NS	0.026
C20:5n3 (EPA)	4.56	4.05	4.16	4.45	**	NS	0.277
C22:1ω11	1.04	0.93	0.96	1.01	*	NS	0.073
C22:5ω3	2.38	2.19	2.18	2.40	NS	*	0.159
C22:6ω3 (DHA)	12.69	13.66	12.96	13.39	**	NS	0.468
Σω3	25.41	25.59	25.00	26.01	NS	NS	0.757
Σω6	18.57	18.99	18.88	18.69	*	NS	0.289
ΣSFA	15.75	15.33	15.80	15.28	NS	NS	0.634
ΣMUFA	39.49	39.37	39.59	39.27	NS	*	0.213
ΣPUFA	44.76	45.30	44.61	45.44	NS	NS	0.706
EPA+DHA	17.24	17.72	17.12	17.84	NS	NS	0.593

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 <3%. They were utilized to calculate Σ.
* P < 0.05; ** P < 0.01; *** P < 0.001; NS, Not Significant (P > 0.05).
R×S interaction was not significant and, therefore, it is not reported in the present table.

A high ω3/ω6 PUFA ratio is an important feature for nutrition as an index of the lipid quality, and it was accompanied by the reduction in chronic inflammatory diseases such as cardiovascular disease, obesity, non-alcoholic fatty liver disease, inflammatory bowel disease, rheumatoid arthritis, and Alzheimer's disease (Patterson, Wall, Fitzgerald, Ross, & Stanton, 2011).

Furthermore, the relationship between the intake of EPA+DHA fatty acids and cardiovascular health in healthy populations was recently underlined by the EFSA (2010). Likewise, Swanson, Block, and Mousa (2012) assured the importance of EPA and DHA for proper fetal development and its effects on many aspects of cardiovascular function including inflammation, peripheral artery disease, major coronary events, and anticoagulation. According to World Health Organization (WHO, 2008), the recommended daily intake of EPA+DHA for adult males and non-pregnant/non-lactating adult females is 0.250 g/day, with insufficient evidence to set a specific minimum intake of either EPA or DHA alone. For adult pregnant and lactating females, the minimum intake for optimal adult health and fetal and infant development is 0.3 g EPA+DHA/day. The present finding revealed that a portion (100 g) of the raw or cooked burger, even though affected by the two different recipes and by the duration of the frozen storage time, however contained EPA+DHA that accounted for 0.400 g, of which almost 0.300 g was represented by DHA and about 0.100 g by EPA. Therefore, our results emphasized that one piece of cooked burger (100 g) could provide more than the suggested daily intake of EPA and DHA with a higher percentage of DHA than EPA, as suggested.

Lipid oxidation patterns

Regarding lipid stability in the raw samples, Table 5 illustrates the CD and TBARS values according to the recipes and the storage time. Results revealed that the recipe significantly affected lipid

oxidation in terms of both the primary and secondary oxidation products here considered. The R1, with more European sea bass, had higher CD value and TBARS than R2. Since the formulations only differed for the fish species amount, it seemed reasonable to attribute the difference in the oxidative status to the kind of flesh utilized for burgers' production. Indeed, a previous study showed that sea bass meat is much more prone to be oxidized than that of trout by the mechanical treatment (Secci et al., 2016). Besides, the dominance of trout in R2 recipe, which was characterized by lower oxidative status property due to carotenoid content, could be the responsible for these differences (Secci et al., 2016). Concerning the storage, CD values of the raw samples increased significantly during the storage period in line with Piccolo et al. (2014). Nevertheless, TBARS value significantly decreased at the end of the storage. This could be related to a delay in the occurrence of secondary oxidation, assured by the fact that PUFA did not change at the end of the storage, resulting from the antioxidant properties of lemon juice added in both the recipes. Moreover, the low storage temperatures are optimal for preserving fish from oxidative deterioration, as highlighted by other studies (Baron, Kjærsgård, Jessen, & Jacobsen, 2007; Choubert, Brisbarre, & Baccaunaud, 2011). Another explanation could be the undetectable interaction of malondialdehyde with the decomposition products of protein (Hernández-Herrero Roig-Sagués, López-Sabater, Rodríguez-Jerez, & Mora-Ventura, 1999) which determined a decrease in MDA-eq. level in samples analysed. Tokur, Polat, Beklevik, and Özkütük (2004) who evaluated the lipid stability in fish burgers of tilapia (*Oreochromis niloticus*) during eight months of storage at -18 °C noticed that TBARS value did not change significantly during the first 7 months of storage. After that, a sharp increase was found. They explained this fluctuation by the TBARS likelihood to combine with some biological compounds in the fish muscle. The present results concerning TBARS were in line with Mahmoudzadeh et al. (2009), who noticed a significant reduction in TBARS value of deep flounder (*Pseudorhombus elevatus*) fish burger at the end of five months of frozen storage at -18^{o}C.

Table 5. Primary (CD, mmol Hp/kg sample) and secondary (TBARS, mg MDA/kg sample) oxidation products of raw fish burger recipes at T0 and after 90 days of frozen storage. Data are expressed as mean values (n=3) and the Root Mean Square Error (RMSE) is reported for each item.

	Recipes (R)		Storage (S, days)		R	S	RMSE
	R1	**R2**	**0**	**90**			
CD	0.200	0.176	0.178	0.197	**	*	0.010
TBARS	0.980	0.810	1.310	1.020	**	***	0.150

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, Not Significant ($P > 0.05$).
R×S interaction was not significant and, therefore, it is not reported in the present table.

The ABTS, DPPH, and FRAP parameters have been widely used to check the ability of compounds to act as free radical scavengers, hence to assess the antioxidant activity in food matrix (Mancini et al., 2015). Table 6 shows the antioxidant capacity for raw fish burger recipes immediately after their preparation (T0) and after 90 days of frozen storage (T90). The R1 and R2 raw burgers had significantly different values of ABTS and DPPH. Although these differences could be attributed to the specific essential antioxidant system of the muscle of the two fish species that compose the recipes (Martínez-Álvarez, Morales, & Sanz, 2005), it should be noted that R2 samples presented significant lower level of ABTS and a lower oxidative status than R1 samples. This let hypothesize that the antioxidant capacity of R2 acted during the early stages of MSM handling, i.e. during the preparation f fish burgers, thus defending the food matrix from oxidative damages from the beginning.

Table 6. Antioxidant capacity expressed as ABTS (mmol Trolox /kg sample). DPPH (mmol Trolox /kg sample) and FRAP (mmol Trolox /kg sample) for raw fish burger recipes at different storage time (T0 and T90). Data are expressed as mean values (n=3) and the Root Mean Square Error (RMSE) is reported for each item.

	Recipes (R)		Storage (S, days)		R	S	RMSE
	R1	**R2**	**0**	**90**			
ABTS	0.82	0.65	0.83	0.64	***	***	0.055
DPPH	0.31	0.32	0.32	0.31	**	**	0.006
FRAP	0.44	0.44	0.46	0.41	NS	***	0.017

** P < 0.01; *** P < 0.001; NS, Not Significant (P > 0.05).
R×S interaction was not significant and, therefore, it is not reported in the present table.

The storage at negative temperature for 90 days significantly reduced the antioxidant capacity of raw burgers by reducing the values of the three parameters assessing the antioxidant activity. The same trend was also reported by Secci et al. (2016) for European sea bass and rainbow trout meat during the frozen storage.

Conclusions

The physical and nutritional quality and the lipid stability of the ready-to-cook products differently formulated based on two different relative amounts of MSM fish species have been investigated during frozen storage and after the oven-cooking. The formulated recipes differed for color, fatty acid composition, and oxidative status. Overall, the recipe containing more rainbow trout appeared redder than the other, as indeed expected, and contained less MUFA and SFA. However, while looking at the amount of EPA + DHA contained in 100 g of raw and cooked burger, a value of ~ 0.400 g was found irrespective to the recipe formulation and storage. Frozen storage was confirmed as a suitable method for preserving fish products from the loss of nutritional quality due to the oxidative phenomena. Further studies could focus on the development and enhancement of the recipes taking due account of the acceptance characteristics of the different niches of consumers.

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Other contributions

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Guest Editors: Massimo Trabalza-Marinucci (Coordinator), Cesare Castellini, Emiliano Lasagna, Stefano Capomaccio, Katia Cappelli, Simone Ceccobelli, Andrea Giontella

orange) were assessed in both fresh and frozen semen. Semen quality parameters recorded in fresh semen show a good quality soon after collection. After thawing, no significant effect of the equilibration time (10 *vs* 30 min) was observed for all the sperm quality parameters assessed. However, slightly higher values were observed with 10 min equilibration compared to 30 min: sperm motility = 33.5% *vs* 31.8%, SMD = 40.7% *vs* 38.7%, viability = 36.3% *vs* 35.9%. These results lead us to assume that the equilibration time of 10 minutes is sufficient to allow dehydration of sperm cells. The development of an effective freezing protocol is required to create a sperm cryobank to support the *ex situ in vitro* conservation of the original population of Mediterranean brown trout in the Biferno river.

P074

Comparison of two basic extenders on the *in vitro* post-thaw quality of rabbit semen

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Semen cryopreservation is a strategic tool to secure genetic diversity. Research efforts have focused on developing freezing protocols to improve the cryopreservation of rabbit semen by the reduction of sperm cryoinjuries. Since a lack of knowledge about the use of basic extenders, we tested the effect of a commercial extender (Cortalap®) compared to Tris-citrate-glucose (TCG) on the *in vitro* post-thaw quality of rabbit semen. Six pools of semen (4 ejaculates/pool) were collected from 40 adult rabbit bucks of Bianca Italiana breed from the Italian Rabbit Breeders Association (ANCI-AIA, Volturara Appula (FG), Italy), an aliquot from each pool was used for the analysis of fresh semen, the remaining part of pooled semen was cooled at 5 °C for 90 minutes. Each pool was split into two equal aliquots, and each of them were diluted to a ratio 1:1 (v:v) with a freezing extender composed of TCG or Cortalap® both containing 16% of dimethylsulfoxide and 0.1 M sucrose. The diluted semen was packaged into 0.25 mL plastic straws, equilibrated at 5 °C for 45 min. Semen was frozen at heights of 5 cm above liquid nitrogen for 10 min, then straws were transferred into liquid nitrogen for storage at -196 °C. Sperm samples were thawed at 50 °C for 10 seconds. Sperm motility (phase contrast microscopy), viability (SyBr-PI), acrosome integrity (FITC-PSA) and DNA intactness (Acridine orange) were examined on fresh and post-thawed sperm. Sperm variables among the fresh and cryopreserved semen were compared by ANOVA, followed by Duncan's comparison test, the level of significance was set at $p \leq .05$. Results showed that the cryopreservation process impaired the post-thaw quality of rabbit semen compared to fresh semen ($p < .05$). However, the quality of frozen-thawed semen was affected by the freezing extender. In fact, the post-thaw semen quality was significantly improved in semen samples diluted in the Cortalap® extender compared with TCG for total and progressive motility (43.4 ± 1.4 *vs* 36.8 ± 1.5 and 36.5 ± 1.1 *vs* 30.2 ± 1.9), sperm viability (52.5 ± 1.8 *vs* 44.6 ± 2.1) and acrosome integrity (37.5 ± 0.6 *vs* 30.9 ± 1.2). The present results show that the Cortalap® extender provided better *in vitro* condition to preserve sperm integrity (membrane and acrosome) and function (motility) during the cryopreservation process. However, further studies are needed to confirm these results *in vivo*.

P075

Advantages and disadvantages of mechanical separation process applied to fishery industry

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Fish represent an important source of nutrients, such as essential fatty acids and minerals, so numerous technological strategies have been adopted for exploiting the huge amount of no directly marketable specimens without altering fish nutritional quality. Recently, mechanical separation process (MSM) have been demonstrated to be successfully applied to fish by-products for increasing processing yield. The present study aims to compare the impact of mechanical separation with manual mincing applied on farmed European sea bass, gilthead sea bream, rainbow trout, and wild Atlantic horse mackerel by evaluating technological yield and nutritional quality of obtained meat. Eighteen fish for each species were subdued to the two different treatments. MS process yield was found higher than the manual one when applied to sea bass, sea bream, and trout (58, 63%, 70% respectively against 39, 40, and 53%). Horse mackerel instead was better separated by manual operation. MS seemed to slightly (around 2%) but significantly increase water content and decrease minerals in all the species except sea bass. Interestingly, even Ca tended to decrease confirming the efficiency of the mechanical separation process in muscle separation from the skeletal part of the fish, which is the main source of Ca. Hopefully, lipid fraction of the considered species remained

unaltered. Few differences in C18:2n6 (1.18 *vs* 1.00 g/100 g total fatty acids), and C22:1n11 (0.27 *vs* 0.20 g/100 g total fatty acids) were found for horse mackerel minced and MSM meat. Globally, seawater species confirmed to be richer in PUFA n3 than freshwater species being around 55, 33, and 38 g/100 g total fatty acid in mackerel, sea bass and sea bream, whereas 21 g/100 g total fatty acids were found in trout. Processing may promote oxidative damages as confirmed by TBARS content of MSM samples, however sea bass and sea bream were found the most susceptible by doubling their TBARS content compared to minced samples. Trout and horse mackerel were mostly unaffected even if TBARS values of the latter exceeded 6 mg/100g muscle. maybe because of both intrinsic lipid susceptibility and killing/handling procedure after fishing. In conclusion, mechanically separation process can be adopted in order to exploit unmarketable fish, however not all the species shown to be equally prone. Globally, sea bass and trout were the most suitable from a technological and qualitative perspectives.

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P076

On-growing turbot (*Scophthalmus maximus*, Linnaeus 1758) in a land-based aquafarm in Southern Italy: preliminary results

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Turbot (*S. maximus*) is considered a possible candidate to increase the number of farmed fish species and contribute to diversify Italian aquaculture production. Rearing techniques, developed in France and Spain, highlighted good growth performances for this species, while breeding attempts in Italy, carried out in the past, failed due to the high water temperatures reached in the tanks during the fattening cycle in the summer. The present study aims to evaluate the feasibility of turbot rearing in a land-based aquafarm located in the Campania region, South of Italy. 7000 turbot juveniles (initial weight 7.5 g) were purchased from a specialized French hatchery (France Turbot) and placed in a rectangular concrete raceway (27x7 m) at the Soc. Coop. "Acquamarina" located in Villa Literno (CE, Italy) in December 2015. The tank was adapted to the species and fitted with a shading net. Fish were fed commercial diets (Biomar, Spain). Dissolved oxygen (>8 mg/l) and temperature were monitored daily, while pH (7.3 ± 0.1), salinity (37‰) and TAN (<5.5 mg/l) were determined monthly. The farm sources its water from wells, with a constant temperature of 18 °C, with slight seasonal fluctuations (16 °C-20 °C) in tank, still contained within the ranges tolerated by the species thanks to a proper management of water renewal and to the shading. In addition, the low pH and temperature values allowed un-ionized ammonia to be kept under control and at acceptable levels for the species (0.04 mg/l)."

Monthly, a sample of fish, representative of the population, was weighed to determine the average weight of the subjects, the FCR and the SGR. At the beginning and during the trial, a sample of subjects was sacrificed to evaluate the whole body and the fatty acid profile of the fillets. After 445 days of rearing, fish reached an average weight of 770 g, with a FCR of 1.15 and a SGR of 1.04. These performances allow us to hypothesize the achievement of the minimum commercial size (1 kg in Italy) within 18 months of rearing. These preliminary results suggest that in particular environmental conditions and adopting appropriate farming solutions, on-growing turbot in Italy is feasible. This could act as a catalyst to revitalize a sector, such as fish farming, which has been in difficulty in recent years especially in the Campania region.

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Sensory comparison of mussels (*Mytilus galloprovincialis*) farmed in the Adriatic and Sardinian Sea

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Italy, with Spain and France, is a top producer of molluscan shellfish in the EU; the national production of bivalve mollusc (111,000 tons) accounts for 63% of the Italian aquaculture

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Physico-chemical changes occurring in clean label fish burgers during frozen storage

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Two 'clean label' formulations for fish burgers made with mechanically separated fish meat were chemically and physically characterised during 90 days of frozen storage (T90). The formulations differed in the ratios of European sea bass to rainbow trout (50:50, R1; 30:70, R2). The formulations were added with lemon, salt, water, and potato flakes to obtain products containing 72% of water. The protein and lipid contents were not affected by the presence of the different amount of fish species. Overall, even the fatty acid composition of R1 and R2 was slight affected. The sum of the polyunsaturated (PUFA) ω6 fatty acids was higher in R2 than in R1 (18.99 vs 18.57g/100g of total fatty acids) while no differences in the sum of PUFA ω3 emerged. The higher amount of trout in R2 seemed to better preserve the product from lipid oxidation, as revealed from the lower content of secondary oxidative products (thiobarbituric acid reactive substances, TBARS) found in the R2 than in the R1, being 0.81 and 0.98 mg MDA/kg sample, respectively. The high amount of trout in R2 significantly increased the shear stress of the fish burgers (4.09 and 4.99 N, in R1 and R2 respectively). Frozen storage showed to be a good way for food preservation. Indeed, proximate and fatty acid composition of fish burgers were not altered for 90 days. A low oxidative profile of fish burgers emerged by evaluating both conjugated dienes (CD) and TBARS content in the products immediately prepared (T0) and after 90 days of storage. On the other hand, the recipes showed a dramatic decrease in their shear stress during the storage, being 7.62 N at T0 and 3.30 N at T90. In conclusion, the recipe with more trout (R2) showed less changes in physical quality and had more oxidative stability during frozen storage.

Physico-chemical changes occurring in clean label fish burgers during frozen storage

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BACKGROUND

Nowadays, cconsumers are increasingly focusing on healthy, natural and simple ingredients, and minimally processed products. Beside, the food industry concerns about sustainable production through reducing food waste have gained more importance. Mechanical separation process (MSM) is considered a successful technique for exploiting no directly marketable fish, such as European sea bass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) damaged or undersized. However, a depletion of the quality of fish products can be affected by both processing and storage. Hence, the objective of this study was to investigate how the physical, chemical and nutritional quality of two "clean label" fish burgers, based on mechanical separated meat from European sea bass and rainbow trout, could be affected by the formulation and the frozen storage.

MATERIALS & METHODS

Not directly marketable specimens of:
European sea bass
Rainbow trout

MSM technique

Ingredients (g per 100g)	R1	R2
MSM sea bass	35.875	52.675
MSM trout	35.875	22.575
Potato flakes	8.31	8.31
Water	18.11	14.61
Lemon	1.00	1.00
Salt	0.83	0.83

Sea bass:trout ratio (%) 50:50 30:70

FISH BURGER

CLEAN LABEL

TREATMENTS
Recipes
R1 (n. 12 burgers)
R2 (n. 12 burgers)
Storage
0 day (T0)
90 days at -18 °C (T90)

Chemical analyses
- Proximate composition (AOAC, 2012)
- Total lipids (Folch et al., 1957)
- Fatty acids profile (Morrison & Smith, 1964)
- Conjugated dienes, CD (Srinivasan et al., 1996)
- TBARS (Vyncke, 1970)

Physical analysis
- Texture as shear stress

RESULTS

The protein and lipid contents in R1 and R2 were not affected either by the recipes or the frozen storage. The higher trout content in R2 significantly increased its shear stress. Both R1 and R2 showed a dramatic decrease in shear stress values during the storage (**Tab. 1**).

Tab. 1. The proximate composition (g/100g sample) and shear stress of fish burger recipes at T0 and after 90 days of storage (T90)

	Recipes		Storage	
	R1	R2	0	90
Moisture	72.17b	72.55a	72.45	72.27
Protein	13.77	13.74	13.62	13.89
Ash	1.85	1.93	1.92	1.86
Total lipids	5.56	4.52	4.70	5.38
Shear stress, N	4.09b	4.99a	7.62a	3.30b

Tab. 2 reveals that the fatty acid composition of R1 and R2 burgers was slightly affected by the recipe formulation. The sum of the polyunsaturated fatty acids (PUFA) ω6 was higher in R2 than in R1 while no differences in the sum of PUFAω3 emerged. Storage did not modify the fatty acid composition. **A portion** (100g) of each of the two burger recipes contained **0.400g of EPA+DHA** (recommended intake by FAO/WHO: 0.250g/day), even after 90 days of frozen storage.

Finally, lipid oxidation products are presented in **Tab. 3**. The R2 burgers seemed to better preserve the product than R1 ones, having lower content of the primary oxidative products (CD) and the secondary oxidative products (thiobarbituric acid reactive substances, TBARS). R1 and R2 burgers showed low oxidative profile, based on CD and TBARS contents, either at T0 or after 90 days of storage.

Tab.3. Conjugated Dienes (CD) and TBARS of fish burger from the two recipes at T0 and after 90 days of frozen storage.

	Recipes		Storage	
	R1	R2	0	90
CD, mmol Hp/kg sample	0.200a	0.176b	0.178b	0.197a
TBARS, mg MDA-eq./kg sample	0.980a	0.810b	1.310a	1.02b

Tab. 2. Fatty acid profiles (g FAs/100g total FAs) of fish burger recipes after 90 days of frozen storage.

	Recipes		Storage	
	R1	R2	0	90
C14:0	1.58	1.46	1.58	1.47
C16:0	10.36	10.06	10.38	10.04
C16:1ω7	2.45a	2.28b	2.39	2.33
C18:0	2.92	2.96	2.97	2.91
C18:1ω9	30.23b	30.64a	30.63a	30.25b
C18:1ω7	2.31a	2.28b	2.30	2.29
C18:2ω6	15.60	15.77	15.75	15.61
C18:3ω3	4,17a	4.06b	4.10	4.13
C20:1ω9	2.22a	2.07b	2.11	2.19
C20:4ω6	0.92b	1.00a	0.96	0.96
C20:5ω3 (EPA)	***4.56a***	***4.05b***	***4.16***	***4.45***
C22:1ω11	1.04a	0.93b	0.96	1.01
C22:5ω3	2.38	2.19	2.18b	2.40a
C22:6ω3 (DHA)	***12.69b***	***13.66a***	***12.96***	***13.39***
ΣPUFAω3	25.41	25.59	25.00	26.01
ΣPUFAω6	18.57b	18.99a	18.88	18.69
ΣPUFA	44.76	45.30	44.61	45.44
ΣMUFA	39.49	39.37	39.59a	39.27b
ΣSFA	15.75	15.33	15.80	15.28

CONCLUSION

The two '**clean label**' formulations for fish burgers made with mechanically separated Mediterranean fish meat resulted in fish burgers of good stability and high nutritional value, also after frozen storage. However, the recipe with **more trout** showed less change in physical quality and had **more oxidative stability** during the frozen storage. Further studies could focus on developing and enhancing the recipes by microbiological and consumers acceptance features.

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General conclusions

The overall purpose of this study was to produce new fish products (fish burger) obtained by means of mechanical separation (MS) process applied to non-directly marketable different species of fish from Italian aquaculture (European sea bass, gilthead sea bream and rainbow trout), and evaluate the physical, chemical, nutritional and sensory properties of the obtained fish burgers.

In conclusion, the present PhD thesis showed that:

- Mechanical separation process presented good results in terms of yield without much modification of the micro- and macro-nutrient contents. Nevertheless, yield of the process and nutritional values of the meat recovered by MS differed according to species-specific characteristics. In this regard, rainbow trout had the highest processing yield in both manual separation and MS processes compared to European sea bass, and gilthead sea bream. The yield of MS process was higher than the manual one in these species (42 and 45 g/100 g, respectively for sea bass and sea bream against 39 and 40 g/100 g) on the contrary to rainbow trout (50 g/100 g against 53 g/100 g) due to high residual left on the drum (5 g/100 g).

- Fatty acids composition and, consequently, the calculated atherogenicity and thrombogenicity indexes did not alter in the whole fish, minced fish burger, and MSM-fish burger of the three fish species.

- Four products resulting from four clean label formulations could emerge from a mixture of fresh mechanically separated fish meat and simple natural ingredients, which are characterized by high nutritional value and are different in sensory properties and protein content. The differences in sensory profile resulted from the fish to potato ratio, while the prevalence of rainbow trout over the sea bass did not considerably modify the burger characteristics.

- The fish burger formulations could meet the tastes and expectations of different consumer groups, and a burger of 100 g provided more essential fatty acids than the recommended daily intake, regardless of the formulation.

- Ready-to-cook products can be developed from two different ratios of MSM rainbow trout and sea bass fish. The two burger formulas had a good quality and a high nutritional value. However, the recipes with more trout showed superior physical features and better oxidative stability.

 Storage at -18 °C for 90 days of the burgers obtained with the clean label recipes confirmed to be a good way to preserve burgers from oxidative deteriorations and maintain their nutritional quality despite some changes in physical and chemical characteristics.

In conclusion, the study carried out through different steps of the research showed the feasibility to produce clean label fish burgers starting from not directly marketable species through mechanical separation process. Few and simple ingredients such as lemon, salt and potato flakes were utilised for the preparation of high nutritional quality fish burgers which demonstrated to be stable during frozen storage. The fact that the highest stability was obtained in fish burgers containing a high proportion of rainbow trout could be of interest for seafood industry due to the lowest economical value of this species in comparison with the more expensive seawater aquacultured species (European sea bass).