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THE EFFECT OF DIFFERENT MARINATION PROCEDURES ON THE QUALITY OF DRIED BLUE WHITING (Micromesistius poutassou)

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ABSTRACT

In this study, the influence of marinates containing different combinations of soy sauce, sorbitol, sucrose, and citric acid on blue whiting before and after drying were studied. The effects on sensory attributes (odour, flavour and texture), microbial quality (Total Viable Counts, Listeria and coliforms), physico-chemical profile (pH, a_w and Total Volatile Basic Nitrogen) and proximate composition (moisture, protein, sodium chloride and fat) were evaluated. For comparison, fresh (not marinated) blue whiting was used as a control. The results showed that the different marinade ingredients and amounts exerted distinct effect on the sensory attributes of dried blue whiting. The sweetness flavour was significantly intensified by the sucrose combined with soy sauce, whilst citric acid enhanced significantly the intensity of sour flavour. Soy sauce also had a slight influence on odour attributes. Rubbery texture was found in the marinated fish, whilst the control ended with more dry texture, as well as more dried fish odour and flavour. The marinade ingredients also had an effect on colour; soy sauce enhanced the red colour whilst the citric acid decreased the redness in the products. After drying, lower level of TVB-N was found in the dried marinated fish compared with the control-dried sample. Higher counts of bacteria were found in the control group after drying than in the marinated groups; this may have been caused by the marination but also that the washing may have reduced the amount of bacteria. Citric acid reduced the pH of the fish at the marination stage leading to substantially lower pH in the dried fish compared with the control. The aw and

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Fernaneds, C. 2018. *The effect of different marination procedures on the quality of dried Blue whiting (Micromesistius poutassou)*. Nations University Fisheries Training Programme, Iceland [final project]. http://www.unuftp.is/static/fellows/document/carina16prf.pdf moisture content was found to be the lowest in the dried control sample, suggesting that the carbohydrates which were taken up during the marination in the other fish samples bound some water.

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ABBREVIATIONS

 $a_w - Water Activity$

- BHT Butylated Hydroxytoluene
- CFU Colony-Forming Units
- DGP Direcção Geral das Pescas
- EC European Commission
- FAO Food and Agricultural Organization of the United Nations
- FEP Fluorinated Ethylene Propylene
- GDA Generic Descriptive Analysis
- GMP Good Manufacturing Practice
- GRAS Generally Recognized as Safe

IA - Iron Agar

- ICES International Council for the Exploration of the Sea
- INDP -- Instituto Nacional de Desenvolvimento das Pescas
- ISK Iceland Krona
- ISO International Organization for Standardization
- LOG Logarithm
- MRI Marine Research Institute
- pH Potential of Hydrogen
- PPM Parts Per Million
- PUFA Poly-Unsaturated Fatty Acid

PV - Peroxide Values

- RH Relative Humidity
- **RPM** Revolutions Per Minute
- SSO Specific Spoilage Organisms
- TCA Trichloroacetic Acid
- TL Total Length
- TVB-N Total Volatile Basic Nitrogen
- TVC Total Viable Count
- VIS/NIR Visible/Near-Infrared
- WHC Water Holding Capacity

1 INTRODUCTION

The economic and social benefits of fishery products are globally recognized. Furthermore, fish consumption also brings several nutritional benefits to human populations. It contains a good selection of minerals and vitamins; also, the proteins contain all the essential amino acids in the right proportions for a balanced diet (Murray, 1983). About 50 to 60 percent of daily protein needs of an adult can be supplied by 150 g portion of fish. In countries with a high population density, in which total protein intake may be low, fish protein may represent a very important nutritional component. It accounted, in 2010, for 6.5 percent of all protein consumed and 16.5 percent of the global population's intake of animal protein (FAO, 2014a).

Fisheries and aquaculture has been an important source of food since ancient times (FAO, 2014b). According to a preliminary estimate, the average world fish consumption in the 1960s was 9.9 kg/per capita per year and had increased to 19.2 kg/per capita per year in 2012. Additionally, in the last five decades, world fish production has increased at an annual average rate of 3.2 percent, and surpassed the world population growth by 1.6 percent (FAO, 2014a).

The proportion of world fish production used for direct human consumption has been increasing since the early 1990s, and in 2012 more than 86 percent (136 million tonnes) of world fish production was utilized for direct human consumption (FAO, 2014a). However, because fish is a highly perishable food product, it is associated with some diseases transmitted through food. Thus, to achieve the benefits of fish consumption, safety and quality must be guaranteed through the entire production chain. Food safety, which represents the main concern of the food industries, is crucial to ensure food and nutritional security throughout the world (FAO, 2014c). FAO underlines the importance of food production and consumption in every society, and further asserts that it can have economic, social and even environmental consequences in the societies.

Due to its intrinsic characteristics, fish meat deteriorates easily. Fish processing methods are often used to prolong the shelf life of fishery products, preventing the action of mechanisms that lead to their deterioration. Processed products are defined by the Regulation (EC) No 852/2004, article 2, paragraph (o) as "foodstuffs resulting from the processing of unprocessed products. These products may contain ingredients that are necessary for their manufacture or to give them specific characteristics." (European Comission).

Fishery products can be processed in several forms into a wide range of products (FAO, 2014a). Salted, dried and smoked fish are examples of processed products marketed and appreciated all over the world. In 2012, 12 percent (16 million tonnes) of the fish caught worldwide for human consumption, was utilized in dried, smoked or other cured forms (FAO, 2014a). As well as salting, drying, smoking, and canning, marination is a widely-known food preservation technique. It is used in several cultures to produce a very specific flavour in food. Marination is a process in which the product is submerged for a pre-determined time at refrigeration temperatures in marinade previously prepared (Eysteinsson, 2016). In Europe, marination is popular and marinated products, along with smoked products, are processed in Eastern and Central Europe, mainly in Poland and the Baltic States (FAO, 2014a). In addition to species traditionally used for marination such as herring, some others species are also processed as marinated such as eels, hake, mackerel, cod, sea salmon, dogfish and shrimps (Shenderyuk and Bykowsky, 1994). In Iceland, as well as in some others European countries such as Ireland and Turkey, some trials have been carried out on underutilized fishery products to optimize this processing method (Baygar *et al.*, 2010; Fagan *et al.*, 2006).

The total world production for dried fish is 3.140.000 tonnes, where Iceland and Norway are the main producers, followed by African and Asian countries (Arason, 2003). In Iceland, dried fish has been a staple food of Icelanders for centuries, being the main food in the country in the old days. Stockfish has always played an important role for the country's economy. In the 13th century, it became one of the most important export products, and in the later years it has been exported to Nigeria and Italy (Icelandic Ministry of Fisheries and Agriculture, 2016). Despite the great interest in dried fish production for human consumption, the country also produces dried pet food, a new and growing industry, and dried seaweed. Indoor drying using geothermal energy, instead of oil and electricity, has been the main drying method used in the fishing industry for salted fish, cod heads, small fish, and other products (Arason, 2003).

Blue whiting (*Micromesistius poutassou*), is a small marine pelagic fish, belonging to the cod family. In Iceland, only a small amount of the total catch is used for human consumption, and then the product is frozen at sea. Most of the catch is processed domestically into fish meal and oil. When frozen at sea the species is processed and gutted on board large freezer vessels (Icelandic Ministry of Fisheries and Agriculture, 2016). Economically, this species represents an important product caught and processed in the country (Eysteinsson, 2016). In 2015, from the Icelandic blue whiting catch, of about 215 thousand tonnes, only 163 tonnes were frozen at sea for human consumption, and about 214 thousand tonnes were reduced to fish meal and oil (Table 1). In the same year, the total value of the blue whiting catch was estimated at around 5,7 billion ISK, with the value for domestic processing catch estimated as 5.6 billion ISK. While the blue whiting export value was estimated as 101.4 million ISK, the frozen product at sea for human consumption was estimated as only 8.5 million ISK (Statistics Iceland, 2016).

Being of the cod family, blue whiting has, as cod and haddock, low levels of fat content, and is light in colour, therefore well suited to processing. However, the fish is caught far from land and therefore it is difficult to ensure adequate shelf-life for on-land processing, and due to its small size, it has been considered purely as a fish for feed and oil. Also, undesirable processing attributes and intrinsic softness, poor freezing and storage capabilities are very important blue whiting attributes that make it difficult for use for human consumption by conventional means. In addition, economic considerations as the cost of processing compared to product value on the market may limit the utilization of blue whiting for human consumption (Icelandic Ministry of Fisheries and Agriculture, 2016; Jóhannsson, 2006 *cit in* Eysteinsson, 2016).

In Cape Verde, fish is the main animal protein consumed by the population (Direcção Geral das Pescas e Instituto Nacional de Desenvolvimento das Pescas, 2004). Although the ten islands that form the archipelago are surrounded by the Atlantic Ocean, fishery resources are, in general, not significant in terms of quantity when compared to global volumes. However, some species of great economic importance are found in its waters. Lobsters, some pelagic and demersal fish, as well as migratory species such as tuna fish, are some examples of halieutic resources found in the country's seas (Oceanic Development, 2010). In Cape Verde, due to its privileged geographical position, the sea can provide many potential economic activities. The marketing of salted, dried, smoked or marinated fish are some examples of such activities with high value potential for large fish processing industries, as well as for small fishing communities in Cape Verde. Sun drying is one of the first and most popular fish preserving methods used in the country. However, it is carried out mainly on surplus fish from a large catch. The demand for sun dried fish is only significant at a certain period of the year, in the "Lent" season, and only three of the nine inhabited islands of the

archipelago have some tradition for fish drying. Demersal species such as the grouper (*Cephalopholis taeniops*), small pelagic species such as the juvenile bigeye scad (*Selar crumenophthalmus*) and black mackerel (*Decapterus macarellus*) are used, as well as large ones such as albacore (*Thunnus alalunga*) and wahoo (*Acanthocybium solandri*).

Despite the privileged geographic position of Cape Verde, the already known nutritional and gastronomic attributes of preserved fish and their good acceptance in the domestic market, from the total fish capture only a very small fraction, less than 3%, is preserved (salted, dried, or in brine). Thus, fresh fish is the most frequently available in the domestic markets and canning is the main activity of the processing industry (Direcção Geral das Pescas e Instituto Nacional de Desenvolvimento das Pescas, 2004). Consequently, except for canned fish, there is a noticeable lack of processed raw material in the country.

The main aim of this study was to optimize a marinating processing technique with the purpose of obtaining a new value-added product, marinated dried blue whiting, for Iceland and for export. Another objective of this project was to learn about fish marinating process and in-door drying to be able to apply the technique in Cape Verde, thereby contributing to valorisation and better use of raw materials through the introduction of new fish processing methods in the country.

The study had the following specific objectives:

- To combine different marination time, temperature, and ingredients (soy sauce, sorbitol, sucrose, and acids), to determine and define the most appropriate marination technique to produce a high-quality marinated-dried blue whiting. For this, sensory and microbial evaluations, along with physicochemical and proximate analysis were carried out on the raw material, after marination and on the final products;
- To analyse the applicability of marination-drying process in Cape Verde, considering the socio-economic aspects of the country. For this, a comparison between the raw material, procedures, materials, and components used during this study and the existing conditions in Cape Verde was done.

2 LITERATURE REVIEW

2.1 Blue Whiting

2.1.1 Biology and distribution

Micromesistius poutassou (Risso, 1827), or blue whiting (Figure 1), is a pelagic marine fish, which belongs to the cod family, Gadidae, being one of the two species in the genus *Micromesistius*. It can be found at depth range between 150 - 3000 m (Svetovidov, 1986), but usually at 300 - 400 m, in temperate waters around 14°C (Cheung *et al.*, 2013). It is distributed in the Northwest Atlantic Ocean, more specifically in southern Greenland, off southeast Canada, and the north-eastern coast of USA; and in the Northeast Atlantic Ocean, including around Iceland, in the western Mediterranean, and in the south along African coast to Cape Bojador (Cohen *et al.*, 1990), shown in Figure 2.



Figure 1: Blue whiting (*Micromesistius poutassou*). Source: Fisheries.is



Figure 2: Blue whiting distribution map. Source: Fishbase.org

The species makes daily vertical migrations, in which during the day it is near the bottom and at night it is found in the surface waters (Cohen *et al.*, 1990). The larger individuals feed on small fish and cephalopods, but the blue whiting feed predominantly on small crustaceans. They are, however, important prey for many other fish (Icelandic Ministry of Fisheries and Agriculture, 2016). The maximum reported age and length the species can reach is 50 cm TL male/unsexed and 20 years, respectively (Cohen *et al*, 1990). The average size after reaching sexual maturity is 29-32 cm, but individuals as small as 18-20 cm (one-year-old individuals) have been recorded (Eysteinsson, 2016).

Blue whiting is a white fish in which the bulk of fat, mostly composed of Poly-Unsaturated Fatty Acid (PUFA), is stored mainly in the liver. The fat content in the muscle is always low, usually below 1%. Fluctuations in its chemical composition, noticeable mainly in the liver, is related to differences of water and fat content due to spawning season (Murray, 1983; Eysteinsson, 2016). The species has lean white meat and it is traded as fresh and frozen fish, and processed as oil and fishmeal (Cohen *et al.*, 1990). It is an abundant species in Icelandic waters. Blue whiting became, along with capelin, the major species targeted by pelagic fisheries in Iceland, after the herring stock collapse in the late 1960s (Icelandic Ministry of Fisheries and Agriculture, 2016). As can be seen in Table 1, only pelagic trawls are used to catch the fish.

2.1.2 Capture production

In 2012, the global fishery production in marine waters was 79,7 million tonnes (FAO, 2014a). In the world, blue whiting was among the ten most fished species and the stock was mainly caught in the northeast Atlantic (Icelandic Ministry of Fisheries and Agriculture, 2016). After strong variations from 1990s to 2005, the blue whiting capture reached very low levels in 2010, but from 2012 catches have been increasing. Thus, the International Council for the Exploration of the Sea (ICES) advised an increase in the total allowable catch by 64 percent for 2013 and 48 percent for 2014, based on a spawning stock biomass that almost doubled from 2010 to 2013 (FAO, 2014a).

In Iceland, fishing of blue whiting started in small quantities, in 1972. However, in the following years it became a very important economic species (Eysteinsson, 2016). After almost two decades of very little fishing effort, large-scale blue whiting fishing began in 1998. In 2003, the catch by Icelandic vessels reached 500.000 tonnes (Icelandic Ministry of Fisheries and Agriculture, 2016). In 2014, the catch of this species by Icelandic vessels was 182.777 tonnes, increasing by 17.6% in 2015 (Statistics Iceland, 2016). Statistical data from 2015 of blue whiting catch in Iceland waters can be seen in Table 1.

Year	Catch by Icelandic	Catch by t	type of landing and s (tonnes)	pecies	Catch b processing (to:	by type of g and species nnes)	Catch by type of fishing gear and species (tonnes)
	vessels	For	Landed abroad	Frozen	Frozen	Reduction	Pelagic trawl
	(tonnes) domestic for fish		for fishmeal and	at sea	at sea		
		processing	oil production				
2015	5 214.954 211.231 3.560		154	163	214.713	214.923	

Table 1: Statistical data from the blue whiting catch on 2015 (Statistics Iceland, 2016).

2.2 Fish Freshness and Quality

Fish is highly perishable, with a higher deterioration probability than other food of animal origin. This is related to some intrinsic characteristics in fish tissue such as a high water activity, nutrient content, phospholipid content, presence of enzymes with rapid destructive action in the tissues and viscera and a pH close to neutrality (Soares and Goncalves, 2012). Huss (1995), pointed out autolysis, bacterial activity and rancidity as the three main reasons for the spoilage and quality deterioration of fresh fish. The fat oxidation, or rancidity, takes place usually after autholytic and bacterial activity, in which high temperature or light expure can increase its rate (Quang, 2005). Fatty species, as mackerel, are the most affected by rancidity because of the high fat content (Love, 1982).

Fresh fish is popularly defined as the fish which is preserved only by cooling in a temperature close to 0° C. On one hand, handling procedures greatly influence the maintenance of raw material freshness. On the other hand, raw material freshness intended for processing greatly influences the final product quality. Thus, to maintain safety and quality characteristics of fish products, handling procedures must include all operations from capture to its consumption. That means the prevention of contamination by microorganisms and foreign substances, thus reducing the spoilage rate and slowing down the physical damage of the edible parts of the fish (Huss, 1995). Fish freshness is the attribute that represents the maintenance of similar proprieties to that of the live fish. Sensory, microbial, and physicochemical methods can be used to assess the fish freshness and quality (Olafsdottir *et al.*, 1997).

Sensory evaluation can assess the effect of raw material handling procedures on the quality (Huss, 1995). Boziaris (2014), points out some disadvantages of the method, such as it requires highly trained personnel to be reliable, therefore it is not attractive as a routine method. However, sensory evaluation can produce fast and reliable results compared with many other methods which is a great advantage, making sensory evaluation the most commonly used in the food industry. Sensory evaluation can provide a reliable estimate of freshness state of seafood and quality of the fish at various stages in the value chain, e.g. through examination of the general outer appearance of eyes, skin, gills, flesh texture, and odour of the fish skin, gills and flesh of raw fish or flavour, odour and texture of cooked fish (Martinsdóttir *et al.*, 2001).

In fresh lean or non-fatty fish species, such as blue whiting, autolytic changes cause the initial loss of quality, while bacteria action causes mainly their spoilage (Huss, 1994). However, only a fraction of the bacterial population, the Specific Spoilage Organisms (SSO), usually induces the spoilage. The SSO growth causes loss of sensory features and shelf life reduction of the product, while the pathogen microorganism's growth, on other hand, causes health consumer risks, for their presence or for their toxins production (Caldera, 2013). Microbial methods allow the determination of a large

variety of microorganism, providing relevant information on the microbial quality and safety of fish and others fishery products. These methods are used to detect pathogenic bacteria (*Salmonella, Staphylococcus aureus, Listeria monocytogenes, E. coli*) or indicator organisms of faecal pollution, such as *E. coli*, or other types of general contamination or poor practices, such as coliform bacteria and total viable count (Sciortino and Ravikumar, 1999).

The determination of the chemical spoilage parameters related to microbial growth are more pratical for rotine exams, since microbiological results are retrospective (Dainty, 1996). Traditional chemical techniques have the disadvantage of being usually sample destructive; they are costly and time consuming, although a range of non-destructive automated and physical instrumental methods are available, such as VIS/NIR spectroscopy and electronic nose (Boziaris, 2014).

2.3 Fish Processing Methods

Processing methods are employed in the food industry with the main objective of preserving the food quality and safety. Regulation (EC) No 852/2004, article 2, paragraph (m) defines processing has "any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes" (European Comission).

Before fish processing, important steps such as bleeding, gutting, icing or freezing, should be carried out on the boats immediately after fish capture. Such measures help to prevent or minimize the microbial and enzymatic activities, and the rancidity in case of fatty species. Several processing methods are used all over the world. Along with salting and smoking, fish drying and marination are traditional methods that can be applied, either single or in combination, to produce a stable product, with special organoleptic characteristics of high nutritional value (Boziaris, 2014). The hurdles and objectives of traditional seafood processing methods targets on this study can be seen in Table 2.

Process	Hurdle	Objective
Salting	Low a _w	Inhibition of microbial growth
Drying	Low a _w	Inhibition of microbial growth
Marination	Low pH, organic acids	Inhibition and/or inactivation of microorganisms

2.3.1 Fish marination

Recently, seafood production countries have been focused on marination and drying of underutilized fish (Eysteinsson, 2016). Marination is the process of submerging foods in a solution, which can provide the development of flavours and textures in the final product. Marinades are used for both meat and fish. Considered as a semi- preservative method for fish (Arason *et al.*, 2014) marinated fish products have a short shelf-life and must be kept cold during processing until consumption (Köse, 2010). A variety of fish species, in different sizes and shapes, such as headed, gutted and filleted, can be used in fish marination (Köse, 2010). Crustaceans, bivalves and some fatty fish as mackerel, herring, sardine and anchovies are the main fishery products used (Arason *et al.*, 2014).

Different ingredient combinations can be used to provide peculiar flavours in the products. The marinade solutions also have the purpose of preventing microbial growth and of inhibiting enzymatic activity. This is due to the effect of salt, low pH and water activity, and the use of antimicrobial agents (Arason *et al.*, 2014). A mixture of salts, sugar, spices, acids, and oil, sometimes phosphates, are the main products used in fish marination (Eysteinsson, 2016). Salt is very important to the process, since, in addition to preventing microbial growth, it also alters some attributes, such as taste, texture and structure of the fish meat (Arason *et al.*, 2014). The fish marination solutions contain usualy 6 to 18 percent of salt (Köse, 2010). The role of sugar in fish marination process is mainly to give sweet taste and the concentration used depends on the products and the markets (Arason *et al.*, 2014). To enhance the flavour, organic acid substances such as acetic, lactic and citric acid, as well as pH reducing adjuncts as soya souce, are often incorporated into acid marinated processes (Yusop *et al.*, 2010).

Different marination methods can be applied, such as cold, cooked, and fried marination, in which the first one is the most commonly used (Arason *et al.*, 2014). The cold marination consists of treating the fish in a marination bath with relatively high vinegar (acetic acid) and salt content (Capaccioni *et al.*, 2011). The salt should be used in sufficient amount to keep the fish flesh firm, while the concentration of the vinegar will determine the degree of preservation. In addition, the fish should be fully immersed in the marinating solution throughout the process, this being one of the most important marinating conditions (Arason *et al.*, 2014).

For a successful final quality of the marinated products, important factors should be considered. The composition of the marination solution, the ratio of fish to liquid and the fish treatment are decisive aspects that may affect the final quality of the products (Meyer, 1965). Arason *et al* (2014) also consider the quality of the raw materials and the ingredients used in the process to be important. On one hand, the lipid content of fish muscle, the catching method and the handling procedures on board may influence the raw material quality, and consequently the marinated product; on the other hand, bad quality ingredients may affect directly the final products. Thus, a proper handling and fast cooling on board and processing after landing, as well as a good quality and proper composition of ingredients used in the marinated products.

2.3.2 Fish drying

Fish drying it is a very popular food preserving method used in many countries. The combination of cold, dry, and low humidity in high latitudes allows the drying fish processing to be very simple and with minimal loss of raw material. However, in some tropical countries, much of the catch deteriorates before it can be consumed, especially in the wet season (Doe and Olley, 1990). A large variety of species can be used for drying, using different drying methods, depending on the species characteristics and on the intended use. Fish drying is fundamentally based on water removal by heating the product. Drying is mainly used to prolong the preservation time. The transfer of heat into the product and the removal of moisture from the surface are two important aspects in fish drying that should be considered (Arason, 2003).

Different methods can be applied, such as sun drying, solar drying, heat pump drying, freeze drying and osmotic dehydration (Nguyen *et al.*, 2014). Several factors influence the choice of the drying method by the community or the fish processor. The consumer's preference for a given product, the climate, the availability of energy sources, the effect of drying method on the nutritive value of the product, and the introduction of improved technology must be considered (Doe and Olley, 1990).

In Iceland, due to climate constraints, indoor drying of fish has been chosen instead of out-door drying. This method was initiated 25 years ago. Before that, traditionally, cod heads were dried hanging on out-door stock racks. In an in-door warm system, small fish or cod heads are dried by blowing warm air over the raw over the raw material with subsequent removal of the moisture from the product. The method has many advantages, among them the fact that is possible to dry the fish all year around, regardless of the weather conditions; also, it takes only a few days, compared to out-door drying in which the fish takes several weeks to be dried. Geothermal energy has been applied in the fishing industry and its use is likely to increase in the future. It has been mainly applied for the drying of small, salted, cod heads, fish frames and other fishery products. In Iceland, indoor drying has been tested in regions where this type of energy is available and is being used by most small dryers (Arason, 2003).

2.4 Food Preservatives

Despite several food preservation techniques, the contamination and deterioration of food by microorganisms is an important problem in several countries of the world (Leistner and Gorris, 1995). Preservatives are used to prevent microbial and chemical deterioration. Food's shelf life can be extended by as much as 200%, preventing or retarding chemical and biological deterioration. Microbial spoilage can be prevented by using antimicrobials, while antioxidants, antibrowning or antistaling agents can be used to prevent chemical deterioration such as browning, staling, and lipid oxidation (Gould and Jones, 1989).

Temperature, water activity, pH, and preservatives are factors with great influence on the microorganism's growth and survival, which are used by the main techniques of food preservation aiming at delaying or preserving microbial growth in food (Gould and Jones, 1989). Some of the food preservatives used as flavouring agents exhibit antimicrobial properties and, in general, the flavouring agents are more antifungal than antibacterial (Hauben *et al.*, 1996). Acids are used in food preservation as acidulants or antimicrobials agents. The microorganism's growth is inhibited by organic acids, through the inactivation or by affecting: the cell wall, cell membrane, metabolic enzymes protein synthesis system or the genetic material (Leistner, 1995). By acting also as chelating agents, organic acids can inhibit lipid oxidation and aid in sucrose inversion. They also have an effective antimicrobial function due to their both ability to depress pH below the growth range of microorganisms and metabolic inhibition by the undissociated acid molecules (Feng and Huang, 2001).

Acetic and citric acids, despite being inhibitants to mold growth, are mostly used as acidulant (Simpson *et al.*, 1995). Acetic acid is a better inhibitor of bacteria than of yeasts and molds (Leistner, 1995). In foods, vinegar with 5% of acetic acid is most widely used as marinades for meat, poultry, and fish (Knorr, 1995) and is also used in salad dressings, sauces and mayonnaise as flavouring agent. It is Generally Recognized as Safe (GRAS) when used at a level not more than the amount reasonably required to accomplish the intended effect (Lewis, 1989). Citric acid, on the other hand, used as a standard to evaluate the effects of other acidulants in food, is the most widely used organic acid in food industry (Knorr, 1995) as acidifier, curing accelerator, dispersing, flavouring and sequestrant agent in cured comminuted meat food product, meat (dried), etc. GRAS when used in accordance with the Good Manufacturing Practice, GMP (Lewis, 1989). Sorbitol, also known as Glucitol, is used in food as nitrite sweetener, sequestrant, stabilizer, flavouring, thickener, texturizing, curing agent, etc., in chewing gum, chocolate, candy (hard and soft), cough drops, etc. It is GRAS, with limitations, when used in accordance with the GMP (Lewis, 1989). Sucrose is used

in hog carcasses, meat, and poultry in food as flavouring or hog scald agent. It is GRAS when used in accordance with GMP (Lewis, 1989). Despite the high concentration (six times) needed for the same effect as salt, it can act in a similar way to salt exerting antimicrobial action, and is used in many food preparations (Vega-Mercado *et al.*, 1996). Soy sauce is a seasoning agent with a distinct aroma suggestive of meat extracts and salty taste. It is applied worldwide in cooking and eating, and one of the most widely Oriental fermented products consumed in Asiatic countries as a condiment and coloring agent in food preparations (Murooka and Yamshita, 2008; Luh, 1995). Traditionally, it has been used in beef marinades rather than fish marinades (Eysteinsson, 2016). In addition to certain bioactive compounds, it contains taste components such as amino acids and polyols, and related coumpounds such as flavour components (Onishi and Suzuki, 1966; Onishi and Suzuki, 1970; Kataoka *et al.*, 1997). Soy sauce also has antioxidant activity (Ando *et al.*, 2003).

3 MATERIALS AND METHODS

3.1 Raw Material

About 20 kg of frozen, headless, and gutted blue whiting (*Micromesistius poutassou*) were used in this study. The samples were obtained from Norlandia, an Icelandic fish processor, and kept in frozen storage at -18° C. Before the beginning of the experiments, the samples were thawed at low temperature, 0 to 4°C, for about 24 hours.

3.2 Experimental Design

3.2.1 Initial experiment

Before the main experiment, pre-trials were carried out to set up the best marination procedure for the main experiment. The pre-trials were carried out from 19th to 23rd December 2016, using small pieces of raw material (Figure 3). It consisted of trials using different marination combination of the ingredients: soy sauce, sorbitol, sucrose, citric and acetic acids, and water. The marination time was also determined during the pre-trials. Blue whiting samples, frozen and kept at -18°C, were thawed at 0-4°C for 24 hours. The samples were divided into six groups, based on marinade ratios, and then marinated for 12, 24, 48 and 72h. A fresh fish sample was used as control. In addition to the fish, the brine was also analyzed. For the brine control, six fish-free marinade solutions samples, with different marinade ratios and cold storage (12 and 72 hours), were used.



Figure 3: Frozen blue whiting pieces.

Physico-chemical analysis was carried out on the raw material (control), the marinated fish, and the marinade solutions (brines). The pH, moisture and salt content were measured in all fish and brine samples. The protein content was measured on both marinated fish and marinade solutions after 12 and 72 hours. The water activity (a_w) was also measured in all fish samples. The a_w , pH, moisture, and salt content were also measured in the fish control sample and in the brine controls after 12 and 72 hours.

3.2.2 Main experiment

To determine the quality and chemical composition on marinated-dried blue whiting, samples from the same batch were used. Small pieces of blue whiting were thawed and divided into five sample groups: one not-marinated, and four different groups of marination (Figure 4). While the not-marinated sample was dried directly, the other samples were marinated before drying. The marination time was 48 hours, since the marinated groups showed that equilibrium was reached after this period of incubation, during the pre-trial.



Figure 4: Flowchart demonstrating the blue whiting production process and measurement plan.

3.2.3 Sampling

In the main experiment, three sampling points were carried out, as can be seen in Figure 4. Sensory, microbial, physicochemical, and proximate analysis were performed on the raw material. The control and marinated samples were analyzed with regard to pH and a_w, and microbial and proximate analysis were carried out as well. On the last sampling point, GDA, TVC, *Listeria monocytogenes*, coliforms, a_w, colour and moisture, fat, protein and sodium chloride contents were measured in dried samples.

From raw material, 8 pieces were taken to carry out the microbial, physico-chemical, and proximate analysis, and 28 pieces for the sensory evaluation and training of the sensory panel. 4 pieces from the control sample and each marination group were taken for microbial analysis. Finally, 8 pieces of each marinated-dried group were taken to carry out the microbial, physicochemical, and proximate analysis, and for the sensory evaluations, 28 pieces were taken from each final product groups. Therefore, for this study, a total of 176 pieces of blue whiting were used.

3.2.4 Marination and group descriptions

For the marination, the samples were divided into four different marination groups, M1, M2, M3 and M4, which varied in content of ingredients as seen in the Table 3. A fifth group was used as a control, which was not incubated in a marinade solution. The incubation time was 48 hours, for all the marination groups. For this experiment, the same ingredients as in the pre-trial were used, except no acetic acid was used. The purpose of using soy sauce was to provide flavour and salt into the fish flesh, while sorbitol and sucrose was for flavour and texture, and the citric acid for reducing the pH, but also to affect the shelf-life of the final product.

	Ingredients/Ratios							
Trials	Blue whiting	Sucrose	Citric Acid (0.5%)					
Marination 1	2.500	1625	375	1500				
Marination 2	2.500	2062.5	375	750	312.5			
Marination 3	2.500	2032.5	375	750	312.5	30		
Marination 4	2.500	2500	375		625	0		

Table 3: Marinade ingredients and ratios used during blue whiting marinade incubation.

3.2.5 Drying

The blue whiting drying was carried out at an Icelandic fish drying company, Hardfisksalan. Each group samples were placed individually into a drying rack (Figure 5), and then dried in a drying cabinet (Figure 6) for four days. Afterwards, the final products were placed into bags and then transported to the laboratory to perform the laboratory analysis. The four dried post-marination groups were denominated by "MD" (marinated-dried), and "CD" (control-dried) for the control sample group.





Figure 5: Blue whiting placed on the drying racks.

Figure 6: Blue whiting samples in the drying cabinet.

During the drying process, temperature, humidity, and air speed were monitored and recorded. Inside the drying cabinet with 1.5 tonnes capacity (Figure 7) the temperature was around 17 °C. Throughout the drying period, the average air speed was approximately 3 m/s, and the relative humidity (RH) was around 44.3%.



Figure 7: Fish drying cabinet.

3.3 Sensory Evaluation

3.3.1 Generic Descriptive Analysis (GDA)

The method, as introduced by Lawless and Heymann (2010), was applied to evaluate the sensory attributes on blue whiting samples, to determine their freshness. A group of ten panellists of Matis's sensory panel performed the evaluations. In order to obtain better results, the process was divided into two steps: a. panellists training; and b. sensory evaluation.

a. Panellists training: Before carrying out the sensory evaluation sessions, the panellists were trained in the sensory attributes of marinated and dried blue whiting, according to international standards (ISO 8586:2008). Two sessions were carried out during two sampling days to perform the training on the five groups (CD, MD1, MD2, MD3, and MD4). Two different groups were used in the first session (CD and MD3), and the other three (MD1, MD2, and MD4) groups in the second session. Two pieces were attributed to each panellist

to perform the training on GDA for odour, flavour and texture attributes of marinated and dried blue whiting.

b. Sensory evaluation: Both the raw material (frozen/thawed pieces of blue whiting) and the final products were evaluated with sensory evaluation. The fresh fish was cooked (Figure 8) and then evaluated in one sensory session. The sensory evaluation of the final products was done in triplicate for marinated-dried group samples, and in duplicate for CD and MD4 group samples. A total of five sensory evaluation sessions were carried out. The sessions were performed in Matis sensory laboratory, using individual booths for each panellists and normal light (Figure 9).



Figure 8: Cooked blue whiting.



Figure 9: Individual cabin for sensory evaluation.

The freshness state of the raw material was evaluated by 8 panellists through the examination of 13 sensory attributes, on a 15 cm unstructured line scale (from "none" to "much") which in data analysis was converted to numbers from 0 to 100. The sensory attributes and their definition are shown in Table 8, in Appendix 1. To perform the sensory evaluation on the marinated and dried blue whiting, each 15 sensory attributes were evaluated by 10 panellists. The sensory attributes and their definition are shown in Table 4.

3.4 Microbial Analysis

Total Viable Counts (TVC), *Listeria monocytogenes* and Coliforms were performed to determine and quantify the microbial activity on the blue whiting samples. Analysis were performed on fresh fish, marinated fish, and marinated-dried fish, in duplicate. The data was expressed as a logarithm of the number of colony-forming units (Log cfu/g). Minced fish (20 g) was mixed with 180 g of cooled Maximum Recovery Diluent (MRD, Oxoid, UK) in a stomacher for 1 min. Successive 10-fold dilutions were done as required.

	Sensory attribute	Scale	Definition
Odour			
	Sweet	none much	Sweet odour
	Soy sauce	none much	Odour of dark soy sauce
	Dried fish	none much	Odour of dried fish/processed fish
	Rancid	none much	Rancid fish oil
Flavour			
	Dried fish	none much	Flavour of dried/processed fish
	Sweet	none much	Sweet flavour
	Salt	none much	Salty flavour
	Sour	none much	Sour flavour
	Soy sauce	none much	Flavour of dark soy sauce
	Rancid	none much	Rancid flavour
	Bitter	none much	Bitter flavour
Texture			
	Firm	soft firm	Firmness of sample, evaluated in first bite
	Rubbery	none much	Rubbery, elastic texture
	Tender	tough tender	Evaluated by chewing
	Dry	moist dry	Dry: completely dry, no moisture

 Table 4: Generic Descriptive Analysis evaluation form (Lawless and Heymann, 2010). Each attribute was rate on a scale of 0-100.

3.4.1 Total Viable Counts (TVC)

Total viable psychrotrophic counts (TVC) were performed on iron agar (IA) as described by Gram *et al.* (1987) with the exception that 1% NaCl was used instead of 0.5% with no overlay. Counts of Specific Spoilage Organisms (SSO) were evaluated on IA. Plates were spread-plated and incubated at 17 °C for 5 days. Counts of all colonies (both white and black) on IA gave the number of total count, and counts of black colonies gave the number of SSO.

3.4.2 Listeria monocytogenes

Enrichment Listeria broth base (UVM Formulation Oxoid CM0863) at 30 °C for 24 hours was done. The inoculation was done into Fraser broth at 37 °C, for up to 48 hours. Both primary and secondary enrichment cultures were streaked onto Oxford (Listeria Selective agar base Oxoid CM0856) and OCLA (Chromogenic Listeria agar base Oxoid CM1084B + supplements) agar at 37 °C for 48 hours. Confirmation tests were done on five colonies from each plate and included Gram-staining, catalase, and motility. Species identification included haemolysis on Blood agar and testing on API Listeria (System for the identification of Listeria, bioMérieux SA/France) (NMKL 136, 2010).

3.4.3 Coliforms

The Most Probable Number (MPN) method was used. The pre-enrichment was done in Lauryl Sulphate Tryptose (LST) broth, at 37 °C for 48 hours and confirmation tests were done in Brilliant Green Lactose Bile (BGLB) broth for total coliforms at 37 °C for 48 hours, and in EC broth for faecal coliforms at 44 °C for 24 hours. To perform the completed test for *E. coli*, each gassing EC tube was gently agitated, removed a loop ful of broth in tube of tryptone broth and incubated for 24

hours at 44 °C. Test for indole by adding 0.2-0.3 mL of Kovacs'reagent was done. Appearance of distinct red colour in upper layer was a positive test (NMKL 96, 2009).

3.5 Physico-chemical measurements

3.5.1 Total Volatile Basic Nitrogen (TVB-N)

A steam distillation method was used, described by Malle and Poumeyrol (1989), for the TVB-N determination. The measurements were done on the raw material and final products. For the extraction, 100 g of blue whiting muscle was mixed with 200 mL of 75% aqueous trichloroacetic acid solution, homogenized in a blender for 1 min. and then filtered through a Whatman no. 3 filter paper. The distillation was performed using a Kjeldahl-type distillator (Struer TVN). Into a distillation flask, 25 mL of filtrate was transferred and 6 mL 10% NaOH were added. The distillate was collected into an Erlenmeyer flask containing 10 mL 4% boric acid, and placed under the condenser for the titration of ammonia for 4 minutes. The boric acid solution turned green when alkalinized by the distilled TVB-N, which was titrated with aqueous 0.0372 N sulphuric acid solution (H2SO4) using 0.05 mL graduated burette. The complete neutralization was obtained when the colour turned grey/pink on the addition of a further drop of sulphuric acid. The results were expressed as mg N/100 g.

3.5.2 Water activity (a_w)

An AQUA LAB Water Activity Meter were used to measure the water activity in fresh fish, marinated and marinated-dried blue whiting samples. About 2 g of samples were filled in a clean and dry measurement plate and placed into the instrument. The a_w was automatically measured after the program started. The analysis was performed in duplicate.

3.5.3 Acidity (*pH*)

Measurements of pH in fresh, marinated and marinated-dried fish were carried out using the Bragadottir *et al.*, (2007) method. Five grams of sample was mixed with 20 mL of deionized water and stirred for 5 min. prior to measurement, with combined electrode (SE 104- Mettler Toledo, Knick Berlin Germany) connected to a portable pH meter (Portamess 913, Knick, Berlin, Germany).

3.5.4 Colour

The colour was measured in the raw material and in the final products. Three samples from the raw material and from each final product group were selected, in which four different points were evaluated. A Minolta Chroma Meter CR-400 (Minolta Camera Co., Ltd, Osaka, Japan) was used to measure the intensity of the flesh colour, using the CIE Lab system. The instrument recorded 3 variables L*, a*, and b*, in which L* represented the lightness variable, ranging from 0 (black) to 100 (white); a* represented the redness, ranging from red (a⁺) to green (a⁻); and b* variable represented the yellowness, ranging from yellow (b⁺) to blue (b⁻). Whiteness value was also calculated for all sample groups, according to the formula (Shaviklo *et al.*, 2012):

Whiteness =
$$L * -3b *$$

3.6 Proximate Analysis

Moisture, sodium chloride, protein and fat content were performed to evaluate the proximate composition in the fish samples.

3.6.1 Moisture

The moisture content was determined as the percentage of weight loss during drying at 103 °C, according to ISO 6496:1999. Moisture content was measured in fresh and marinated-dried blue whiting samples. A 5.0 g of sample was weighed and placed in an aluminum foil dish which was prepared with a thin layer of sea-sand and a glass rod. The samples were mixed thoroughly with the sand. The glass rod was kept on the dish and then left to dry for 4 ± 0.1 hour in the oven at 103 °C. The dish was removed from the oven and allowed to cool to ambient temperature in a desiccator for about 15 minutes. The dry matter content was calculated by extracting the moisture content.

3.6.2 Sodium chloride content

The sodium chloride or salt content in fresh and marinated-dried blue whiting samples was determined according to AOAC (17th ed. 2000 no 976.18). Soluble chloride was extracted from the samples with water. Upon addition of nitric acid, the solution was titrated with silver nitrate and the end point was determined potentiometrically.

3.6.3 Protein content

Protein content was determined by the Kjeldahl method (ISO 5983-2:2005). The protein content was measured in fresh and marinated-dried blue whiting samples. A 5.0 g sample was digested by sulphuric acid in the presence of copper as a catalyst. Then, the sample was placed in a distillation unit, 2400 Kjeltec Auto Sample System. The acid solution was made alkaline by a sodium hydroxide solution. The ammonia was distilled into boric acid and the acid was simultaneously titrated with diluted H2SO4. The nitrogen content was multiplied by the factor 6.25 to get the ratio of crude protein.

3.6.4 Fat content

Total lipids were extracted from 25 g of samples ($80\pm1\%$ water) with methanol/chloroform/0.88 % KCl(_{aq}) (at 1/1/0.5, v/v/v) according to the Bligh and Dyer (1959) method. The samples were weighed into 250 mL FEP plastic bottles intended for organic solvents and water was added as necessary. 25 mL of chloroform and 50 mL of methanol were added and homogenized (Ultra-Turrax T-25 basic, IKA, Germany) for 2 min in ice bath. Additional 25 mL of chloroform was added and homogenized for 1 min followed by 25 mL 0.88% potassium chloride solution and homogenized for 1 min, and centrifuged for 20 min at 2500 rpm at 0-5 °C. The lower chloroform phase containing the lipids was then filtered via disodium sulphate on a glass filter under suction. The suction flask was rinsed well and made up to mark in a 50 mL volumetric flask. The lipids were then calculated by evaporating the chloroform under nitrogen gas. The results were expressed as gram lipid per 100 g wet muscle. The lipid content was carried out on fresh and marinated-dried blue whiting samples.

3.7 Statistical Analysis

One-way ANOVA (Analysis of Variance) was carried out on the results to conduct data summaries and multiple comparisons with post-hoc corrected t-tests, between the sample groups, in MS - Excel 2016 program. To determine if sample groups differ significantly from the control sample, one-sample tests (t-test, Wilcoxon, and single-case) was performed.

To carry out the statistical analysis on sensory data, a GLM (general linear model) corrected for panellists use of scale was performed at NCSS 2000' software (NCSS, Utah, USA). Duncan's post hoc test was used to analyse statistical differences between the sample groups, and FIZZ' software (Version 2.50B, Biosystémes) to collect the data. Panelcheck V1.4.0' software (Nofima, Tromsø, Norway) was used to monitor panellist's performance. Significance of difference was defined at the 5% level (p<0.05), with marginal significance if 0.05<p<0.10.

4 **RESULTS**

4.1 Sensory and microbial quality of raw material and final products

4.1.1 Sensory evaluation

Raw Material

The sensory results from thawed and cooked blue whiting indicated that the product was rather dry and tough, with a trace of sweet and shellfish odours, and a metallic but no bitter flavour. A trace of spoilage sour odour and flavour was detected, as well as frozen storage odour and flavour. TMA odour and flavour was obvious (Table 9 in Appendix 1).

Final Products

The results of sensory evaluation of blue whiting final products are shown in Figure 10. Significant differences (p<0.05) were found for the flavour and texture attributes in blue whiting dried products, whereas no significant differences were found in terms of odour. The MD1 group had a significantly lower sweet taste compared to the MD4 group. Also, the MD1 group had lower intensity of soy sauce odour and flavour, and a very firm texture, and marginally firmer compared to the other groups (0.05). The intensity of the sweet odour was obvious, but not very strong, and higher in the MD3 group, with no significant differences from the other groups. However, a higher intensity of sour flavour, as well as a significantly higher sour flavour were detected in this group. Furthermore, this group had a significantly drier texture when compared to the MD4 group, and lower intensity of rubbery texture. The MD4 group, marinated only with soy sauce and sucrose, had the lowest intensity of sour flavour, a significantly higher sweet flavour and more moist texture compared to MD3 group, as well as the highest intensity of soy sauce flavour of the marinated-dried groups.



Figure 10: Sensory profile of blue whiting final products (o- = odour; f- = flavour; t- = texture).

The MD4 group was compared to the control-dried sample (Figure 11). The MD4 group was significantly different from the control-dried sample in most sensory attributes (p<0.05). The control had a significantly higher intensity of dried fish odour and flavour, dry texture, and salt flavour. The MD4, in turn, showed a significantly higher intensity of soy sauce odour and flavour, sweet flavour, and rubbery texture, with marginal significances (0.05) regarding the sweet odour and sour flavour. MD4 group had is higher intensity of rancid flavour.



Figure 11: Comparison between control-dried and MD4 groups (o- = odour; f- = flavour; t- = texture).

4.1.2 Microbial activity

The total viable count (TVC) in the samples after the marination and drying processes can be seen in Figure 12. The TVC found in the control sample was 5.6 log cfu/g. After the marination procedure,

the values slightly decreased, in which the M2 group showed the highest count, 5.4 log cfu/g, while the lowest, 4.4 log cfu/g counting, was observed in the M3 group. Black colonies counts were below 20 cfu/g in M2 and M3 groups; both control and M1 group got 3.0 log cfu/g; and M4 group presented 2.3 log cfu/g. After sampling drying, TVC were high in all marination groups, but the black colonies counts were below 20 cfu/g in all groups. The TVC in the control-dried was higher (8.3 log cfu/g) than in any marinated-dried groups. Of the marinated-dried groups, TVC was highest in MD4 or 7.3 log cfu/g.



Figure 12: Total viable count in the control, marinated and dried blue whiting samples.

The *E. coli* counts were below 0.3 cfu/g in all sample groups and no *Listeria monocytogenes* was detected in the raw material (control sample), nor in the samples after marination and drying procedures. However, total coliforms were detected in M2, M4, and MD4, the highest value, 1.5 cfu/g in MD4 (Table 5).

Method	Samples/Count results (cfu/g)									
	Control	M1	M2	M3	M4	Dried	MD1	MD2	MD3	MD4
Total coliforms	< 0.3	< 0.3	0.9	< 0.3	0.4	< 0.3	0.4	< 0.3	0.4	1.5
Listeria monocytogenes	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
E. coli	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3

Table 5: Microbial (total coliforms, *E. coli*, and *L. monocytogenes*) counts in blue whiting samples during different marination (M1-M4) and after drying (MD1-MD4).

4.2 Physico-chemical profile of raw material, and marinated and dried blue whiting

A sample of thawed blue whiting, that did not undergo marination process, was used as a control sample. After thawing, physical and chemical measurements were done in the control sample, and the results can be seen in the Table 6.

	Analysis								
Sample	pН	a_w	Moisture (%)	Protein (%)	Sodium chloride (%)	Fat (%)	Dry Matter (%)	TVB -N (mg N/100g)	
Raw material	7.5	0.99	78.8	20.2	0.7	0.1	21.2	32.7	

Table 6: Initial Physico-chemical profile and characteristics of raw blue whiting.

4.2.1 *Physical properties*

Acidity (pH)

The average pH in the control sample and after marination and drying, as well as in brine samples can be seen in Figure 13. Significant differences in pH were observed in blue whiting samples. After the marinade incubation, the pH values decreased significantly (p-value <0.05) for M3 (the group containing citric acid in the marinade solution) compared with the control sample, while the pH of M1, M2 and M4 remained similar as the control sample. As well as after the marination incubation, MD3 group showed a significantly lower pH value (p<0.05) than the control and other marinated groups. As well as in fish samples, same behavior was found in brine samples, in which the acid group, B3, obtained the lowest average value, 4.6, compared with the rest of the brine groups, in which the values were around 6.6.



Figure 13: pH values in blue whiting (raw, marinated and dried) and brine samples.

Water activity (a_w)

The average a_w values for marinated and marinated-dried blue whiting samples are shown in the Figure 14. After the marinade incubation, the average water activity value of all marinated groups was slightly lower than that of the control value, or 0.97 and 0.99 respectively. No significant differences were observed between marinated groups. However, after drying, the water activity drastically decreased. Significant differences were found between the control and the samples after

drying. The sample dried without been previously incubated into a marinade solution (control-dried group) obtained lowest value, 0.45. The marinated-dried groups had higher water activity, but no significant difference was observed between marinated groups.



Figure 14: Water activity in the control, marinated and dried blue whiting samples.

Colour

The average lightness observed for each sample can be seen in the Figure 15. Significant differences (p-value <0.05) were found for lightness (L* value) in blue whiting sample groups. The final products obtained higher L* values than the value found for the control sample. The highest L* value was observed in MD1 group, 80.0, which was significantly higher than the control, 58.4. The MD3 group presented the second highest lightness, 76.2, followed by MD2, with 75.4. The lowest, 62.5, L* value was obtained by MD4. No significant differences for lightness were found between the marinated-dried blue whiting groups.



Figure 15: Average L* values in control and marinated-dried blue whiting. Small letters show significant differences (p<0.05) between samples.

The average whiteness observed for each blue whiting final product can be seen in the Figure 16. The control sample presented highest whiteness than that found in marinated-dried blue whiting groups, but with no significant differences. However, the control-dried sample showed a significant highest (p-value <0.05) whiteness compared with marinated-dried groups. Between the marinated-groups, the MD4 presented the lowest whiteness, and the highest was found in MD3 group, followed by MD2, and finally, MD1. No significant differences for whiteness were found between the marinated-dried blue whiting groups.



Figure 16: Whiteness in control and marinated-dried blue whiting samples. Small letters show significant differences (p<0.05) between samples.

The average a* values obtained by the control and the dried products can be seen in Figure 17. Significant differences were observed for redness (a* value) in the group of samples. The Control group showed negative a* value (a⁻), while the groups after drying showed positive a* values (a⁺). MD1 group exhibited a significant higher (p-value <0.05) redness than the control, control-dried and MD3 groups.



Figure 17: Redness in fresh and marinated-dried blue whiting samples. Small letters show significant differences (p<0.05) between samples.

As well as for a* values, significant differences were found for yellowness (b* value) in blue whiting samples. The marinated-dried groups showed highest b* values than the control and control-dried groups. When compared to all sample groups, the control exhibited the lowest yellowness value, with MD1 and MD2 groups presenting significantly higher (p-value <0.05) values. Same was observed between dried groups, with the marinated-dried groups showing higher b* values than the control-dried sample, but no significant differences were found. The average b* values (yellowness) for each sample are shown in Figure 18.



Figure 18: Yellowness in fresh and marinated-dried blue whiting samples. Small letters show significant differences (p<0.05) between samples.

Total Volatile Basic Nitrogen (TVB-N)

The average TVB-N values found for control, control-dried and marinated-dried sample groups can be seen in Figure 19. Despite no significant differences were found between the control and marinated-dried groups, the highest TVB-N values were obtained in samples after drying. The control-dried group had a significantly higher (p-value <0.05) TVB-N value than the marinateddried groups. Among the marinated-dried groups, the MD4 group obtained the lowest value, 36.1 mg N/100g, while the MD1 group showed the highest, 60.1 mg N/100g, followed by MD3, 58.8 mg N/100g, and MD2 with 40.8 mg N/100g.



Figure 19: TVB-N in the control sample and blue whiting final products.

4.2.2 Chemical composition

Moisture

The average moisture content in blue whiting after the marination and drying process can be seen in Figure 20. Significant differences (p-value <0.05) were observed in moisture values in blue whiting sample groups. Among the marinated groups, the M4 showed the highest moisture content, 75.1%, and the lowest value, 70.0%, was obtained by M3 group. The moisture values in marinated groups were lowest than that found in the control group, 78.8%, however with no significant differences. After drying, the average moisture values in the samples decreased significantly. The control moisture value was significantly higher (p-value <0.05) than the marinated-dried groups. No significant differences were found between the marinated-dried groups. The lowest moisture value, 14.6%, was observed in MD3 group, and MD2 showed the highest value, 19.3%, followed by MD4, 18.0%, and MD1, with 15.0% of moisture content.



Figure 20: Moisture content in the control, marinated and dried blue whiting samples.

Sodium Chloride Content

The average sodium chloride content in blue whiting after the marination and drying process can be seen in Figure 21. Blue whiting group samples showed significant differences (p-value <0.05) for the sodium chloride content. Except for M3 group, that presented 0.2% of sodium chloride, all marinated groups obtained higher values than the control, 0.7%. M1 group had the highest value, 1.3%, followed by M2 and M4 groups, both with the same value, 1.1% of sodium chloride content. However, no significant differences were found between the control and marinated groups. The sodium chloride content in the final products increased significantly (p-value <0.05) compared to the marinated groups. The highest value was found in MD1 and MD3 groups, both with 3.0%, followed by MD2 and MD4 groups, with a sodium chloride content of 2.9%. Significant differences (p-value <0.05) were also found when comparing control-dried and marinated-dried groups, in which the average value found for control-dried, 2.0% of sodium chloride, was lower than the marinated-dried groups.



Figure 21: Sodium chloride content in the control, marinated and dried blue whiting samples.

Protein Content

The average protein content values observed in blue whiting after the marination and drying process, and in brine samples can be seen in Figure 22. Significant differences (p-value <0.05) were observed for the protein content in blue whiting samples. After the marinade incubation, the protein content was significantly lower than that of the control sample. M3 group reached the highest value, 16.8%, and the lowest was found in M2 group, 15.1% of protein content. After the drying process, however, the values increased significantly (p-value <0.05), when compared to those found in marinated fish groups. The control-dried sample achieved the highest value, 75.8% of protein content. As well as after the marination step, the MD2 final product group remained with the lowest average value, 42.3% of protein content. The highest value was obtained in MD1, 49.9%, followed by MD3, 46.0%, and finally MD4 group with 45.4% of protein content. Among brine samples, the acid group, B3, had the lowest protein content or 1.5%, B4 group showed 1.6%, and in both brine groups B1 and B2, the average values were slightly higher, 1.7% of protein content.



Figure 22: Protein content in the control, marinated and dried blue whiting samples.

Fat Content

The average fat content values observed in blue whiting after the marination and drying process can be seen in Figure 23. No significant differences were found in the blue whiting samples regarding the fat content. After the incubation into the marinade solution, all blue whiting groups showed higher values than that found in the control, 0.1%. Three of the four marinated groups, M2, M3 and M4, obtained the same value, 0.3%, while M1 reached the higher value, 3.5% of fat content. After drying, the protein content in the final products had slightly increased, except for the M1 group, in which the value decreased to 1.1%. Despite this, no significant differences were found between the dried blue whiting groups.



Figure 23: Fat content in the control, marinated and dried blue whiting samples.

Mass Balance

The mass balance for the blue whiting samples was followed on protein basis through the processing (marination and drying; Table 7). The protein yield stands for the total amount of protein in the fish after drying as a percentage of the protein in the marinated fish (fresh for the control fish). High protein yield was observed in all the groups or about 90 - 107%. The amount of carbohydrates in the marinated and dried blue whiting was estimated by subtracting from the dry matter, protein and salt content. The carbohydrate compounds in the raw material was 0.3%. After marination, the highest carbohydrate level was observed in M3 group, 12.7%, followed by M1, 9.8%, M2, 8.8%, and M4, with the lowest percentage 7.2%. After drying, the control-dried sample had the lowest carbohydrate, 7.8%, while the higher value was observed in the acid group (M3) with 36.4%, followed by M2, 35.5%, M4, 33.7%, and the lowest in M1 group, 32.1%. The results are shown in Appendix 2.

		_			
Sample	Before	After	In brine	After	Yield of protein
	marination	marination		drying	(%)
Raw material	530.0 g	NA	NA	504.1 g	95
M1-MD1	505.0 g	435.6 g	52.9 g	464.8 g	107
M2-MD2	505.0 g	432.8 g	52.7 g	412.8 g	95
M3-MD3	505.0 g	450.9 g	48.7 g	408.0 g	90
M4-MD4	505.0 g	474.4 g	48.9 g	424.9 g	90

Table 7: Mass balance on protein basis during processing.

5 DISCUSSION

5.1 Sensory and microbial quality of raw material and final products

In this study, significant differences were found mainly for flavour attributes in blue whiting final products. Sucrose had a distinct effect on the flavour attributes of marinated-dried products. Significantly less sweet taste was found in MD1 group since no sucrose was added to the marinade solution, in comparison with the MD4 group, marinated in a higher level of this sucrose. However, this group had higher soy sauce flavour value. The addition of sucrose to the marinade solution increased the concentration gradient between the product and the marinade which allows a faster diffusion of other solutes into the product (Deumier, 2000). As well as sucrose, the citric acid added to the marinade solution had an effect on the sensory attributes of MD3 group, which had more sour, flavour and drier texture. The MD1 group had a very firm texture, with marginal differences compared to the other groups, possibly because of the influence of sorbitol added in higher level than in the other marinade solutions.

The marination had a significant effect on the final products in terms of odour, texture, and flavour attributes, since one marinated-dried sample, the MD4, was used to compare with the control-dried sample sensory attributes. Thus, the marinated-dried group had significantly higher intensities of soy sauce odour and flavour and sweet flavour. Moreover, the control-dried had a significantly higher intensity of dried fish odour and flavour, and dry texture than MD4, which had a rubbery texture due to the marinade uptake during the marinade incubation. In addition, a higher salt flavour intensity

was found in the control-dried sample. Similar results were found in a previous study with marinateddried blue whiting (Eysteinsson, 2016), which related that the moisture loss causes the sodium chloride crystallization because of the full saturation of the aqueous phase on the surface of the product, leading to a saltier taste. Bitter flavor, and rancid odour and flavour were not detected in a significantly level in the blue whiting final products.

Total Viable Count (TVC) in raw blue whiting used in this study was 5.6 \log_{10} cfu/g. After the marinating processes, lower TVC was detected in all sample groups. The marinade incubation for 48 hours induced a visible reduction of TVC in the group marinated with 0.5% citric acid, which achieved a reduction rate of 1.2-logs in comparison with the control raw material. No significant effect of marination process on the reduction rates, however, were observed for the other sample groups. A reduction rate of 0.5-logs was achieved by both marinated groups with soy sauce and 41% sorbitol, and with soy sauce and sucrose; while only 0.3-logs was achieved by the group marinated with soy sauce, 41% sorbitol, and sucrose. In previous studies, a considerable reduction on TVC was reported after sardine fillets marination (Kilinc, 2003), and reduction rates of 1.55- and 1.7-log were detected for Pacific saury (*Cololabis saira*) marinated with 2 and 3% acetic acid, respectively (Sallam *et al.*, 2007).

The TVC increased in all sample groups during the drying process (4 days at 17° C, in an indoor chamber). The TVC increased the most in control-dried by 2.7-logs, in comparison to the raw material. Of the marinated-dried groups, the highest TVC increase was found in MD4 by 2.2-logs, followed by MD3, 1.4-logs, MD2, 0.6-logs, and the lowest in MD1 by 0.4-logs. A higher concentration during the drying period due to loss of water may have caused this increase. The drying process is not lethal per se to microorganisms, although some of them are destroyed by this process, and many types may be recovered from dried foods, indeed, especially if proper practices are not followed in the drying steps. Thus, bacterial endospores, yeasts, molds, and many Gram-negative and Gram-positive bacteria may survive and multiply (Jay *et al.*, 2005). However, despite this increase, the final products TVC counts did not exceed 100,000 per gram, which is the goal for dried foods (Jay *et al.*, 2005) and was in accordance with the guidelines for pathogens in seafood (Huss, 1994; Table 5-6).

Specific Spoilage Organisms (SSO) were also found in raw material, as well as in samples after marinade incubation for 48 hours. The SSO number was reduced after the drying process, with all final products showing black colonies counts below 20 cfu/g, which can be considered a satisfactory number, according to the guidelines for ready-to-eat foods (Forsythe, 2011; Table 6.14). This reduction is important to prevent spoilage (Caldera, 2013).

Microbial indicators, such as coliforms, are often used to assess food safety and sanitation (Jay *et al.*, 2005). In this study, the highest coliforms counting, 1.5 cfu/g, was detected in a dried sample group. According to Jay *et al.* (2005), it is generally agreed that the coliform count of dried foods should be zero or nearly so. However, low numbers of coliforms are permitted in sensitive foods at numbers ranging 1 to not over 100/g. In both marinated and dried blue whiting products, the results for *E. coli* were in accordance with the guidelines for pathogens in seafood (Huss, 1994; Table 5-6), also with the guidelines for ready-to-eat foods (Forsythe, 2011; Table 6.14). No *L. monocytogenes* was detected, which satisfies the general agreement that little or no growth of this organism should be tolerated in food (Huss, 1994), although Forsythe (2011) pointed out an acceptable number of 20- $<10^2$ (guidelines for ready-to-eat foods, Table 6.14).

5.2 Physicochemical profile of marinated and dried blue whiting

In this study, while three groups of sample were marinated with soy sauce, one group was marinated with citric acid, in addition to soy sauce to lower pH. The pH of raw blue whiting was 7.5. After the marination, the three soy marinated groups had a pH of around 7.0 while the citric acid + soy sauce marinated group had a pH of 5.1. The pH measured in the marinade solutions was also lower, 4.6, in the acid group when compared with the other marinade solutions which had a pH of 6.6 (B1 and B2) and 6.1 (B4). After the marinating process and drying, the acid group had a pH of 5.5. Despite the decrease in pH resulting from marinating and from drying process in all group of samples, Arason *et al.* (2014) recommend that the pH of the marinade solution should be kept lower than 4.5, since at acidic pH ($1.0 < pH \le 4.5$) all food poisoning bacteria and most spoilage bacteria are prevent from growing. According to the same author, acetic acid is the most efficient, and the most frequently used acid for this purpose. Previous studies confirm significant reduction in pH using acetic acid into the marinade solutions. Baygar *et al.*, (2010), reported a sea bass fillets pH decreased after 5 hours, with significant changes, after 36 hours' storage in marinade solution containing acetic acid. Sallam *et al.* (2007) also reveled a significant pH reduction, about 2 units, after Pacific saury (*Cololabis saira*) marination in acetic acid.

The water activity (a_w) in raw blue whiting used in this study was 0.99. The marination processing showed no significant effect in the aw. Notwithstanding the marinated blue whiting products presented slightly lower aw in comparison with the raw material, no significant differences was found, as well as among the marinated groups. Drying processing, on the other hand, had a significant effect in the final products a_w in which the values decreased significantly compared to the control raw material. After drying, a significant lowest aw, 0.45, was achieved by control-dried sample, compared to the raw material. Comparing the marinated and marinated-dried groups, the group marinated with soy sauce and sorbitol had the highest reduction rate, from 0.97 to 0.64, followed by soy sauce, sorbitol and sucrose group, 0.98-0.68; and soy sauce, sorbitol, sucrose and citric acid group, 0.97-0.70. The lowest a_w reduction rate was observed in the group marinated with soy sauce and sucrose, from 0.98 to 0.73. In a previous study, Eysteinsson (2016), also reported a decrease in water activity after drying processing in marinated and dried blue whiting. To avoid microbial growth, dried fish products water activity should be maintained below the critical value of 0.60 (Perera and Rahman, 1997). However, although yeasts and moulds organisms are more tolerant at a reduced a_w and can grow at value above 0.62, the pathogenic bacteria cannot grow at a_w below 0.85-0.86 (Rahman and Labuza, 2007).

In this study, the lightness, whiteness, redness, and yellowness did not differ significantly between the final marinated products, but significant differences were observed between these groups and the control group. The final marinated products had higher lightness, redness, and yellowness, but lower whiteness, compared to the control group. The MD1 group had a significantly higher lightness, redness and yellowness values from the control groups, possibly due to the combination of soy sauce and a higher level of sorbitol used in the marinade solution. The MD4 group exhibited the lowest lightness, whiteness and yellowness values, probably because a higher level of sucrose in the marinade solutions. The marinade ingredients had an impactful effect in the final products for redness, in which these groups exhibited a higher red colour, while the control sample showed a lower redness value. The acid group (MD3) showed a lower redness value indicating that addition of citric acid to the marinade solution decreased the redness in the products. In a previous study of marinated-dried blue whiting (Eysteinsson, 2016), similar trend was observed for higher lightness

and redness of the final product related to soy sauce. However, according to Eysteinsson (2016), the combination of high level of soy sauce and lemon juice seemed to increase the redness in the products. The different color changes observed in the final products can be explained by the different combinations of ingredients in the marinade solutions. Moreover, food containing reducing sugar undergo a colour change known as *Maillard* reaction and these compounds are directly involved in nonenzymic browning (Jay *et al.*, 2005). Soy sauce, the main ingredient used in this research, contains about 5% reducing sugars (Luh, 1995).

In this study, higher TVB-N content was detected in both control-dried and marinated-dried blue whiting groups, compared to the raw material. The control-dried sample had a significantly higher TVB-N content. No significant differences were observed between the marinated-dried groups. According to the Commission Regulation (EC) No 2074/2005, the limit of TVB-N content for species of the Gadidae family, in which blue whiting belongs, is 35 mg of nitrogen/100g of flesh. These results were in line with a previous study with marinated-dried blue whiting (Eysteinsson, 2016). In the study, the higher TVB-N value found after marination was explained by the presence of soy sauce as the factor that influence the increase of TVB-N after marination, since it contains 1.5% total nitrogen, of which about 45% are in amino acids form that can influence the TVB-N formation. Eysteinsson (2016) also reports, in a previous study using the same warm indoor drying method, the formation of TVB-N of the control group, due to higher temperature and longer processing time, which increase the TVB-N rate in the final products.

5.3 Drying post-marination influence in the profile of final products

In this study, drying had a dramatic effect on the profile of the final products. The greatest influence of drying occurred in the M1 group, since after drying the main changes in chemical composition were observed for this group. Higher reduction rate of moisture and lower sodium chloride uptake, as well as a higher protein, compared to the other marinated-dried groups were observed. This group also had a great reduction rate in the fat content, despite not statically significant. These results can possibly be related to the higher sorbitol content combined with soy sauce, and the fact that no sugar was added in the marinade solution, affected by drying processing. The addition of citric acid and sucrose, combined with lower sorbitol content seemed to play an opposite effect, since the M3-MD3 group had the lowest loss of moisture and higher sodium chloride uptake. Different results for moisture and sodium chloride contents were reported in a similar study with marinated-dried blue whiting (Eysteinsson, 2016), in which the high loss of moisture was related to a higher sodium chloride content. However, many factors can influence the moisture and sodium chloride content in foods. On one hand, the water diffusion in foods is controlled by factors such as the size of water molecule and of the pore in the product, and the presence of other molecules that water can collide with in the vapour phase (Labuza and Hyman, 1998), on the other hand, factors such as species, thawing, brine concentration, fillet thickness, fish size can influence the salt uptake (Baygar et al., 2010).

In food products, drying seems to have negative impacts on the nutritional properties. Changes in the proteins and lipids of the fish muscles leads to a decrease in the fish nutritional value. On one hand, during drying process, a protein denaturation/aggregation leads to changes in amino acid composition, protein solubility and digestibility. On the other hand, lipid oxidation can lead to a degradation of fatty acids as PUFAs (Boziaris, 2014). Significant changes in the protein nutritional value can be caused by the interaction between lipid oxidation products and protein constituents such

as amino acid and peptides (Surono, 1991). Moreover, changes in the texture during drying process can develop due to loss of water and changes in proteins and lipids of the fish muscle (Boziaris, 2014).

The different combinations of the ingredients used in the marinade solutions had a visible effect on the mass balance of the blue whiting during processing. After marination, the acid group (M3-MD3) had lower moisture and sodium chloride contents, and higher protein content, compared to the raw material. A decrease in the pH muscle leads to a reduction in the repulsion of structures within the myofibril, which allows that structures to pack more closely, and thus resulting in a decrease on water holding capacity (WHC), and consequently to water losses from the muscle. Also, low concentration of NaCl and low pH can affect the WHC, increasing the water loss (Hussain, 2007). This group reached the highest carbohydrate compounds, estimated on the base of the results of dry matter, moisture, and salt contents, while the raw material had the lowest, since it did not undergo marination.

5.4 Project applicability in Cape Verde

In addition to development of an innovative and added-value product for human consumption in Iceland, for both national market and for export, it is intended that this study can also be very important for Cape Verde. Fisheries products have great importance for the Cape Verdean's diet both because of its good nutritional composition, and because it is inexpensive compared to beef or pork meat, which causes it to be consumed by all social classes in the country. Therefore, adding value to a very appreciated and consumed product could be positively accepted by Cape Verdean's society.

Regarding the fish marinating aspects, it is a preservation method that can certainly be applied in the country. The marinade solution ingredients can easily be purchased as well as the fish species recommended for the best marination results. Considering the overexploitation of some fish species in the country, this project can be applied for poorly exploited fishery resources and thereby alleviating pressure on target species traditionally exploited as Atlantic mackerel or some tuna species. Besides that, because of the low diversification in fish processing methods, as was said before, the techniques performed within the scope of this study can therefore be adopted to improve the fish processing in the country.

In this project, to complement the marination and produce a safe final product, the fish was dried for four days, using the geothermal and electric energy. In the case of Cape Verde, the sun drying may be applied as a better option in view of the many problems with electric energy that the country has faced in the last years. Additionally, since marinated and dried fish is not an industrial fishery product, but mainly canned fish, it can be an innovative fishery product, produced on an industrial scale for national consumption as well as for export, bringing social and economic benefits to the country.

6 CONCLUSIONS AND RECOMMENDATIONS

The microbiological quality of blue whiting after processing was considered satisfactory and the products presumptively safe to eat, considering the low number of SSO, which are related to the quality and process indices; the absence of *Listeria* and a low number of *E. coli*, both pathogenic bacteria related to food safety index; the low number of total viable count and coliform bacteria, which are indicators of general contamination or poor processing practices.

The marination had a visible impact on the sensory attributes of the final products. The soy sauce together with sucrose intensified the sweetness flavour; soy sauce also had an important influence on the odour attribute; citric acid enhanced the intensity of sour and bitter flavours, also the texture. The marinade uptake was responsible for a higher rubbery texture compared to the control-dried sample, which ended with higher dry texture, as well as higher dried fish odour and flavour.

The marinated-dried blue whiting tended to be higher in lightness, redness, and yellowness, but lower whiteness, compared to the control. Drying and marinade ingredients played an important influence in these colour changes. Soy sauce, a reducing sugar agent, had an impactful effect in the redness, enhancing the red colour, while the citric acid, in turn, seems to have an inverse effect, decreasing the redness in the products.

Different combinations and amounts of the marinade ingredients led to visible changes in the mass balance of protein, sodium chloride, moisture, carbohydrates compounds, as well as in the protein yield, during marination and drying processing.

For future studies on marinated and dried blue whiting, several attributes should be considered. The soy sauce should be used at a low level, in order to minimize chemical changes, since the reducing sugars are involved in nonenzymic browning leading to colour changes in the products. The moisture content should be kept at a proper level during marination to avoid microbial growth, but at the same time, decreasing the hardness of the product after drying. Considering the lack of tradition related to sweet-savoury dishes in the Cape Verdeans' culinary, less amount of sugar compounds should be used in the marinade solutions in order to minimize the sweetness flavour on the final products.

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APPENDICES

Appendix 1: Sensory analysis in blue whiting.

	Sensory attribute	Scale	Definition	
ODC	DUR			
	Sweet	none much	Sweet odour	
	Shellfish, algae	none much	Shellfish, algae odour, characteristic fresh odour	
	Frozen storage	none much	Frozen storage, cold storage odour	
	TMA	none much	TMA, dried fish, amine	
	Spoilage sour	none much	Spoilage sour, sour milk, vinegar, butyric acid	
FLAVOUR				
	Metallic	none much	Characteristic metallic flavour of lean, white fish	
	Sweet	none much	Characteristic sweet flavour of lean, white fish	
	Frozen storage	none much	Frozen storage, cold storage, cardboard, rancid	
	Bitter	none much	Bitter flavour	
	Spoilage sour	none much	Spoilage sour flavour	
	TMA	none much	TMA, dried fish, amine	
TEX	TURE	-		
	Juicy	dry juicy	Dry - draws juice from mouth	
	Tender	tough tender	When chew a few times	

Table 8: Generic Descriptive Analysis scale for cooked blue whiting.

Table 9: Generic Descriptive Analysis results for cooked blue whiting (score scale: 0-100). Average values of 8 trained panelists.

	Sensory attribute	Average score
ODOUR		
	Sweet	16
	Shellfish, algae	19
	Frozen storage	16
	ТМА	23
	Spoilage sour	9
FLAVOUR		
	Metallic	10
	Sweet	20
	Frozen storage	16
	Bitter	5
	Spoilage sour	4
	ТМА	27
TEXTURE		
	Juicy	24
	Tender	38

Appendix 2: Proximate composition and dry matter.

	Marinated fish							
Samples	Water content (%)	Dry matter (%)	Protein (%)	Salt content (%)	Carbohydrates (%)			
Raw material	78.8	21.2	20.2	0.7	0.3			
M1	73.4	26.6	15.5	1.3	9.8			
M2	75.0	25.0	15.1	1.1	8.8			
M3	70.3	29.7	16.8	0.2	12.7			
M4	75.1	24.9	16.6	1.1	7.2			

Table 10: Proximate composition and dry matter in marinated blue whiting.

Table 11: Proximate composition and dry matter in dried blue whiting.

	Dried fish						
Samples	Water content (%)	Dry matter (%)	Protein (%)	Salt content (%)	Carbohydrates (%)		
Control-dried	14.4	85.6	75.8	2.0	7.8		
MD1	15.0	85.0	49.9	33.7	32.1		
MD2	19.3	80.7	42.3	2.9	35.5		
MD3	14.6	85.4	46.0	3.0	36.4		
MD4	18.0	82.0	45.4	2.9	33.7		