

## EFFECTS OF TURMERIC ON QUALITY ATTRIBUTES OF BRINE SALTED DRIED ATLANTIC POLLOCK (*Pollachius virens*)

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### ABSTRACT

Fish is a major source of protein but is highly perishable and so preservation methods to slow down spoilage are crucial. Salt drying is a simple traditional method used to preserve fish in Sri Lanka. Partial substitution of salt with spices as preservative could result in better products of salted dried fish due to antioxidant, and antimicrobial attributes of particular spices. Further, it may improve food attractiveness by adding flavours and colours. In this study, the effects of turmeric in salted dried fish production at 1% and 2% brine concentrations were studied during the processing after drying of Atlantic pollock (*Pollachius virens*). The effects on sensory attributes (appearance, odour, flavour and texture), microbial quality (Total Viable Counts and Specific Spoilage organisms), physico-chemical profile (water activity, water content, salt content, pH, TVB-N, Fat content, FFA, PV and TBARS) were evaluated. The results showed that the turmeric had a clear masking effect on spoilage odour and flavour of the dried product. The fillets were still fresh after brining, but after incubation and drying, a clear difference in spoilage flavour and odour was seen between turmeric treated samples and samples brined in turmeric free salt solution. Turmeric also influenced colour as a natural colour enhancer. After drying, lower level of TVB-N was found in the dried turmeric treated fish compared with the turmeric free dried sample. The addition of turmeric did not have an impact on water content, salt content, water activity, fat content and yield, but the 2% brined samples had higher salt content, higher yield, lower water content and lower water activity compared to 1% brined samples. The FFA values increased and phospholipids decreased during the processing, but no significant variation was observed between the sample groups. The PV and TBARS values indicated that turmeric acted as prooxidant instead of antioxidant for the turmeric concentration used.

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## TABLE OF CONTENTS

1	INTRODUCTION .....	7
1.1	Background and rationale.....	7
1.2	Objectives.....	8
2	LITERATURE REVIEW .....	9
2.1	Atlantic pollock.....	9
2.1.1	Biology.....	9
2.1.2	Distribution .....	10
2.1.3	Capture and production.....	10
2.2	Fish quality.....	10
2.2.1	Sensory evaluation .....	11
2.2.2	Microbiological evaluation .....	11
2.2.3	Biochemical Evaluation .....	11
2.3	Lipid Oxidation .....	12
2.4	Fish preservation .....	12
2.4.1	Salted dried fish production.....	12
2.4.2	Use of spices in salted dried fish production .....	13
2.5	Turmeric .....	13
2.5.1	Utilization of turmeric.....	13
2.5.2	Limitations in use of turmeric.....	14
3	RESEARCH METHODOLOGY .....	14
3.1	Raw materials.....	14
3.1.1	Fish.....	14
3.1.2	Turmeric.....	14
3.1.3	Salt .....	15
3.1.4	Test with salted dried fish sample from Sri Lanka .....	15
3.1.5	Effects of unfiltered versus filtered turmeric brine on fillet appearance .....	15
3.1.6	Selection of salt and turmeric concentrations for main trial .....	15
3.2	Main experiment .....	16
3.3	Physio-chemical analysis .....	18
3.3.1	Weight and Colour measurements .....	18
3.3.2	Moisture content .....	19
3.3.3	Water activity.....	19
3.3.4	Salt content.....	19
3.3.5	Acidity (pH level) .....	19

3.3.6	Protein yield .....	19
3.3.7	TVB-N .....	19
3.3.8	Lipid content .....	20
3.3.9	Free Fatty Acids .....	20
3.3.10	Phospholipids .....	20
3.3.11	TBARS .....	21
3.3.12	Peroxide value .....	21
3.4	Microbiological analysis .....	21
3.4.1	Sample preparation .....	21
3.4.2	Total plate count (TPC) and Specific spoilage organisms .....	21
3.5	Sensory Analysis .....	22
3.6	Statistical analysis .....	23
4	RESULTS AND DISCUSSION .....	23
4.1	Pretrial results .....	23
4.1.1	Test with sample from Sri Lanka .....	23
4.1.2	Testing the turmeric powder samples .....	24
4.1.3	Effect of filtered brine containing turmeric on fillet appearance .....	24
4.1.4	Selection of brine and turmeric concentration for main trial .....	25
4.2	Main experiment .....	27
4.2.1	Physiochemical profile of raw material .....	27
4.2.2	Physiochemical analysis .....	28
4.2.3	Microbiological analysis .....	37
4.2.4	Sensory analysis .....	38
5	CONCLUSIONS .....	39
6	LIST OF REFERENCES .....	42
7	APPENDIX .....	45

## LIST OF FIGURES

Figure 1. Atlantic pollock ( <i>Pollachius virens</i> ) .....	9
Figure 2. Drying cabinet at Matis .....	16
Figure 3. Flowchart demonstrating the salted fish production process and measurement plan .....	17
Figure 4. Arranging pollock fillets in drying racks.....	17
Figure 5. Fish fillets arranged in drying cabinet .....	18
Figure 6. Taking colour measurements using a Minolta chroma meter CR-400.....	18
Figure 7. Individual cabin for sensory evaluation .....	22
Figure 8. Cooking fish fillets for sensory evaluation.....	22
Figure 9. Marinated /brined 24 hours in unfiltered 10% brine solution with 2% turmeric powder.....	25
Figure 10. Marinated/brined 24 hours in filtered 10% brine solution with 2% turmeric powder .....	25
Figure 11. The fillets marinated in 0.5%, 1% and 2% turmeric concentrations .....	26
Figure 12. Yield change with salt concentration of brine .....	26
Figure 13. Changes in b value of fish fillets during processing.....	27
Figure 14. The percentage of the yield change during the processing of brined dried pollock in different conditions of brining applied .....	28
Figure 15. Average L values for different sample groups at different sampling points .....	29
Figure 16. Average a values for different sample groups at different sampling points.....	29
Figure 17. Average b values for different sample groups at different sampling points.....	30
Figure 18. Changes in moisture content of fish while processing the brine salted dried pollock .....	31
Figure 19. Changes in water activity of pollock during the brined dried fish production .....	31
Figure 20. Changes in salt content of pollack while processing.....	32
Figure 21. Changes in pH level of pollock while processing .....	33
Figure 22. The TVB-N value of the raw material and the final products of pollock.....	33
Figure 23. Changes in lipid content of pollock while processing.....	34
Figure 24. Changes in FFA of pollock while processing .....	35
Figure 25. Changes in phospholipids content of pollock while processing.....	36
Figure 26. Changes in peroxide values (PV) of pollock while processing.....	36
Figure 27. Changes in Thiobarbituric acid reactive substances (TBARS) of pollock while processing .....	37
Figure 28. Changes of total bacteria count in Pollock fillet during the processing .....	38

## LIST OF TABLES

Table 1. Nutrition facts of saithe.....	10
Table 2. The information on turmeric samples tested and the tests carried out.....	14
Table 3. Scale used to evaluate strength of spoilage odour, spoilage flavour and salty taste..	23
Table 4. Water content, salt content and water activity values of salted dried fish sample from Sri Lanka.....	24
Table 5. Microbiological analysis results of turmeric samples.....	24
Table 6. Values of water activity, water content and salt content in fillets brined in different salt concentrations.....	27
Table 7. Physiochemical profile of raw material .....	28
Table 8. Changes of SSO in pollock fillet during the processing.....	38

## ABBREVIATIONS

aw	- Water Activity
CFU	- Colony-Forming Units
DHA	- Docosahexaenoic acid
DNA	- Deoxyribonucleic acid
DRCB	- Dichloran Rose-Bengal Chloramphenicol Agar
EPA	- Eicosapentaenoic acid
FAO	- Food and Agricultural Organization of the United Nations
FEP	- Fluorinated ethylene propylene
FFA	- Free Fatty Acids
GDA	- Generic Descriptive Analysis
IA	- Iron Agar
ISO	- International Organization for Standardization
LOG	- Logarithm
MFARD	- Ministry of Fisheries and Aquatic Resources Development
NARA	- The National Aquatic Resources Research and Development Agency
NOAA	- National Oceanic and Atmospheric Administration
PAB	- Pseudomonas Agar Base
pH	- Potential of Hydrogen
PV	- Peroxide Values
RPM	- Revolutions Per Minute
SLS	- Sri Lanka Standard
SSO	- Specific Spoilage Organisms
TCA	- Trichloroacetic Acid
TEP	- 1,1,3,3-tetraethoxypropane
TSC	- Tryptose Sulphite Cycloserine Agar
TVB-N	- Total Volatile Basic Nitrogen
TVC	- Total Viable Count
UV	- Ultraviolet
WHO	- World Health Organization

## 1 INTRODUCTION

### 1.1 Background and rationale

Fish is a major source of protein and it provides livelihood for millions of people who are engaged in harvesting, handling, processing and distribution. Fish is highly perishable, which can be noticed by rapid decline of its quality after harvest, if handled improperly and not processed on time (Mahmud, et al., 2008). The spoilage mechanisms can be driven by microbial growth, enzymatic activities or chemical reactions. Thus, the preservation methods aimed to stop the various spoilage patterns should target these three triggers in different approaches (Mahmud, et al., 2008). The preserved fish products help to meet the demand of fish protein during off seasons of fishing, utilize excess catch and improve consumer preference in terms of taste, flavour and quality.

Sri Lanka is an island located in Indian ocean and known as “Pearl of the Indian Ocean”. It is 65,610 km<sup>2</sup> in surface area. In Sri Lanka, the fisheries sector is important in development of economic and social lifestyle by providing direct and indirect employment opportunities for about 560,000 people and livelihoods for more than 2.7 million people in coastal communities (NARA, 2017).

Sri Lankan fisheries sector affords more than 60% of the animal protein consumed by people in the country (NARA, 2017). Commonly fish is purchased in Sri Lanka as fresh, dried or canned. In year 2016, the per capita fresh, dried and canned fish consumptions in Sri Lanka were 11.8, 3.6, 1.4 kg/year respectively (MFARD, 2018). This shows that a significant amount of the fish protein requirement is met by dried fish.

Fish preservation techniques in practice around Sri Lanka include chilling, freezing, salting, drying, smoking, salting and drying, fermenting, and canning. Salting and then drying is a combined, simple, traditional and commonly used method to preserve fish in Sri Lanka (Ariyaratna, 2011). The principle of salting process is preserving food by removing the moisture and creating an environment unsuitable for microbial growth. Most bacteria cannot grow in high salt concentrations (Moncel, 2019). Salt content of dried fish samples taken from the markets ranged from 14.05% to 17.41% which is above the Sri Lankan Standard (SLS), accepted maximum salt content of 12% (Nuwanthi, Madage, Hewajulige, & Wijesekera, 2016). Dried fish production in Sri Lanka uses large amounts of salt because the drying takes a long time and salting is used to hinder spoilage during processing and handling as well as to shorten the drying time.

Salt molecules are composed of both sodium and chloride, in which sodium makes up to 40% weight of the molecule. Sodium (Na<sup>+</sup>) is micronutrient essential for life and the recommended level of sodium intake by the World Health Organization (WHO) is maximum of 2 g per day for adults (≥16 years old) equivalent to 5 g of salt (NaCl) per day. Use of excess amount of salt can have negative health effects (Dharshini, Priyadarshini, Baskaran, & Raj, 2018). High levels of sodium consumption contribute to increased blood pressure and a consequent higher risk of cardiovascular and renal disease. Because of the positive correlation between sodium intake and the incidence of hypertension and diabetes, especially in older people, dried fish production with low salt has become a critical requirement for public health security. Production of dried fish with low salt could reduce the salt intake of the general public and

therefore have beneficial public health impacts (Nuwanthi, Madage, Hewajulige, & Wijesekera, 2016).

Partial substitution of salt with spices as preservative could reduce the salt content of dried fish. There are many spices which are suitable salt replacements due to their antioxidant activity, bioavailability enhancement, antimicrobial, hypolipidemic, antidiabetic, anti-inflammatory, anticarcinogenic, neuroprotective, antiallergic, antibacterial, antifungal and antiaging activity. They help fight against diseases such as osteoporosis, DNA damage and heart diseases (Shilpa, Soni, & Pandya, 2018). In addition, spices improve food attractiveness by adding flavours and colours. The current experiment is to investigate the potential of using turmeric in fish preservation in Sri Lanka.

Turmeric is a promising source of natural antioxidants, as indicated by high content of polyphenols, flavonoids, tannins, and ascorbic acid and by its considerable DPPH free radical-scavenging activities and fluorescence recovery after photobleaching values (Tanvir, et al., 2017). Turmeric has also been found to have antimicrobial properties and can be used to prevent of bacterial growth (Niamsa & Sittiwet, 2009).

The main fish species used in salted dried fish production in Sri Lanka are trevally, Spanish mackerel, sardinella and skipjack tuna (Anonymous, 2013). The lipid content of the above species in Sri Lanka is 1.5%, 3.3%, 9.4% and 16.2% respectively (Devadason, et al., 2016). Among those, trevally (*Caranx ignobilis*) is the most common fish processed in the north of Sri Lanka. Trevally has a selling price and is in high demand in Northern Sri Lanka. A similar species to trevally it in appearance, size, texture and colour of muscles and composition, Atlantic pollock (*Pollachius virens*) was selected for this research. Fat content significantly changes quality of raw and salted dried fish products as due to the influence of oxidation (Azhar & Nisa, 2006). So, the Atlantic pollock being a lean fish with fat content of about 0.2 – 0.3% is suitable for the present study as having similar fat content as trevally fish ( (Gashti, 2002). The Atlantic pollock is readily available in Iceland.

Most studies carried out on the use of turmeric in salted dried fish production have been based on dry salting method. A comparative study to analyse salting procedures for salted dried herring shows that brining before dry salting increases the weight in fish fillets and protein yield and decreases the salt uptake compared to single dry salting method (Ariyaratna, 2011). This may be due to the uniform and effective diffusion of salt into the fish fillets. Therefore, the present study will use wet salting (brining) to test the effect of turmeric on sensory, microbiological and physico-chemical changes in salted dried fish.

No literature work was found during a literature search regarding effects of turmeric on quality or shelf life of brine salted dried fish and therefore the present study is the first attempt to study the effect of turmeric on quality attributes of brine salted fish. The learning method and knowledge that will be gained during this study will be applied to increase knowledge with the aim of improving the quality of salted dried fish production in Sri Lanka.

## 1.2 Objectives

The objective of the present study was to assess the quality changes in salted dried lean fish (Atlantic pollock) with and without turmeric by sensory, microbiological and physicochemical analysis. More specifically, the objective was:



- To characterize the sensory, microbiological and physico-chemical changes of salted dried fish produced with and without addition of turmeric.
- To estimate the advantages and disadvantages of using turmeric to reduce the salt content in the salted dried fish.

## 2 LITERATURE REVIEW

### 2.1 Atlantic pollock

#### 2.1.1 Biology

Atlantic pollock (*Pollachius virens*) also known as saithe, coalfish, coely, green cod, boston bluefish is an active, schooling fish occurring in inshore and offshore waters to above 200 m depth. It belongs to the same family as cod and haddock but is distinguished from other species of the genus *Gadus* by its dark colour (Figure 1). Its growth is rapid and can reach a total length of nearly 130 cm, but common size is from 30 to 110 cm. The maximum age is 25 years (EUMOFA, 2018). It grows up to 15 kg. But it grows fast at first until they sexually mature between the ages of 3 and 6. They spawn multiple times per season (NOAA, 2017).

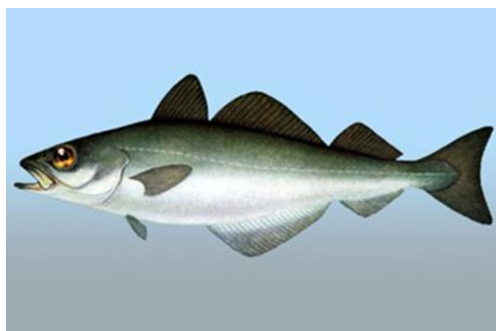


Figure 1. Atlantic pollock (*Pollachius virens*)

Source: gingason.is

The pollock is 19.3% protein in the edible portion of the fish. The pollock is chosen for the study as it is a lean fish and with a fat content of 0.9 g in the edible portion of 100 g of the fish (Table 1) with higher proportion of unsaturated fats.

Table 1. Nutrition facts of saithe

<b>Saithe (raw) – Nutrient content per 100 g edible portion</b>	
Energy	86 kcal
Protein	19.3 g
Fat - total	0.9 g
Fatty acids - saturated	0.2 g
Fatty acids - unsaturated	0.5 g
Omega 3	465 mg
Sodium	122 mg

Source: responsiblefisheries.is

### 2.1.2 Distribution

Although it can be found from the Arctic Ocean to the Mediterranean, pollock is commonly a northern fish. It is found mainly in the eastern and western Atlantic. It usually enters coastal waters in spring and returns to deeper water in winter. Smaller fish in inshore waters feed on small crustaceans and other small fish, while the larger pollock prey predominantly on other small fish. This fish usually arrives into coastal waters during the spring and return to deeper waters during winter (EUMOFA, 2018).

### 2.1.3 Capture and production

The 2016 commercial landings of Atlantic pollock totalled more than 5.7 million pounds and were valued at \$ 6.5 million. Pollock is increasing in popularity with recreational anglers that have traditionally targeted other *Gadus* species like cod and haddock. Fishing occurs year-round, peak landings are from November through January (NOAA, 2017). Atlantic pollock is utilized fresh, dried, salted and dried, smoked, canned and frozen. These products can be eaten steamed, fried, broiled, boiled, microwaved and baked.

## 2.2 Fish quality

Fish is regarded a healthier meat option due to its protein, high content of long chain polyunsaturated fatty acids and micro-nutrients. However, fish is one of the most highly perishable food products (Jeyasanta, Sinduja, & Petterson, 2016). During handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product. Shelf life is defined as the period, under defined conditions of storage, for which a food product remains safe and fit for use (Alasavar, Abdullah, Fereidoon, & Alex, 2002). Fish spoilage results from a complex activity involving physical, chemical and microbiological actions of deterioration. Off odours and off flavours, slime gas production, discolouration and soft texture are characteristics of spoilage in fish (Lakshmanan, 2000). Sensory, microbiological and chemical methods have been used to assess freshness and quality of fish during handling and storage.

### 2.2.1 *Sensory evaluation*

Sensory evaluation is the method used to determine the freshness and quality of fish and fish products at different stages in processing. The signs of deterioration in quality can be detected efficiently by use of the senses to judge the quality of fish by means of sight, smell, taste or touch (Lakshmanan, 2000). Sensory evaluation should be carried out by trained personnel in the reception or processing halls of fish factories or at the auction site. However, sensory evaluation of cooked fillets should be carried out in rooms with special facilities (Martinsdottir, 2010).

The codex 1999 guidelines for the sensory evaluation of fish and shellfish in laboratories describe facilities, procedures and training of assessors and can be used as a basis for practising sensory evaluation in the fisheries sector. The effects of the surroundings should be reduced as much as possible where the sensory evaluation is carried out. Lighting and ventilation are important and noise level, foreign odours and distracting elements should be minimized (Martinsdottir, 2010).

In sensory evaluation, species and the features of the product may interfere with the assessment. Fresh fish will normally be assessed through appearance and odour. Fish change in appearance during spoilage in ice and it is not usually difficult to accurately grade iced fish by appearance alone. It is recommended to assess the frozen fish in the frozen state. The assessor should note the nature and state of wrappings and glazes and the product should be examined for any discolouration and dehydration. Cooked samples should be held in a closed container until reaching a comfortable tasting temperature and kept warm unless they are assessed immediately. The products which are not warm should be slightly heated before assessing (Codex, 1999).

### 2.2.2 *Microbiological evaluation*

Microbiological examinations of fish products evaluate the possible presence of bacteria or organisms of public health significance and give an impression of the hygienic quality of the fish including temperature abuse and hygiene during handling and processing. The total number of bacteria, specific spoilage organisms and pathogenic bacteria present in a fish product is an indicator of the quality of the product. The determination of total bacteria count is used mainly to assess the microbial quality. Microbial activity is responsible for spoilage of most fresh fish products and gives information on shelf life of the product (Lakshmanan, 2000).

### 2.2.3 *Biochemical Evaluation*

The biochemical and chemical methods for evaluation of seafood quality are related to quantitative standards. The establishment of tolerance levels of chemical spoilage indicators eliminates the need to base decisions regarding product quality on personal opinions. Total volatile basic amines (TVB) is one of the most widely used measurements of seafood quality. The highly unsaturated fatty acids found in fish lipids are very susceptible to oxidation. The primary oxidation products are the lipid hydroperoxides. These compounds can be detected by chemical methods, generally by making use of their oxidation potential. The biochemical/chemical methods may best be used in resolving issues regarding products of marginal quality and these indicators have been used to replace time-consuming microbiological methods (Huss, 1995).

## 2.3 Lipid Oxidation

Lipid oxidation is one cause of deterioration and spoilage, causing rancidity in food by affecting its odour, flavour and appearance. This is a serious issue in all species, more so when the fish is fatty. Lipid oxidation can occur at different stages of the production chain such as during processing and storage causing quality deterioration including off-odour and off-flavour. Moreover, it can cause loss in essential fatty acids, vitamins, consumer acceptability and economic value (Azhar & Nisa, 2006). Progression of lipid oxidation depends on different factors, such as the amount of lipid present, the degree of unsaturated fatty acids in the muscle, salt content and storage conditions of products (Nguyen, Thorarinsdottir, Thorkelsson, Gudmundsdottir, & Arason, 2012).

Free fatty acids (FFA) accumulate in fish muscle due to enzymatic hydrolysis of lipids, and the increase of FFAs reduce the quality of the fish. The functional fatty acid components such as EPA and DHA are also released from polar lipids or triacylglycerols to the free fatty acid fraction by hydrolysis (Kaneniwa, Yokoyama, Murata, & Kuwahara, 2004). Several methods have been used to measure primary (hydroperoxides) and secondary (TBARS) oxidation products in foods for determining the degree of quality changes. Sensory evaluation can be useful to detect rancidity resulting from lipid oxidation.

## 2.4 Fish preservation

Processing methods are employed in the food industry with the main objective of preserving the food quality and safety. For processing fish products, important steps such as bleeding, gutting, icing or freezing should be carried out on the boats immediately after fish capture. This helps prevent or minimize the microbial and enzymatic activities, and the rancidity in case of fatty species (Boziaris, Kordila, & Neofitou, 2011). Decomposition of components of biomolecules in a fish cause spoilage. For decomposition to occur, bacteria require different levels of moisture, temperature, oxygen, and time. Food preservation works by removing at least one of these four conditions.

The various preservation methods used in fisheries are categorized into three types, namely; physical, chemical and bio-preservation. The physical methods deal with three distinct spoilage control measures: moisture control (drying), thermal control (chilling and freezing) and non-thermal control technologies (high pressure processing, packaging technologies, irradiation, ozonation, pulsed electric fields and oscillatory magnetic fields treatments). Chemical methods of fish preservation include curing, smoking and use of natural antimicrobial preservatives, organic acids and salts. Bio-preservation methods reduce the growth of undesirable microorganisms by favouring the growth of competitive and antagonistic microorganisms (Mahmud, et al., 2008).

### 2.4.1 Salted dried fish production

Salting in combination with drying is an important preservation method around the world. In many developing countries, salted and dried fish is an important source of low-cost dietary protein. The main factors influencing the quality of dried salted fish is freshness of fish, salting method, salting period, brine concentration, texture of salt and drying kinetics. Salting and sun drying reduces the water content, which means that some of the water-soluble vitamins may be lost or lowered, while most of the nutritional value remain the same (Jeyasanta, Prakash, & Patterson, 2016).

Salting is used because most bacteria, fungi and other pathogenic organisms cannot survive in a higher salty environment, due to the osmotic pressure that salt creates. Any living cell in an environment with high concentrations of salt will become dehydrated through osmosis and die or become inactivated. This extraction of water from the product causes a decrease in the water activity. Thus, results in a decreased activity of bacteria and enzymes (Oliveira, Pedro, Nunes, & Costa, 2012).

Generally, the water activity factor in fish is close to 1 and it can be decreased down to 0.8 and 0.7 after heavy salting and drying. Only some so called 'salt-loving' (halophile) bacteria can grow in water activities down to 0.75 (Sampels, 2015). The effectiveness of drying can be assessed by measuring water activity in the product. Water activity indicates how much free water is in the product or how much water is available for chemical reaction and microbial growth (Kristjansson, 2013).

#### *2.4.2 Use of spices in salted dried fish production*

Generally, salt removes water from fish flesh. Salt binds the water and therefore lowers water activity as the fish is dried. Spices and herbs can also help to remove water by forming bond with fish and add some nutrients and bioactive compounds which prevent the growth of micro-organisms. Thus, this process can be recommended as a preservation technique in large scale dry fish production (Alex & Eagappan, 2017). Combination of salt and spices can produce good quality dried products with excellent sensory characteristics and satisfactory percentage of protein and fat. This method can be used to produce good quality dried product for domestic consumption as well as export (Rahman, et al., 2017).

Nahid et al., (2017) investigated the combined effect of salt and turmeric powder along with smoke-drying on the production of smoke-dried products from types of fish and their nutritive value. Combining salt and turmeric treatment with smoke-drying was shown to have a positive and significant effect on the proximate, chemical and mineral composition of those freshwater fishes and reduces bacterial load and making them nutritionally suitable for all (Nahid, Latif, Chakraborty, Farid, & Begum, 2017). A study on anchovy treated with salt, pepper, turmeric and Ajwain showed that the spices improved the quality in terms of appearance, rancidity, saltiness, texture and general acceptability and shelf life of the final products (Alex & Eagappan, 2017).

## **2.5 Turmeric**

Turmeric is an underground rhizome which imparts a distinctive flavour to food, but it is also used to provide food with a deep yellow colour and sometimes called "Indian saffron". Curcumin is the main active ingredient in turmeric. It has powerful anti-inflammatory effects and is a strong antioxidant. The curcumin content in turmeric is 3% by weight. In the form of this fine, dried, yellow powder, turmeric is mostly sold to customers in developed countries. Currently, India is the major producer of turmeric, and it is also the major user of its own production. Other producers in Asia include Bangladesh, Pakistan, Sri Lanka, Taiwan, China, Myanmar, and Indonesia. Turmeric is also produced in the Caribbean and Latin America: Jamaica, Haiti, Costa Rica, Peru, and Brazil (Plotto, 2004). In Sri Lanka, turmeric is easily available and at a low cost.

### *2.5.1 Utilization of turmeric*

Turmeric is part of Sri Lankan culture: it is an important ingredient in curry dishes; it is also used in many religious observances, as a cosmetic, a dye, and it enters in the composition of

many traditional remedies. Turmeric is grown in wet and intermediate zones of Sri Lanka and is used as an ingredient in the preparation of most of the dishes in Sri Lankan cuisine including salted fish curry. Turmeric and isolated compounds from turmeric have been shown to have a variety of beneficial pharmacological effects. These include antioxidant, antiarthritic, antimutagenic, antitumor, antithrombotic, antibacterial, antifungal, antiviral, nematocidal, choleric and antihepatotoxic activities (Shilpa, Soni, & Pandya, 2018).

### 2.5.2 Limitations in use of turmeric

The contamination of microorganisms in spices may cause serious problems for the food industry. The contamination may occur during harvesting, handling, transportation and storage. The utilization of spices, e.g. turmeric, which is contaminated with microorganisms in food production and preservation would accelerate the putrefaction of manufactured food products. Mould, bacillus, clostridium, and other bacteria growth under unfavourable conditions trigger quality changes and occasionally mycotoxin production. In many countries, fumigation with ethylene oxide, heat sterilization and gamma irradiation have been tried with varying degrees of success to sort out this issue (Juri, Ito, Watanabe, & Tamura, 1986). So, when selecting turmeric, attention should be paid to the risk of contamination and sterilization technique used in its production.

## 3 RESEARCH METHODOLOGY

### 3.1 Raw materials

#### 3.1.1 Fish

Fresh pollock fish fillets (n =14) were collected on 17<sup>th</sup> of December 2019 for the pre-trial, and 65 fresh pollock fish fillets were collected on 21<sup>st</sup> of January 2020 for the main experiment. All fillets were in iced storage condition at a temperature below 4 °C from the fishing and fish processing company Brim Ltd. located in Reykjavik, Iceland. Then fish fillets were cleaned using water and used for the salted dried fish production.

#### 3.1.2 Turmeric

To select turmeric fulfilling standards on microbial contamination, three turmeric powders were tested, outlined in Table 2 below. The turmeric powder samples were tested for *Bacillus cereus*, *Clostridium perfringens*, total count bacteria, yeast and moulds.

Table 2. The information on turmeric samples tested and the tests carried out

Turmeric samples	Best before date (yyyy/mm/dd)	Microbiological tests carried out
TRS Asia's finest foods	2021/02	<i>Bacillus cereus</i> , <i>Clostridium perfringens</i> , total count bacteria, yeast and moulds
Prima	2022/09	Total count bacteria
Pottagaldrar	2022/07/11	Total count bacteria

#### *Bacillus cereus*

The method used is based on the known amount of sample being put on Blood agar and grown at 30°C for 24 to study haemolysis. Likely colonies are sown on *Bacillus cereus* selective agar and grown at 30°C for 24 hours to investigate mannitol and lecithin activity (NMKL 67, 2010).

*Clostridium perfringens*

The method is based on isolating *Cl. perfringens* by cultivation on TSC agar in anaerobic conditions. The TSC agar consists of Perfringens agar base (PAB) and cycloserine. In PAB, sodium metabisulfite and ferric ammonium citrate were used as an indicator of sulphite reduction in sulphide in *Cl. perfringens* that form black colonies. Cycloserine solution makes the agar selective towards *Cl. perfringens*. Black colonies of TSC agar were streaked and properties such as gram stain, shape, mobility, lactose fermentation and hemolysis on blood agar was examined (NMKL 95, 2009).

*Total count bacteria*

The aerobic plate count was determined by preparing dilution series of the sample material according to general microbiological principles, followed by pour plating into Plate count agar medium in Petri dishes.

The samples were incubated under aerobic conditions at  $30.0 \pm 1.0$  °C for  $72 \pm 6$  hours.

The number of viable aerobic microorganisms per gram of sample was calculated from the number of colonies counted on selected plates and by multiplying the number of colonies obtained by the dilution factor (NMKL 86, 2013).

*Moulds and yeasts*

The Colony Forming Units (CFU) level was determined by preparing dilution series of the sample material according to general microbiological principles, followed by spread plating onto Dichloran Rose-Bengal Chloramphenicol Agar (DRCB-Agar) medium in Petri dishes. The samples were incubated at  $22.0 \pm 1.0$  °C for 5 days. Moulds and yeasts were counted separately and the number per gram of sample was calculated from the number of colonies counted on selected plates and by multiplying the number of colonies obtained by the dilution factor (NMKL 98, 2005).

*3.1.3 Salt*

Industrial food grade “Kronan” table salt purchased from the Kronan supermarket in Vinlandsleid, Iceland was used for the project work.

*3.1.4 Test with salted dried fish sample from Sri Lanka*

Before the pretrial, 100 g sample of salted dried trevally fish bought at the Sri Lanka market which were brought to Iceland and its composition (salt, water, water activity) was analysed.

*3.1.5 Effects of unfiltered versus filtered turmeric brine on fillet appearance*

The small fish fillet pieces of eight were immersed as two groups for 24 hours in 10% (w/w) brine solution with 2% (w/w) turmeric. One of the solutions was filtered by using laboratory test sieve having 125 µm mesh size before using it as marinade. This was carried out to observe the uniformity and distribution of turmeric around fillets of fish. The ratio of fish to brine was 1:1. The samples were evaluated by means of visual sensory evaluation to see the uniformity and distribution of turmeric around fillets of fish.

*3.1.6 Selection of salt and turmeric concentrations for main trial*

Before the main experiment, pre-trials were carried out to set up the best procedures for production of salted dried fish with turmeric. The pre-trials were carried out using 12 fish fillets

as raw material. The pre-trials were carried out using different concentration of brine solution (2%,4%, 6%, 8%, 10%) and turmeric powder (0.5%, 1%, 2%). The fish fillets were immersed in the different concentrations of brine solution separately with fish weight to brine weight in the ratio of 1:1 with different concentration of turmeric added to each for 24 hours at chilled storage (temperature 0 - 4 °C). Then fish fillets were drained well after 24 hours and stored at 7 °C for 2 days. Then, fish were evaluated for the uniform distribution of turmeric. The group of fillets dipped in same turmeric concentration which is having uniform turmeric was selected and dried at  $30.0 \pm 2.0$  °C in a small drying cabinet at Matis (Figure 2).



Figure 2. Drying cabinet at Matis

The samples were measured for colour, salt content, water activity, and sensory evaluation. Small pieces of each of the four sample groups were then cooked with 10 times weight of water for 20 minutes in medium flame and evaluated again by the trained sensory panellists. The most suitable salt and turmeric concentrations were selected based on these results.

### 3.2 Main experiment

Of the 65 fillets obtained, five fillets were used for the study of fresh raw material and the remaining 60 pollock fillets were divided into four groups (Figure 3). The main objective of the study will be to evaluate the effect of turmeric extract in salted dried fish production on sensory, microbial and chemical quality.



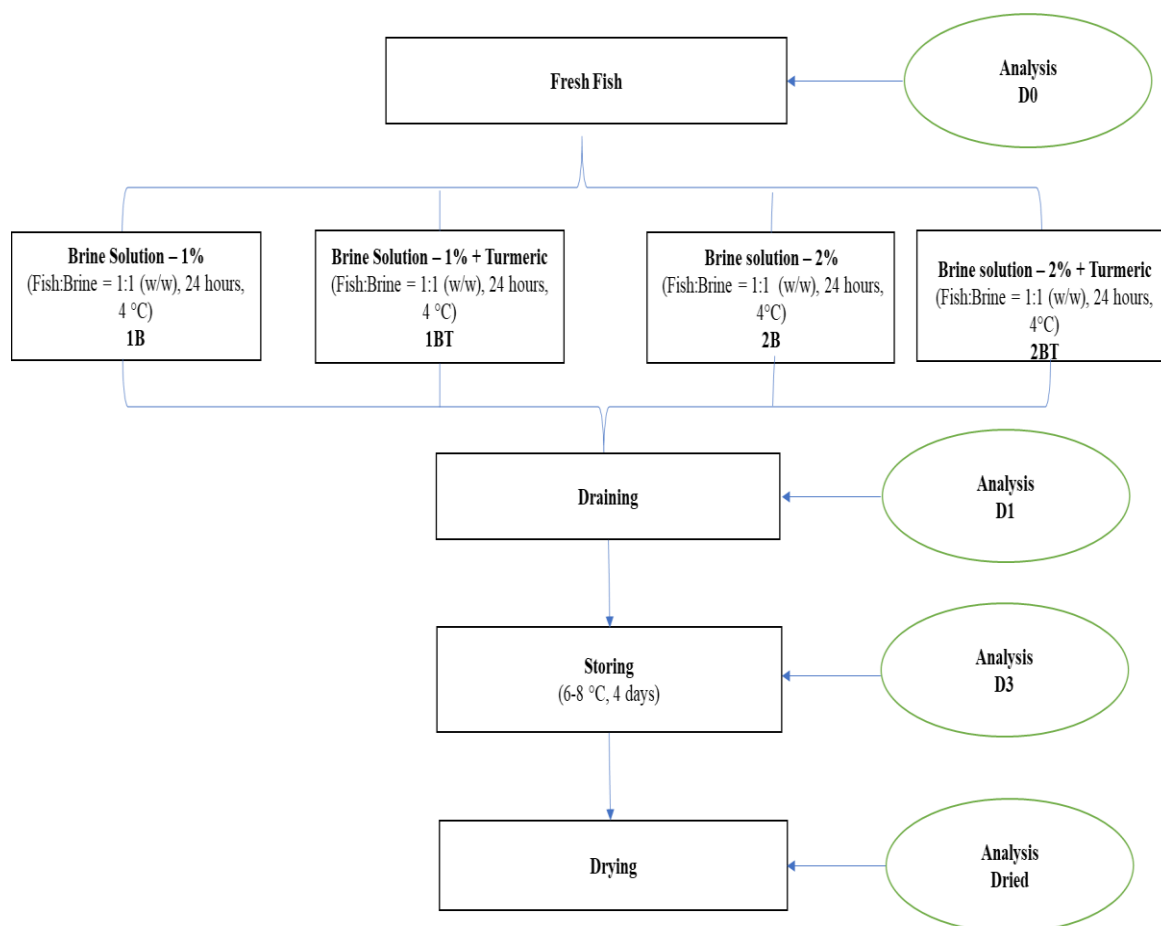


Figure 3. Flowchart demonstrating the salted fish production process and measurement plan

The 1% and 2% (w/w) brine solutions were prepared, mixed well in a plastic container and kept overnight for the salt to fully dissolve at 0 - 4 °C. The four groups of fish fillets were immersed separately into 1% and 2% brine solutions with and without turmeric using fish to brine in the ratio of 1:1 in terms of weight for 12 hours at 0 - 4 °C. Then, fish were drained well after 24 hours and stored at 7 °C for 2 days. Then, the fish were dried at  $20.0 \pm 2.0$  °C in drying cabinet in Icelandic fish drying company, Hardfisksalan for 7 days (Figures 4 and 5).



Figure 4. Arranging pollock fillets in drying racks



Figure 5. Fish fillets arranged in drying cabinet

Initially, fresh fillet samples were analysed for physicochemical, microbiological and sensory characteristics. Thereafter sampling was done after brining, after incubation for two days and after drying and the samples were analysed for changes in yield, water content, colour, pH, water activity, microbiological and sensory characteristics. Protein content was analysed only for the fresh samples and TVB-N analysis was carried out with fresh samples and dried samples. Lipid content, phospholipids, FFA, TBARS and peroxide value analysis were done for fresh, incubated for two days and dried samples. Salt content analysis was done for fresh, brined and dried samples. Each analysis was done as a duplicative study.

### 3.3 Physio-chemical analysis

#### 3.3.1 Weight and Colour measurements

Four fillets from each experimental group were separated, tagged, and were used for weight and colour measurements in each trial. The colour was measured in the raw material and in the final products (Figure 6). Five colour measurements were taken from each fillet, from the front part, to the tail part. 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 ( $b^-$ ).



Figure 6. Taking colour measurements using a Minolta chroma meter CR-400

### 3.3.2 Moisture content

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 dried samples. Approximately 5.0 g of homogenized sample was weighed in a crucible using an analytical balance. Then, samples were dried in a drying oven at 103 ± 2.0 °C for four hours and allowed to cool for 30 minutes in a desiccator before recording the final weight. Three samples were prepared from three fish fillets for determination of moisture. Results were expressed to the nearest 0.01% using the following equation.

$$W1 = \frac{M3 - (M5 - M4)}{M3} * 100$$

Where,

W1 = Percentage of moisture present in fish muscle in grams.

M3 = Weight of the sample in grams.

M4 = Weight of the crucible in grams.

M5 = Total weight of the sample and crucible after drying in grams.

### 3.3.3 Water activity

An AQUA LAB Water Activity Meter was used to measure the water activity in fresh fish, brined, stored for 2 days and dried 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.

### 3.3.4 Salt content

The sodium chloride or salt content in 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.

### 3.3.5 Acidity (pH level)

The pH values were measured using blended samples of fish fillets with a portable pH meter (Portamess 913, Knick, Berlin, Germany). The pH meter was calibrated for pH-4, pH-7 and pH-10 before starting measurements.

### 3.3.6 Protein yield

Protein content was determined by the Kjeldahl method (ISO 5983-2:2005). The protein content was measured in fresh pollock 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 Kjeltac 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 H<sub>2</sub>SO<sub>4</sub>. The nitrogen content was multiplied by the factor 6.25 to get the ratio of crude protein.

### 3.3.7 TVB-N

The steam distillation method of Malle and Poumeyrol (1989) was used for the TVB-N determination. Fish fillet of 50 g was mixed with 100 ml of 75% aqueous trichloroacetic acid solution, homogenized in a blender for 1 minute and then filtered through a Whatman no. 3 filter paper. The distillation was performed using a Kjeldahl-type distillatory. Into a distillation flask, 25 ml of filtrate was transferred and 6 ml 10% NaOH was added. The distillate was collected into an Erlenmeyer flask containing 10 ml 4% boric acid and was placed under the

condenser for the titration of ammonia for 4 minutes. The boric acid solution was turned green when alkalinized by the distilled TVB-N, and then titrated with aqueous 0.0372 N sulphuric acid solution (H<sub>2</sub>SO<sub>4</sub>) 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 TVB-N content was calculated by the following formula and the results will then be expressed as mg N/100 g (Malle & Poumeyrol, 1989)

$$\text{TVB - N} \left( \text{mg} \frac{\text{N}}{100 \text{ g}} \right) = \frac{14 \frac{\text{mg}}{\text{mol}} * a * b * 300}{25 \text{ ml}}$$

Where,

a: Volume of sulphuric acid (ml)

b: Normality of sulphuric acid

### 3.3.8 Lipid content

Total lipids were extracted with methanol/chloroform/0.88% KCl (aq) (at 1/1/0.5, v/v/v) according to the Bligh & Dyer method (Bligh & Dyer, 1959). The samples were weighed into 250 ml fluorinated ethylene propylene (FEP) plastic bottles and distilled water was added according to correction table. For the dried fish samples, the water was added and kept overnight inside the refrigerator at 0-4 °C. 25 ml of chloroform and 50 ml of methanol were added and homogenized for 2 minutes in an ice bath. Additional 25 ml of chloroform was added and homogenized for 1 minute followed by 25 ml 0.88% potassium chloride solution and homogenized for 1 minute, 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 was made up to mark in a 50 ml volumetric flask. The lipid content was then calculated by evaporating the chloroform under nitrogen gas. The results were expressed as gram lipid per 100 g wet muscle.

### 3.3.9 Free Fatty Acids

Free fatty acid content was determined by the method of Bernardez, *et al.*, (2005), based on complex formation with cupric acetate-pyridine, followed by absorbance reading at 715 nm (UV-1800 spectrophotometer). About 3 ml of the lower phase resulting from fat extraction (Bligh & Dyer, 1959) was added in a screw cap culture tube. Any solvent present was removed at 550 °C using nitrogen jet. After cooling down, 3 ml of cyclohexane was accurately added swirled to dissolve the sample. About 1 ml of cupric acetate-pyridine reagent was added and vortexed for ~40 seconds. After centrifugation at 2000 rpm for 10 minutes at 4 °C, the upper layer was read at 710 nm in spectrophotometer. The concentration in the sample was calculated as µmol oleic acid based on a standard curve spanning a 0-20 µmol range. The FFA results were expressed as g FFA/100 g lipids.

### 3.3.10 Phospholipids

Phospholipid content of the fish muscle was determined on the lipid extraction (Bligh and Dyer 1959) by using a spectrophotometric method, based on complex formation of phospholipid with ammonium ferro thiocyanate, followed by absorbance reading at 488 nm (UV-1800 spectrophotometer). About 10 µl of the lower phase resulting from fat extraction (Bligh & Dyer, 1959) was added into 15 ml tube, followed by 1.99 ml of chloroform. Thiocyanate reagent (1 ml) was added and vortexed for ~40 seconds. After centrifugation at 2000 rpm for 5 minutes at 4 °C, the lower layer was read at 488 nm in spectrophotometer. The concentration in the sample was calculated as g phospholipids per 100 g lipids based on a standard curve

prepared from stock solution of phosphatidylcholine in chloroform spanning a 0-150 µg/ml range. The results were expressed as g phosphatidylcholine equivalents per 100 g of total lipid extracted.

### 3.3.11 TBARS

Thiobarbituric acid-reactive substances were measured using the method of Lemon (1975) with modifications. A 5.0 g sample was homogenised with 5 ml of 7.5% trichloroacetic acid (TCA) using an Ultra-Turrax homogeniser (Kika Labortechnik, T25 basic, Staufen, Germany) for 10 seconds and again 5 ml TCA was added and homogenized. For the analysis of dried fish samples, about 20 ml of TCA was added for homogenizing as in two series of 10 ml and kept for one hour at 0-4 °C before centrifuging. The homogenate was then centrifuged at 5000 x g for 20 minutes at 4 °C. Then the supernatant was filtrated and transferred into 15 ml tubes. A mixture of 0.5 ml of the filtrated supernatant was added into Eppendorf tubes, followed by 0.5 ml of 0.02 M thiobarbituric acid solution and heated in a water bath at 95 °C for 40 minutes. The heated samples were cooled down on ice and absorbance values were read at 530 nm. The results were expressed as µmol malondialdehyde per kg sample (µmol MDA/kg) and calculated using a standard curve prepared from 1,1,3,3-tetraethoxypropane (TEP).

### 3.3.12 Peroxide value

Lipid hydroperoxides were determined with some modifications in ferric thiocyanate method (Santha and Decker 1994). Total lipids were extracted from 5.0 g of samples with 10 ml ice-cold solvent (included chloroform: methanol (1:1) solution, containing 500 ppm BHT to prevent further peroxidation during the extraction process). 5.0 ml of sodium chloride (0.5 M) was added into the mixture and homogenized for 30 seconds and then centrifuged at 5100 rpm for 5 minutes (TJ-25 Centrifuge, Beckmann Coulter, USA). For the analysis of dried fish samples, about 20 ml of ice cold solvent and 10 ml of sodium chloride were added for homogenizing and kept for one hour at 0-4 °C before centrifuging. The bottom layer (chloroform layer) was collected and transferred into 15 ml test tube. 500 µl of bottom layer were collected into Eppendorf tubes and added with 500 µl solvent stored at room temperature, followed by 5 µl of ammonium thiocyanate (4 M) and ferrous chloride (80 mM) mixture (1:1). The mixture was then vortexed, incubated for 10 minutes at room temperature and read at 500 nm (Tecan Sunrise, Austria). A standard curve was prepared using cumene hydroperoxides. The results were expressed as µmol lipid hydroperoxides per kilogram sample.

## 3.4 Microbiological analysis

### 3.4.1 Sample preparation

The fillets were minced, and 20 g were mixed with 180 g of cooled maximum recovery diluent in a stomacher for 1 minute at normal speed. Successive 10-fold dilutions were done as required.

### 3.4.2 Total plate count (TPC) and Specific spoilage organisms

1 ml of 1/10 dilutions were transferred using a pipette to Petri plates and melted Iron agar at 45 °C was poured on the plates and the content was mixed to solidify. After solidification, the plates were covered with a thin layer of Iron agar then incubated at 22°C for 48 hours. All the microbiological analysis was conducted in duplicate and data expressed as a logarithm of the number of colony (white and black)-forming units (log cfu/g). All plates were enumerated, and colonies recorded as colony forming units (cfu/g). Counts of all colonies (both white and black)

on Iron agar represent the number of total count and counts of black colonies represent the number of specific spoilage organisms (NMKL 184, 2006).

### 3.5 Sensory Analysis

Product evaluation was done in the pretrial in two sessions. The appearance, odour, flavour and texture were evaluated by three trained panellists of the dried pollock before and after cooking, results summarised by panel leader.

Five evaluation sessions were carried out in the main experiment using sensory facilities at Matís, Reykjavík (Figure 7). Samples of cooked pollock were evaluated fresh before brining, immediately after brining and after storage for two days at 7°C. Dried saith was evaluated before and after cooking.



Figure 7. Individual cabin for sensory evaluation

Four trained panellists participated in the evaluation on each sampling day (ISO, 2014). Each sample of cooked saith (before drying) was ~50 g of crosscut loin, cooked for 6 minutes in a steam oven (Convotherm Elektrogeräte GmbH, Egging, Germany) (Figure 8) and presented warm to the panellists in a 140 ml aluminium box with a transparent plastic lid.



Figure 8. Cooking fish fillets for sensory evaluation

All samples were coded with three-digit numbers and presented in a random order to the panellists. Each panellist received two samples of each sample group. The first sample was evaluated individually, and the panellists were instructed to describe the odour, appearance, flavour and texture of the sample. Spoilage odour, spoilage flavour and salty taste were evaluated on a 5-point scale from “not detected” to “very strong” (Table 3).

Table 3. Scale used to evaluate strength of spoilage odour, spoilage flavour and salty taste.

Scale	Definition
0	not detected
0.5	threshold
1	trace
2	weak
3	moderate
4	strong
5	very strong

After individual evaluation, the panellists discussed the results while being presented with another sample, to reach a consensus on the description of the sample. Panellists also gave each sample a score on Torry freshness scale for lean white fish (Shewan, Macintosh, Tucker, & Ehrenberg, 1953). Two fillets were evaluated of dried uncooked saith. The panellists first evaluated the intact fillets, then samples were removed from one of the fillets and evaluated. Pieces were cut out of fillets of dried pollock and cooked in water at ratio fish: water = 1:20 for 30 minutes. The pieces were transferred to aluminium boxes and presented to the panellists in the same manner as the cooked fresh saith.

Short Generic Descriptive Analysis (GDA) was carried out on cooked fish fillets with the participation of four trained panellists. The panellists evaluated spoilage characteristics and salty taste on a scale. After that, the panellists discussed the other sensory characteristics of each sample and described them in text. The data is numerical for the GDA attributes but in text for other sensory characteristics.

### 3.6 Statistical analysis

Data were analysed using MS - Excel program and two-way ANOVA (Analysis of Variance) was carried out on the colour measurements and significance of difference was defined at the 95% level ( $p < 0.05$ ).

## 4 RESULTS AND DISCUSSION

### 4.1 Pretrial results

#### 4.1.1 Test with sample from Sri Lanka

The results of measurements of the salted dried trevally fish sample from Sri Lanka sample showed that the range of water content of the fillets was between 16% - 19%. So that the fish fillets were very dry. The water activity of the product was also satisfactory as it was less than 0.75 (Table 4). The salt content seemed to be light salted fish but seems to be in an acceptable level as according to the Sri Lanka Standard accepted maximum salt content is 12% on dry basis (Nuwanthi, Madage, Hewajulige, & Wijesekera, 2016).

Table 4. Water content, salt content and water activity values of salted dried fish sample from Sri Lanka

Analysis	Thick Fillet pieces	Thin Fillet pieces
Water content	18.5% ± 5%	16.7% ± 5%
Salt content	3.5% ± 5%	3.8% ± 5%
Water activity	0.7238	0.7110

In order to simulate the properties of the trevally sample from Sri Lanka, it was suggested to focus on production of salted dried fish with turmeric having water content below 20% and salt content of 2.5% and 3.5% to create a salty environment, though concentrating on a product with low saltiness. Water activity of the dried fish should be within 0.65 - 0.75 to create a negative environment for microbial activity.

#### 4.1.2 Testing the turmeric powder samples

For the TRS Asia's finest foods turmeric sample, *Bacillus cereus*, *Clostridium perfringes*, yeast and mould count obtained were within the recommended range limit for the spices, although the total count was extremely high (Table 5). Two turmeric samples were tested for total count in 1 g at 30 °C. Among those, "Prima" turmeric powder total count was also high, but "Pottagaldrar" had a much lower total count. Therefore, it was decided to use Pottagaldrar turmeric for the experiment purchased directly from company in bulk of 1 kg. Grounded turmeric powder obtained from Pottagaldrar ehf. located in Kopavogur, Iceland was used for all other experiments. The company use the spice of turmeric which is imported from India and the product is steam-treated to achieve the low microbe count but neither fumigated with ethylene oxide or propylene oxide nor treated with ionising radiation.

Table 5. Microbiological analysis results of turmeric samples.

Analysis	TRS Asia's finest foods	Prima	Pottagaldrar
<i>Bacillus cereus</i>	<20		
<i>Clostridium perfringes</i> in 1 g	<10		
Total count in 1g at 30 °C (cfu/g)	4,800,000	110,000	300
Yeast in 1 g	<20		
Molds in 1 g	160		

#### 4.1.3 Effect of filtered brine containing turmeric on fillet appearance

From the observation of the drained fillets which had been marinated in 2% turmeric solution marinated for 24 hours, a lighter colour and more uniformity in appearance was seen in the filtered solution (Figure 9) than in the unfiltered solution (Figure 10). This was probably due to the precipitation of the sediment particles of turmeric which is present in the unfiltered solution. Therefore, it was recommended to use filtered solution as the product of dried fish from this would probably be preferred in the market.





Figure 9. Marinated /brined 24 hours in unfiltered 10% brine solution with 2% turmeric powder



Figure 10. Marinated/brined 24 hours in filtered 10% brine solution with 2% turmeric powder

#### *4.1.4 Selection of brine and turmeric concentration for main trial*

The turmeric was not distributed evenly after marinating in a solution with 0.5% or 1% turmeric concentration. Comparatively, fillets marinated in 2% concentration of turmeric seemed to be an attractive product (Figure 11). The four fillets brined in 4%, 6%, 8% and 10% salt concentrations with 2% turmeric were selected and dried to carry out further physiochemical analysis.



Figure 11. The fillets marinated in 0.5%, 1% and 2% turmeric concentrations

The weight of the dried product decreased significantly but, when comparing among groups the dried fish product yield seemed to increase with increasing salt concentration of brine from 4% - 10% (Figure 12).

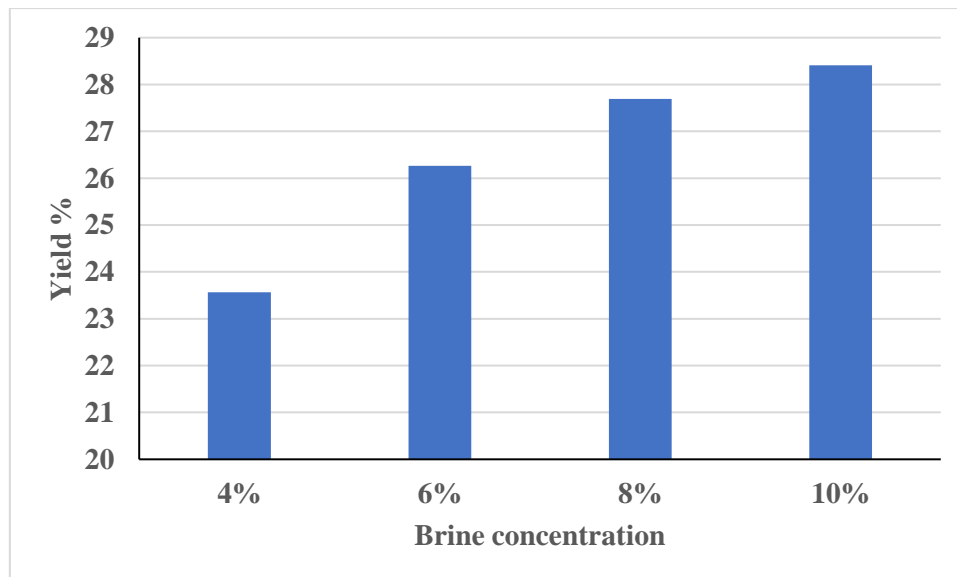


Figure 12. Yield change with salt concentration of brine

The b value of the product increased when the brine solution contained turmeric (Figure 13). This shows a yellow colouration of the product.

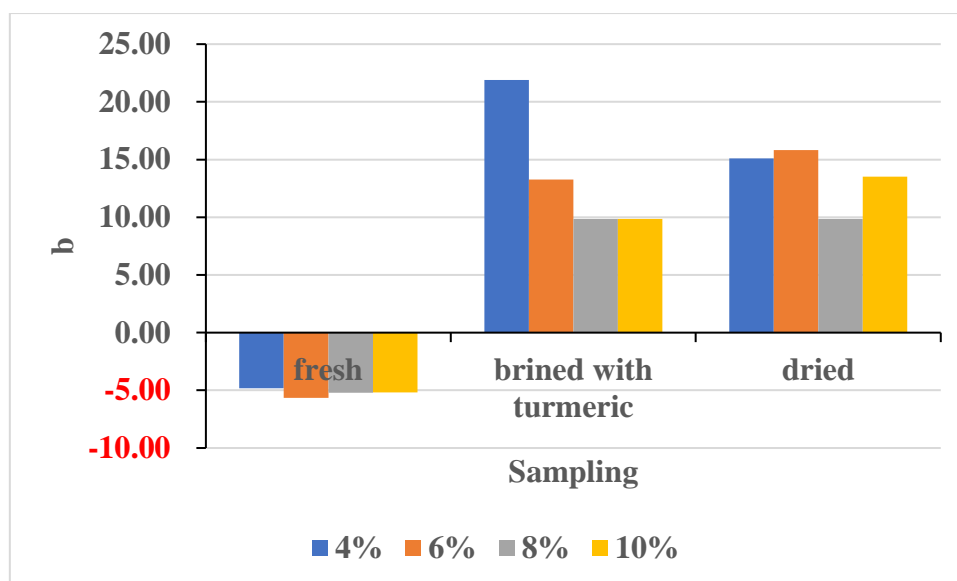


Figure 13. Changes in b value of fish fillets during processing

The values of water activity and water content was lower than the expected water activity of 0.7- 0.75 and water content of 20% (Table 6). There was no significant pattern in change of water activity and water content as the fillets brined in low salt concentrations of 4% and 6% dried too much. The salt content in fish flesh increased with the salt concentration of the brine solutions. Even the lowest salt concentration used in this study resulted in a very high salt content of the product.

Table 6. Values of water activity, water content and salt content in fillets brined in different salt concentrations

Salt concentration of brine	Water activity	Water content (%)	Salt content (%)
4%	0.6171	11.88	8.5
6%	0.6367	14.28	11.7
8%	0.6762	21.58	12.3
10%	0.6557	18.99	13.6

## 4.2 Main experiment

### 4.2.1 Physiochemical profile of raw material

Physical and chemical measurements were done in sample of fresh pollock and the results can be seen in the table 7 below. The fish flesh consists of water as the main constituent and the water activity value is very high. This fish consists of 19.2% of protein. The fat content value of the fish is low (0.90), this is a lean fish as trevally fish in Sri Lanka which has a lipid content of around 1.5%. The freshness of fish can be detected through the analysis of volatile products, such as TVB-N, total bacteria counts, specific spoilage bacteria and sensory evaluation. The TVB-N value of raw material was 15.55 mg N/100g which is low compared to the acceptable limits of 35 mg N/100g for pollock. These results indicate that the fish used for processing was fresh and in a good condition.

Table 7. Physicochemical profile of raw material

Sample	Moisture content (%)	Dry matter (%)	Water activity	Salt content (%)	pH	Protein (%)	Fat (%)	TVB-N (mg N/100 g)
Raw material	78.68	21.32	0.99	0.4	6.36	19.15	0.90	15.55

#### 4.2.2 Physicochemical analysis

Generally, the total yield changed during the processing (Figure 14). It increased after brining and decreased after drying. The change yield for the above results was calculated with reference to the raw material. The value of raw material was taken as zero and positive values show the gain in yield and negative values show loss in yield. The increase in yield at the brine salting step was not significant. The drying process resulted in huge loss in yield with no significant difference between the different sample groups. The yield decreased by 75% from the raw material.

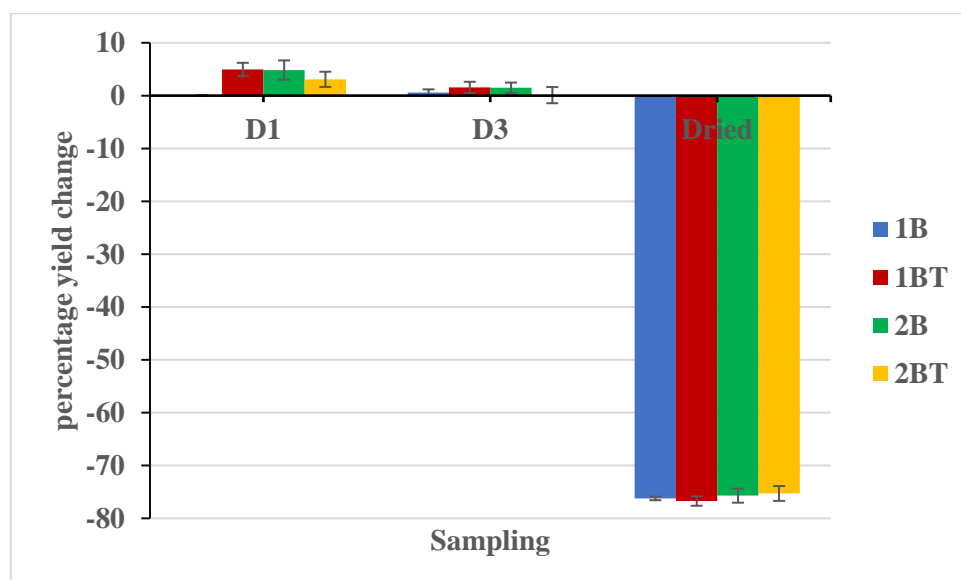


Figure 14. The percentage of the yield change during the processing of brined dried pollock in different conditions of brining applied

(sample groups: 1B =1% salt, 1BT =1% salt with 2% turmeric, 2B=2% salt) and 2BT=2% salt with turmeric; sampling points: D1=after brining, D3 =after incubating for 2 days at 7 °C and dried).

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) during the processing. The final products obtained lower  $L^*$  values than the value for the raw samples. The highest  $L^*$  value was observed in D3 (~50), which was significantly higher than the dried sample (~40). No significant differences for lightness were found between the different sample groups.

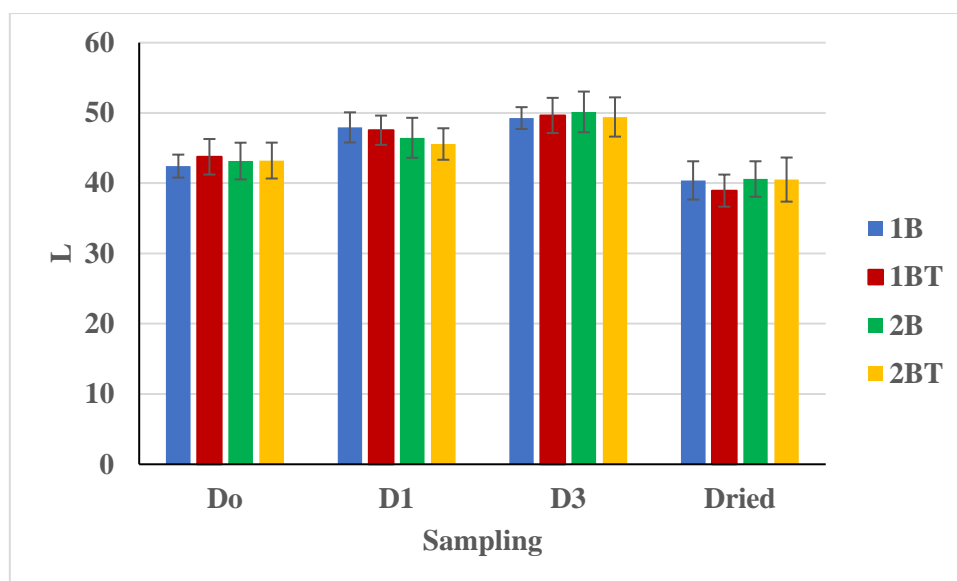


Figure 15. Average L values for different sample groups at different sampling points

(sample groups: 1B =1% salt, 1BT =1% salt 2% turmeric, 2B=2% salt) and 2BT=2% salt 2% turmeric; sampling points: D0 =fresh raw material, D1= after brining, D3 =after incubating for 2 days at 7 °C and dried).

The average  $a^*$  values obtained by the different sample groups at different sampling points can be seen in Figure 16. Significant differences were observed for  $a^*$  value among the group of samples ( $p=0.016$ ). The groups with turmeric addition showed negative  $a^*$  values ( $a^-$  green), while the other groups showed positive  $a^*$  values ( $a^+$  red). Though, the groups with turmeric after drying showed positive  $a^*$  values ( $a^+$  red). Significant differences were observed for  $a^*$  value during the processing ( $p=0.007$ ). The groups with turmeric addition showed negative  $a^*$  values ( $a^-$  green) on D1 and D3, while the other groups showed positive  $a^*$  values ( $a^+$  red) D1 and D3. Though, the groups with turmeric after drying showed positive  $a^*$  values ( $a^+$  red) it is lower (1.1-1.3) than without turmeric groups (3.3-3.6).

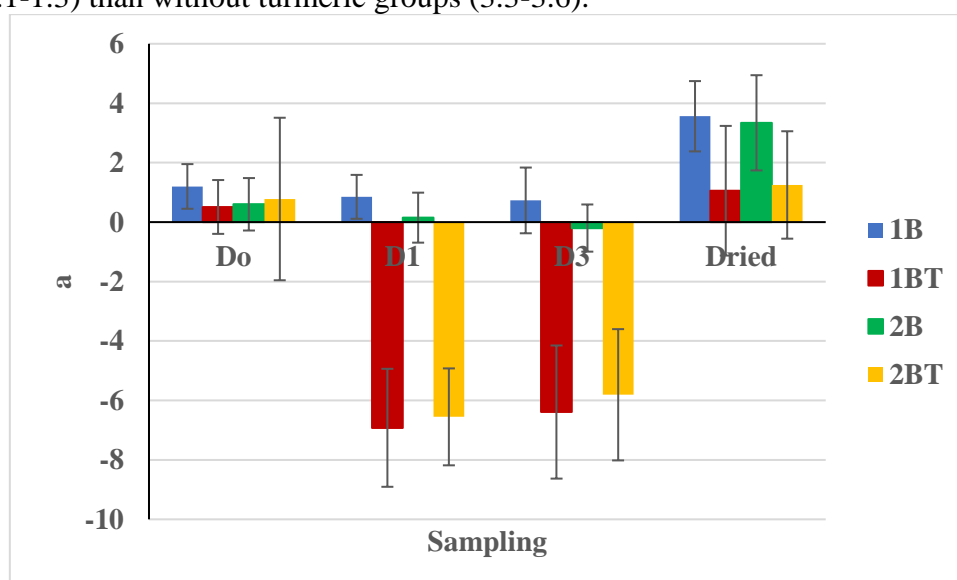


Figure 16. Average  $a^*$  values for different sample groups at different sampling points

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D1=after brining, D3 =after incubating for 2 days at 7 °C and dried).

As for  $a^*$  values, significant differences were found for yellowness ( $b^*$  value) in different groups of pollock samples. The turmeric added groups showed positive  $b^*$  values ( $b^+$  yellow), while non turmeric added groups showed negative  $b^*$  values ( $b^-$  blue). The turmeric added groups are significantly different from the other groups ( $p=0.022$ ). The average  $b^*$  values (yellowness) for each sample are shown in Figure 17.

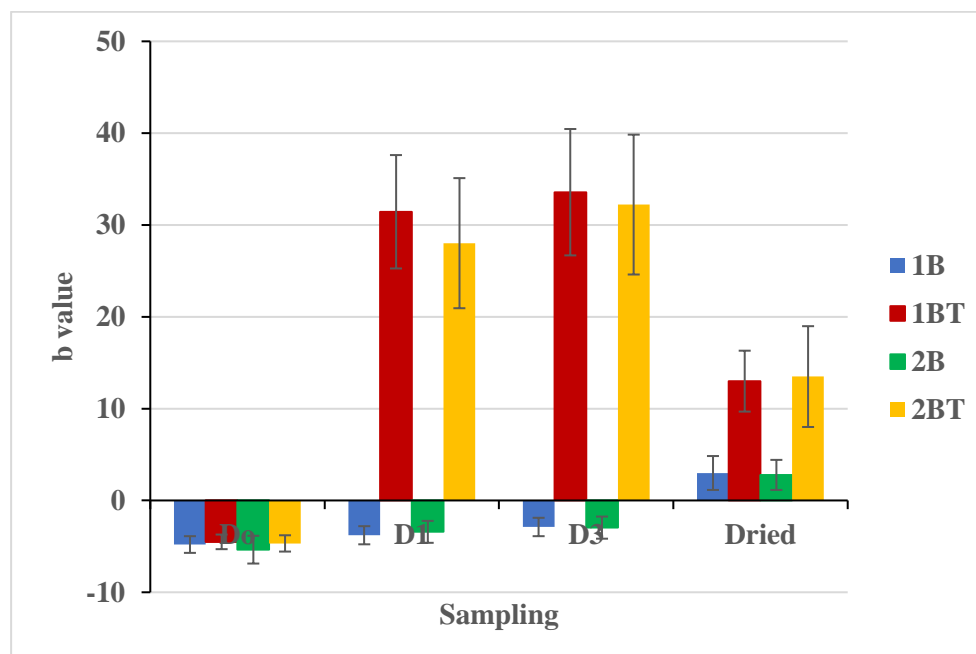


Figure 17. Average  $b$  values for different sample groups at different sampling points

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D1=brined, D3 =after incubating for 2 days at 7 °C and D13=dried).

In this study the  $L$ ,  $a$ ,  $b$  values differed significantly during the processing, and significant differences were observed in  $a$ ,  $b$  values among sample groups. The dried product with turmeric addition is yellowish which could aid in masking the visibility of development of spoilage colour in the product.

The average moisture content in pollock for the four sampling points during the whole process can be seen in Figure 18. No significant differences were found between the different sample groups. The moisture values on D1 and D3 were similar to moisture content found on D0, ranging from 78 to 81%. After drying, the average moisture values in the samples decreased significantly. The dried groups with 2% brine solution showed slightly lower moisture content than the other groups, however with no significant differences. The lower moisture values in the dried fish produced ranged from 14-16%.

A quality dried fish product with an expected shelf life of around 9-10 months should have moisture content below 20% (Gopakumar, 1997). The dried fish samples have a lower moisture content than 20%, ensuring a long shelf life of the product.

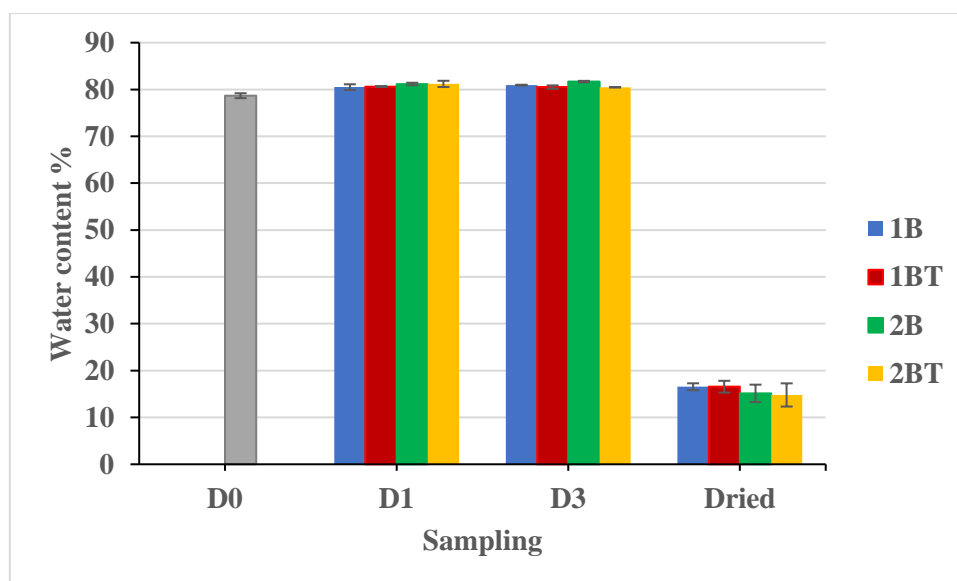


Figure 18. Changes in moisture content of fish while processing the brine salted dried pollock

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D1=after brining, D3 =after incubating for 2 days at 7 °C and dried).

After the brining and incubation, the average water activity value of all sample groups was similar to the value of the raw material, or 1.0. However, after drying, the water activity drastically decreased (Figure 19). Significant differences were not found between the different sample groups after drying. However, a trend was observed for slight differences in water activity between different salt concentrations of brine groups. The dried pollock using 2% brine gave the lower value compared with 1% brined.

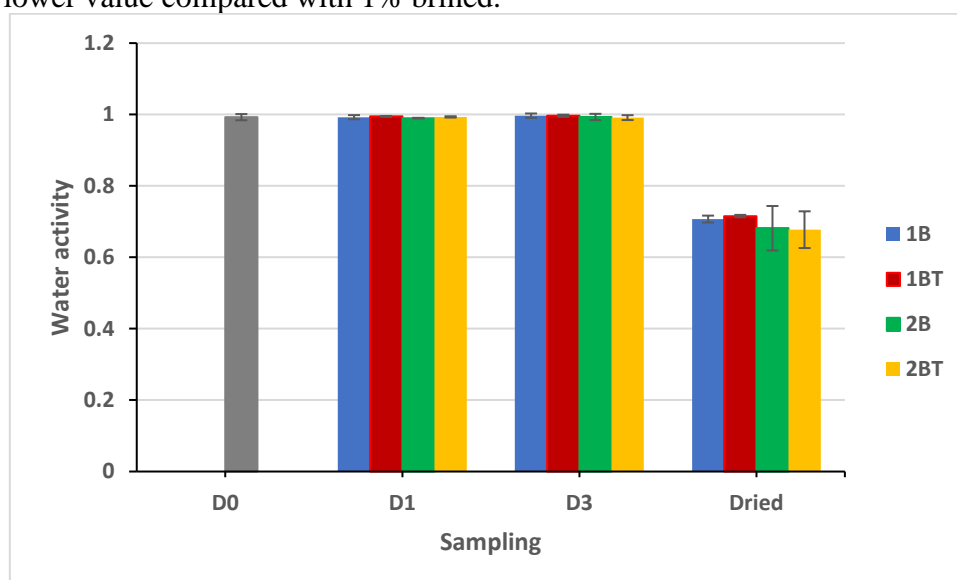


Figure 19. Changes in water activity of pollock during the brined dried fish production

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D1=after brining, D3 =after incubating for 2 days at 7 °C and dried).

The decrease in water activity after drying creates environment which is not suitable for microbial activity. To avoid microbial growth in dried fish products, water activity should be maintained below the critical value of 0.60 (Rahman & Labuza, 2007). However, although yeast and mould organisms are more tolerant at a reduced aw and can grow at value above 0.62, the pathogenic bacteria cannot grow at aw below 0.85-0.86 (Rahman & Labuza, 2007). This shows that the dried products in this project create an unsuitable environment for pathogenic bacteria.

The proportion of salt in the fish increased slightly after brining and increased drastically after drying because of dehydration. The salt content was higher in the groups brined with comparatively higher salt concentration. After brining, the salt content of pollock muscle ranged from 0.5 to 0.6% in the 1% brined group and 0.7 to 0.8% in the 2% brined group. After drying, the salt content of pollock muscle ranged from 2.25 to 2.35% of the 1% brined group and 3.0 to 3.5% of the 2% brined group. The brine salt concentration showed an important effect on the salt uptake into the muscle (Figure 20).

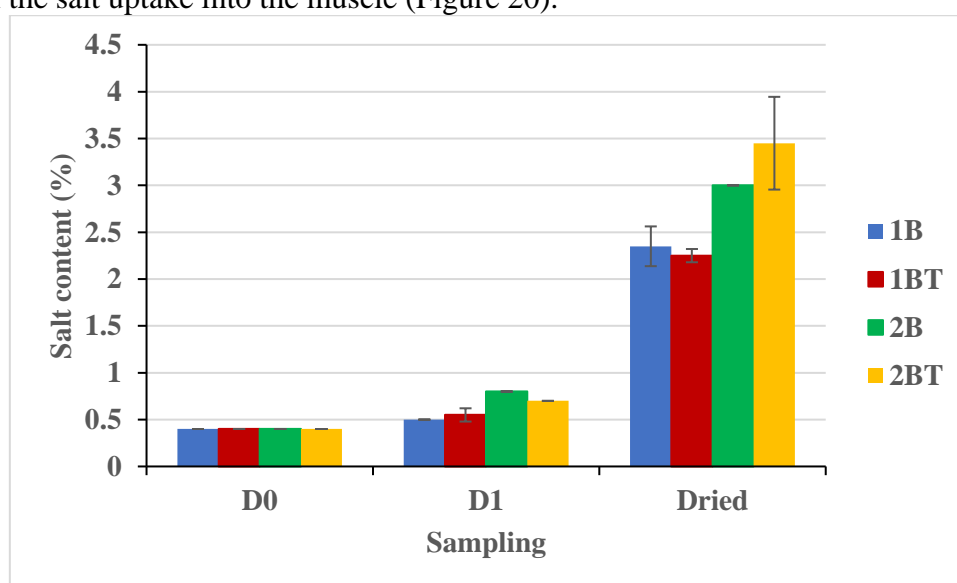


Figure 20. Changes in salt content of pollack while processing

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D1=after brining and dried).

Salt penetrates fish muscle by osmotic pressure. The rate of salt uptake and water release depends on the differences of salt concentration and quality of the fish muscle. The main features of salting are the removal of some of the water from the fish flesh and its partial replacement by salt. During salting, salt uptake and water exudation are mutually dependent. This is revealed in this study, as salt content in muscle decreased with an increase in water activity value and moisture content.

The pH in fresh fish is almost neutral. The pH 7.35 is the upper limit of acceptable of a fish (Anonymous, 1986). The pH generally increased in all groups during incubated storage period. Even though, there is an increase in pH of fish it was within the recommended limitations (Figure 21).



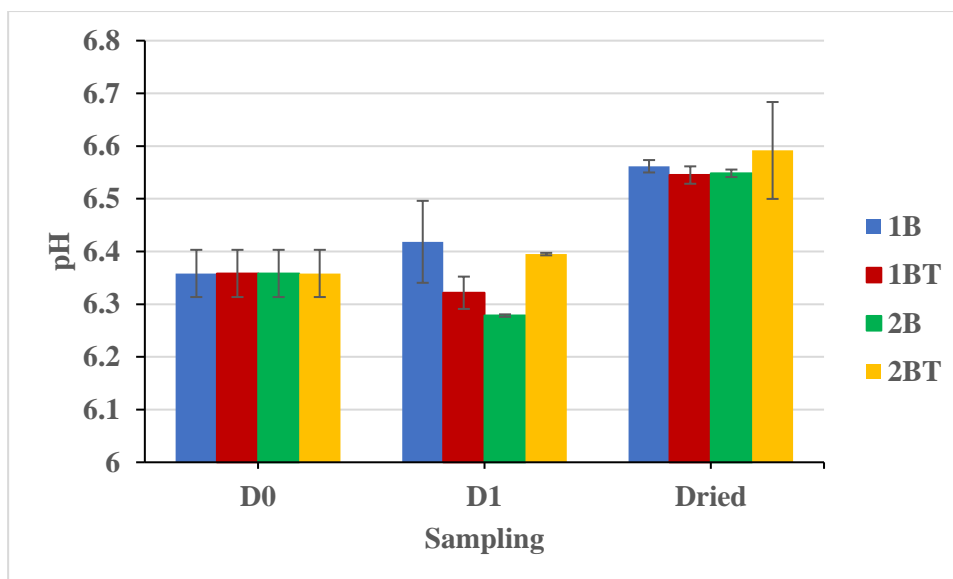


Figure 21. Changes in pH level of pollock while processing

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D1=after brining and D3 =after incubating for 2 days at 7 °C.

The average TVB-N values found for raw material and dried samples can be seen in Figure 22. A drastic increase in TVB-N was observed between the D0 and dried, the highest TVB-N values were obtained in samples after drying as the fish fillets were incubated two days at 7 °C to trigger the spoilage condition. The sample groups with turmeric addition into brine had a slightly lower TVB-N value than the groups without turmeric. Among the different dried sample groups, the 2BT group obtained the lowest value, 237.1 mg N/100g, while the 2B group showed the highest, 258.9 mg N/100g, followed by 1B, 241.3 mg N/100g, and 1BT with 237.7 mg N/100g.

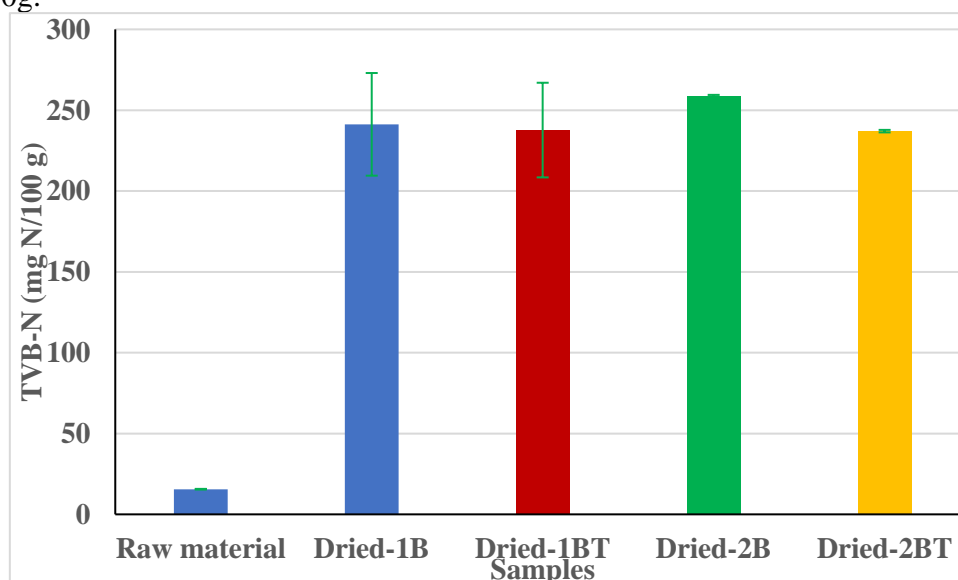


Figure 22. The TVB-N value of the raw material and the final products of pollock

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material and dried).

The total volatile basic nitrogen (TVB-N) is one of the most widely used measurements of seafood quality and is thought to be an important parameter for determining the freshness of fish products. It is a general term which includes trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with seafood spoilage (Huss, 1995).

Total volatile bases (TVB) is a group of biogenic amines formed in non-fermented food products during processing and storage and is a commonly used estimate of spoilage. Thus, the results indicate that the fish was somewhat spoiled after the incubation period and that treatment with turmeric might have decreased spoilage rate in the fish.

It was evident that the lipid content of the final dried products was always higher than before drying because of the decrease in water content after drying. The raw fish lipid content was 0.9% which infers that the fish selected for the study is a lean fish. In addition, the lipid content of fish remained rather stable during brining as there was no significant variation in the moisture content. Meanwhile, the lipid content increased drastically during drying to 4-5% (Figure 23). As moisture content decreased with higher salt concentration of brine, the fish brined with higher salt concentration had higher percentage of fat.

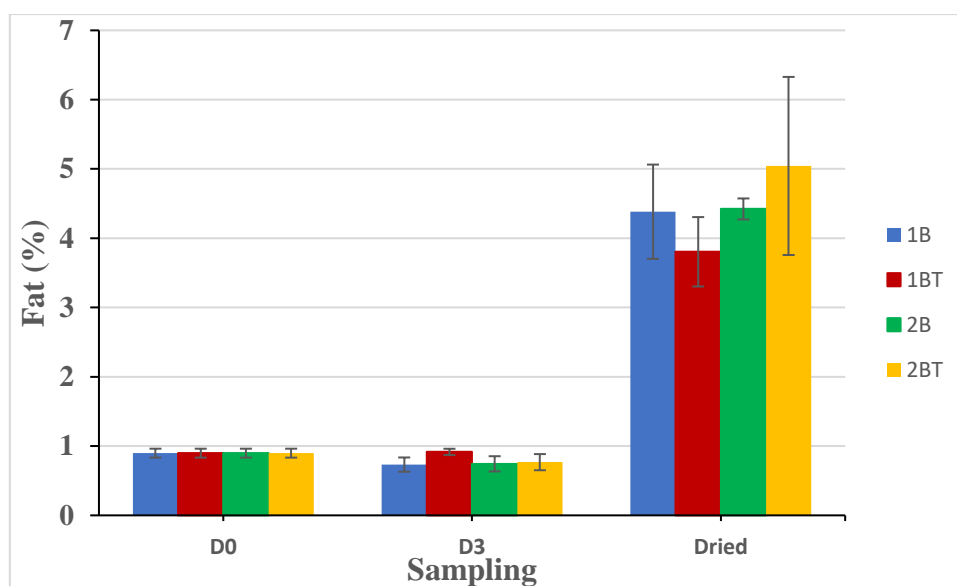


Figure 23. Changes in lipid content of pollock while processing

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D3= after incubating for 2 days at 7 °C and D13=dried).

The FFA concentrations increased during the processing from the initial raw material with 4.8 (g FFA/100g lipid) in raw fish. There was no significant difference in FFA content between the four groups after brining and after drying. However, there was a slight decrease in FFA value for those groups with turmeric addition (Figure 24).

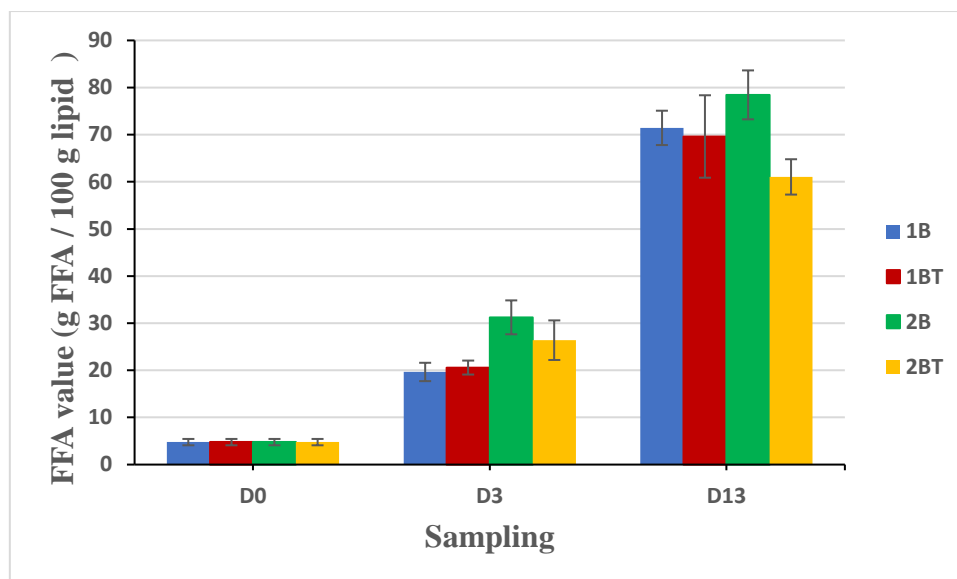


Figure 24. Changes in FFA of pollock while processing

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D3= after incubating for 2 days at 7 °C and dried).

FFA is an indicator of hydrolytic activity due to lipolytic enzymes. Usually this will increase during handling, processing and storage. High levels of free fatty acid is an indication of microbial spoilage activity. The acceptable limit of FFA is 2 - 5% (Bimpo, 1998). In dead fish, lipases from internal organs might be released into muscle, where lipids are localized. In delayed fishes there is an increase in the release of lipases into the muscle. During preparation such as eviscerating, filleting and washing, some enzymes might be discarded but some are still associated with muscle. When the fish is subjected to salt the remained enzyme is reactivated and hence lipid hydrolysis can take place and resulted in the formation of free fatty acids from glycerides and phospholipids (Karungi, Byaruhanga, & Muyong, 2004).

The phospholipids concentrations decreased during the processing from the initial raw materials with 23.6 (g phospholipids/100g lipid) in raw fish. There was no significant difference in phospholipid content between the four groups after brining and after drying (Figure 25).

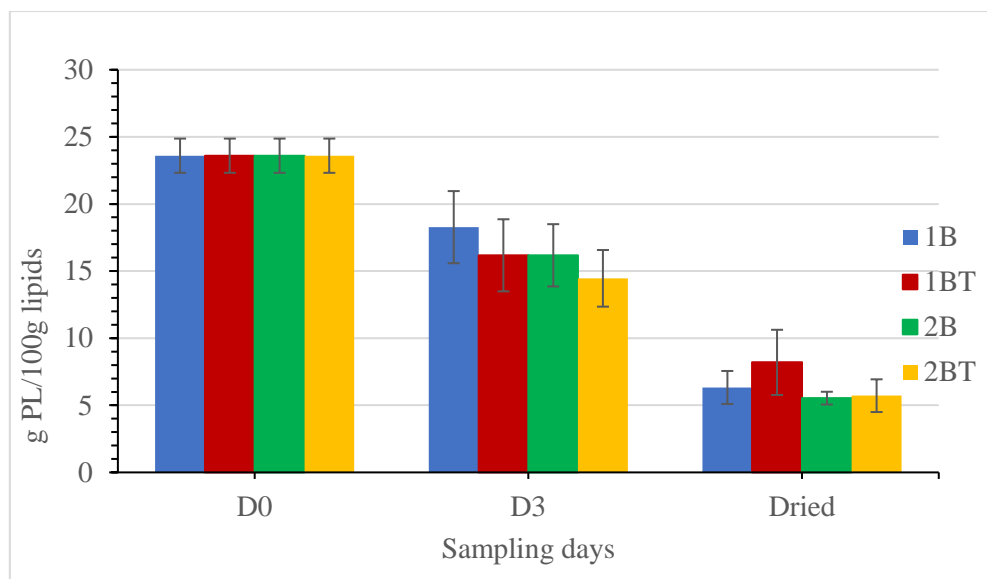


Figure 25. Changes in phospholipids content of pollock while processing

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D3= after incubating for 2 days at 7 °C and dried).

Lipid hydroperoxide (PV) and thiobarbituric acid reactive substance (TBARS) are used to indicate the lipid oxidation in food products. In general, during and after processing, the PV of fish products increased compared to the initial raw materials. This presented clearly for groups with turmeric with an increase of approximately 4 times in D3 and 10 times in dried. However, the PV value of those groups without turmeric was relatively stable (Figure 26).

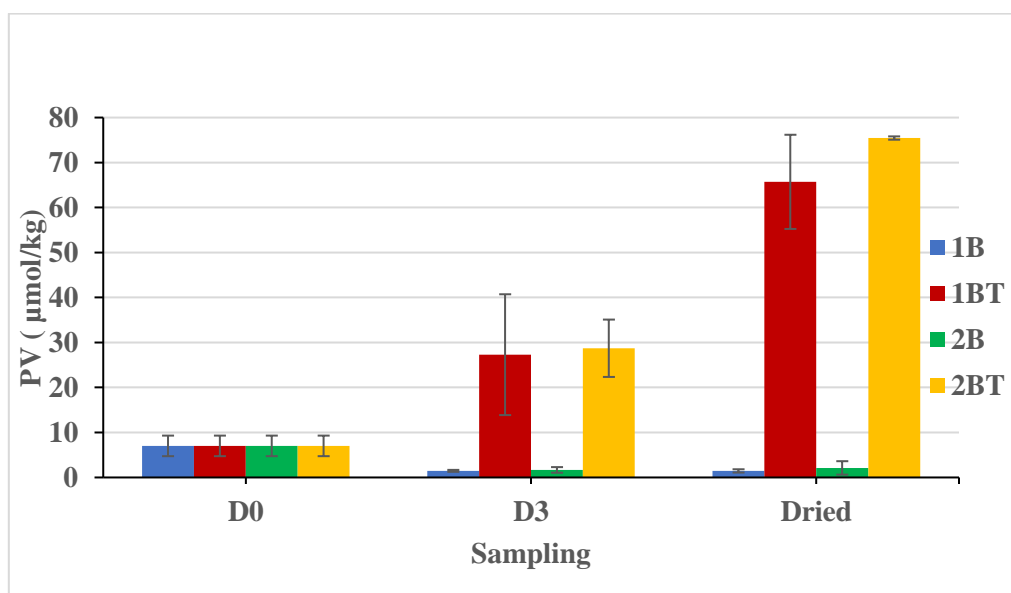


Figure 26. Changes in peroxide values (PV) of pollock while processing

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D3= after incubating for 2 days at 7 °C and dried).

The TBARS value remain stable from D0 to D3 at a level of 2.5  $\mu\text{mol}/\text{kg}$  and increased 10 times after drying (Figure 27). High temperature used accompanying drying process for a long time can be the most important cause for the formation of lipid hydroperoxide and TBARS in the products. Lipid oxidation is catalysed by light, heat, trace metals or enzymes and involves free radical generation. Free radicals propagate autoxidation by reacting with oxygen to form hydroperoxides, which break down to generate other new free radicals and TBARS. In the above case it seems to be strange that the PV values of 1B and 2B seem to be stable but TBARS appeared at the end product. This might be caused by a PV peak during the incubation or drying periods during which no sampling was made and would therefore have been missed. Else, there may be other substances which formed the aldehyde.

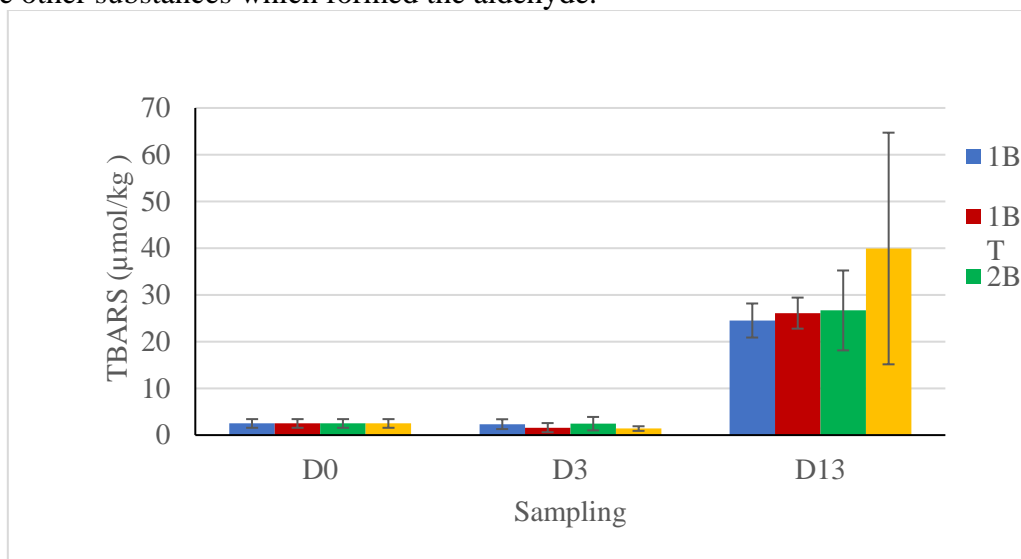


Figure 27. Changes in Thiobarbituric acid reactive substances (TBARS) of pollock while processing

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D3= after incubating for 2 days at 7 °C and dried).

The antioxidant potential of turmeric depends not only on its structure but also on the pH of the localized micro-environment. At pH 3–7, turmeric acts as an extraordinarily potent H-atom donor. The quick release of H-ion from the turmeric is very much beneficial for its antioxidant action (Malik & Mukherjee, 2014). The pH at this condition is suitable for turmeric to be an antioxidant. However, in this experiment the turmeric might have played as a prooxidant. Another significant study shows that the prooxidative activity of turmeric depends on its dosage and the corresponding prevailing chemical conditions in the vicinity. While at low concentrations, turmeric shows antioxidative activity, at higher concentrations, it shows prooxidative activity (Banerjee, Kunwar, Mishra, & Priya, 2008). This indicates that the concentration used in this study has resulted in a prooxidative activity of the turmeric.

#### 4.2.3 Microbiological analysis

The total viable count (TVC) in the raw material was  $9.3 \times 10^2$  cfu/g. According to ICMSF 1986, TVC below  $5 \times 10^5$  cfu/g is the acceptable limit for the fresh fish. This indicates that the raw material selected for the study was in a good condition. On D3, the TVC was between  $3-6 \times 10^7$  cfu/g which is above the limitation which indicates that the product was somewhat spoiled after the incubation period. After the drying process, the values slightly decreased to the range of  $2-5.5 \times 10^3$  cfu/g which is below the acceptable limit of  $1 \times 10^5$  for dried fish. From D0 to D3,

that the values are slightly lower for the turmeric treated groups with the group of same brine concentration used (Figure 28). Drying process removes water from fish and make an unsuitable environment for the growth of microbes.

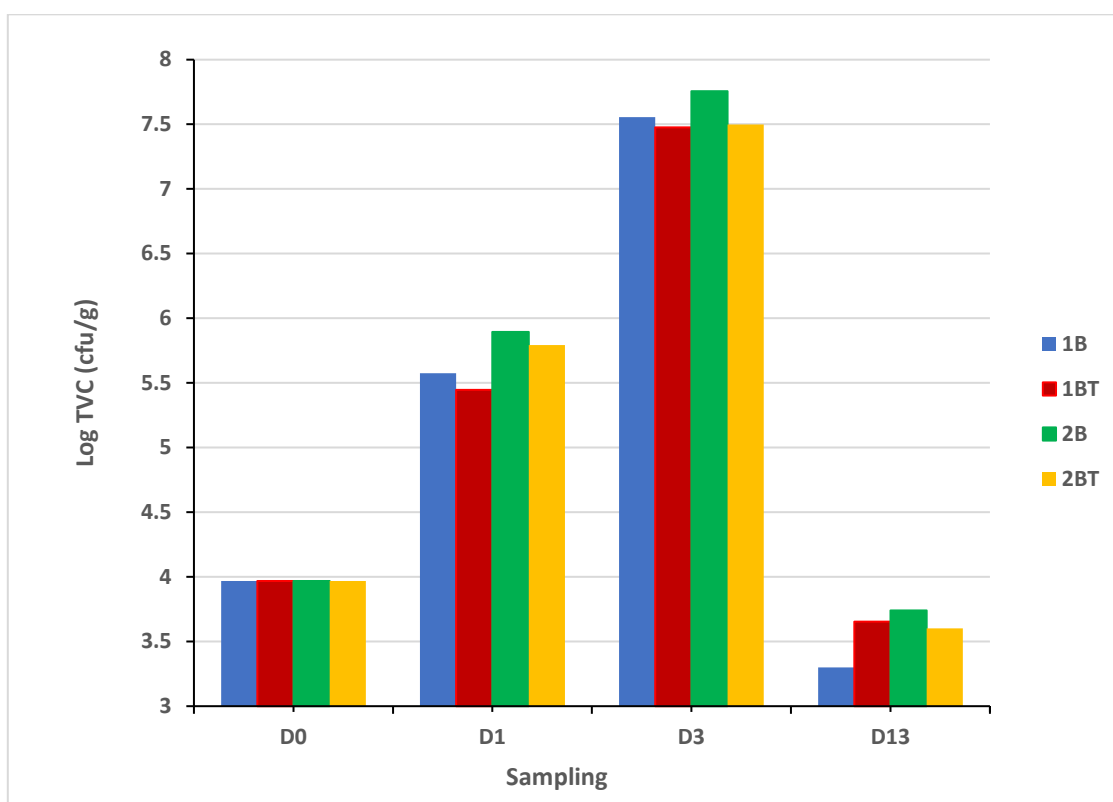


Figure 28. Changes of total bacteria count in Pollock fillet during the processing

(sample groups: 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D1=after brining, D3 =after incubating for 2 days at 7 °C and dried).

Black colonies count which depicts specific spoilage organisms (SSO) were  $6.3 \times 10^2$  cfu/g in raw material. This is illustrated in Table 8, below, where 1B =1% brined, 1BT =1% brined with turmeric, 2B=2% brined) and 2BT=2% brined with turmeric; sampling points: D0 =fresh raw material, D1=after brining, D3 =after incubating for 2 days at 7 °C and dried. The count increased after incubation and dropped again after drying. But any specific trend in change of count is not observed among different sample groups.

Table 8. Changes of SSO in pollock fillet during the processing

	D0	D1	D3	Dried
<b>1B</b>	630	1650	104500	<1000
<b>1BT</b>	630	1750	115500	<1000
<b>2B</b>	630	1300	565000	<1000
<b>2BT</b>	630	2600	405000	<1000

#### 4.2.4 Sensory analysis

The pollock was evaluated as fresh at the start of the experiment and fresh after brining. After incubation, a clear difference was seen between turmeric treated samples and samples brined in turmeric free salt solution. The turmeric treated samples received a Torry score of 5.5 and 6

which is at, or close to, the end of shelf life, but samples brined in salt solutions without turmeric received scores around 4 which indicates that the fish had reached the end of shelf life and was showing clear spoilage characteristics. Salty taste of the pollock before drying was rather weak in all samples but slightly stronger in samples brined in 2% salt solution than in samples brined in 1% solution. The turmeric treated samples were bright yellow in colour which reached only a few millimetres into the fish flesh.

After drying, the fish had a very hard and tough texture. The colour of salt brined samples was dark and heterogeneous, and the turmeric treated samples were dark yellow. It took a lot of effort to remove pieces from the fish fillets to evaluate. Clear processing odour and -flavour had developed in the fish during drying, described as sour and reminding of dried fish or half-dried fish, a traditional Icelandic product (*siginn fiskur*). However, the panel agreed that these were not clear spoilage characteristics but rather as expected for processed and/or dried fish. However slight spoilage odour and flavour was detected in some samples. Spoilage and processing odour and flavour was less detectable in turmeric treated samples, both before and after cooking of the dried fish, but the turmeric did not mask completely sour aftertaste of the cooked fish and gave a slightly bitter aftertaste. Salty taste was rather weak in the dried fish, both before and after cooking, but was slightly stronger in fish brined in 2% salt solution than in 1% solution. Texture of the cooked dried fish was very tough, and it was very difficult to chew the fish.

## 5 CONCLUSIONS

The sample groups showed differences in colour as turmeric is a natural dye. Drying played an important influence in the moisture content, water activity, yield, acidity (pH) and lipid content. The addition of turmeric led to decrease in rate of spoilage of the dried product during the processing which is confirmed by its low TVB-N value compared with samples without turmeric. The turmeric concentration used in this study made that act in a negative manner as antioxidant which lead to lipid oxidation of fish which is not favourable. The microbial quality of pollock after processing was considered satisfactory and the products presumptively safe to eat, considering the low number of SSO and TVC. Before drying, turmeric appeared to reduce total bacteria count during the processing.

Brining with 2% turmeric had a clear masking effect on spoilage odour and flavour of pollock after incubation, as well as spoilage and processing odour and flavour after drying of the fish. The turmeric-treated fish was bright yellow before drying and dark yellow after drying. The yellow colour was mostly limited to the surface of the fish and intruded only a few millimetres into the fish flesh. Turmeric-treated samples had a clear turmeric odour and flavour, but the turmeric did not have effect on the texture of the pollock. Only minor effect of different salt concentration of brine was seen on the pollock. Samples brined with 2% salt solution had a slightly saltier taste than samples brined with 1% solution, but salty taste was rather weak in all samples. The texture of dried pollock was very hard and tough and the texture of cooked dried pollock was described as “tough as a shoe sole” and the samples were very difficult to chew.

In addition to development of an innovative and added-value product for human consumption for both national market and for export, it is intended that this study can also be very important for Sri Lanka. Fisheries products have great importance for the Sri Lankan's diet both because

of its good nutritional composition, and because it is inexpensive compared to 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 Sri Lanka's society using turmeric as it is used commonly in cooking and considering its health benefits. Regarding the fish marinating aspects, it is a preservation method that can certainly be applied in the country. The turmeric used for marinating can also easily be purchased as it is produced in Sri Lanka. Besides that, the techniques performed within this study can be adopted to improve the fish processing in the country. In this project, to produce a safe final product, the fish was dried using the geothermal and electric energy. In the case of Sri Lanka, sun drying may be applied as a better option in view of the many problems with electric energy in the country.

For future studies on salted dried fish production with addition of turmeric, several attributes should be considered. The turmeric concentration should be used at a low level with varying concentrations, in order to minimize lipid oxidation in the products and to trace out both anti-oxidative and pro-oxidative property of turmeric. There is limitation in tracing the actual lipid oxidation due to the lack of sampling during the storage for spoilage and during the drying period. Therefore, more sampling points should be used to follow the actual change in the amount of primary and secondary oxidation products. Some changes can be incorporated into the processing method which would decrease the hardness of the product after drying as product resulted in the present study seemed to be with tough texture in the sensory analysis.



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## APPENDIX

**Panellists' descriptions of sensory characteristics of saith samples****Fresh pollack**

Fresh saith received a Torry score of 9. The odour and flavour were fresh and characteristic for the species, no spoilage was detected. The colour was light and homogeneous, no precipitations were seen, and the samples split easily into flakes when pressed. The texture was juicy, soft and tender.

**After brining**1% salt

Odour: sweet, fresh, characteristic for the species, no spoilage odour.

Flavour: sweet, characteristic for the species, no spoilage flavour, salty taste from weak to moderate.

Appearance: light, homogeneous, falls into flakes when pressed.

Texture: juicy, soft, tender.

Torry: 8,5

2% salt

Odour: sweet, weaker, slight off-odour, a trace of spoilage odour, described as a processing odour TMA like.

Flavour: sweet, slightly salty, spoilage flavour just detectable, salty taste from trace to weak.

Appearance: light, homogeneous, falls into flakes when pressed, some samples darker and more heterogeneous in colour.

Texture: soft, juicy, tender

Torry: 7

1% salt, 2% turmeric

Odour: turmeric odour, no spoilage odour.

Flavour: turmeric flavour, fresh, sweet, slightly salty, the turmeric seems to increase the sweet flavour, salty taste from trace to weak, no spoilage flavour.

Appearance: bright yellow surface

Texture: soft, juicy and tender.

Torry: 9

2% salt, 2% turmeric

Odour: Strong turmeric odour, no spoilage odour.

Flavour: Strong turmeric flavour, sweet, slightly salty, metallic flavour, no spoilage flavour

Salty taste: from weak to moderate.

Texture: soft, juicy, tender, slightly drier than other samples.

Torry: 8

**After incubation period**1% salt

Odour: TMA odour, strong spoilage odour

Flavour: moderate spoilage flavour, a trace of salty taste

Torry: 4

2% salt

Odour: TMA odour, sour, moderate to strong spoilage odour.

Flavour: off-flavour, TMA flavour, Moderate to strong spoilage flavour, weak salty taste

Torry: 4

1% salt + 2% turmeric

Odour: Turmeric odour and a trace of TMA, spoilage odour at threshold.

Flavour: spoilage flavour at threshold, slightly bitter aftertaste, salty taste at threshold

Torry: 6

2% salt + 2% turmeric

Odour: Turmeric odour and a trace of TMA, spoilage odour at threshold.

Flavour: spoilage flavour just detectable, salty taste at threshold.

Torry: 5,5.

**Dried pollack**1% salt

Odour: strong fish odour, not spoilage odour but a little sour.

Appearance: dark, heterogeneous, clear blood stains

Flavour: sour, dried fish, not much TMA, not spoiled

Texture: very hard and tough.

2% salt

Odour: some samples had fishmeal odour and TMA but some had sour odour and off-odour

Appearance: similar as 1% salt but more heterogeneous, slight blue hue in some places, very heterogeneous colour.

Flavour: slight sour flavour, slight TMA, slightly saltier than the 1% sample

Texture: very hard and tough

1% salt, 2% turmeric

Odour: weak odour, strong spice odour but also fish odour.

Appearance: yellow, heterogeneous

Flavour: Quite strong turmeric flavour, dried fish flavour, not sour flavour.

Texture: Very hard and tough, but less than other samples.

2% salt, 2% turmeric

Odour: one of the fillets had spoilage sour odour and a strong TMA odour.

Appearance: similar as 1% salt, 2% turmeric.

Flavour: strong turmeric flavour, slightly salty, slight sour flavour, slight off-flavour (aftertaste)

Texture: very hard and tough, more tough than other samples.

**Dried and cooked pollack**1% salt

Odour and flavour characteristic for processed fish.

Odour: some spoilage sour, as half dried fish (siginn fiskur), trace of spoilage odour

Appearance: heterogeneous colour, dark colour, looks like wet dried fish.

Flavour: like well processed half dried fish, TMA, dried fish flavour, salty taste and spoilage flavour between trace and weak.

Texture: Tough as a shoe sole.

2% salt

Odour: sour, wet dried fish, half dried fish, weak spoilage odour

Appearance: as samples of 1% salt.

Flavour: dried fish flavour, sour aftertaste, spoilage flavour on threshold, weak salty taste.

Texture: Tough as a shoe sole

1% salt, 2% turmeric

Odour: sour spice odour, half dried fish, fermented, weak spoilage odour.

Appearance: yellow

Flavour: Spice flavour at first but dried fish flavour and sour flavour develop when chewing, a trace to weak salty taste.

Texture: as of other samples.

2% salt, 2% turmeric

Odour: strong spice odour, also dried fish odour, not very sour, weak fish odour, a weak spoilage odour which disappears quickly.

Appearance: yellow

Flavour: a weak salty taste, fish flavour, sour aftertaste, spoilage flavour on threshold

Texture: Softer than other samples but still very tough.