EFFECT OF NATURAL ANTIOXIDANT SOURCES ON THE QUALITY UPGRADATION OF PROCESSED PRODUCTS FROM INDIAN MACKEREL

(Rastrelliger kanagurta)

Thesis submitted to

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By

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Certificate

This is to certify that this thesis entitled "Effects of natural antioxidant sources on the quality up gradation of processed products from Indian Mackerel (Rastrelliger Kanagurta)" is an authentic record of research work carried out by Smt. Beena Sulochanan under my supervision and guidance in the School of Industrial fisheries, Cochin University of Science and Technology, in partial fulfillment of the requirements for the degree of Doctor of philosophy of the Cochin University of science and Technology and no part thereof has been submitted before for any degree.

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DECLARATION

This is to certify that this thesis entitled " Effect of natural antioxidant sources on the quality up gradation of processed products from Indian mackerel (Rastrelliger kanagurta)" is a bonafide record of research carried out by me under the supervision and guidance of Prof.(Dr.)Saleena Mathew, Director, School of Industrial Fisheries, Cochin University of Science and Technology, in partial fulfillment of the requirements for the PhD degree of Cochin University of Science and Technology and that no part of it has previouly formed the basis for award of any degree, diploma, associate ship, fellows hip or other similar recognition in any University or Institution.

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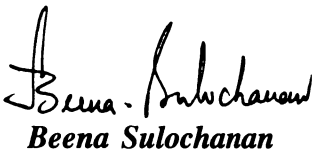

Beena Sulochanan

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CHAPTER 1
INTRODUCTION

GENERAL INTRODUCTION

1.1 Mackerel (*Rastrelliger kanagurta*)

Indian Mackerel (*Rastrelliger kanagurta*) is a major pelagic fishery along the South West Coast of India. The abundance is restricted to Kutch, Karnataka and Goa coast. The recorded landing of the fish for the year 2006-07 is 1,41,918 tons. Consumption of the fish is either locally as fresh fish or as frozen products. Considerable quality of mackerel are exported to Thailand (7170.83 tons) and Malaysia (3,790 tons). Though, in limited quantities it is exported to European countries USA and UAE also. The total quatity of mackerel exported from India during the year 2006-07 is 12,118.37 tons (Source: MPEDA). The market prospects in the international scenario too show that mackerel fishery is gaining importance. The Scottish Pelagic Sustainability Group has just entered a formal process of assessment for Western Mackerel fisheries according to the Marine Stewardship Council (MSC) standard. If approved, the mackerel fishery will be the first major pelagic tank vessel fishery in the world to be certified as well managed and sustainable. Plate 1.,1 shows the photograph of Indian mackerel taken as the raw material for the whole study.

Mackerel as a fatty fish has all necessary protein, vitamins and minerals in the desired proportions. In mackerel, the dark muscle is rich in lipids compared to light muscle. The lipid content of belly flap is remarkably higher compared to other parts. Like mackerel, commercial use of fatty fish species has been limited by the susceptibility of the fish to oxidative reaction of its lipids. In addition to the high concentration of highly unsaturated fatty acids, there also exists many prooxidants in the muscle tissue of mackerel.

1.2 Lipid oxidation

It is generally accepted that lipid oxidation occurs through a free radical process whereby the first step involves an extraction of H from a fatty acid (LH) molecule to produce a free radical (L*). Molecular oxygen, whose two unpaired electrons are spin forbidden to interact directly with unsaturated fatty acids, can readily react with the free radical formed, to produce a lipid peroxy free radical, (LOO*). The LOO* may then abstract a H from another fatty acid (LH) to produce another fatty acid free radical. This process is termed as the propagation stage. Termination occurs when two free radicals interact.

It has been suggested that hydroxy free radical (OH*) or something very similar to it can function as the initiator in lipid peoxidation. The hydroxy free radical can be produced by the reaction of transition metals with the reduction products of molecular oxygen, super oxide and hydrogen peroxide. Iron is of primary importance in fish tissue. Heame pigments may be activated by the hydrogen peroxide to initiate lipid oxidation.

Fats, oils and lipid-based foods undergo several degradation reactions both on heating and on long term storage. The main deterioration processes are oxidation reactions and the decomposition of oxidation products which result in decreased nutritional value and sensory quality. The retardation of these oxidation processes is important for the food producer and, indeed, for all persons involved in the entire food chain from the factory to the consumer. Oxidation may be inhibited by various methods including prevention of oxygen access, use of lower temperature, inactivation of enzymes catalyzing oxidation, reduction of oxygen pressure, and the use of suitable packaging.

Another method of protection against oxidation is to use specific additives, which inhibit oxidation. These are correctly called oxidation inhibitors, but nowadays are mostly called antioxidants. These inhibitors represent a class of substances that vary widely in chemical structure and have diverse mechanisms of action. The most important mechanism is their reaction with lipid free radicals, forming inactive products. Additives with this mechanism are antioxidants, in the proper sense. Usually, they react with peroxy or alkoxy free radicals, formed by decomposition of lipid hydroperoxides. Other inhibitors stabilize lipid hydroperoxides, preventing their decomposition into free radicals. Decomposition of hydroperoxides is catalysed by heavy metals, and consequently metal chelating agents also inhibit oxidation. Some substances called synergists demonstrate no antioxidant activity in themselves, but they may increase the activity of true antioxidants. Finally, singlet oxygen oxidises lipids many times faster than the common triplet oxygen, and consequently singlet oxygen quenchers also have an important inhibitory effect on lipid oxidation. The free radicals formed are found to be detrimental to human health and is the causative factor for many diseases.

1.3 Biogenic amines

Seafood has gained popularity and market shares in most of the countries due to being exotic, tasty, light and healthy. This trend has been questioned by another trend as consumers are becoming

more aware of safety and food poisoning. Quality has increasingly included concern on safety and this has highlighted the importance of temperature effects on bacteria and their activities in fish and shellfish. Production of biogenic amines especially histamine, is both a spoilage and a safety concern, and much progress has been made in identifying the factors controlling the process and the bacteria which may be involved. Much work has been done on the bacteriological effects of temperature changes during handling and storage of fish.

Biogenic amines have been defined chemically as aliphatic, alicyclic and heterocyclic organic bases of low molecular weight. Biogenic amines are formed by the action of bacterial enzymes (decarboxylases) on substrates such as free aminoacids. The presence of free aminoacids, microorganisms that can decarboxylate the aminoacids, and favorable conditions for growth of microorganisms are the factors that govern the formation of biogenic amines in certain fish species like mackerel, herring, tuna, sardines and anchovies. Important biogenic amines in fish are histamine, putrescine, cadaverine, tyramine, spermine and spermidine. Since such amines are formed by the bacterial flora of food material, prevention of bacterial growth or inhibition of such amine production would be very important for food safety. As some spices and herbs have been reported to possess antimicrobial activity in food spoilage bacteria, the use of certain spices to prevent the bacterial growth and thereby inhibit amine production was considered. Although considerable data are available on the growth of various bacteria in the presence of spices, data on their effects of the metabolic products are still scarce.

1.4 Antioxidative effects of various herbs and spices

Spices occupy an important position in the Indian culinary scene. Among the 52 Indian spices, pepper, ginger comes in the nine spices that have been regarded as major spices and the remaining as minor spices. Spices have various effects when used in foods. Not only do they impart flavour , pungency and colour characteristics, they also possess antioxidant, antimicrobial and neutraceutical values. The demand of spices is increasing the world over,for culinary purposes due to changed food habits, and as constituents in various pharmaceutical preparation of different system of medicine.

A large number of reports concerned with the antioxidative activity of herbs, spices and tea have been published. Antioxidant activity varies according to the country in which the plant was grown.

The early research also recognized that the antioxidative capacity of herbs, spices and tea, or of their extracts, depends on the substrate used in the evaluation.

The essential oils from a number of herbs and spices were also studied for antioxidative activity, e.g. oregano, rosemary, sage, clove, coriander, cumin, fennel, thyme, marjoram, laurel, peppermint, basil, cinnamon, nutmeg and black pepper. Although the compounds in the essential oils of some of them possess antioxidant activity, the aromatic character of these compounds limits the use of the essential oils as antioxidants in foods.

Some of the more popular synthetic antioxidants used are phenolic compounds such as Butylated Hydroxy Anisole (BHA), Butyled Hydroxy Toluene (BHT), Tertiary Butyl Hydroxyquinone (TBHQ) and Propyl Gallate (PG). Therefore, synthetic antioxidants in use are subjected to a limit of 0.02% of the fat or oil content of the food. They have been very thoroughly tested for their toxicological behaviors; still new toxicological data impose some caution in their use. In this context, natural products appear as healthier and safer than synthetic antioxidants.

The use of preservatives is therefore, an important fact of food product regulation and ensuring food safety. In recent times, as demand has increased for a wide range of different processed fishery products, and as challenging lifestyles require products with longer shelf lives, use of preservatives has become essential. However, the current trend is to use chemical preservatives in food in minute quantities, which has implications for the storage and safety of food products. Many countries have strict regulatory controls on use of chemical preservatives. In this context, the present study is of immense significance、

The present study is undertaken to determine the inhibitory effect of the selected spices on the biogenic amine production in mackerel and also their role as a potent antioxidant source in the preservation of mackerel and its products.

1.5 Major Objectives

- To study the antioxidant effect of spice extracts of rosemary, ginger, pepper and clove in comparison with a synthetic antioxidant BHA (Butylated Hydroxy Anisole) on the quality characteristics of chilled and frozen stored mackerel.

- To study the textural characteristics of treated and cooked samples of mackerel.
- To study the effect of spice extracts on inhibition of biogenic amine formation in fresh fish
- To study the effect of spices on the quality and storage stability of dried products from mackerel.
- To formulate some value added products from treated samples of rosemary extracts.

Plate 1.1 Indian Mackerel *(Rastrelliger kanagurta)*

CHAPTER 2

CHEMISTRY OF ACTIVE CONSTITUENTS OF SPICES

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2.2 Rosemary

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2.1 Introduction

The mere mention of a natural antioxidant brings about an association with spices and herbs, in that product developer utilize spice and herb extracts as replacements for synthetic antioxidants. This chapter will provide information regarding the geographical distribution and description of rosemary, ginger, pepper, and clove; the extraction methods of the active components and structural components of the active antioxidants.

2.2 Rosemary

Botanical name: *Rosemarinus officinalis*

2.2.1 Description and distribution:

It is a dense evergreen highly branched shrub growing up to one meter, with almost cylindrical leaves, two to four cm long by one to three mm thick, having inrolled margin, which are dark green in the upper side and silver stripped underneath. The plant blooms throughout the year and abundantly in spring. The flowers nestle in clusters at the terminal of the branches (Plate 2.1).

Rosemary grows wild and is also cultivated in Yugoslavia, Spain, Portugal, France and Europe as well as in California in U.S.A. It is a native of Southern Europe and grows wild on dry rocky hills in the Mediterranean region. It has been suggested as suitable for cultivation in temperate Himalayas and Nilgiri Hills with dry to moderately moist climates. Its lovely name 'Rosemary' joins two latin words meaning 'dew of the sea', because it thrives best where fog rolls in from the sea, as in the case along with its native Mediterranean region. The colour of the dried herb is brown green. The crushed rosemary however has an agreeable and fragrant, spicy aroma with a camphoraceous note. The taste has fragrant, spicy, pungent, bitter and camphoraceous notes.

2.2.2. Antioxidant constituents of Rosemary

From time to time studies have been undertaken to evaluate the anti oxygenic properties of spices. However it was Chipault et al., (1956) who by a systematic investigation compiled the "Antioxidant index" of spices which led to the finding that rosemary, its petroleum extracts, tops the list in retarding lipid oxidation. The art of steam distilling the essential oil from the over ground parts of rosemary is known for centuries. All parts of the plant except the woody stem are exploited for oil of rosemary and is distilled in most countries from freshly harvested plants.

(a) (b)

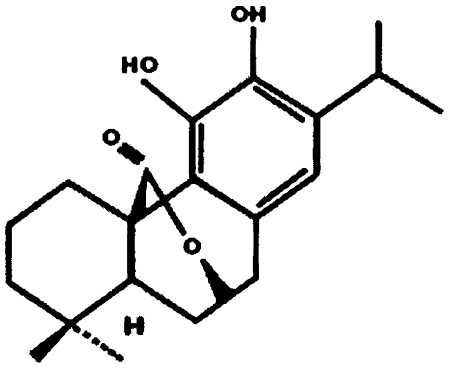
Plate 2.1 Rosemary *(Rosmarinus officinalis)*

(a) Rosemary flower (b) Rosemary leaves

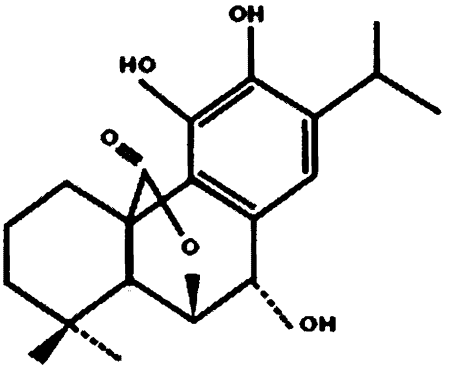
Extensive research has been done to unmask the source of antioxidant activity of rosemary. A number of compounds from the herb have been identified as having this property and there by include: -

1) Carnosic acid
2) Rosemanol
3) Epi Rosmanol
4) Iso Rosmanol
5) Rosmadiol
6) Rosmariquinone
7) Rosemaridiphenol
8) Rosemarinic acid
9) Carnosol

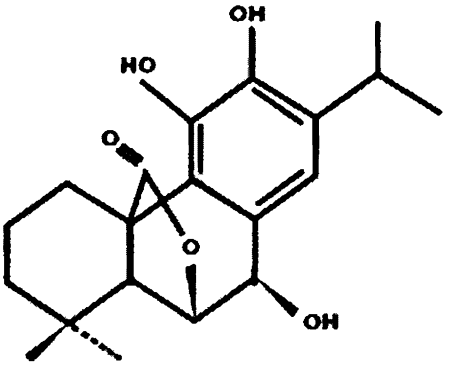
The chemical structures of these compounds are given in Figure 2.1



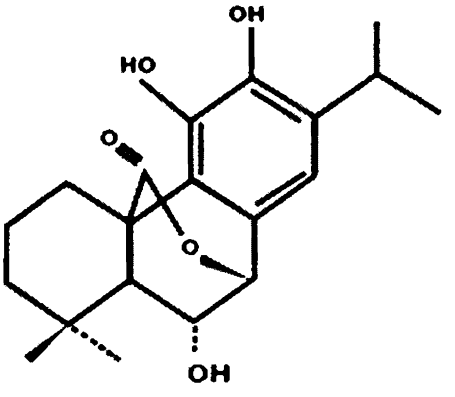
Carnosol



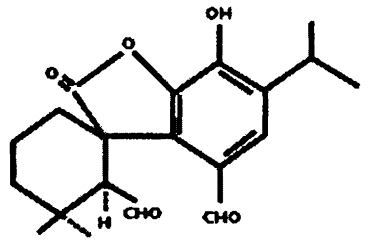
Rosmanol



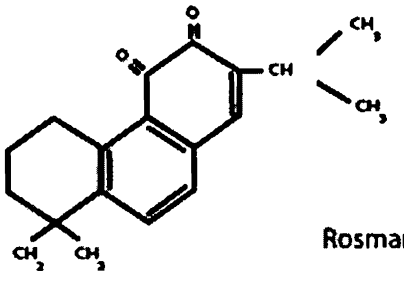
Epirosmanol



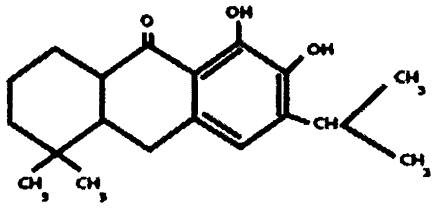
Isorosmanol



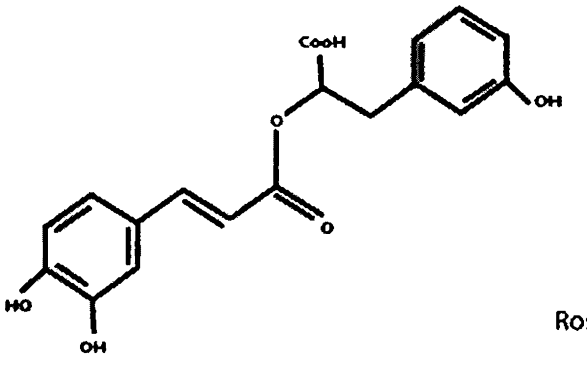
Rosmadial



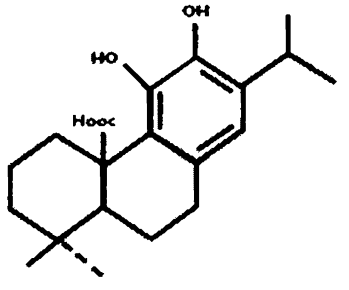
Rosmariquinone



Rosmaridiphenol



Rosmarinic acid



Carnosic acid

Figure. 2.1

Of these carnosol is an artifact derived from carnosic acid. From an oxidation inhibition point of view, some of these compounds equal or surpass Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT) and tocopherol. Of the spices, rosemary is outstanding as a rich source of tremendously valuable and versatile antioxidant. The antioxidant principles of rosemary have stolen the limelight and thrown a challenge to synthetic antioxidants, which are vigorously contested by numerous food laws.

The use of extract from rosemary spice as a natural antioxidant was first reported by Rac Ostric-Matijasevic (1955). In 1973, a patent was issued to Berner et al (1973), for the extraction of rosemary with oil. Later, chang et al. (1977) reported a patented process for the extraction of rosemary and sage, followed by a vacuum steam distillation of the extract in an edible oil or fat to obtain an odourless and flavourless natual antioxidant. Its antioxidant activity was demonstrated in both animal fats and vegetable oils. Further more, it was able to improve the flavour stability of soyabean oil, as well as potato chips.

Procedure for preparing an odorless and flavorless antioxidant as described by Chang et al. (1977) is epitomized in the flow chart (Figure 2.2).

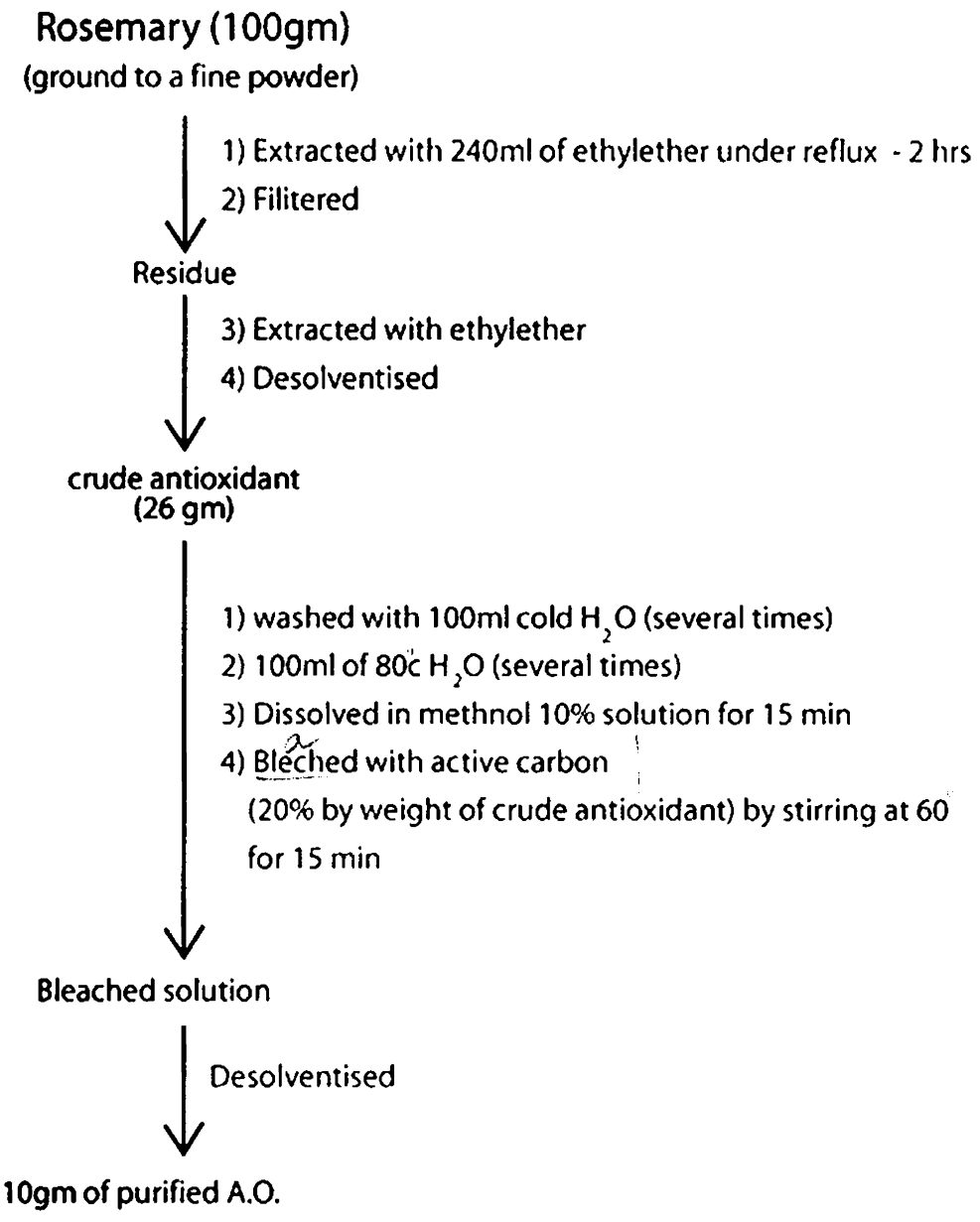


Figure 2.2 Flow chart for the preparation of rosemary antioxidant (Chang et al 1977)

100gm of rosemary that had been ground to a fine powder were extracted with 240 ml of diethyl ether under refluxing conditions for 2 hrs. The mixture was filtered and the residue could be extracted again with fresh solvent. The combined filtrate was freed of solvent to yield up to 26 g of crude antioxidant depending on the number of extractions.

The crude antioxidant was washed with 100ml of cold water several times, and then with 100ml of 80°C water several times. It was then dissolved in methanol (10% solution) and bleached with active carbon by stirring at 60°C for 15min.up to 20% wt of the crude antioxidant may be used. The bleached solution was freed of solvent to yield approximately 10g of purified antioxidant.

Bracco et al., (1981) also reported the use of double step, falling film molecular distillation to obtain an active antioxidant from rosemary extract.

This involves the microionisation of the herb in edible oil. e.g., ground nut, peanut oil. The antioxidants are transferred to the liquid phase. This is followed by a cleaning operation, either by filtration or centrifuging and molecular distillation on falling film or centrifugal system to collect the low molecular weight, active components, which deodorises and partially bleaches them. The antioxidants thus retrieved are subjected to column chromatography using solvents of increasing polarity. The fractions obtained were further investigated by mass spectrometry and UV absorption. The various stages are epitomized in the flowchart (Figure 2.3).

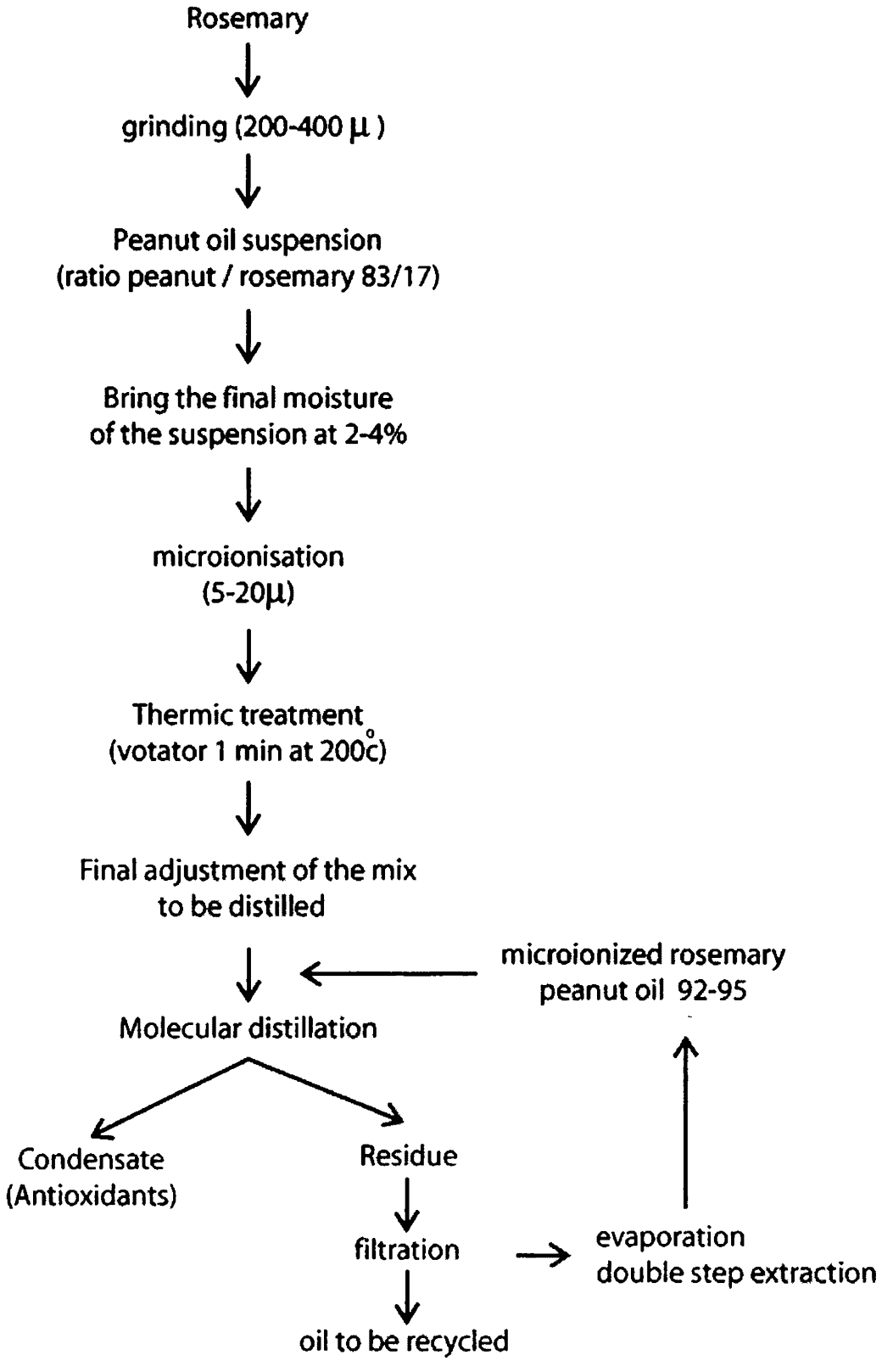


Figure 2.3: Flow chart for the recovery of Active antioxidant of Rosemary (Bracco et al., 1981).

Wu et al., (1982) has also reported the fractionation and identification of carnosol one of the active antioxidant components in the extract of rosemary. The scheme of preparation is shown in flow chart (Figure 2.4).

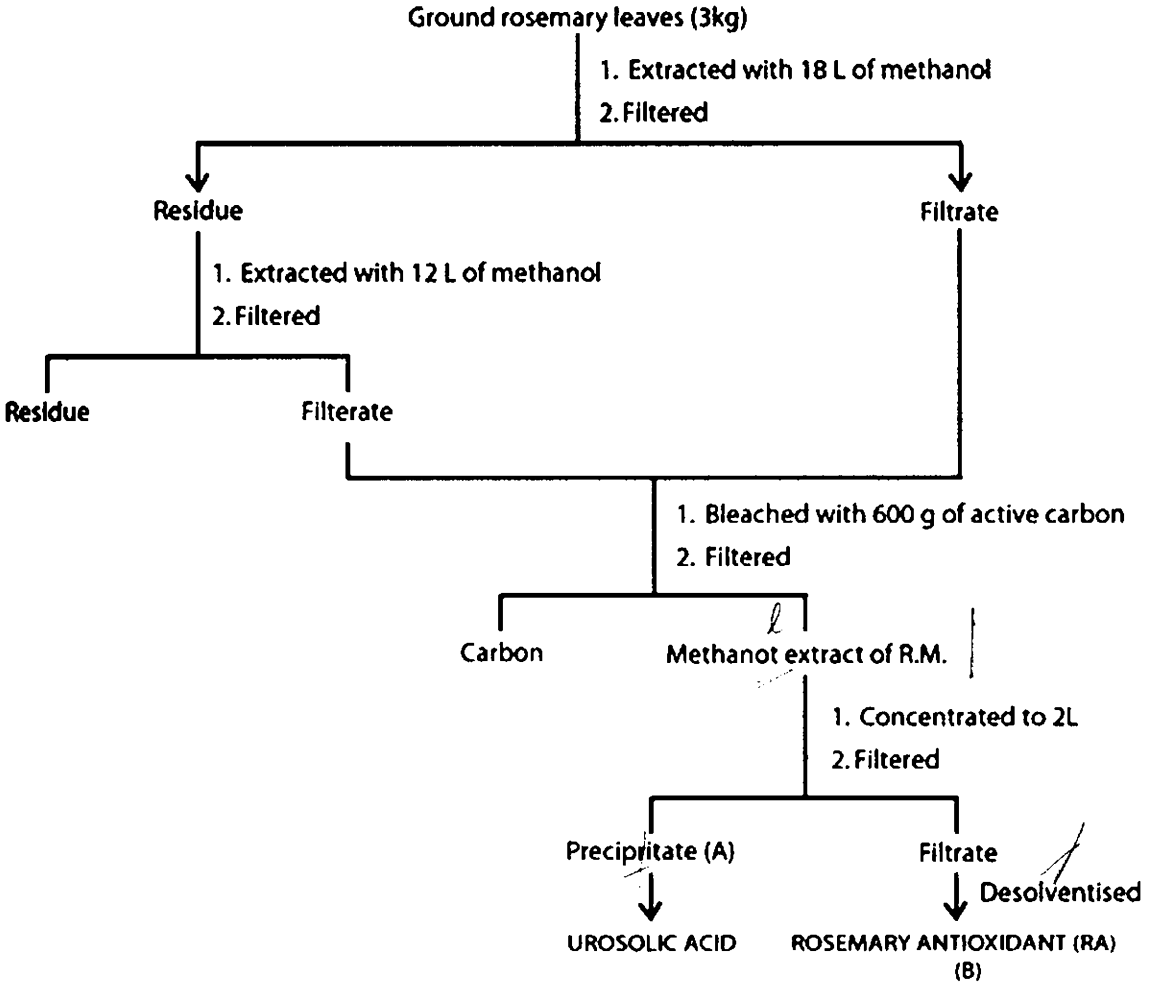


Figure 2.4: Flow chart for the preparation of Rosemary antioxidant.

Three kg of rosemary leaves that had been ground to a fine powder was extracted with 18L of methanol at 60°C for 2 hr. The mixture was filtered and the residue was extracted with 12L of fresh methanol. The combined filtrate was bleached with 600g of active carbon and then filtered to yield a light brown filtrate. The methanol solution was then concentrated to about 2.L by rotary evaporation and then filtered to remove theprecipitates (A) The filtrate was freed of solvent to yield 3.5% of rosemary antioxidant (B).

2.2.3 Fractionation of Rosemary Antioxidant (RA)

RA (10g) was first separated in to 7 fractions using glass column (id, 1.75 inches length, 23 inches) packed in the activated silicic acid. The column was eluted by stepwise gradient elution, using 5% ether in hexane, 10% ether in hexane, 25% ether in hexane, 50% ether in hexane, 75% ether in hexane, pure ether and pure methanol.

Each fraction was then rechromatographed on the same silicic acid column to yield a total of 16 fractions (Figure 2.5).

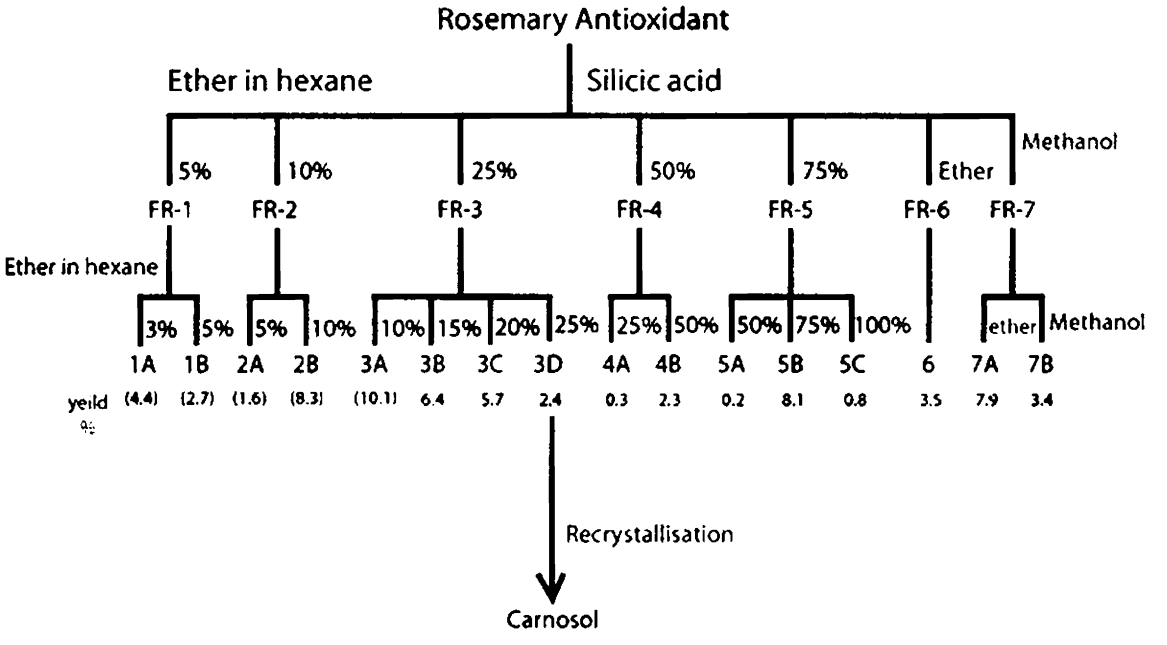


Figure 2.5 Chromatographic fractionation of rosemary antioxidant.

In order to elucidate the chemical structure of the components responsible for the antioxidant properties of RM, it was fractionated by repeated column chromatography with silicic acid using stepwise gradient elution. 7 primary fraction and 16 sub fractions was obtained. After further crystallization and spectral analysis (IR and NMR) the structure of carnosol was confirmed.

2.3 Ginger

Botanical name: *Zingiber officinale*

2.3.1 Description and distribution

It is a tropical perennial herb of the Zingiberacea family. Elongated, multibranched, irregular fleshy and pungent, its rhizome is prized for its healing properties. Though originated in tropical Asia it is widely grown in India, Jamaica, China, Hawaii, Australia, and Nigeria. (Plate 2.2).

2.3.2 Active constituents of Ginger

Ginger contains 1.5% - 3% essential oil, fixed oil 2-12%, starch 40-70%, protein 6-20%, fibre 3-8%, ash up to8%, water 9-12%, pungent principles and other saccharides, cellulose colouring matter and trace minerals (Ridley, 1912; Govindrajan, 1982; Purseglove et al., 1981; Weiss, 1997; Langner et al., 1998).

The essential oil is composed mainly of sesquiterpene hydrocarbons. This group compounds to about 50%-66% of the volatile oil. Oxygenated sesquiterpene are present up to 17%, and the remainder is composed of monoterpene hydrocarbons and oxygenated monoterpenes. Of the sesquiterpenes hydrocarbons, about 20% - 30% is (-)á- Zingiberene, up to 12% (-)â bisabloene, up to 19% (+) -ar- curcumene and up to 10% is farnesene (Weiss, 1997). A sensory study showed that â sesquiiphellandrens and ar-curcumen were the major contributors to the "ginger flavour", whereas á - terpieol and citral contributed a lemony flavour. The high lemon flavour and high citral content is apparent is Australian ginger. This product contains up to 19.3% citral versus up to 4% in other sources.

There have been numerous studies concerning the pungent components of ginger and what contribute to that pungency (Purseglove et al., 1981). Fresh ginger contains gingerol, which can be described as a series of compounds with the general structure (1-4 hydroxy - 3 - methoxy

(a) (b)

Plate 2.2 Ginger *(Zingiber officinale)*

(a) Ginger plant (b) Ginger Rhizome

phenyl) - 5 hydroxyl alkan - 3- one. Theyare mainly condensation products of Zingerone with saturated straight chain aldehydes of chain length, 6, 8, and 10. These are described as (6) (8) and (10) gingerols. The structure of the pungent principles derived from ginger rhizome are as shown in Figure 2.6

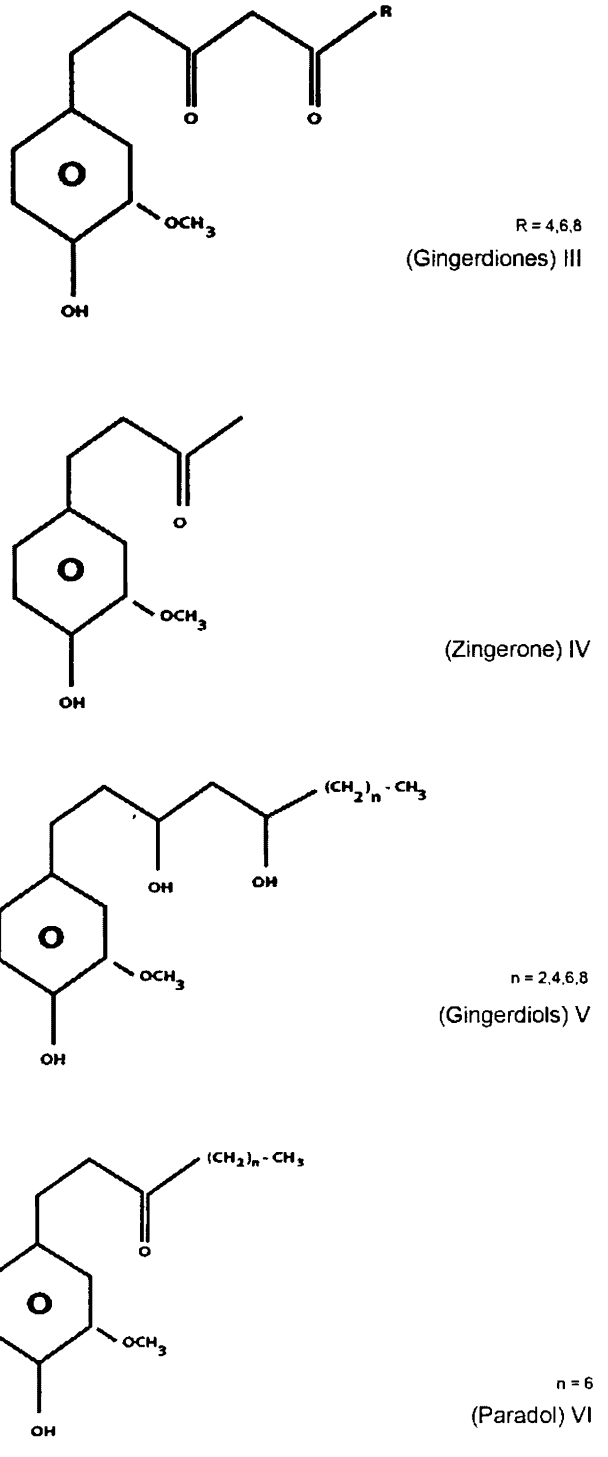


Figure 2.6: Structure of the pungent principles of ginger.

GingerolsI			Shogoals II		
n= 1, (3)	-	gingerol	n= 2, (4)	-	Shogaol
n = 2, (4)	-	gingerol	n= 4, (6)	-	Shogaol
n = 3, (5)	-	gingerol			
n = 4, (6)	-	gingerol	n= 6, (8)	-	Shogaol
n = 6, (8)	-	gingerol	n= 8, (10)	-	Shogaol
n = 8, (10)	-	gingerol			
n = 10, (12)	-	gingerol			

Ginger has been reported to exert antioxidant activity (Hirahira et al., 1974; Hirosue et al., 1978; Lee et al., 1982; Jitoe et al., 1992) but there have been few published reports on its active components. Fuijo et al. (1969) reported that the antioxidant activity of the pungent principle, zingerone and shogoal, in dehydrated pork. Lee and Ahn (1985) examined the effectiveness of ginerol in a â carotene - linoleic acid - water emulsion system.

2.3.3 Extraction and fractionation

Dried steamed rhizomes of ginger (995 g) were ground and extracted five times with CH_2Cl_2 (Dichloro methane) (2 L each) and subsequently thrice with 2 litres of methyl alcohol at room temperature (Kikuzaki and Nakatani, 1993). The combined CH_2Cl_2 extract concentrated to yield a brown viscous residue. This extract was separated by steam distillation to obtain a volatile and non volatile oil. The latter was subjected to column chromatography on silica gel to give 11 eluted fractions using the benzene - acetone solvent system. The combined, methanol extract was freed from the solvent. The steps in extraction and fractionation are shown in Fig. 2.7

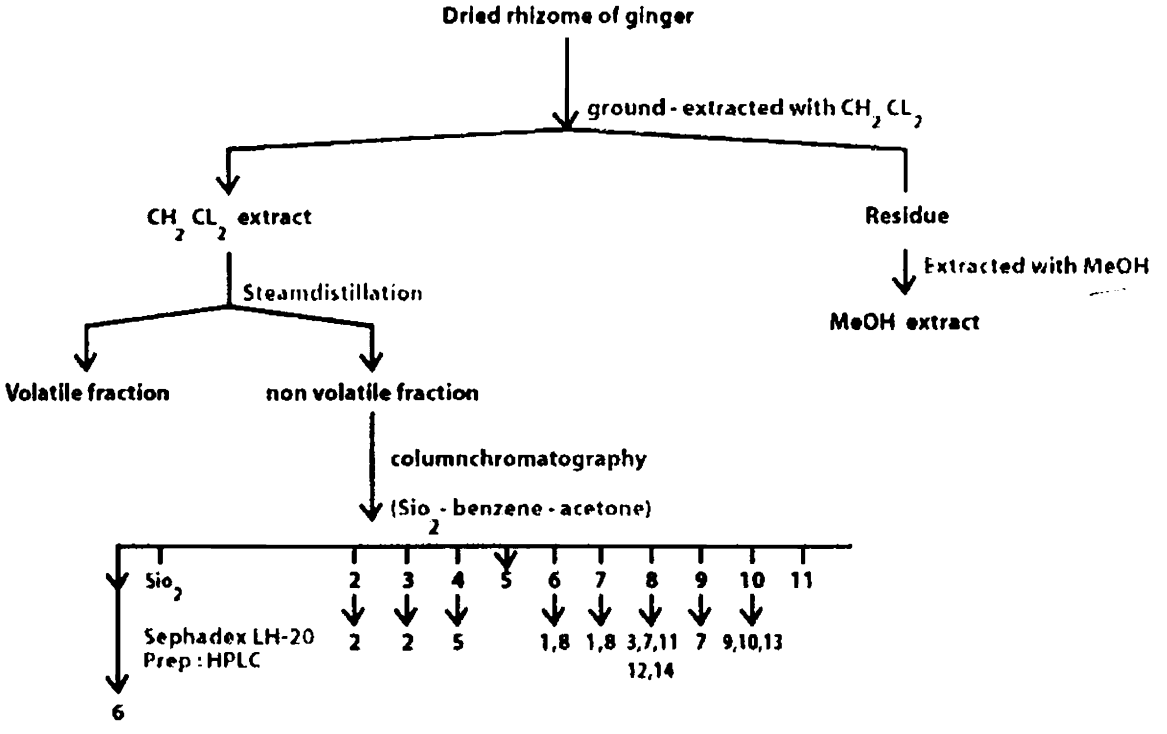
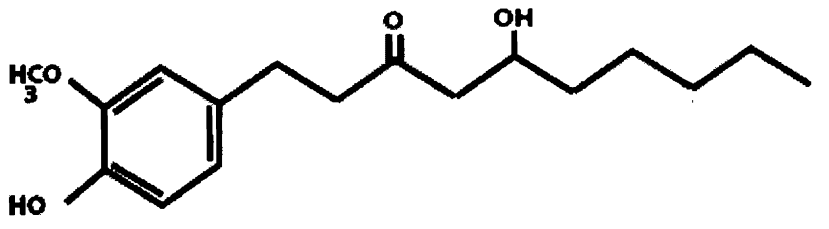


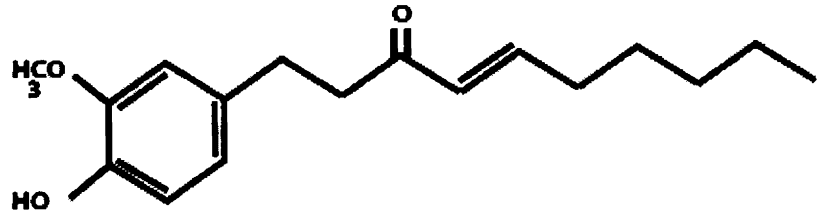
Figure 2.7: Flowchart for the extraction and fractionation of Ginger rhizome.

The fractions with positive activities (fraction 1-10) were purified by column chromatography on silica gel and sephadex LH - 20 and by preparative HPLC. Gingerol (1) was isolated from fraction 6 and 7 and - shogal (2) mainly from fractions 2 and 3 Gingerdiol analogues (compounds 3 and 5) were obtained from fraction 8 and 4 respectively, and dehydrogingerone (6) was isolated from fraction 1. Seven diaryl heptanoids (7-13) were obtained from the more polar fraction, 7 to 10; and curcumin (14) the first known isolated compound from *Zingiber officinale* was obtained from fraction 8.

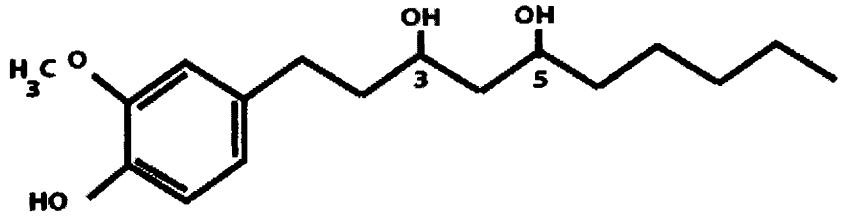
The compound tested and isolated for antioxidant activity was structurally classified in to five types, according to the substitution pattern of the side chains as follows (Figure 2.8).



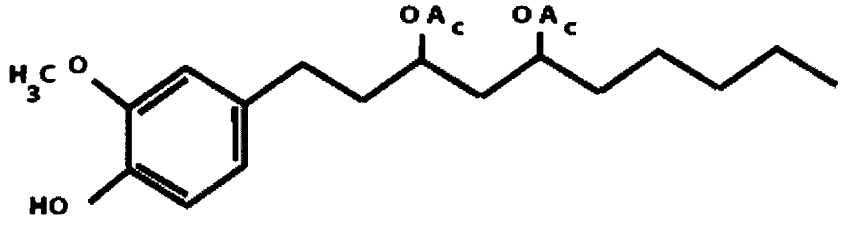
(1)



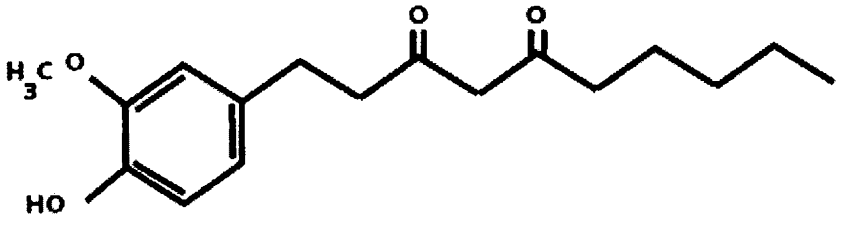
(2)



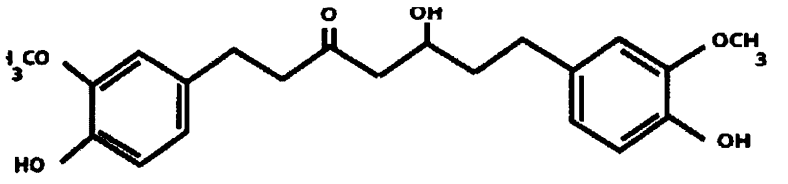
3; 3R 5S
4; 3S 5R



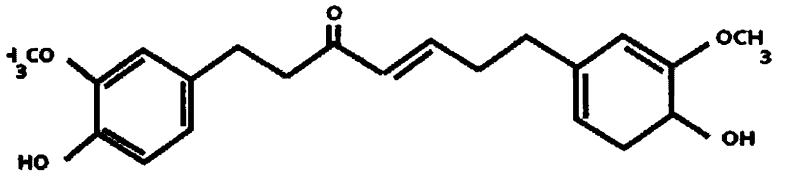
(5)



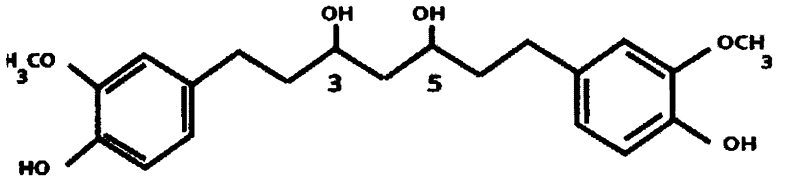
(6)



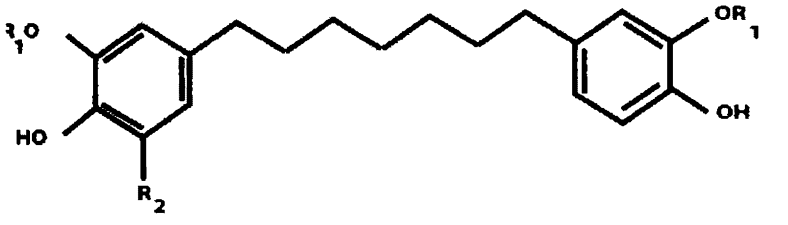
(7)



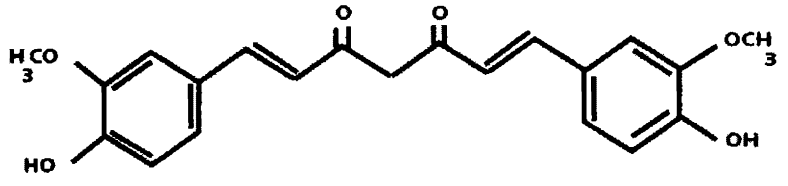
(8)



9 ; 3R, 5S
10; 3S, 5S



11 : $R_1 = CH_3$ $R_2 =$ H, 3R, 5S
12 : $R_1 = CH_3$ $R_2 = OCH_3$,
13 : $R_1 = R_2 =$ H, 3S, 5S



(14)

Figure 2.8: Structure of compounds isolated from ginger.

Gingerol related compounds (1-6) delayed oxidation of linoleic acid, and based on low absorbance values their activities were higher than that of á tocopherol. Efficiency tended to increase in the order 6<4< (6) - gingerol (1) < (6) shogaol (2) < (6) - gingerdiol (3) < 5. The antioxidant activity of the diaryl heptanoids with two 4- hydroxy - 3 methoxy phenyl groups (7-11 and 14) at the same concentration was compared. They appeared to exert greater antioxidant potential than tocophenol, and showed a tendency to be more active than gingerol related compounds (1-6) in each of the corresponding five stages. The antioxidant effect increased in the order 14<10<7<8d"11, which suggested that the substitution pattern of side chain was important in antioxidant activity. The difference in activity among these compounds increased as concentration decreased. We could summarize the activity increases in the order 6<1d"4<2d"3<5, that is for the side chains, 1-ene, 3, 5 Dione<5 dihydroxy -3one d"3S, 5S-diol<4-en-3 one <3R, 5S-diol< 3R, 5S-diacetate, together with results for the diaryl heptanoids.

Comparisons of the activity of 11 with that of 12 or 13 indicated that a compound with a 4- hydroxy -3 methoxy phenyl group appeared more active than those with a 4- hydroxy -3, 5- dimethoxyphenyl group; or 3,4 dihydroxy phenyl group. This suggests that the efficiency was also dependent up on substituents on the benzene ring.

2.3.4 Extraction of Ginger Oleoresin

Ginger oleoresin is a dark brown viscous liquid that has a warm, spicy, sweet, and very rich odour and sharp pungent flavour. On dilution, the oleoresin, affords a characteristic ginger, fresh, sweet, aromatic, rooty spicy and warm note and with a strong pungent sensation (Govindarajan, 1982).

The oleoresin is obtained from dry ginger rhizome by solvent extraction. For the successful recovery of oleoresin with acceptable physico chemical organoleptic properties, the integrity of the raw material, its technique of drying and physical modification prior to extraction are important.

With commercial grade ginger as substrate, yields of oleoresin ranged from 3.5-11% with 15-30% volatile oil have been realized using solvents which include methanol ethanol, isopropanol, acetone, ethyl acetate, methylene chloride, ethylene dichloride, mixed solvents, acetone-H_20 combination and supercritical CO_2.

Kinetics of the extraction with different solvents has been examined. Fast reaction rates are realised by solvents of low viscosity. Ethanol retrieves from the rhizome upto 20 percent of oleoresin, relatively low in volatile oil and pungent principles admixed with other extractives. The ethanolic extract is called as gingerine (Ridley, 1912).

2.3.5 Pharmacological cum healing profile.

Kirtikar et al., (1984) have capsuled the herbal remedies of ginger. The rhizome is an appetiser, useful in diseases of heart and throat, indigestion, asthma, bronchitis, dyspepsia and inflammations. Ginger provides relief in piles, rheumatism, headache, and lumbago pain. For the eyes, the rhizome gives luster, remedies the opacity of the cornea. The beneficial pharmacological applications are due to the cumulative contribution of the individual properties of action of the constituents present in ginger. In this respect, the pungent principles play a notable role (Yamahara et al., 1992) in which (6) gingerol and (6) - paradol are most potent.

Ginger is a warming herb with powerful ability to stimulate heart muscle, which accelerates blood supply. Consequently, cellular metabolic activity is improved and this contributes to the relief of cramps and tension. Ginger acts directly on the digestive tract and releases constipation cramps and flatulence (Yamahara et al., 1990; Kikuzaki, 1993). Ginger can reduce cholesterol concentration in a cholesterol rich diet (Tanabe et al., 1993). Lowering of cholesterol may be attributed to the antioxidant potential of some of the constituents in the spice (Jitoe et al., 1992). Ginger is endowed with antioxidant activity that enables it to preserve lipids and reduce lipid peroxidation in biological system. For this reason, ginger in addition to imparting flavor is competent to offer health benefits by inhibiting lipid per oxidation.

Antioxidants are increasingly linked to the prevention of certain cancers and coronary heart disease, as well as their more established role in preserving lipid based food. Studies include role of components such as gingerol inhibiting linoleic acid oxidation (Kikuzaki and Natakani, 1993); extending the shelf life of meat (Ziauadin et al., 1995), dehydrated pork (Fujio et al., 1969) and fermented meat sausage (Al-Jaley et al., 1987).

Ginger has antimicrobial activity due to the presence of gingerols, e.g. in relation to *Bacillus subtillis* and *Escherichia coli* (Yamada et al., 1992) and *Mycobacteriun* (Galal, 1996).

Ginger has a known influence on the eicosanoid cascade which influences such functions as wound healing, inflammation and platelet aggregation and is involved in conditions such as arteriosclerosis (Srivasta, 1986; Sakawa, 1987; Kiuchi et al., 1992).

Ginger has beneficial effect on the digestive system enhancing gastro intestinal motility and is used traditionally for the treatment of stomach ache, vomiting and indigestion (Yamahara et al., 1990). It has also been investigated for its gastro protectant and anti ulcer activity (Yamahara et al., 1988; Yamada et al., 1992; Yoshikawa et al., 1994).

2.4 Pepper - White and Black

Botanical name: *Piper nigrum*

2.4.1 Description & Distribution

White and Black pepper are both from the same plant- *Piper nigrum*, which is indigenous to the Malabar coast of Southern India.

Black Pepper is the unripe, dried fruit, while white pepper is the mature berry in which the hull is removed. Green pepper corns are immature berries. These are usually freeze dried or mechanically air dried. Pepper is grown on vines, which are usually supported by trees in the wild or wooden stakes if they are cultivated.

Malabar black pepper is generally an aromatic pepper with a high piperine level. Sarwak pepper is from Malayasia, along the north western coast of Borneo. Brazilian pepper is lower quality than Indonesian and Indian varieties.

Black pepper is harvested by hand, when the berry is green and dried in the sun on the spikes. They are heaped in piles to promote a browning reaction caused by fermentation, turning the berries dark, and then raked to allow uniform drying (Plate 2.3).

White pepper is harvested differently. The bright red berries are picked and removed from the spikes and packed in to bags and soaked in slow running water. This loosens the pericarps or hull from the core of the berry. After 2 weeks of soaking, the berries are crampled to remove the rest of the hull and the cores are washed and sun dried.

(a) (b) (c)

Plate 2.3 Pepper *(Piper nigrum)*

(a) Pepper vine (b) Ripe berry (c) Dried pepper corns

2.4.2 Chemical and physical specification.

There are two main components of black and white pepper the volatile oil and the pungent components commonly known as piperine. The volatile oil level in black pepper is usually higher than in white pepper. The hull of the pepper contains fibre and some essential oil. This essential oil is removed during processing in to white pepper. Black pepper contains about to 0.6%-2.6% volatile oil, depending on the source, but usually contains 2%-5% in good quality pepper berries. White pepper contains 1.0%-3% volatile oil. The maturity of the berry can influence volatile oil content.

The volatile oil content increases up to the level in a green pepper corn, and then deceases with maturity. The essential oil contains a large number of compounds. The main compounds present are á pinene, â pinene 1-á-phellandrene, â- caryophyllene, limonene, sabines –delta- 3 carene.

The main pungency component of pepper is piperine. There has been some debate over the years as to whether piperine was the component, which caused pungency, or not.

Piperine is the trans, transforms of 1- piperoyl piperidine. Other minor pungent alkaloids present are piperidine, piperyline, piperolein A and B and piperamine. Piperine content increases with the maturity of the berry.

Piperine can occur in four isomers when synthesized. The structure of piperine is shown in Fig. 2.9.

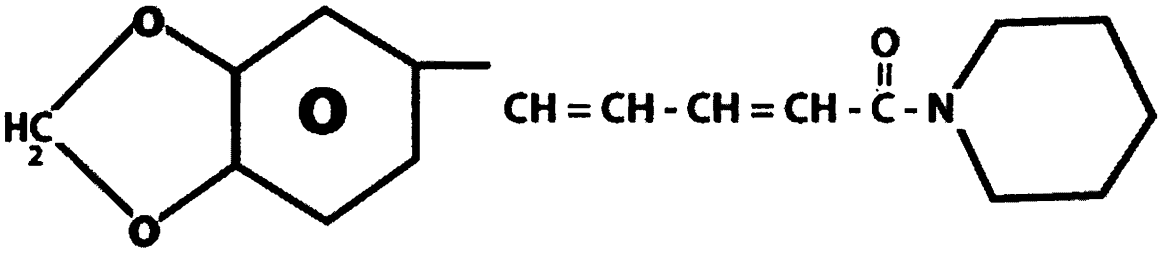


Figure 2.9: Structure of Piperine.

The isomers have different configuration at the double bonds as follows.

Piperene - Trans – trans

Iso piperine - Cis-trans

Iso chavicine - Trans-cis

Chavicine - Cis-cis.

None of the isomers have the high pungency level of piperine. It has been suggested that there is a slow photo isomerisation of piperine to its isomers during storage, thus decreasing its pungency. Black pepper oleoresin and essential oil are both available. The oleoresin varies from source and is available in a variety of strengths with regard to volatile oil and piperine content

2.4.3 Isolation of oleoresin of black pepper

The manufacturing of black pepper oleoresin through homogenization of pepper oil obtained by steam distillation and the solvent extraction of the distillation residue is a versatile procedure.

One of the pre-requisites for successful oleoresin production is the availability of pepper oil. By steam distilling coarse, ground or flaked pepper berries, the oil is collected. The yield composition and aroma profile of the oil vary depending on the spice. Berries of 4 ½ to 5 months maturity are rich in piperine and essential oil.

The distilled spice oil has about twice the amount of low boiling terpenes and only two thirds the amount of sesquiterpenes as compared to their amounts in the extracted oil. For an acceptable flavour, partial elimination of monoterpenes is necessary. During distillation, the oil may be divided in to three parts (a) initial fraction mainly of $C_{10}H_{16}$ hydrocarbons, (b) intermediate with minimum amount of sesquiterpenes, (c) heavy oil rich in sesquiterpenes.

Certain proportion of the three fractions is pooled in a manner to give upgraded oil with acceptable aroma profile. When steam distillation of the spice is not pushed deliberately to the end, a good portion of the heavy oil is retained in the residue from which it is recovered by solvent extraction. A finished oleoresin can be built up from the three distillation fractions, individually or collectively together with the extracted oil.

Piperine is in the 'solubles' extracted from the residue after distillation. Piperine constitutes the overwhelming proportion of the alkaloid mixture that gives the pungent flavour to black pepper. It is sensitive to heat, so that steam distillation provokes its decomposition and additional degradation occurs during desolventisation.

Steam distillation cum-extraction is a volatile route for the manufacture of standard resins. This is accomplished by mixing the essential oil and solvent extractive in the required proportion. Piperine is not very soluble in oleoresin of whole pepper. By centrifugation, substantially all of the undissolved piperine is retrievable as a dry solid residue, leaving a dark oily fluid, referred to as supernatant fraction containing some piperine. The liquid oil fraction consists essentially of liquid volatile oil, liquid non volatile oil and also dissolved piperines.

2.4.4 Antimicrobial activity

Pepper is known to be antibacterial. Two new phenolic compounds reported to be present in green pepper but absent in black, were tested for their antibacterial activity against the foodborne pathogens, *Salmonella typhimurium, Staphyloccus aureus, Bacillus cereus* and *Escherichia coli.* The compounds 3,4-dihydroxyphenyl ethanol glucoside (A) and 3,4-dihydroxy-6-(N-ethylamino) benzamide (B) were found to inhibit the growth of all of the four bacteria. (Pradhan et al., 1999). Dorman and Deans (2000) assessed the antimicrobial activity of volatile oils of black pepper (*Piper nigrum*) against 25 different genera of bacteria. These included animal and plant pathogens, food poisoning and spoilage bacteria. The volatile oils exhibited considerableinhibitory effects against all the organisms, while their major components demonstrated various degrees of growth inhibition. Ejechi and Akpomedaye (2005) studied the activity of essential oil and phenolic acid extracts of pepper fruit against some food-borne microorganisms *Staphylococcus aureus, Salmonella sp., Pseudomonas aeruginosa., Proteus sp., Escherichia coli., Enterococcus faecalis., Serratia sp., Bacillus sp., Clostridium sp., Penicillium sp., Aspergillus flavus,* which were susceptible to the extracts with a minimum inhibitory concentration (MIC) range of 1.0-4.0 mg/ ml. The essential oil inhibited the foodborne organisms better than the phenolic acid

Yamazaki et.al.(2007) studied and characterized the antioxidant activity of extracts from Japanese pepper fruit. The antioxidant activity of the methanol extract from Japanese pepper fruit was found to be equal to that of á-tocopherol and stable under heat treatment. The main compounds

that gave a significant antioxidant activity from the methanol extract were identified to be hyperoside (quercetin-3-0-galactoside) and quercitrin (quercetin-3-o-rhamnoside) as determined by HPLC, mass spectrometry, UV/VIS spectroscopy, and TLC. Evaluation of radical-scavenging activities of hyper oxide and quercitin from Japanese pepper fruit using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method revealed that hyper oxide and quercitrin scavenged DPPH radical strongly. Hence they concluded that Japanese pepper fruit showed the presence of strong antioxidants, namely hyperoside and quercitin.

Oboh et.al (2007) studied invitro the ability of aqueous extracts of ripe (red) and unripe (green) hot peppers to prevent 25 μM Fe^{2+} induced lipid peroxidation in rat's brain; assessed using TBARS (Thiobarbituric acid reactive species). They found that the inhibitory effect of pepper on lipid peroxidation of both basal and Fe^{2+} induced lipid per oxidation and Fe^{2+} chelating effect of the extracts were dose dependent.

2.5 Clove

Botanical Name: *Eugenia caryophyllus*

2.5.1 Description, Distribution and Economic importance

Clove is one of the most ancient and valuable spices of the Orient, known as far as 100 B.C. This spice was later known to the Chinese. Clove was imported in to Europe in 1265.

In India, clove was introduced in 1800 AD by the East India Company. By far the biggest clove producing region in the world today in Zanzibar, followed by Pemba, Madagascar and Indonesia clove in also produced in Malaysia, Srilanka and Haiti but not in commercially significant quantities.

A tropical plant, the life zone of clove falls between 20° North to 20° south of the equator, with even distribution of rainfall from 150-200 cm and tolerates acid soils to pH 4.5.

The clove leaf is nearly elliptical in shape 7-13 cm long and from 3.6 cm wide, smooth with dark green upper surface. The leaves are very aromatic, long full of minute oil glands, just visible with an ordinary lens as green dots on lower surface (Plate 2.4).

(a) (b)

Plate 2.4 Clove *(Eugenia Caryophyllus)*

(a) Branch of a clove tree (Dried clove bud)

2.5.2 Processed products

2.5.2.1 Clove Bud oil

This is derived from the dried buds by steam distillation, (yield 16%) contains as its main constituents, free Eugenol (70-90%), Eugenol acetate and Caryophyllene. Although these substances amount to some 99% of the oil, they are not responsible for the characteristic fresh and almost fruity note of the pure clove oil.

2.5.2.2 Clove stem oil

The chemical composition of the oil derived from clove stem (yield 4-5%) has not been investigated as thoroughly as that of commercially much more important clove bud oil, which is used widely in food products and in pharmaceuticals. The percentage of free eugenol present in clove – bud oil occurs also in the stem oil, but in somewhat different proportions (Fig.2.10)

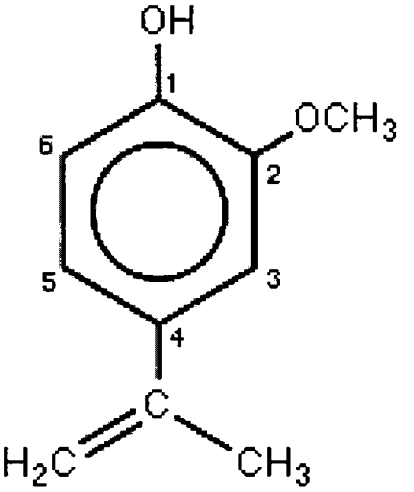


Figure 2.10 EUGENOL

2.5.2.3 Clove leaf oil

Clove leaf oil (yield 1-2%) usually contains a somewhat lower percentage of total Eugenol to that present in clove bud oil. The trace substance methyl –n- amyl ketone for example, which imparts characteristic, almost fruity odour to the bud oil, occurs in leaf oil, probably in even more minute quantities than in the stem oil. Quality or chemical composition of clove bud stem and leaf oil with particular reference to their aroma quality, has been studied and the found antioxidant activity in clove and thyme. Kramer (1985), through the use of thin layer chromatography, ultraviolet (UV), Infrared (IR), mass spectrometry (MS) and HPLC, determined quantitatively that gallic acid and Eugenol were 1.26g and 3.03g respectively in 100gm of clove.

2.5.3 Extraction procedure

150gm of ground clove was packed in to a glass chromatography column (500mmx35mm). Two litres of petroleum ether were percolated through the column from rotary evaporator reservoir above, to remove fat and much of the colour pigment. This was followed by 2litres of 80% ethanol to extract the phenolic compounds and sugars. The ethanol extract was concentrated on a rotary evaporator and extracted three times with ethyl acetate to remove the phenolic compounds and polar organics. Finally the remaining ethanol solution was extracted three times with ethyl ether to remove the nonpolar organic compounds. The steps in extraction are shown in Fig. 2.11.

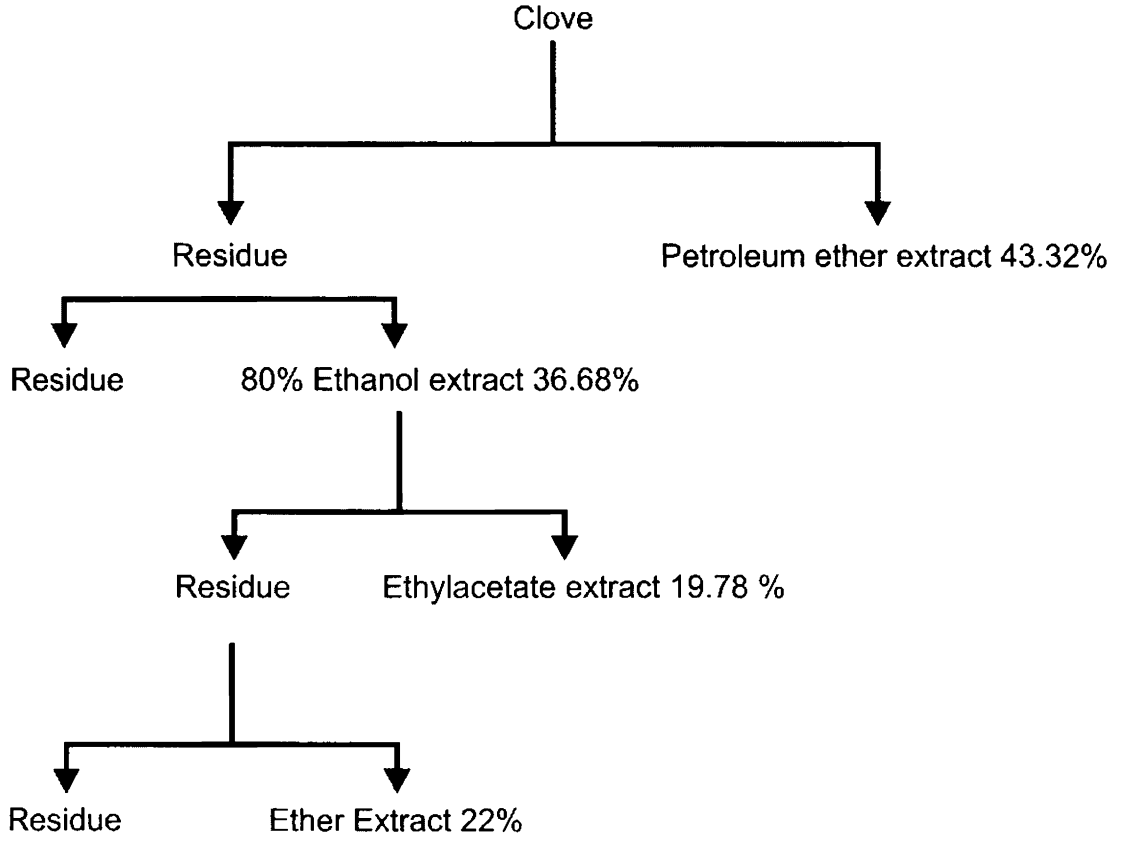


Figure 2.11: Flowchart for the extraction of clove.

CHAPTER 3

EFFECT OF SPICES ON CHILLED AND FROZEN STORAGE OF MACKEREL

3.1 Introduction

Fish has long been regarded as one of the basic sources of animal protein and were it not for the fragility of fish bones, archaeology would probably rank fish more highly in lists of foods from prehistoric times. The basic preservation technologies for fish were probably first drying and later salting, with or without smoking, as dictated by climate and local resources. Strong food flavours were a daily fact of life through the middle ages and although fresh fish was at times available, oxidation of fats was probably barely recognized as a problem in most fishery products.

But in recent times, with the advent of technology the most popular method of long term preservation of fish is by frozen storage. Prolonged frozen storage is known to lead to the deterioration in the quality of fish and shell fish items. Quality changes in fish include lipid oxidation, particularly in fatty fish and protein denaturation and textural changes due to interaction of proteins with oxidized lipids resulting in toughening of texture. For the long term storage of fish fillets, chilling alone has limitations in appreciable extension of shelf life and combination of chilling with other treatments incorporating permitted food additives have been applied to further augment the shelf life.

Antioxidants which include the phenolic compounds such as BHT (Butylated Hydroxy Toluene) and BHA (Butylated Hydroxy Anisole) have been used in the seafood industry. In recent years, there has been an increasing concern about the safety of synthetic food additives including the possible toxicity of the synthetic chemicals used as antioxidants. Naturally occurring compounds particularly spices and herbs have been chosen as a safe alternative to synthetic antioxidants. Much work has been focused on the antioxidant potential of spices on meat and meat products. The objective of the present study is to evaluate the efficacy of the use of naturally occurring spice compounds for maintaining the quality characteristics of fatty fish, mackerel, under chilled and frozen conditions.

3.2 Review of Literature

3.2.1. Muscle food as a substrate for lipid oxidation

Oxidation of lipids is a reaction of major significance in all living tissue and has both beneficial and detrimental consequence for the well being of living organism. (Sevanian and Hochstein, 1985). In muscle derived foods, the fine control mechanism that exist to control lipid oxidation

reactions *in vivo* are less effective and lipid oxidation proceeds in a comparatively uncontrolled manner (Sies, 1986).

After death the lipids in fish are subject to two major changes, lipolysis and autoxidation. Of the two processes, autoxidation is the most important, particularly in the deterioration of frozen fish products causing change in flavour (Banks, 1939), colour (Jones, 1962) and possibly textural changes of the lipid fraction in muscle foods (Sikorski et al., 1976). The polar phospholipids contain the highest proportion of unsaturated fatty acids and it has been established that this fraction as opposed to neutral lipid fraction, is primarily responsible for lipid oxidation in muscle foods.

3.2.2. Frozen storage of fish

Studies on mackerel (*Rastrelliger kanagurta*) of medium (4%) and high (11%) lipid contents, quick frozen individually (IQF) and as blocks (BF) and stored at -23°C showed that block frozen mackerel had higher frozen storage shelf - life than individually quick frozen samples based on sensory evaluation (Nair et al., 1976). Investigations carried out by Garg et al., (1982) on contact plate frozen stored (-18°C) ghol *(Pseudosciaena diacanthus)* in the fillets form indicated that ghol fillets remained in a highly acceptable condition up to 20 weeks and later acceptability steeply declined.

Chinnamma et al., (1995) observed that quick frozen mackerel *(Rastrelliger kanagurta)* stored at -10°C, -20°C and -30°C revealed that as the storage temperature was lowered a proportional extension of shelf life was obtained. Development of rancidity was found to be a limiting factor for acceptability when assessed by sensory and chemical parameters like free fatty acid and thiobarbituric acid value.

Frozen stored pink perch *(Nemipterus japonicus)* and oil sardine (*Sardinella longicieps)* showed significant ($p<0.05$) increase in peroxide value (PV), thiobarbiturc acid (TBA) number and FFA. While sardine showed a greater rate of lipid oxidation, hydrolysis of lipids to FFA was more pronounced in pink perch. In sardine, oxidized and hydrolyzed products of lipids had an equally adverse impact on protein solubility. Hydrolysis of lipids and consequent accumulation of FFA and development of rancidity due to oxidation of the lipids were found to be the major problems during frozen storage of sardine (Gopakumar et al., 1978).

3.2.3 Factors affecting the rate of lipid oxidation

The influence of triglycerides on the development of rancidity was shown to depend upon the degree of unsaturation and the length of time in frozen storage. The relationship between oxidation of polyunsaturated fatty acid (PUFA) of the phospho- lipids and development of rancidity was confirmed by many workers (Watts, 1962 and Greene, 1969). Balogun and Talabi, (1984) have concluded that autoxidation of the triglycerids, principally in the adipose tissues, is responsible for the development of rancidity in raw frozen tuna.

In fish, oxidation is made even more complicated by the presence of protein (Sikorski et al., 1976; Fisher and Deng, 1977) especially heme proteins.

The most unsaturated lipids in all fish are the phospholipids, but they do not oxidise rapidly and this is caused by the physical disposition of the lipids making it difficult for them to participate in the oxidation chain reaction (Hardy et al., 1979). Exposed lipid surface of fillets and gutted fish will oxidise more rapidly than lipids embedded in tissue thorough which oxygen may diffuse only with difficulty (Bito and Kiriyama, 1973; Bligh and Regier, 1976). In fish oils, oxidation will proceed quite readily at ambient temperature (Smith et al., 1972). In wet fish between 0°C and ambient temperatures, oxidation does not appear to be a dominant spoilage factor (Smith et al., 1979). In sardine and mackerel rancid flavours have been reported to affect acceptability (Madhavan et al., 1972). Oxygen is mobilized by fish at these temperatures with a competition for it between microorganism, enzymes and lipids (Smith et al., 1972). Whole fish, when they are exposed and subjected to autoxidation, microorganism that are present which can interact with the oxidised lipid, can possibly affect the oxidation rate. This explains as to why certain fatty species such as trout and gutted mackerel, will oxidise at temperature above 0°C. (Madhavan et al., 1970; Hansen, 1972) where as others such as herring remain relatively unaffected.

Studies on conditions of cold storage have shown important consequences, if dehydration occurs then the rate of oxidation was increased, where as storage under condition of low water loss by packaging glazing or freezing in water had a protective influence (Tarr, 1948).

Lipid oxidation in fish is a free radical process, consisting of initiation and propagation steps. A number of components are normally present in fish tissues which serve as pro-oxidants or antioxidants to both the initiation and propagation steps (Hultin, 1988).

3.2.4. Effect of heme iron

Studies of Decker and Hultin, (1990) and Phillipy, (1984) showed that reducing compounds, such as ascorbate and glutathione decreased with post mortem storage of fish muscle. This decrease will affect the process of lipid oxidation in post mortem muscle. When the temperature of any membrane is lowered sufficiently, its phospholipids bilayer will pass from the liquid crystalline to gel state. Heme iron has been proposed as an initiator and promoter (Kanner and Hard, 1985; Rhee et al., 1987) of lipid oxidation in raw meats. In fish, low molecular weight iron is released from a precursor molecule, probably ferritin with time of storage. (Kanner et al., 1987; Decker and Hultin, 1990). The process is greatly accelerated if the muscle tissue has been frozen and thawed. When the muscle cells tend to be weak to maintain components in the reduced state, myoglobin and hemoglobin (Fe^{2+}) are oxidised to metmyoglobin and methemoglobin (Fe^{3+}). This in turn can react with hydrogen peroxide to produce (Fe^{4+}) which can initiate lipid oxidation (Kanner et al., 1987; John et al., 1989).

Tippeswamy et al., (2007) identified possible non controllable and controllable factors responsible for the occasional lack of heamoglobin (Hb) mediated lipid oxidation of washed cod mince. Among noncontrollable factors were initial peroxide value (PV), level of tocopherol and structure. Among controllable factors were washing, pH, moisture, Hb (haemoglobin) level and light during storage of food.

3.2.5 Effect of lipid oxidation products on protein structure

The myofibrillar protein particularly myosin of many species may be altered by the intraction with different types of lipids or lipid oxidation products during frozen storage (Saeed et al., 1999). This interaction caused considerable changes in some functional properties and in the texture of fish muscle (Howell, 1999).

Exposure of protein to peroxidising lipids of their secondary breakdown products can produce changes in proteins, including loss of enzyme activity, polymerization, insolubilisation, scission and formation of lipid protein complexes. (Sikorski et al., 1976; Sikorski et al., 1990).

Lipids, especially oxidized lipids, may affect the hydrogen bonds and hydrophobic interactions in the proteins of frozen fish. The fatty acid character of lipid molecules exerts a surfactant effect on

protein surfaces, leading to hydrophobic interaction and protein unfolding, thus exposing interior groups for reaction. Further more the carbonyl groups of oxidised lipids may participate in covalent bonding, leading to the formation of stable protein-lipid aggregates.

Jahrenback and Litjemark, (1975) shows the results of model experiments on protein lipid, interaction which gives circumstantial evidence for the effect of ordinary lipid on fish protein. Lipid protein interaction involves two basic mechanisms (Schaich and Karel, 1975). The first mechanism involves protein amino condensation reactions, involving lipid oxidation, break down products, such as malonaldehyde and amino groups. The second mechanism involves the reaction of proteins with lipid oxidation products, which results in the formation of protein centered free radicals (Saeed et al., 1999).

3.2.6. Lipid oxidation in cooked system

In cooked meats, work about the relative pro-oxidant rules of heme and non heme iron in lipid oxidation have been done (Sato et al., 1971). Love and Pearson (1974); Verma et al., (1985) and Apte and Morrissey, (1987) found that iron was released from heme pigments following cooking and proposed that the resultant increase in non heme iron was responsible for the rapid oxidation of stored cooked meats. Studies by Kristensen and Andersen, (1997) show that there was a negligible increase in free iron at the expense of heme iron in myoglobin solutions heated up to 90°C. They also showed an increase in pro-oxidant activity of myoglobin around its thermal denaturation temperature (60-90°C) and attributed this to exposure of the catalytic heme group to lipid hydroperoxides. At levels relevant to meat and meat products, the pro-oxidant activity of heme iron in cooked meats exceeds that of free iron. Other studies using model system have shown that ferritin, when heated may promote lipid oxidation. The other major iron-containing fraction in muscle is the insoluble hemosiderin fraction, which accounts for as much as 30% of total iron in beef and 60% in chicken (Hazell, 1982). In a study in which the relative contributions of different iron-containing fractions to lipid oxidation were found in beef, pork and fish. Apte and Morrissey, (1987) concluded that haemosiderin did not play a significant role in lipid oxidation. Rhee, (1987) has pointed out that the greater susceptibility of cooked meat towards lipid oxidation may also be due to the disruption of meat tissues by cooking; thus bringing the lipid substrate and catalyst in to closer contact.

Similar studies by Tichivangana and Morrissey, (1985) revealed that only in raw fish metmyoglobin was a significant pro-oxidant, whereas significant increases in TBARS, were seen with pork, chicken, turkey and fish in the presence of ferrous iron.

The cooking process leads to thermal denaturation of antioxidant enzymes and enzyme inactivation is believed to contribute in part to lipid oxidation in cooked meats (Lee et al., 1996).

3.2.7. Factors influencing oxidation in Muscle Foods

3.2.7.1. Temperature

A number of extrinsic factors influence lipid oxidation in muscle foods, such as temperature, light etc. Hultin, (1994) showed that increased dissolved oxygen at lower temperatures may offset the effect of reduced temperature on the rate of lipid oxidation, and in fish, frozen thawed muscle may oxidise more than the non frozen tissue (Decker and Hultin, 1990). Lipid oxidation in raw meats in refrigerated storage can be greatly accelerated if the meat has been restructured (O' Grady et al., 1997) In the absence of particular condition that promote lipid oxidation in refrigerated raw meats, quality deterioration due to microbial growth may preceed oxidative deterioration (Monahan et al., 1992). In frozen storage both microbial deterioration and oxidative deterioration are retarded in raw meats but lipid oxidation leading to rancidity occurs with increasing storage time (Bremner et al., 1976).

3.2.7.2. Phosphate

Polyphosphate improves texture, processing/cooking yield and storage stability of meat products (Molins, 1991). Sodium tripolyphosphate (STPP) had no antimicrobial effect in temperature abused; frozen raw ground beef and refrigerated raw pork containing salt (Chu et al., 1987).

3.2.7.3 Ascorbates

Meat products treated with ascorbic acid and sodium ascorbate have inhibited lipid oxidation and preserved desirable meat flavours (Rhee et al., 1997). Kanner et al., (1987) observed that ascorbic acid at low concentration (up to 10-3 M) reduced copper to its catalytically active form, stimulating lipid oxidation in a â - Carotene – linoleate model system.

3.2.8 Herbs and spices as sources of antioxidants

The anti oxidant activity of spices has been known for more than 50 years. (Chipault et al., 1952). They evaluated the anti oxidant properties of 72 kinds of spices, their petroleum ether extract and found 32 spices to retard the oxidation of lard. In their study, the anti oxidant activities of ground sage and rosemary were particularly strong, and oregano, thyme, turmeric, and nutmeg possessed relatively strong antioxidant activities.

The most active substances are produced from rosemary (*Rosmarium officianlis. L.*) Chang et al., 1977) and from Sage *(Salvia officianalis. L.)* Both of them contain carnosol and carnosic acid as active constituents. Naturally occurring compounds in rosemary extracts have been reported to exhibit anti oxidant properties greater that BHA and equal to BHT (Wu et al., 1982). Addition of rosemary extract to simulated minced turkey meat has been shown to provide increased protection from oxidation during cooking. Three more antioxidant substances from rosemary, all phenol diterpene compounds, were also isolated and determined the structural formula for each. Houlihan et al., (1985) have isolated more compounds of rosemary possessing superior antioxidant property to BHA but less effective than BHT. Shelef et al., (1980) has studied the sensitivity of common food borne bacteria to the spices; sage, rosemary and all spice.

Bracco et al., (1981) has described the recovery of anti oxidants from spices and vegetable material. The effect of the essential oil of cinnamon and cloves and their primary constituents, cinnamic acid and eugenol respectively on mold growth and of aflatoxin production has also been studied (Gourama et al., 1995; Rajkumar and Berwal, 2003).

Spices are widely used in a variety of food products. Many spices including cloves, cinnamons, Black pepper turmeric ginger, garlic and onions exhibit anti oxidative activities in a variety of food system (Al – Jalay et al., 1987).

Several investigators in Japan reported that ginger and ginger extract added to lard or other food showed reasonably strong anti oxidant activity (Saito et al., 1976). Lee and Ahn, (1985) investigated the properties; of antioxidant substances in ginger rhizome and their effectiveness as a source of antioxidants in fresh, frozen or pre cooked patties. Ramanathan and Das, (1993) has studied the effectiveness of spices and other related natural products as anti oxidants when present in salted cooked fish.

Ahn et al., (2002) have investigated the comparative antioxidant activity of a grape seed extract and a pine bark extract in cooked ground beef. Yu et al., (2002) conducted studies to determine the potential benefits of water soluble rosemary extracts on stability and quality of cooked turkey products.

Sanchez et al., (2003) conducted studies to determine if natural antioxidant rosemary, oregano and borage alone or in combination with vitamin C, were effective in delaying lipid and myoglobin formation in pork meat, thereby stabilizing meat colour.

3.2.9. Nutritional and health benefits of synthetic antioxidants

Antioxidants are defined by the United states (U.S.) Food and Drug Administration (FDA) as substances used to preserve food by retarding deterioration rancidity or discolouration due to oxidation, (2) code of Federal Regulations (CFR) 170-3 (0) (3).

Stuckey, (1972) has attributed the inhibitory effect of these anti oxidants, to their donation of electrons or hydrogen to fat containing a free radical, and to the formation of a complex between the antioxidant and the fatty acid. Most of the above studies pertain to meat and meat products while, the effect of spices on the storage stability of fatty fish during frozen storage is very limited.

3.3 Materials and methods

3.3.1 Raw Materials

3.3.1.1 Fish

Fresh mackerel was procured from Munambam Harbour, Kochi. The size of fish was around 13-14 cm with 6 – 7 numbers/kg. The fish was washed, iced and transported to the freezing plant of Matsyafed, Govt. of Kerala, Kochi, where the initial treatment, freezing and frozen storage were carried out.

3.3.1.2 Spice oleoresins and synthetic antioxidant

Four different spice oleoresins *viz,* Rosemary, Ginger, Pepper and Clove were used for the study. These were obtained from M/s Synthite Chemicals, Kolenchery and Butylated Hydroxy Anisole (BHA) was used as the synthetic antioxidant. Solutions of oleoresins of the spices and BHA were prepared in two concentrations of 0.02% and 0.05%.

Plate 2.5 Oleoresin Samples of spices

3.3.1.3 Polythene tubes and master cartons

Polythene tubes of 200 guage were used to pack the treated samples meant for frozen storage studies. The fish samples after treatment were stored in 5 ply master cartons after proper labeling.

3.3.2 Sample Preparation

The procured fish was divided into three lots, depending on the method of study.

A. Whole fish for iced storage studies

B. Whole fish for frozen storage studies

C. Fish fillets for frozen storage studies.

A. Whole fish for iced storage studies

Fish was dip treated with four spice extracts and BHA of 0.02% concentration for 20 minutes. A control sample was also taken without any spice extract or synthetic antioxidant treatment. Throughout the preparation and dip treatment, the temperature was maintained at 5°C. The dipped samples were individually packed in polythene tubes and stored in layers of ice for study. There was no direct contact of the fish surface with ice. Each set of treated fish were kept in ice on separate trays. The trays were kept in a chill room at 5°C. From this sampling was done every alternate days for a period of 10 days.

B. Whole fish for Frozen Storage Study

Whole fish for frozen storage studies were categorized into two lots. One for dip treatment and the other for glaze treatment.

i) Dip treatment

Samples of fish were dip treated at two different concentrations, 0.02% and 0.05% for 20 minutes with each spice oleoresin as well as BHA along with two control samples. The samples were frozen in a tunnel freezer at - 40°C for four hours. The frozen samples were packed in polythene tubes individually, and stored in master cartons. The product was stored in a cold store at temperature -20°C. Sampling was done immediately (0 month) and further at regular intervals of two months for a period of nine months.

ii) Glaze treatment.

For glaze treatment, the fish was subjected to freezing in a tunnel freezer, and on the subsequent day, the frozen samples were subjected to uniform application of glaze. For this the frozen samples were dipped in two different concentrations (0.02% and 0.05%) of treatment solutions. The samples dipped in 0.02% concentration were given two coatings of glaze for a period of 5 seconds each. The samples were frozen in between glazing. The samples dipped in 0.05% concentration were given a single dip for 2 seconds only.

C. Fish fillets for frozen storage studies

Fresh mackerel was filleted to skin on fillets. The fillets were subjected to dip treatment with 0.005% spice extracts, following the method of treatment as mentioned in the whole fish study, but the time of dip was 10 minutes. The controls along with the treated samples were frozen in a tunnel freezer at - 40°C for four hours. The frozen fillets were wrapped and sealed in polythene tubes, packed in master cartons and stored in the cold store at -20°C. Samples were drawn at zero time and at regular intervals of two months for analyzing various parameters of lipid oxidation.

In all the above sampling methods triplicates were taken at every stage.

3.3.3 Analysis of Biochemical parameters

3.3.3.1 Proximate Composition

The proximate composition of the tissue *viz* moisture, protein, fat and ash of mackerel was analysed according to AOAC, (1995).

3.3.3.2. pH

10g of sample after the treatment along with control was blended with 90ml of distilled water and the pH of the resultant suspension was measured (AOAC, 1995). A digital pH meter (Cyber Scan pH-500 MERCK) was calibrated with standard buffers of pH 4.0, pH 7.0 and pH 9.0 (SIGMA).

3.3.3.3 Peroxide Value

Peroxide value was determined by the method of AOCS, (1999). 10 g of treated fish muscle was ground well with anhydrous sodium sulphate to remove moisture. It was then transferred to an iodine flask, and extracted with small quantities of chloroform, filtered and made up to 100ml.

10ml of the extract was taken in a pre weighed petridish and evaporated to dryness. The weight of the petridish was taken again, to determine the weight of the residue in the petridish. Another 10ml of the extract was pippetted in to a dry iodine flask. About 20ml of glacial acetic acid and 1 ml of saturated potassium iodide solution was added. The mixture was kept in the dark for 15min. The liberated iodine was diluted and titrated against $N/_{100}$ sodium thiosulphate solution, using 1% starch as indicator.

Calculation

$$\text{Peroxide Value (milleq/Kg of fat)} = \frac{\text{Volume of } Na_2S_2O_3 \times \text{Normaility of } Na_2S_2O_3 \times 1000}{\text{Weight of sample in 10ml extract.}}$$

3.3.3.4 Thio barbituric acid value (TBA)

Thiobarbituric acid reactive substances (TBARS) was performed as described by Tarladigs et al., (1960). 10g of treated sample was mixed with 50 ml distilled water in a waring blender for 2 min. The mixture was transferred quantitatively in to a Kjeldahl flask by washing with an additional 47.5 ml of distilled water. 2.5ml of HCl solution was added to maintain pH to 1.5. A few saddle stones were added to prevent bumping. A small amount of Dow antifoam A was placed on to the lower neck of the flask to prevent foaming. Assembled the apparatus and heated the flask at the highest obtainable heat, on the kjeldahl distillation apparatus. 50ml of distillate was collected, from the moment the boiling begins. The distillate was mixed, and 5ml was pipetted into a glass stoppered tube, with 5ml of TBA (Thiobarbituric acid) reagent. The tubes were stoppered, the content were mixed and immersed in a boiling water bath for 35 minutes. A distilled water TBA reagent blank was also prepared and treated like the samples. After heating, the tubes were cooled in running tap water for 10min. A portion of it was transferred to the cuvette, and the optical density of the sample against the blank was read in a UV/VIS spectrophotometer (HITACHI, U 2800) at 578nm. The reading was multiplied by 7.8 to convert to mg of malonaldehyde per 1000g tissue.

3.3.3.5 Heme iron

Heme iron was determined using the method of Hornsey, (1956). 2g of the samples were transferred into a 50ml poly propylene tube and 9ml of acid acetone (90% acetone + 8% deionised water +

2% HCl) was added. The meat was macerated with a glass rod and allowed to stand for 1 hour in a dark cabinet at room temperature. The extract was filtered with Whatman filter paper No: 42 and the absorbance (A) was read at 640nm against the acid acetone blank.

Total pigments, as acid hematin, were calculated using the formula: Total pigment (ppm) = A x 680 and heme iron was calculated as follows (Clark et al., 1997).

$$\text{Heme iron (ppm)} = \frac{\text{Total pigments (ppm)} \times 8.82}{100}$$

3.3.3.6 Met Myoglobin (MMb)

Met myoglobin content was determined by the method of Lopez et al., (2003)

5 g of treated minced tissue was used to determine MMb concentration in each sample. Myoglobin was extracted with cold 0.04M phosphate buffer in the ratio of 1:10 (tissue: buffer). Samples were homogenized for 15 seconds in a Yorco Tissue Homogenizer, at 10,800 rpm. The homogenates were then centrifuged in a refrigerated centrifuge for 30min at 5°C (50,000g). The absorbance of the supernatant was read at 525, 572 and 730nm. Percentage of MMb was determined using the formula of Krzywicki (1979).

MMb (%) = $1.395-(A_{572}-A_{730}) / A_{525}-A_{730}) \times 100$. Samples were kept in ice at all points of assay.

3.3.4. Statistical Analysis

The experimental design was a randomized block design of 5 rows for five treatments and 2 columns for the 2 storage periods. Analysis of variance (ANOVA) was carried out using the generalized linear model procedure. The difference of means between pairs was resolved by using the least significant difference. The level significance was set at $p<0.01$ and $p< 0.05$ (Snedecor and Cochran, 1989)

3.4 Results

The proximate composition of the tissue of mackerel on analysis gave the following values in percentage

Moisture	71.19 ± .0.2
Protein	21.21 ± 0.4
Fat	7.51 ± 0.1
Ash	1.33 ± 0.3

Table 3.1 shows the variation of pH for the frozen samples of mackerel fillets dip treated with 0.005% spice extracts and there was no significant difference between periods as well as between treatments (Appendix 3.1).

Table 3.1 pH of Mackerel fillets given 0.005% dip and stored at -18°C

Period	1 month	4 months	5 months	6 months
CNT	**6.707**	**6.497**	**6.347**	**6.250**
RM	**6.697**	**6.663**	**6.167**	**6.450**
GIN	**6.483**	**6.560**	**6.213**	**6.220**
PEP	**6.903**	**6.763**	**6.662**	**6.387**
CLO	**6.900**	**6.907**	**6.270**	**6.313**
SYN	**6.920**	**6.433**	**6.433**	**6.423**

3.4.1 Peroxide value

Fig 3.1 shows the changes in peroxide value of treated and chill stored samples given dip treatment at 0.02% concentration. There is significant difference between treatments ($p<.05$) [Appendix 3.2(a)]. In the ANOVA for comparison between samples of control and rosemary, the difference is significant ($p<.05$) [Appendix 3.2 (b)]. Rosemary and clove treated samples shows a lower peroxide value at end of 10 days storage in chilled condition. For samples in chilled storage there is significance between samples stored between 10 days and 8 days period. Significant difference is shown between treatments ($p<.05$) [Appendix 3.2(c)].

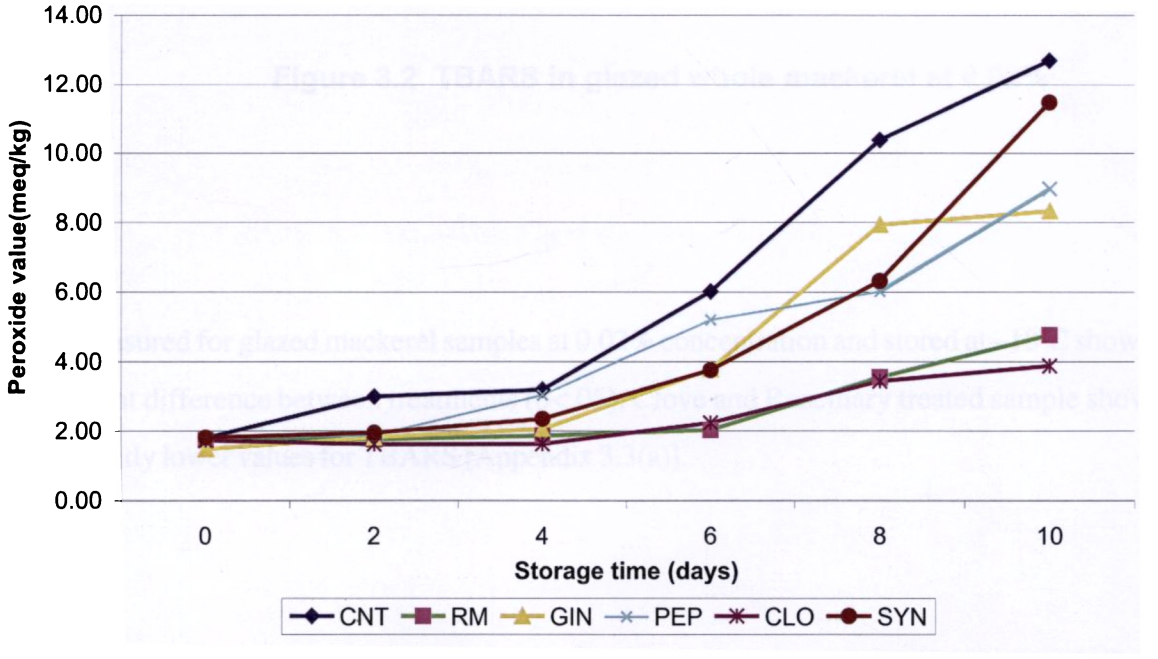


Figure 3.1 Change in peroxide value of chill-stored treated samples at 0.02%(dip)

3.4.2 Thiobarbituric acid reactive substances (TBARS)

Fig 3.2 shows the variation obtained for glazed mackerel (0.02 %) stored at –18°C. ANOVA shows significant difference between treatments ($p<.05$) during the period of 11 months storage, clove and rosemary treated samples shows lower values [Appendix 3.3 (a)].

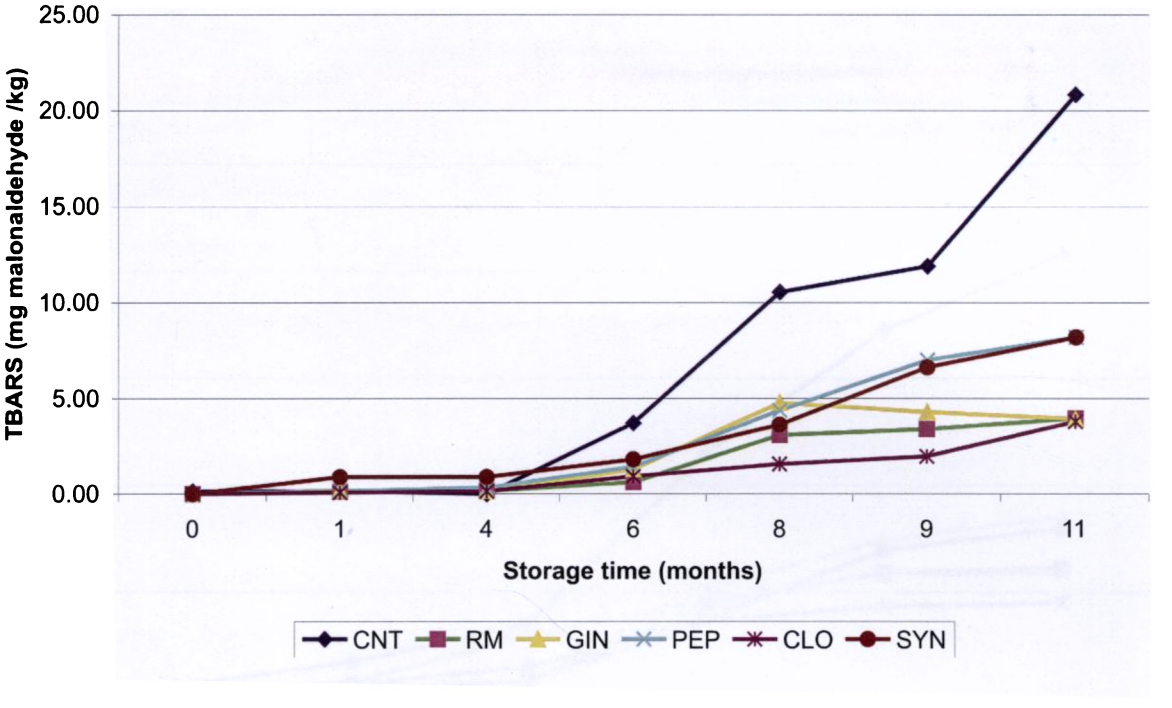


Figure 3.2 TBARS in glazed whole mackerel at 0.02%

TBA measured for glazed mackerel samples at 0.02% concentration and stored at –18°C shows significant difference between treatments ($p<.05$). Clove and Rosemary treated sample show significantly lower values for TBARS [Appendix 3.3(a)].

Fig 3.3 shows the variation of TBARS for the sample of glazed mackerel at 0.05% concentration and stored at -18^0C, shows significant difference between treatments and storage time ($p<.05$). 11 months stored samples shows significantly higher values than 7 months. Control samples showed significantly high TBARS value compared to the treatment samples [Appendix 3.3 (b)].

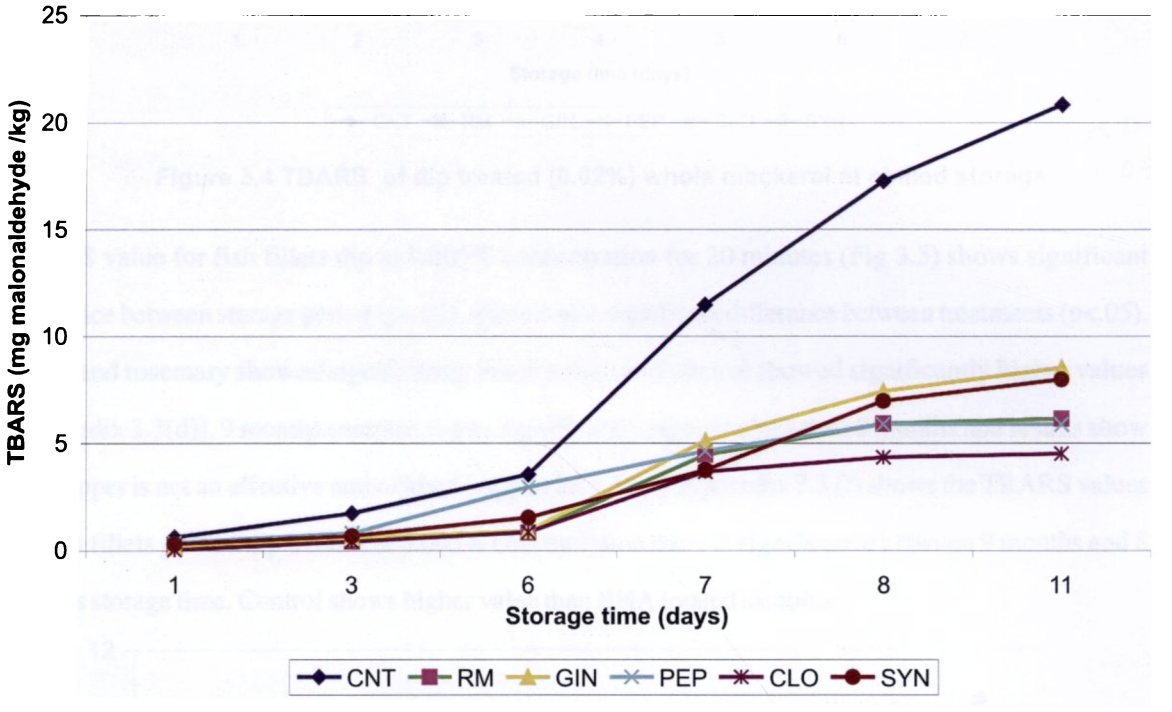


Figure 3.3 TBARS of glazed whole mackerel at 0.05%

Fig 3.4 shows the variation in TBARS obtained from dip treated mackerel (0.02%) stored in ice. There is significant difference between treatments ($p<.05$). The values of clove and rosemary are significantly lower than control [Appendix 3.3 (c)].

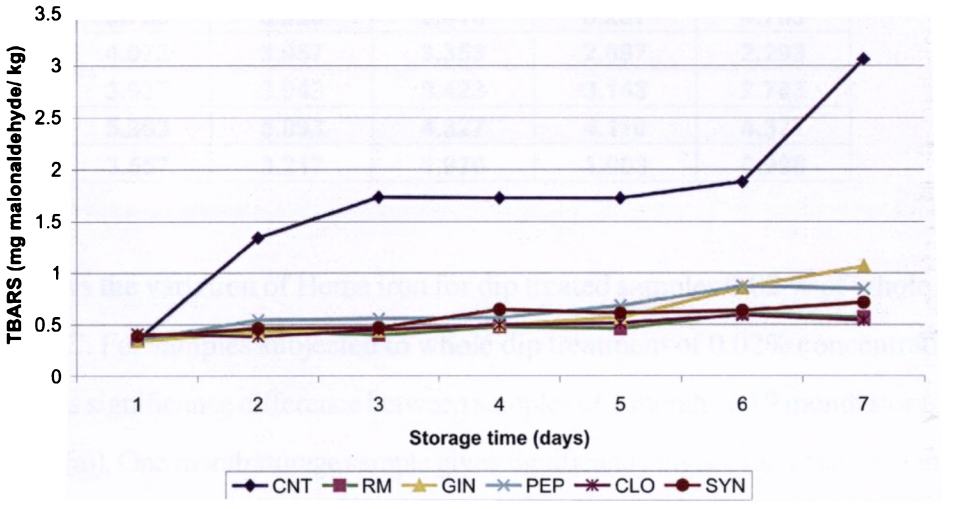


Figure 3.4 TBARS of dip treated (0.02%) whole mackerel at chilled storage

TBARS value for fish fillets dip at 0.005% concentration for 20 minutes (Fig 3.5) shows significant difference between storage period ($p<.05$) , there is also significant difference between treatments ($p<.05$). Clove and rosemary showed significantly lower values and control showed significantly higher values [Appendix 3.3(d)]. 9 months samples shows significantly higher values than 8 months and results show that pepper is not an effective anti oxidant [Appendix 3.3 (e)]. Appendix 3.3 (f) shows the TBARS values for fish fillets given a dip treatment 0.005% concentration there is significance between 9 months and 8 months storage time. Control shows higher value than BHA treated samples.

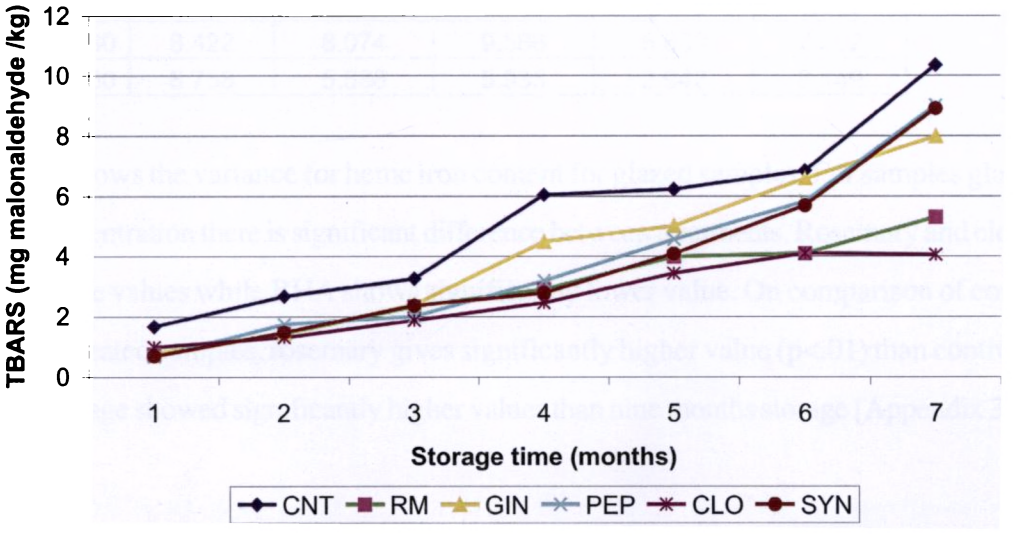


Figure 3.5 TBARS of frozen dip treated mackerel fillets(0.005%) stored at -18 °C

3.4.3 Heme iron

Table 3.2 Heme Iron content of treated (0.02 % dip) and stored at -18°C

Period	0	1 month	3 months	5 months	7 months	9months
CNT	6.830	4.900	3.927	2.850	2.490	2.669
RM	7.010	5.750	5.030	5.010	5.297	4.783
GIN	6.230	4.073	3.957	3.353	2.897	2.293
PEP	6.700	3.937	3.943	3.423	3.143	2.783
CLO	6.800	5.963	5.093	4.327	4.110	4.377
SYN	6.750	3.557	3.217	1.970	1.003	0.998

Table 3.2 shows the variation of Heme iron for dip treated samples 0.02 % of whole mackerel stored at -18^0C. For samples subjected to whole dip treatment of 0.02% concentration for 20 minutes there is significance difference between samples of 1 month and 9 month storage ($p<.01$) [Appendix 3.4(a)]. One month storage sample gives significantly higher value than 9 month storage sample and there is significant difference between treatments ($p<.01$). Clove and rosemary gives significantly higher value and BHA treatment gives significantly lower values. Control at one month storage gives significantly higher values than 9 months storage samples ($p<.05$) [Appendix 3.4(b)].

Table 3.3 Heme iron content of treated (0.02% glaze) and stored at -18°C

Period	0	1 month	3 months	5 months	7 months	9months
CNT	8.329	8.770	7.842	6.548	4.406	2.659
RM	10.500	9.955	10.442	9.709	8.870	7.111
GIN	6.150	6.092	7.544	5.679	4.257	4.952
PEP	8.170	8.070	5.325	5.461	4.678	4.240
CLO	8.460	8.422	8.074	9.588	6.650	7.592
SYN	8.750	8.738	5.588	5.935	2.942	3.149

Table 3.3 shows the variance for heme iron content for glazed samples. For samples glazed with 0.02% concentration there is significant difference between treatments. Rosemary and clove show high average values while BHA shows significantly lower value. On comparison of control and rosemary treated samples, rosemary gives significantly higher value ($p<.01$) than control. Seven months storage showed significantly higher values than nine months storage [Appendix 3.5(a) and 3.5(b)].

3.4.4 Metmyoglobin

Table 3.4 Metmyoglobin content of glazed samples (0.05 % dip) at -18°C

Period	0	1 months	3 months	5 months	7 months
CNT	0.723	0.905	0.929	0.921	0.985
RM	0.472	0.496	0.674	0.688	0.651
GIN	0.685	0.719	0.482	0.765	0.849
PEP	0.455	0.510	0.600	0.630	0.714
CLO	0.436	0.451	0.548	0.536	0.683
SYN	0.442	0.468	0.815	0.773	0.905

Table 3.4 shows the variation of metmyoglobin, in glaze treated samples stored at –18°C. For samples treated with 0.05% glaze, there is significant difference between five months and seven months storage ($p<.05$). Values of seven months are significantly higher than that in five months. There is also significant difference between treatments ($p<.01$). Clove and rosemary showed significantly lower values than BHA treated samples. In control versus ginger, control shows higher values than ginger ($p<.05$). On comparison, pepper treatments shows significantly lower value than control ($p<.05$) [Appendix 3.6]

3.4.5 Acid haematin pigment

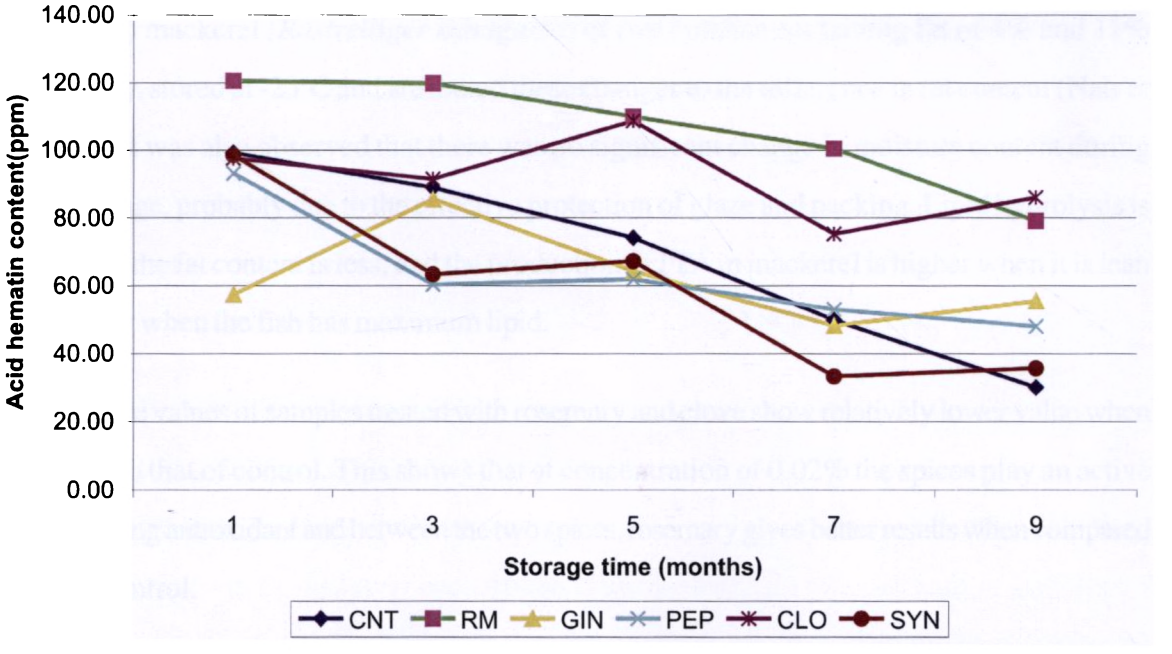


Figure 3.6 Acid hematin pigments of frozen mackerel(0.02% glaze) stored at -18°C

Fig 3.6 shows the variation of total pigments as acid haematin in treated frozen samples. For samples glazed with 0.02% concentration there is significant difference in hematin pigment between storage periods. One month shows significantly higher values than nine months. There is significant difference between treatments ($p<.01$). Clove and rosemary show significantly higher values and synthetic gives very low value. In ANOVA on comparison of control and BHA there is significant difference between months. [Appendix 3.7]

3.5 Discussion

In the process of lipid deterioration, the fundamental reaction is generally accepted to be the process of autoxidation of the unsaturated fatty acids, which are abundant in fish lipids. The highly unsaturated fatty acids are very reactive. During frozen storage oxidative rancidity occurs which is caused by the reaction between oxygen and PUFA, e.g. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), to form hydroperoxides that decompose to volatile aldehydes, ketones and acids responsible for rancid odour. The initial reaction between lipid and oxygen requires a catalyst. Once the reaction has started it becomes, self-propagating and difficult to control (Tall and Harris, 1995; Gopakumar, 2002).

Earlier studies indicated changes in moisture (%) of individually quick frozen (IQF) and block frozen (BF) mackerel *(Rastrelliger kanagurta)* of two batches containing fat of 4% and 11% respectively, stored at -23°C and attributed these changes to the difference in fat content (Nair et al., 1976). It was also observed that there was no significant change in moisture content during frozen storage, probably due to the effective protection of glaze and packing. Lipid hydrolysis is more when the fat content is less, and the production of FFA in mackerel is higher when it is lean and is lower when the fish has maximum lipid.

The peroxide values of samples treated with rosemary and clove show relatively lower value when compared to that of control. This shows that at concentration of 0.02% the spices play an active role as a strong antioxidant and between the two spices, rosemary gives better results when compared to that of control.

3.5.1 TBA values

Thiobarbituric acid reactive substances (TBARS) obtained from mackerel stored at –18^0 C shows that frozen samples glazed at 0.02 % clove and rosemary have significantly lower values for TBARS when compared to control. For frozen whole fish samples glazed at 0.02%, clove and rosemary treated samples have given significantly lower values for TBA when compared to control. Early research (Chipault et al., 1956) has proven that rosemary had remarkable antioxidant effects in lard. In the present study clove showed extremely high antioxidant effect in the case of TBA while control shows significantly higher values compared to rest of treatments, confirming the well pronounced antioxidative effect of spices in treated fish. TBARS values for samples of chilled storage also showed remarkably low values for clove and rosemary. This again highlights the strong antioxidative properties of rosemary. A strong synergistic effect between rosemary extract (0.02 %) and tocopherol (0.05 %) in sardine oil at 30^{0}C in frozen fish meat models has been reported (Wada and Fang, 1992). It has been established that molecule of carnosol and the radicals formed from them participate in the reactions of chain initiations and propagations to a much lower degree than the case with most natural and synthetic antioxidants (Marinova et al, 1991), thereby decreasing the rate of oxidation to a considerable extent. Houlihan et al., (1985) found rosmaridiphenol to be more active than BHA in lard and was equivalent to BHT. Rosemary oleoresin (RO) was superior to BHA and equivalent to BHT. The present result on TBARS values analysed for various chilled and frozen stored products of mackerel supports the view of the antioxidative principles of rosemary oleoresin. Carnosic acid and carnosol showed the ability to chelate the iron and were effective radical scavengers of peroxide radicals (Aruoma et al., 1992). Barbut et al., (1985) studied the effectiveness of rosemary oleoresin (RO) in turkey breakfast sausages. RO was effective as a combination of BHA and BHT with citric acid in suppressing oxidative activity.

Studies show that at the end of 5 months storage, the sample stored at -10°C had 15 times more TBARS value than that stored at -30°C. TBARS value started to increase from the initial stationary phase after 6 months storage in samples stored at -20°C and after 9 months in samples stored at -30°C (Chinnamma et al., 1995). Frozen mackerel stored at -30°C had a shelf life of more than 9 months provided the material is glazed and packed in moisture proof packaging material. Formation of ice glaze on the surface of a myosystem effectively limits the entrance of oxygen and thereby retards autoxidation. Antioxidants provide additional protection against lipid oxidation. Bremner

and Gerald, (1998) showed that oxidation occurs within one month in freshly caught pilchards *(Sardinops neopitchardus)* when stored at temperature of -20°C. In blue whiting fillets during frozen storage (-10°C and -30°C for 1, 3, 5, 7, 9 and 12 months), lipid damage increased showing high value of free fatty acids, peroxide value and conjugated dienes. TBARS index and fluorescence detection showed a good positive correlation with storage time (Aubourg, 1999). Thiobarbituric acid value (TBARS), which is a good index of fat oxidation, was also found to increase in a number of species during frozen storage. In Nile perch (*Lates niloticus*) fillets stored at -13°C, TBARS value and off odor and flavour increased with storage time. Flavour, texture and overall acceptability scores were lower in the samples and decreased with storage time.

Peroxide value shows a general increase during frozen storage. A high positive correlation between rancidity scores of a taste panel and peroxide values was found in frozen fish (Fennema et al., 1973). Awad et al., (1969) observed that the peroxide values increase to a maximum and subsequently decline to a minimum when frozen fish were stored near -10°C. This indicates that they are decomposed faster than they are being formed.

3.5.2 Heme iron

There is a great range in the concentration of haematin components in muscles from different species of fish. These components are present in relatively large concentration in the muscles of fatty fish especially in the lateral band of dark muscles. The characteristic brownish colour of the fish muscles is due to hemoglobin and myoglobin. Fish with highest haematin content are those that are most susceptible to oxidative rancidity. In the lipid oxidation hydroxyl free radicals can be produced by the reaction of transition metals with the reduction products of molecular oxygen like superoxide and hydrogen peroxide. Iron is probably of primary importance in fish tissue. Due to the low solubility of Fe^{3+} at physiological pH almost all cellular iron is complexed. Heme pigments can be activated by hydrogen peroxide to a compound able to initiate lipid oxidation.

The role of metmyoglobin (MetMb) and nonheme iron in accelerating lipid oxidation in cooked meat studied using a model system, containing water extracted muscle residue shows that non heme iron acts as prooxidant in cooked meat, while MetMb has little or no prooxidant activity. Heme compounds may act either as accelerator or inhibitors of lipid oxidation with their action depending on the ratio of the heme to unsaturated fatty acid. There are reports that haemoglobin

dissociation can promote lipid oxidation reactions in tilapia and that heme proteins, myoglobin and haemoglobin and various other derivates are responsible for development of off colours in canned tuna.

The rate and extent of lipid oxidation catalyzed by metmyoglobin in heat treatment was greatest in fish mainly due to level of poly unsaturated fatty acid present in the particular system (Mark et al., 2007). In the samples glazed (0.02 % concentration) there is significant difference between treatments. This probably may be due to the fact that in rosemary treated samples, the heme iron does not dissociate thereby preventing oxidation.

3.6 Conclusion

Freezing is one most effective ways of preservation of fish. The result of fat oxidation indices in the study shows that oxidation can be prevented to a considerable extent by the use of natural compounds. The use of rosemary oleoresin has shown to suppress the oxidative changes in fish muscles. This is well established by the results obtained from the TBARS values of frozen mackerel and products of mackerel stored at -18^{0}C for a period of nine months. Clove also exhibits strong antioxidative effect. On the whole, from the results of various lipid oxidation parameters of samples given, different treatments with spice extracts in comparison with a synthetic antioxidant, BHA and control samples, the spices used could be graded as Rosemary > Clove > Ginger > Pepper, for their antioxidant properties. Among the various pretreatments given as glaze and dip of whole fish and frozen stored at -18^{0}C, the glazed samples with 0.02% spice treatment is found to be most effective. Fillets dip treated with 0.005% concentration and frozen stored gave the optimum results. In this case also, the antioxidant properties was maximum for rosemary followed by clove, ginger, pepper and synthetic antioxidants as confirmed by the TBARS and Peroxide values.

The retention of heme iron, metmyoglobin and acid pigments were also maximum in rosemary treated samples followed by clove explaining the protective effect of the treatment in maintaining the colour and other textural profiles of the samples. The synthetic antioxidant treated samples on frozen storage gave low values of pigments compared to spice treated samples, showing a bleaching action and hence loss of sensory qualities. The fact that the active constituents of rosemary and clove can be made use of for effectively preventing oxidation, thereby maintaining the original characteristics of mackerel has been confirmed.

CHAPTER 4

TEXTURE PROFILE ANALYSIS OF SPICE TREATED SAMPLES

4.1 Introduction

4.2 Review of Literature

4.2.1 Role of muscle proteins

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4.3.3 Texture profile analysis

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4.4.2 Texture Profile Analysis

4.4.1 Organoleptic evaluation

4.5 Discussion

4.6 Conclusion

4.1 Introduction

Musculature of fish contains a great number of muscles that, depending on their anatomical location and activity exhibit structural and compositional differences that lead to different functional properties and processing abilities. Both intrinsic and extrinsic factors of muscle tissue affect the rheological characteristics. Myofibrillar protein and collagen that comprise 70 – 80% of the total protein content control the structure and the specific rheological properties of muscle tissue. Post mortem textural changes are caused by physiochemical changes in the myofibrillar proteins and changes in the extra cellular spaces. Texture is also affected by the pattern of arrangement of structural components and the changes occurring during processing techniques employed. Time-temperature profile also plays a significant role in affecting the textural characteristics of the muscle tissue.

Texture is a complex sensory experience and is also a multi-faceted concept describing the physical properties of foodstuff related to mouth feel and quality. Mouth feel means feelings associated with the process of mastication, salivation, touching with tongue and swallowing the food. Textural variations are complex and changes with moisture, size, temperature, state of surface and structures of foodstuffs. The rheological properties like elasticity, viscosity, visco-elasticity etc. are ideal for evaluation, if these parameters could be correlated with one's mouth feel. Textural judgments are solicited after visual and non-oral examination of food. Flavor and texture of muscle are delicate factors influencing sensory preferences of consumers. Thus, their evaluation is a critical factor in seafood products, as improper processing might lead to poor quality and rheological characteristics, reducing their economic value.

In this study five kinds of dip treated samples of mackerel muscle tissue, were analysed for texture, both instrumentally and by sensory methods which, were subjected to heat treatments at different temperatures.

4.2 Review of Literature

4.2.1 Role of muscle proteins

Most textural properties of seafood products are due to the composition and structure of the muscle proteins (Goll et al., 1977). Muscle proteins (sarcoplasmic and myofibrillar) alter human perception

of fish quality by enzymatic reaction (by the production of sensory compounds like nucleotides and volatile amine compounds) and by direct changes in protein structure that alter tissue properties like juiciness, toughness, gel forming ability and water holding capacity. Chewiness is another function of hardness, cohesiveness and springiness of food (Bourne, 1979). It is another important criterion affected by protein structural changes. Toughness is the most critical quality parameter of tissue. Muscle toughness is a complex property and depend upon the two structural proteins namely connective tissue and myofibrillar protein that give the tissue its mechanical property. Each of the structural components of the connective tissue makes a distinct contribution to the overall toughness of the meat. Hatae et al., (1978) and Niwa, (1992) observed that tenderness does not vary significantly with soluble form of collagen. Proteolysis also alters the association of the muscle fibers and their interaction between protein and water molecules that might account for tenderness and rheological changes in the muscle (Dunajski, 1979).

4.2.2 Texture analysis

Muscle texture could be measured by studying the rheological properties and by observing some of the physical and chemical parameters related to the texture. Texture of fish muscle could be measured by different organoleptic and instrumental procedures. Later techniques include cell fragility tests, changes in protein solubility and water binding capacity (Hamm, 1975). Studies of the rheological properties yielded parameters more closely related to the sensory evaluations. Mechanical methods are suitable for quantifying mechanical texture namely hardness, springiness, cohesiveness, toughness (firmness); chewiness, and (stiffness) resistance to mastication (Szezesniak, 1963). Protein quality was found to influence strain to failure more than rigidity and that water content influences rigidity more than strain to failure (torsion).

Davey and Gilbert, (1974) investigated the effect of cooking temperature on protein - protein interactions, enzyme hydrolysis and textural quality and observed that at temperatures between 55°C and 85°C some tenderizing takes place that could be due to alkaline protease activity. He also indicated that the texture of cooked meat was affected by gelatin derived from the muscle collagen.

4.2.3 Effect of spices on texture

Ginger has widely been used in domestic preparation to enhance taste. Investigations have shown that ginger possess effective tenderization properties when added to meat products (Thompson et al., 1973; Syed Ziaudeen et al., 1995). Naveena and Mendritta, (2001) have studied the tenderizing effect of sheep meat and ginger added samples were more juicer than control.

The objective of the present study is to determine whether the spice treated cooked sample have any enhancing effect on textural quality parameters. No systematic work is reported on the effect of spice treatment on the texture of fatty fishes. Hence in the study attempt is made to investigate the effect of spice treated samples on the textural parameters and organoleptic qualities on thermal treatment at 45°C, 70°C and 100°C.

4.3 Materials and Methods

4.3.1 Raw material collection and sample preparation

Fresh whole Mackerel fish (*Rastrelliger kanagurta*), procured from Munambam Harbour, Kochi, was divided in to six groups as follows. Each group of fish was subjected to dip treatments of specific spice extract. A synthetic antioxidant BHA (Butylated hydroxyl Anisole) was also used. Control sample was not subjected to any sort of treatments. The duration of dip treatment was 5min and 10min with two different concentrations of spice extract (0.02% and 0.05%). The dip treated samples were stored in a refrigerator at 4°C for one hour. Later the fishes were filleted and uniform pieces of size ($2cm^3$) were cut out from the muscle tissue. Care was taken to avoid skin or any small pin bones in the cut tissue. For cooking experiment, the uniform sized fish fillet, was wrapped in 5mm thick aluminum foil and cooked at three different temperatures of 45°C, 70°C and 100°C for three minutes in a thermostatic water bath. It was then cooled and subjected to texture profile analysis, as well as sensory analysis. The cook loss was also calculated. The details of this are given in the flowchart below Fig 4.1

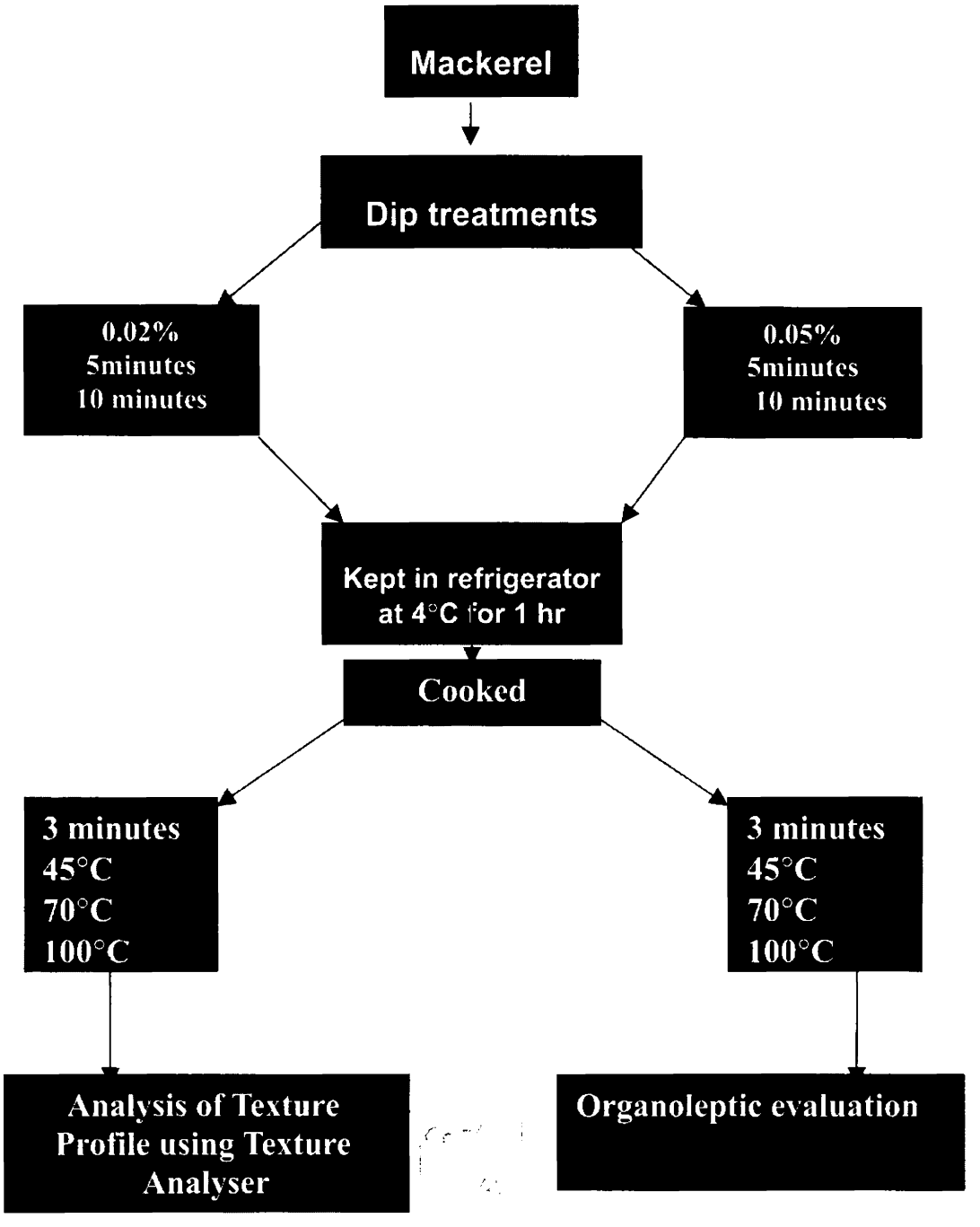


Figure 4. 1 Flowchart showing the treatment and cooking of samples for Texture Profile Analysis and Organoleptic evaluation

4.3.3 Texture profile analysis

Instrumental texture profile analyses of samples were done using Texture Analyzer (Lloyds UK Instruments) according to Bourne, (1978). During measurement a small flat-faced cylindrical probe of 50mm diameter compressed the bite of fish $2cm^3$ twice in a reciprocating motion. The test speed and trigger force were standardized to 15 mm/min and 0.5 N respectively. It imitated the action of the human resembling the two times reciprocating motion involving the repeated compression of sample to its original height between two parallel surfaces and recording force versus displacement. From the force-time curve various textural parameters like hardness, cohesiveness, springiness and stiffness were evaluated since they were statistically significant. Five replicates of measurements were taken for each sample. The maximum force required for the first and second compression denoted the hardness I and hardness 2 and the ratio of the area under the second cycle of compression curve to the area under first cycle compression curve determined the cohesiveness (Bourne, 1978). A typical force time curve is shown in Fig 4.2.

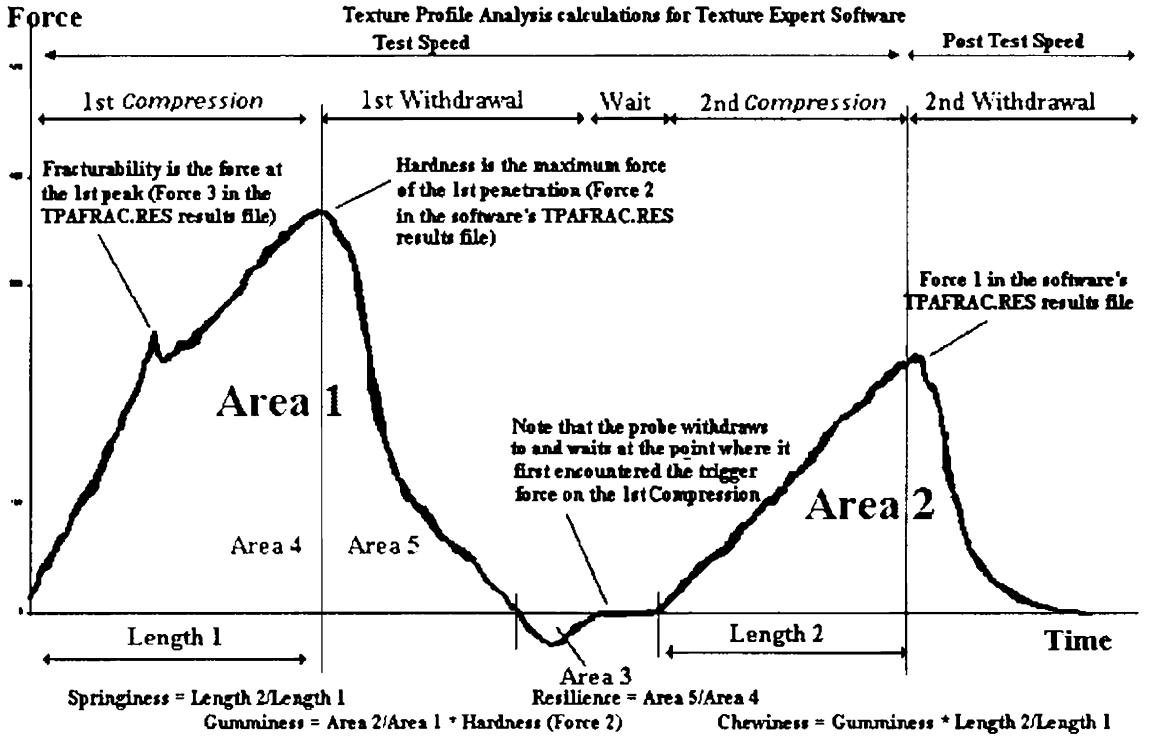


Figure 4.2 Plot showing a typical force-time curve of texture profile analysis

4.3.2 Organoleptic evaluation

A team of six panel members performed the sensory evaluation of the samples and a hedonic scale of 10-point was used for assessment (Borderias et al., 1983). The selected characteristics were tested as defined by Jowitt, (1974).The Performa 1 and 2 for the sensory evaluation is given in Appendix 4.1 and 4.2.The different textural properties evaluated were wateriness (release of water on compression), firmness (force required to compress the material between the molars or between the tongue and palate), elasticity (rubbery mouth-feeling), cohesiveness (extent to which a material could be deformed before it ruptures), juiciness (feeling of liquid in the mouth after chewing 3 to 4 times) and hardness(force required for biting through the sample). The sensory panel also recorded the sensory descriptions of the sample (odour, colour, flavour, touch and overall acceptability scoring) using10-point hedonic scale. Five replicates of each sample were considered.

4.3.4 Statistical analysis

The experimental design was a randomized block design of 5 rows for five treatments and 2 columns for the 2 storage periods. Analysis of variance (ANOVA) was carried out using the generalized linear model procedure. The difference of means between pairs was resolved by using the least significant difference. The level significance was set at $p<0.01$ and $p< 0.05$ (Snedecor and Cochran, 1989).

4.4 RESULTS

4.4.1 Cook loss

Fig. 4.3 shows the cook loss of treated samples cooked at 70°C and 100°C. There was significant difference ($P<0.001$) between samples cooked at 70°C and 100°C after dip treatment of 0.02% concentration for 10 minutes. Cook loss was higher for samples cooked at 100°C. For samples of 0.02% concentration for 5 minutes dip also there was significant difference between temperatures of 70°C and 100°C ($p<0.001$). Samples cooked at 100°C showed a higher cook loss than those at 70°C. Between treatments and cooking temperature there was no significant difference. For samples cooked at 70°C after 5 minutes dip, there was significant difference between concentrations. (Table 4.1).

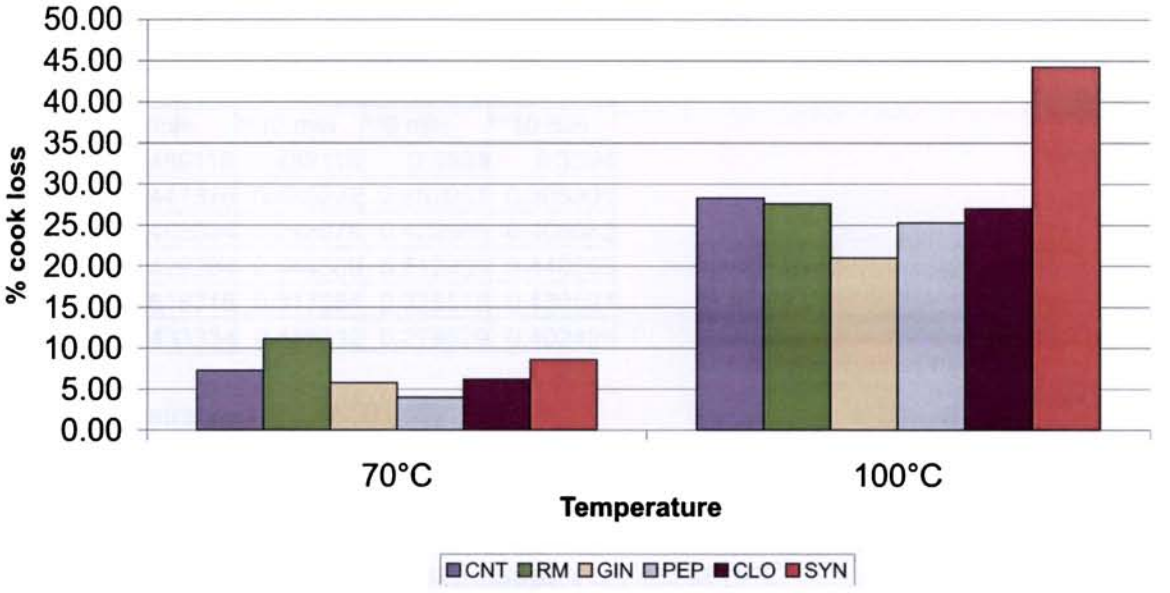


Figure 4.3. Cook loss of treated samples (0.02%) at different temperatures (5 min dip)

Table4.1 Percentage cook loss of dip treated samples cooked at 70 °C

	*0.02%		*0.05%	
Treatment	5min dip	10min dip	5min dip	10min dip
CNT	7.30	7.47	7.80	7.80
RM	11.13	11.90	17.50	9.50
GIN	5.80	3.80	11.80	7.40
PEP	4.00	6.16	12.25	11.80
CLO	6.20	2.65	7.30	6.40
SYN	8.60	5.23	7.90	7.75

4.4.2 Texture Profile Analysis

The experimental data collected were subjected to statistical analysis using two factor ANOVA, wherever the treatment effects were found to be significant, difference was calculated at 5% level and significant effects were identified. The results of the analysis of the data are presented below.

Tables 4.2, 4.3 and 4.4 shows the various texture profile parameters observed for treated and cooked samples of mackerel. Of the all various texture parameter analysis, springiness showed significant results. ($P<0.001$). Samples treated with 0.02% concentration showed significantly high values than those treated with 0.05% concentration. [Appendix 4.1, 4.2, 4.3, 4.4 & 4.5]

Table 4.2 Hardness1 (kgf) of treated samples cooked at 70°C

	*0.02%		*0.05%	
Treatment	**5 min	**10 min	**5 min	**10 min
CNT	0.489118	0.489118	0.3594	0.3594
RM	0.447876	0.485292	0.452053	0.305301
GIN	0.463534	0.298878	0.422865	0.406082
PEP	0.429204	0.444569	0.512889	0.440565
CLO	0.616716	0.317264	0.325618	0.430521
SYN	0.433334	0.416232	0.278629	0.402428

* spice concentration - 0.02% , 0.05%

** Dip time- 5 min , 10 min

Table-4.3 Springiness (kgf) of treated samples cooked at 70°C

	*0.02%		*0.05%	
Treatment	**5 min	**10 min	**5 min	**10 min
CNT	1.08342	1.08342	0.747328	0.747328
RM	1.161738	1.066885	0.938852	0.962377
GIN	1.1306	0.946804	0.984301	0.989557
PEP	1.322671	0.992392	0.989679	1.173643
CLO	1.238252	1.046314	0.958842	0.992791
SYN	1.019194	1.072111	0.861697	1.160238

*spice concentration- 0.02% , 0.05%

**Dip time- 5 min , 10 min

Table-4.4 Cohesiveness (kgf) of treated samples cooked at 70°C

	*0.02%		*0.05%	
Treatment	**5 min	**10 min	**5 min	**10 min
CNT	0.317327	0.317327	0.272548	0.272548
RM	0.281702	0.275566	0.294198	0.237359
GIN	0.327991	0.192748	0.318148	0.277347
PEP	0.288883	0.27972	0.345243	0.296282
CLO	0.345045	0.219687	0.251498	0.263193
SYN	0.270773	0.273126	0.214687	0.286393

*spice concentration- 0.02% , 0.05%

**Dip time- 5 min , 10 min

Table-4.5 Gumminess (kgf) of treated samples cooked at 70°C

	*0.02%		*0.05%	
Treatment	**5 min	**10 min	**5 min	**10 min
CNT	0.159496	0.159496	0.09829	0.09829
RM	0.128142	0.140971	0.137797	0.084027
GIN	0.156702	0.058244	0.138562	0.116751
PEP	0.124288	0.126648	0.177838	0.136029
CLO	0.217494	0.069968	0.084503	0.113202
SYN	0.121464	0.114645	0.068587	0.116627

*spice concentration- 0.02% , 0.05%

**Dip time- 5 min , 10 min

4.4.1 Organoleptic evaluation

Table 4.6: *Overall sensory score of samples treated and cooked at 70°C and 100°C

Treatments	Temperature	
	70 ° C	100 ° C.
Control	6	4.3
Rosemary	8	6.4
Ginger	7.5	4.6
Pepper	8	3.2
Clove	8.7	4.7
Synthetic	5.9	4.9

* Mean of 10x 3 values

From the organoleptic score values of samples cooked at 100°C all the samples irrespective of the treatments were found to show a lower value compared to those cooked at 70°C. Among the treatments; rosemary, pepper and clove showed higher values compared to control.

4.5 Discussion

Cooking plays a significant role in affecting the textural properties of muscle tissue. Certain typical differences were observed between the behaviour of the myofibrillar proteins and collagen at elevated

temperatures. At 60°C collagen fibers become solubilised thus textural changes in flesh at higher temperatures were related to heat denatruration of the myofibrillar protein (Dunajski, 1979). Stanley and Hultin, (1982) indicated that frozen storage brought a slight increase in hardness. Heat induced gelation of myofibriallar protein is an important functional property. The formation of protein network in the gel contributes to the unique textural characteristics and to the functional properties of the product. Springiness and fracture force are inversely related. The functional properties of fish muscle were associated with the ability of the myofibrillar protein to form a three dimensional gel pattern upon heating. The thermally induced interaction of fresh fish muscle occurred in three distinct stages namely 40°C, softening 60°C, and gelation 80°C. It was proposed that setting phenomenon could be due to the hydrophobic interactions. The softening could be related to naturally occurring proteolytic enzymes, and it varied with species and gelation could be due to the covalent intermolecular cross linkages between proteins. Myosin (pre rigor) accounted for most of the gel forming capacity of the myofibril protein system (Xiong et al., 1999).

Springiness is an important parameter for the intrinsic quality of frozen surumi. Stability of the meat protein especially myofibrillar proteins against heat and frozen storage is species dependent. Protein stability of cold water species is much poor than temperate or tropical water species (Okada, 1996). At present fatty fishes are generally not used as raw material for surumi. The meat pH of these fishes is as low as 6 and myofibrillar proteins are likely to suffer from acid denaturation. Recent trend of kamaboko products aims Japanese manufacturers at developing new products which are soft texture. Some of the soft textured products are made by incorporating isolated soy protein into surumi based products.

Incorporation of spices which has a tenderizing effect on texture which is brought by ginger and springiness parameter on texture brought about by pepper cannot be over looked. Though rosemary and clove show good antioxidant properties their contribution to textural parameters seems to be limited. This probably may be due to high volatility of the active constituent of spice during cooking. Further study in this subject is required to assess the textural parameters in frozen storage.

4.6 Conclusion

- A low concentration of 0.02% with a dip treatment time of 5 minutes gave a better result for the springiness parameters of texture.
- Cook loss was higher for samples cooked at 100°C irrespective of the dip treatment time and concentration.
- Though rosemary and clove show good antioxidant properties their contribution to textural parameters seems to be limited.

CHAPTER 5

EFFECT OF SPICES ON THE FORMATION OF BIOGENIC AMINES

5. 1 Introduction

5.2 Review of Literature

5.2.1 Factors affecting amine formation

5.2.2 Physiological and functional activities of biogenic amines (BA).

5.2.3. Effect of storage on production of biogenic amines.

5.2.4. Significance of biogenic amines in human health

5. 3 Materials & Methods

5. 3. 1. Sample preparation

5.3.2 Bacteriological analysis forming bacteria.

5.3.3. Analysis of biogenic amines

5.4 Results

5.4.1. Bacteriological analysis

5.4.2 Biogenic Amine Index (BAI)

5.5 Discussion

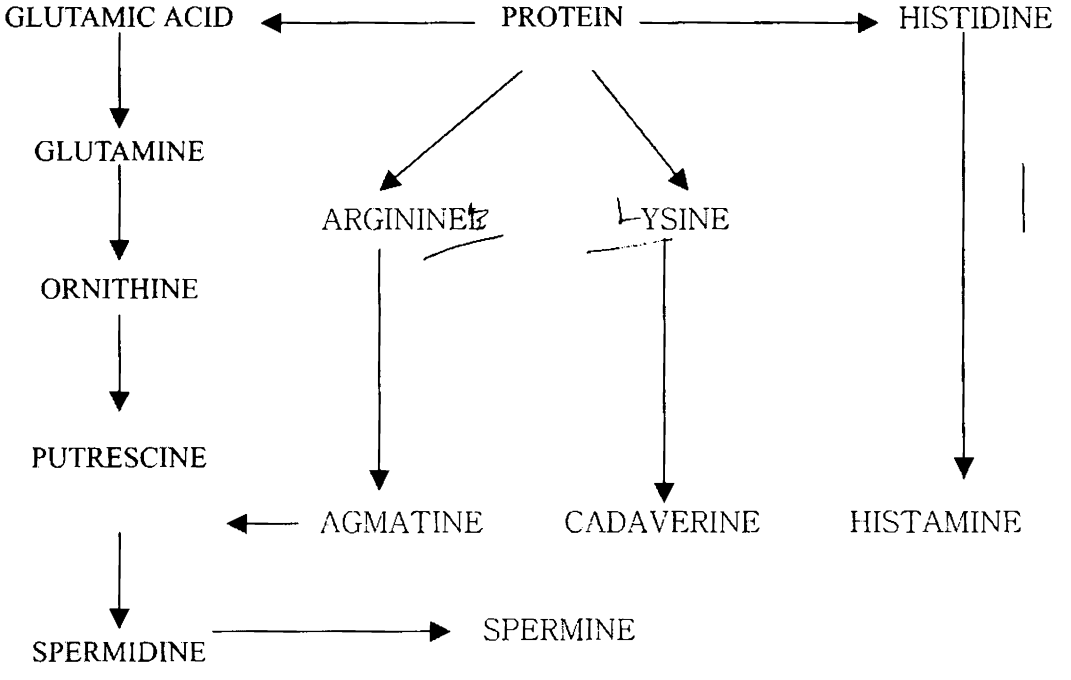
5.6 Conclusion

5.1 Introduction

Biogenic amines are defined as aliphatic, alicyclic and hetrocyclic organic bases of low molecular weight. They are not only biosynthesized in animal and vegetable cells, but also produced by the decarboxylation activity of bacterial enzymes. Biogenic amines have been studied extensively because of their involvement in food borne diseases. The most important biogenic amines occurring in food are histamine, putrescine, cadaverine, spermidine, spermine, tyramine and agmatine.

Scombroid poisoning is a food borne intoxication caused by the consumption of scombroid fish containing hazardous levels of histamine in the muscle tissue. The group of fish most frequently implicated in scombroid poisoning is the family Scombridae, which includes the tuna, mackerel and bonito. Histamine is the main compound responsible for this intoxication and its toxicity is increased by other biogenic amines such as putrescine and cadaverine present in fish. Putrescine is the decarboxylation product of amino acid lysine and cadaverine arises from the decarboxylation of ornithine.

The biological pathway for the formation of nonvolatile amines putrescine, histamine, cadaverine, spermidine, agmatine and spermine from amino acids is as shown below.



Histamine is produced by microbial decarboxylation of the free histidine in the tissue as a result of improper handling of fish. Bacteria containing histidine decarboxylase are the main source of histamine formation in scombroid fish. This enzyme is responsible for the conversion of free histidine in fish muscle to histamine. Under normal circumstances, exogenous amines from food are absorbed and detoxified very quickly in the body by the activity of amine oxidases or by conjugation. In low concentration it is not a health hazard.

Several studies have shown the distribution and classification of the types of bacteria present in many kinds of marine fish, both as natural microflora and spoilage organisms. Of those organisms, which possess the ability to decarboxylate histidine to form histamine, the enterobacteriacea are primarily responsible for the decomposition of the scombroid fish. Among the enteric bacteria reported, *Morganella morganii* has been consistently shown as histamine formers both in fish and culture broth. In addition to those species *Proteus vulgaris, Proteus mirabilis, Clostridium perfringens, Enterobacter aerogenes* and *Vibrio alginolyticus* have all been isolated from skip jack tuna.

Studies have revealed that traditional spices and herbs possess antimicrobial activity on food spoilage bacteria and that, the use of spices to prevent the bacterial growth, thereby inhibiting amine production could be considered as an alternative to artificial food preservation.

The objective of this work is to assess the inhibitory effect, brought about by four spice oleoresin extracts namely Rosemary, Ginger, Pepper, and Clove, along with a synthetic preservative B.H.A. (Butylated Hydroxy Anisole) on the rate of biogenic amine formation in mackerel on storage at ambient temperature.

5.2 Review of Literature

Sea food quality is affected by handling gutting, processing and storage temperature. For the seafood industry, the monitoring of indicator compounds such as biogenic amines, associated with seafood safety is as important as quality assurance. The Food and Drugs Administration United States (1996) has set action levels of histamine as 20 mg/100 gm and 50 mg/100gm for spoilage and hazard respectively.

Biogenic amines have been studied extensively because of their involvement in food borne diseases. Among the biogenic amines histamine is potentially hazardous and is the causative agent of histamine intoxication associated with the consumption of sea food (Morrow et al., 1991).

Scombroid poisoning is a food borne intoxication caused by the consumption of scombroid fish containing hazardous levels of histamine in the muscle tissue (Arnold and Brown, 1978., Behling and Taylor, 1982). Histamine is produced by microbial decarboxylation of the free histidine in the tissue as a result of improper handling of the fish. Numerous bacteria have been reported to possess histidine decarboxylase activity (Taylor et al., 1978) but only *Klebsiella pneumoniae, Morganella morganii* (Kwabata, 1956., Sakabe, 1973) and *Hafnia alvei* (Havelka, 1967) have been indicated as causative organism in the formation of the toxicological significant levels of histamine in fish.

5.2.1 Factors affecting amine formation

The formation of biogenic amines in foods depends on several factors viz temperature and time of storage, pH, oxygen supply, muscle type, effect of pre processing, and different processing steps like salting, smoking, irradiation etc. The use of antimicrobial agents also has influence on the formation of biogenic amines.

5.2.1.1 Temperature

With respect to the influence of temperature on the synthesis of biogenic amines there are different views. Santos et al., (1986) opined that storage temperature did not significantly influence maximum tyramine content in anchovies though refrigeration temperatures delayed the start of the production. Disagreeing with the above information, Diaz et al., (1992) found that histamine and tyramine concentrations increased with the time and storage temperature of chihua cheese. Putrescine biosynthesis by *Enterobacter cloacae* was detected at 20 ° C after 24 h of incubation but not at 10° C, and *Klesbsiella pneumoniae* showed less extensive cadaverine production at 10° C compared to 20° C. Further it is reported that histamine production slowed at 10° C and nearly terminated at 5° C. This is attributed to the slow growth of histamine producing bacteria at low temperatures. No histamine was formed by *Pseudomonas morganii, Pseudomonas vulgaris, or Hafnia* strains after one month of incubation at 1° C (Halasz et al., 1994). Similarly, Klausen and Lund, (1986) reported that amine contents were temperature dependent and were two to twenty times higher at 10° C compared to that at 2° C in both mackerel and herring. In most cases it is proven that there is

direct relationship between biogenic amine formation and time and temperature of storage. So it can be inferred that temperature abuse in highly perishable food items is the main cause of biogenic amine toxicity.

Amine concentrations are unaffected by cooking, with the exception of spermine, which decreased during heat treatment of cooked ground beef at 200° C for 2 h. Histamine is thermally stable during the cooking process (Luten et al., 1992). This has great significance in the thermal processing of foods like canning where if the raw material used contains biogenic amines, the final product will also be containing the same amines without much quantitative changes.

5.2.1.2 pH

The pH level is an important factor influencing amino acid decarboxylase activity. Santos et al., (1986) found a higher tyramine level in mackerel when the pH was low. The conversion of histidine to histamine by Klebsiella pneumonie isolated from skipjack tuna has an optimum pH of 4. Amino acid decarboxylase activity was stronger in an acidic environment, the optimum pH being between 4 and 5.5 (Teodorovic et al., 1994). In such an environment bacteria are more strongly encouraged to produce the amino acid decarboxylase enzymes, as a part of their defense mechanisms against the acidity (Halasz et al., 1994; Teodorovic et al., 1994).

5.2.1.3 Constitutional influences

The amine formation in fishes is also influenced by the constituents in the fishes. In the case of red muscle, more histamine is produced since it contains more histidine. In the case of fishes like tuna and mackerel it is more compared to white muscle fish such as rockfish (Flick et al., 2001).

5.2.1.4. Effect of Processing

In smoking process, hot smoking could effect less histamine production than cold smoking process. Depending on gutting, production of biogenic amines also changes. Whole ungutted fish has more production rate of histamine than fillet of gutted fish (Arnold and Brown, 1978).

Anti microbial agents such as Glycine (10% w/v) sorbic acid (0.1-0.2% w/v) and 10% (w/v) of citric, malic and succinic acids have a diminishing effect on synthesis of biogenic amines (Kang and Park, 1984).

5.2.2 Physiological and functional activities of biogenic amines (BA).

Most of the biological functions of BA are attributed to their polycationic nature. As polycations, they bind non-covalently to negatively charged phospholipids and many types of proteins that directly modulate membrane permeability and play an important role in the maintenance of membrane integrity and in other functions (Srivastava and Smith, 1982).

The Polyamines putrescine, spermidine, and spermine are among the most ubiquitous organic compounds found in nature, and they exist as polycations at physiological pH. Thus, it is not surprising that these simple molecules can intereact with a wide variety of celluar constituents, such as RNA – DNA, nucleotides, proteins, and other acidic substances. Polyamines have been shown to interact with the cellular lipid bilayer and have also been shown to promote membrane fusion.

Histamine possesses a powerful biological function, serving as a primary mediator of the immediate symptoms noted in allergic responses (Stratton et al., 1991). Putrescine, cadaverine and agmatine have been identified as potentiators that increase the toxicity of histamine to human by depressing histamine oxidation (Arnold and Brown, 1978., Halaz et al, 1994).

Polyamines such as putrescine, cadaverine, and spermidine have been reported to be free radical scavengers (Santos, 1996). Lovaas (1991) has demonstrated that amines such as spermine, spermidine and putrescine have marked antioxidant property to polyunsaturated fatty acids. Tyramine possesses also the same property. Amines were also investigated as possible mutagenic precursor, since some amines may act as precursor for other compounds capable of forming nitrosamines, which are carcinogenic to various animals including human (Shalaby, 1996). Putrescine and cadaverine on heating are converted to pyrrolidine and piperidine respectively, from which N-nitroso-pyrrolidine and N-nitroso-piperdine are formed. Therefore, technological processes of food such as salting and smoking seem to induce nitrosamine formation, while cooking (frying) enhances their formation (Doyle et al., 1993). Tyramine, which leads to the formation of mutagenic compounds like 3- diazotyramine induces oral cavity cancer in rats. Secondary amines such as agmatine and polyamines such as spermine and spermidine can also produce carcinogenic N-nitrosoamines in fish, meat and vegetable products.

5.2.3. Effect of storage on production of biogenic amines.

Stiudies have shown that the formation of histamine in Indian mackerel was not significant upto a period of 10 hr at ambient temperature (26° C) reaching an average value of only 7.51 mg/100 gm, and increased significantly thereafter (Vijayan and Balachandran, 1996). Studies by Veciana et al., (1996) showed that high contents of biogenic amines in semi preserved anchovies, was influenced by the hygenic quality of the raw material to be processed, and the storage temperature during shelf life. According to Pacheco et al., (2000) postmortem behaviour of sardine muscle indicated both endogenous and microbial deterioration processes could be controlled during storage at 0° C. Studies by Rossi et al., (2002) reveal that cadaverine could be used either alone or together with histamine as part of quality control programme in skip jack and big eye tuna. Du et al., (2002) confirmed that the change of tuna quality was affected by the bacterial numbers found in the fillets while the increase in aerobic bacteria can serve as a useful indicator of the overall tuna quality, and the presence of a possible histamine or biogenic amine hazard. The increase in histamine producing bacteria contributes to the rapid increase of histamine or biogenic amines contents and health hazard especially to tuna fillets stored at 22° C.

5.2.4. Significance of biogenic amines in human health

Biogenic amines at normal concentrations are not problematic for human beings. Under normal conditions they are catabolised with the help of a number of enzymes. Healthy individuals can oxidize or detoxify dietary polyamiens and biogenic amines by acetylation and oxidation mediated by the enzymes monoamine oxidases (MAO EC: 1.4. 3.4) diamine oxidase (DAO EC 1.4.3.6) and polyamine oxidases (PAO; EC 1.5.3.11). The detoxification system of histamine composed of two distinct enzymes, diamine oxidase and histamine N methyl transferase. However, it apparently fails to detoxify large amounts of histamine that can be ingested with spoiled scrombroid fish. Occurrence of biogenic amines in fish is a very common quality criterion. Generally histamine is the principal biogenic amine of fish followed by cadaverie. As decomposition progresses, together with histamine and cadaverine, other amines such as putrescine, tyramine, agmatine and tryptamine are increased (Yamanaka and Matsumbo, 1989). As with other biological indicators such as TMA, DMA, TVB-N and K value, amines are also used as a potential freshness index in fish and shellfish. Yamanaka and Matsumbo (1989) reported that formation of putrescine, cadaverine and histamine and loss of spermidine and spermine were observed as decomposition of tuna progressed. He

concluded that these amines might serve as quality indicators of tuna. Shakila et al (2003) reviewed and found that histamine alone is not considered as a reliable indicator of decomposition as concentration of its precursor histidine vary greatly in scombroid and nonscombroid fish. The amines, which increase with storage time at normal temperature, are agmatine; histamine, cadaverine, tyramine etc. For these reasons Meitz and Karmas (1978) suggested a freshness index using different amines. According to them Index- (ppm cadaverine + ppm putrescine + ppm histamine)/ (1+ppm spermine + ppm spermidine) they reported that this formula has a simple mathematical design and as decomposition progresses histamine, putrescine and cadaverine rise in their values, while spermidine and spermine fall.

Later, Valle et al. (1996) modified it and gave the term Quality Index (Ql) or Biogenic Amine Index (BAI)

So, Ql or BAI = (Histamine (ppm) + cadaverine (ppm) + Putrescine (ppm) / (Spermidine (ppm) + Spermine (ppm))

Good agreement has been observed between spoilage index and sensory quality.

Even though problems related to biogenic amines are of universal prevalence, very few countries impose limits on biogenic amines particularly on histamine. These limits are determined based on the type of micro flora, their capabilities, condition of spoilage, risk on gastrointestinal diseases, occurrence of amine oxidase inhibitor drugs etc. For this hazard action level (HAL) or defect action level (DAL) has also been set.

A regulatory limit of histamine in fish and fishery products has been established in several countries. Maximum permissible limits for other biogenicamine such as putrescene and tyramine were not prescribed by any regulatory agencies in US, EU and Japan. It was reported that cadaverine and putrescene could be used as freshness indices for fish and shellfish respectively (Shakila et al., 2003). Fish and fishery products containing cadaverine below 15 mg% are considered as good for consumption, 15 -20 mg% indicates potential decomposition and over 20 mg% advanced decomposition. The table given below gives the limits of histamine in fish in different countries.

Regulatory limits on histamine in fish imposed by different countries.

Country	Limit
USFDA	50 mg% (hazard action level)10 -20 mg% (defect action level)
EEC	10 mg% (defect action level)20 mg% maximum allowable limit
Canada	10 mg% indicator composition10 -20 mg% (defect action level)
Germany	20 mg%
Denmark	30 mg%
India	20 mg%
Sweden	20 mg%

In addition to this, Sims et al. (1992) suggested 0.5 mg/kg as threshold limit of cadaverine in fish.

5. 3 Materials & Methods

5. 3. 1. Sample preparation

Fresh Mackerel procured from Munambam Harbour was divided into two lots. Each lot was further divided into six groups. Each group of fish was subjected to a dip treatment of four spice extracts namely Rosemary, Ginger, Pepper, and Clove, along with a synthetic preservative B.H.A. (Butylated HydroxyAnisole) and a Control. The dip treatment was done at spice oleoresin concentrations of 0.05% for ten minutes. The treated samples of fish were arranged on a plastic tray and stored at 28° C ± 2° C. Sampling was done at regular intervals of 4 hours, 9 hours, and 20 hours after dip treatment. Sample controls without any spice extract were also analysed at 0 hour, 4 hours, 9 hours and 20 hours interval. The above treated samples of fish were also subjected to bacteriological analysis for enumerating histamine-forming bacteria. All Samples for bacteriological analysis were processed under aseptic conditions. Samples were also taken for biogenic amine analysis.

5.3.2 Bacteriological analysis

To remove each section, the fish was kept on a clean alcohol disinfected surface. The skin was first peeled away and then a section of muscle tissue was cut out. Care was taken not to contaminate the section with material from gut cavity.

5.3.2.1Culture Media used

a). Tryptone Glucose Beef Extract Agar (TGBEA)

Beef extract	3.0 g
Tryptone	5.0 g
Dextrose	1.0 g
Agar	15.0 g
Distilled Water	1000 ml
pH	7.0 ± 0.1 ml

Added the above ingredients to one litre of distilled water and soaked for 15 minutes. Dissolved the ingredients by gentle heating and sterilized by autoclaving at 121° C for 15 minutes at 15 lbs.

b). Modified Nivens medium: (Niven et al., 1981).

Glucose	0.5 %
Tryptone	0.5 %
Yeast extract	0.5 %
NaCl	0.5 %
CaCO3	0.1 %
Agar	2.0 %
Histidine hydrochloride	1.0 %
Saline	0.9 %
Peptone water	0.1%

Dissolved the ingredients proportionately for the required volume by gentle heating and sterilized by autoclaving at 121° C for 15 minutes at 15 lbs.

5.3.2.2. Total Plate Count (TPC) and enumeration of histamine decarboxylating bacteria. (HDB)

10gm muscle samples were blended in 90 ml peptone water (0.1%) and serially diluted (FDA 1992). A 1 ml aliquot was taken from the diluted samples and mixed with plate count agar for enumeration of TPCs. Another 1 ml aliquot was taken for enumeration of histamine producing bacteria and mixed with Modified Nivens medium (Niven et al., 1981). Enumeration of TPCs and

histamine producing bacteria was carried out in duplicate and plates were incubated at 25° C for 2 days on the Modified Niven's medium. Purple colonies with or without halos were regarded as positive histamine formers.

5.3.3. Analysis of biogenic amines

5.3.3.1 Apparatus:

Quantitative determination of the biogenic amines was conducted using a Waters HPLC system with a Binary pump model M 515, a 600 Gradient mixer solvent delivery system, a dual ë absorbance UV/VIS detector model 2487 and a C 18 Symmetry column (5 µM particle size, 4.6 mm id x 250 mm length column) with a flow rate of 1.5 ml/min. The equipment is provided with column oven, and a manual injector. Data analysis was performed using EMPOWER 2 chromatography software.

5.3.3.2 Reagents:

Biogenic amines standards (Putrescine dihydrochloride, cadaverine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, histamine dihydrochloride and agmatine sulphate) were purchased from Sigma-Aldrich, Poole, and Dorset, UK. HPLC grade Acetonitrile was used as solvent A and deionized ultrapure Millipore water was used as solvent B (Rios and Elix Millipore Water Purification System).

5.3.3.3 Preparation of standard amine solution:

Putrescine dihydrochloride (182.9 mg), cadaverine dihydrochloride (171.4 mg), spermidine trihydrochloride (175.3 mg), spermine tetrahydrochloride (172.0 mg), histamine dihydrochloride (165.7 mg) and agmatine sulphate (175.4 mg) were dissolved separately in 10ml HPLC grade water. A composite standard comprising all the above biogenic amines were also used. The final concentration of free base for each amine was 10 mg/ ml solution.

5.3.3.4 Sample preparation:

Fish muscle (5 g) from all the treated and control samples was taken from the dorsal part of the fish fillet without skin and transferred to a 250 ml centrifuge tube, The sample was homogenized with 20 ml 6% TCA (trichloroacetic acid) for 3 min, centrifuged at 12 000 g for 10 min at 4° C and filtered through Whatman No1 filter paper. The aliquot was made up to 50 ml with distilled water and was stored at -30° C until futher analysis.

5.3.3.5 Derivatization procedure: (Ozogul, 2002)

A stock solution was prepared by dissolving 2% benzoyl chloride in acetonitrile to enhance the reaction with amines. For derivatization of standard amine solutions, 50µl was taken from each free base standard solution (10 mg/ml) and 2 ml of TCA extract for fish samples. One millilitre of 2M sodium hydroxide was added, followed by 1 ml benzoyl chloride (2%) and vortex mixed for 1 min. The reaction mixture was left at room temperature (24° C) for 30 min. The benzoylation was stopped by adding 2 ml of saturated sodium chloride solution and the solution was extracted two times with 2 ml of diethyl ether. The upper organic layer was transferred into a clean tube after mixing and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 500µl of acetonitrile and 20µl aliquot was injected for HPLC analysis.

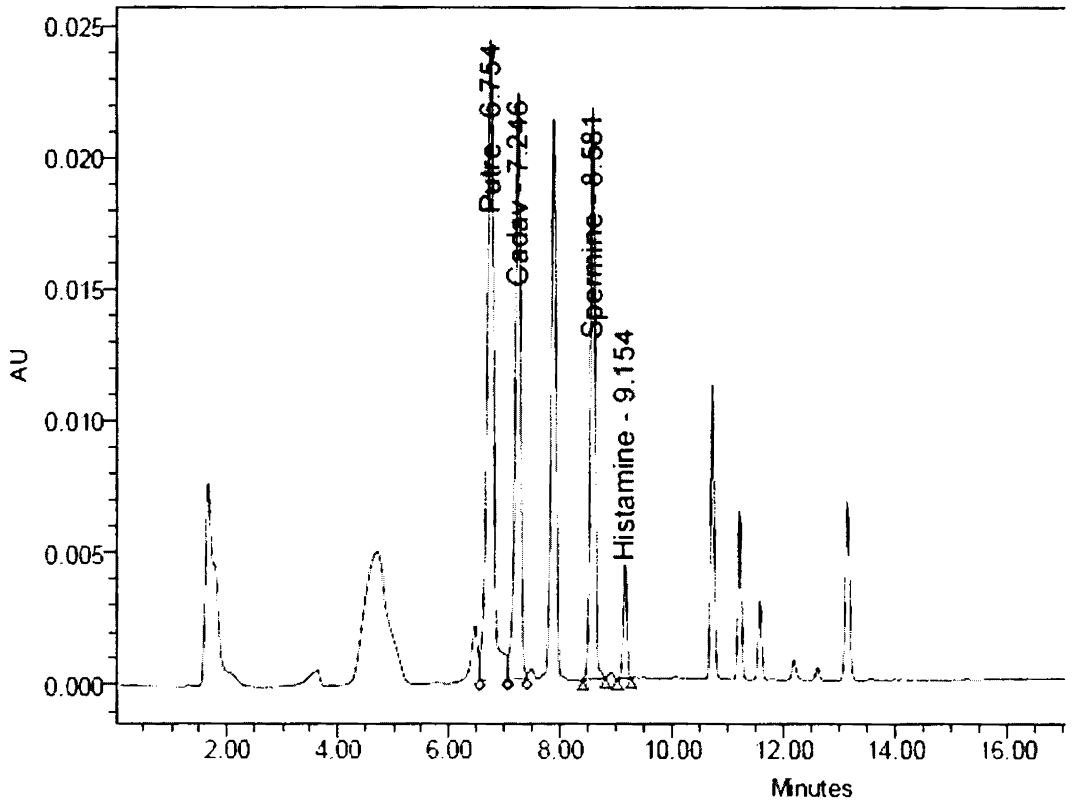
5.3.3.6 Chromatographic conditions

Chromatographic separation made use of continuous gradient elution with acetonitrile (eluant A) and HPLC grade water (eluant B). The gradient started at 80% acetonitrile and was decreased to 20% and finally increased to 80% in 16 min. The total separation time was less than 7 min and the gradient was run for 20 min to ensure full separation. HPLC gradient profile for separation of benzoyl derivatives of biogenic amines is as shown below. Detection was monitored at 254 nm.

A calibration curve for each of the amines in the range of 0-100µg/ml was prepared. Correlation of peak areas of individual amines standards and composite standards with known concentration was calculated after injecting each of the standard amine solutions

HPLC gradient profile for separation of biogenic amines.

HPLC gradient profile					
	Time (min)	Flow rate ml/min	Acetonitrile (%A)	Deionized Millipore Water (% B)	Curve
1	-	1.5	80	20	
2	10	1.5	80	20	6
3	15	1.5	20	80	6
4	16	1.5	20	80	6
5	20	1.5	80	20	6



	Peak Name	RT	Area	% Area	Height
1	Putre	6.754	200830	40.32	24382
2	Cadav	7.246	151078	30.33	22293
3	Spermine	8.581	125809	25.26	21704
4	Histamine	9.154	20316	4.08	4272

HPLC Chromatogram of Standard Biogenic amines.

5.4 Results

5.4.1. Bacteriological analysis

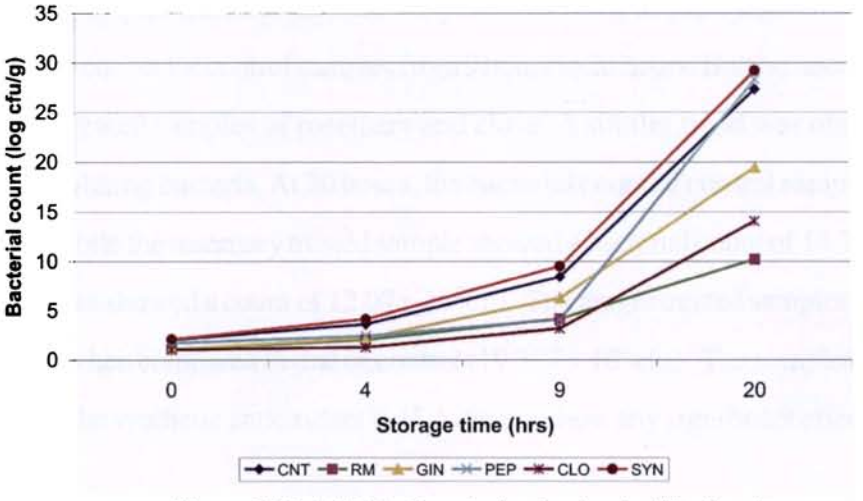


Figure 5.1Total Plate Count of spice treated Mackerel

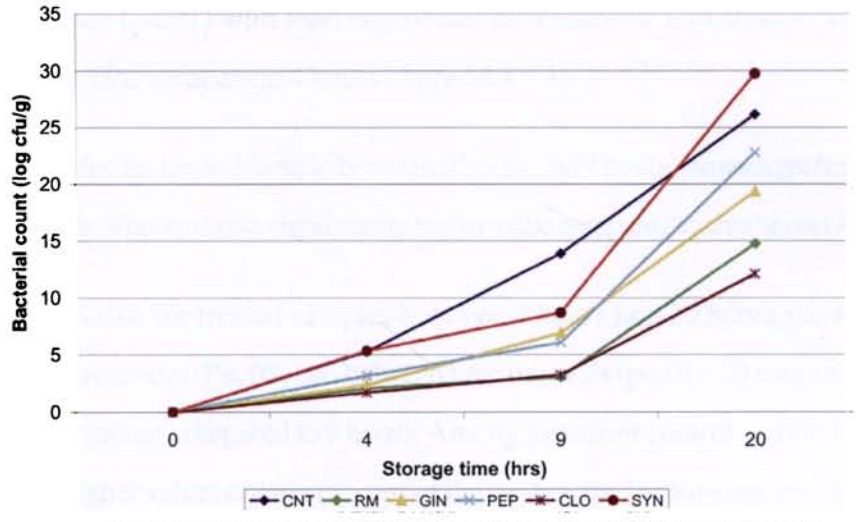


Figure 5.2 Histidine decarboxylating bacteria(HDB) in spice treated Mackerel

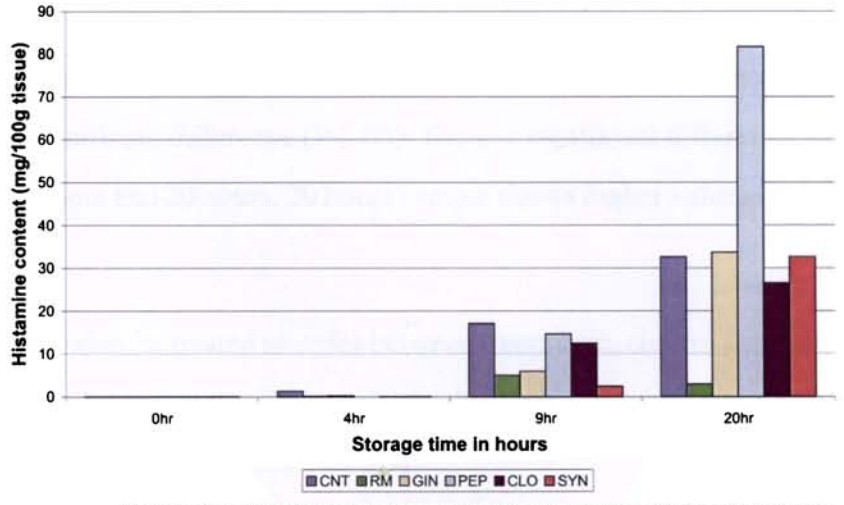


Fig 5.3 Variation in Histamine content in treated samples of mackerel

Fig. 5.1 and Fig. 5.2 show the Total Plate Count (TPC) and Histamine Decarboxylating Bacterial (HDB) count respectively of samples treated with spices. Fig. 5.3 shows the variation in histamine content for treated samples stored at ambient temperature. The total plate count data showed an increase in bacterial counts for control samples from 9 hours to 20 hours. But the microbial growth was inhibited for treated samples of rosemary and clove. A similar trend was observed in the histidine decarboxylating bacteria. At 20 hours, the bacterial count of control sample came upto 26.160 x 10^4 cfu. While the rosemary treated sample showed a bacterial count of 14.773 x 10^4 cfu, clove treated samples showed a count of 12.07 x 10^4 cfu. The ginger treated samples also showed a decreasing trend when compared to that of control (19.397 x 10^4 cfu). The samples treated with pepper extract and the synthetic antioxidant B.H.A did not show any significant effect.

ANOVA of treatment versus storage period between 4 hours and 9 hours shows that there is a significant difference ($p<.01$) with least significant difference of 1.71.9 hours sample shows significantly higher TPC compare to 4 hours (Appendix 5.1).

ANOVA for TPC value for treated sample between 0 hours and 9 hours shows significant difference between time intervals. 9 hours shows significantly higher value compared to zero hours (Appendix 5.2).

ANOVA for HDB value for treated samples between 9 hours and 20 hours shows significant difference between treatment ($P<.05$) and between time intervals ($p<.01$). 20 hours samples shows significantly higher values compared to 9 hours. Among treatment control and synthetic samples gave significantly higher values compare to rest of the treatments. In clove and rosemary, bacterial count is significantly lower compared to others (Appendix 5.3).

ANOVA for HDB value for treated samples between 9 hours and 20 hours in control and rosemary shows there is a significant difference ($P<.05$). There is significant difference in the bacterial count between 9 hours and 20 hours. 20 hours sample shows higher values than 9 hours value (Appendix 5.4).

ANOVA for HDB value for treated samples between treatments, control and ginger between 9 hours and 20 hours shows that there is significant difference ($p<.01$). 20 hours sample shows higher values than 9 hours value (Appendix 5.5).

5.4.2 Biogenic Amine Index (BAI)

Table 5.1 Biogenic amine content of treated samples kept at ambient temperature

	4 hours					
	put	cad	spd	spm	his	agm
CNT	1.753	1.043	0.055	0.015	1.303	27.333
RM	2.913	0.533	0.200	0.019	0.140	23.117
GIN	0.809	0.169	0.020	0.010	0.289	2.817
PEP	0.647	0.130	0.019	0.036	0.000	3.247
CLO	0.120	0.049	0.049	0.000	0.070	0.570
SYN	0.951	0.310	0.047	0.009	0.020	2.313
CNT 0 time	0.083	0.073	0.020	0.009	0.075	0.092

put-Putrescine **cad**-Cadaverine **spd**-Spermidine
spm-Spermine **his**-Histamine **agm**-Agmatine

Table 5.2 Biogenic amine content of treated samples kept at ambient temperature

	9 hours					
	put	cad	spd	spm	his	agm
CNT	4.317	2.123	5.010	0.302	17.020	31.103
RM	0.000	0.000	0.776	0.507	5.017	0.000
GIN	0.000	1.803	0.000	1.057	5.990	168.483
PEP	0.000	2.410	0.000	1.603	14.640	108.127
CLO	0.000	1.813	0.382	0.000	12.403	1.080
SYN	0.000	0.000	0.480	0.643	2.420	0.510

Table 5.3 Biogenic amine content of treated samples kept at ambient temperature

	20 hours					
	put	cad	spd	spm	his	agm
CNT	0.033	0.000	1.650	2.520	32.633	12.367
RM	0.000	4.757	4.870	5.317	2.943	0.000
GIN	9.100	2.267	1.460	1.540	33.627	2.520
PEP	7.040	0.000	3.787	4.753	81.667	0.000
CLO	7.420	4.517	3.533	0.000	26.433	2.140
SYN	6.800	3.243	1.937	0.000	32.643	3.047

Table 5.1, Table 5.2 and Table 5.3 shows the concentration of various biogenic amines, putrescine, cadaverine, spermidine, spermine, histamine and agmatine during ambient storage. The samples were those that were given a dip treatment of 0.05 % concentration for ten minutes. The biogenic amines were analyzed by HPLC. The storage periods were extended well beyond the accepted time for edibility in order to give a full picture of the production of these amines from the fresh fish to the putrid state.

Figure 5.4 shows the result of the Biogenic Amine Index, which was calculated as the sum of putrescine, cadaverine and histamine (Hernandez – Jover et al., 1996). Here again, the inhibitory effect of rosemary and clove are more pronounced than other treated samples of ginger, pepper and B.H.A.

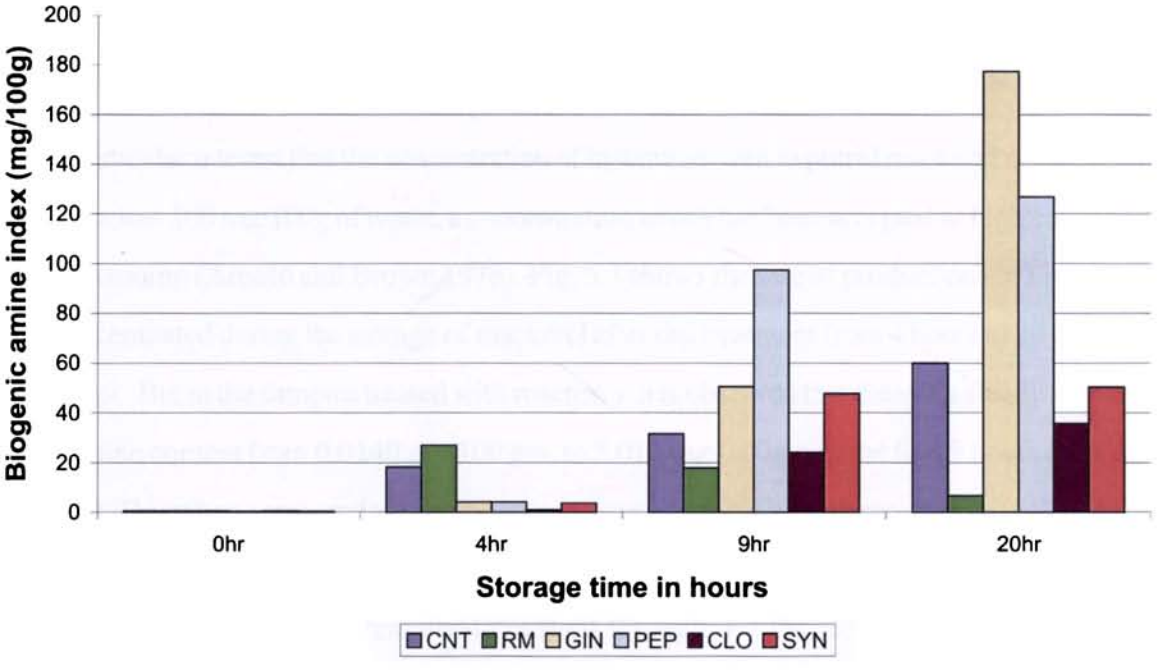


Figure 5.4 Variation in Biogenic amine index of treated samples of mackerel

ANOVA for Biogenic amiene index for treated samples between 4 hours and 20 hours there is significant difference ($p<.05$) between periods. The least significant difference calculated is 14.10 and 20 hour sample gave higher BAI values compared to 4 hours (Appendix 5.6).

5.5 Discussion

The amount and type of amine found in a food sample depends on the commodity and the type of microorganisms present (Santos, 1996). The pre requisite for the production of amines by microorganism include availability of amino acids, presence of decarboxylase positive microorganisms and conditions favourable for bacterial growth. Biogenic amine development is related to fish spoilage and also to tri methylamine Nitrogen (TMA-N) levels and the change in sensory parameters (Halaz, 1994). There is evidence that the concentration of histamine found to be relatively high in viscera (Salguero & Mackie, 1979) and in the region of the belly wall (Kizevetta and Nasodkeva 1972).

It is of particular interest that the concentration of histamine even in putrid mackerel in this study are well below 100 mg/100g of tissue, a concentration which has been accepted as high enough to cause poisoning (Arnold and Brown, 1978). Fig. 5.3 shows the rate of productions of histamine that is accentuated during the storage of mackerel after dip treatment from 4 hours to 20 hours in the control. But in the samples treated with rosemary, it is observed that there is a steady increase of histamine content from 0.0140 mg/100 gm. to 5.017 mg /100gm in the first 9 hours after dip treatment. This when compared to control sample showed a significant decrease in the value of the amine content. Results show that the initial levels of putrescine, spermidine and spermine in fresh samples were dominant and remained constant through out the storage period at ambient temperature, for 9 hours.

Pelagic fish, particularly the fish of the Scombroidae, have usually high levels of free histidine in tissue extracts and it has been found that the potential for histamine production is related to the concentration of free histidine (Edmunds & Eitenmiller, 1975). These results on treated mackerel are similar to those described by Karmas and Meitz (1978) in tuna. According to Du et al., (2002) the change in the quality of tuna fillets were affected by the bacterial count, while the increase in aerobic bacteria can serve as a useful indicator of the overall quality of the fish. It is also confirmed that the presence of a possible histamine producing bacteria contribute to the rapid increase of

histamine or biogenic amine contents leading to health hazards. Studies by Vijayan and Balachandran (1996) also revealed that the formation of histamine in Indian mackerel is not significant up to a period of 10 hours at ambient temp (26°C) reaching an average value of 7.51 mg/100 gm and increased significantly thereafter. The present study also shows a similar trend in histamine content. Results show that the initial levels of putrescine, spermidine and spermine were dominant and remained constant through out the storage period at ambient temperature up to 9 hours. The result agrees with the studies of Baixas-Nogueras et al. (2001). Cadaverine and agmatine were the biogenic amines with the highest concentration at the end of storage in the treated samples as well as control. Cadaverine was the most potential index for the freshness of white prawns, and putrescine was a supplementary index of shrimp of high quality. This can be explained by the fact as other authors have highlighted (Wei et al., 1990, Lopez et. al., 1995) that the enzymatic decarboxylation is enhanced when the concentration of gases is lower thereby increasing the production of amines.

Certain spices that are generally used in Indian Culinary are known to provide distinctive flavours to the food as well as show bacterial inhibitory activity. Bozin et al., (2007) have studied the antimicrobial activity of the essential oils of rosemary. Eugenol, the major essential oil from clove, has been shown to inhibit the extra cellular enzyme productions of bacteria (Thoroski, et.al.1989). The study of Wendakoon and Sakaguchi (1992) on the effect of spices on growth of biogenic amine formation by bacteria in fish muscle showed clove and cinnamon were found to be the most effective. In this present study, rosemary showed the maximum inhibitory effect on histidine decarboxylase, thus confirming to the studies of Bozin et al (2007), highlighting the strong antimicrobial activity of rosemary. It was further observed that after a certain time, the amine levels showed a decrease at 9 hours in the case of all spice treated samples. This could be due to the amine oxidase activity of the bacteria. Some bacteria are able to produce amine oxidases in the presence of mono or diamines (Gale, 1942).

5.6 Conclusion

Biogenic amines (BA) are low molecular weight organic compounds that can be found in various foods and beverages such as wine, beer, fish and meat, normally as a consequence of microbial activity of the free amino acid. Several countries have established regulations of biogenic amines

intake from various kinds of food because amines, especially tyramine and histamine can be toxic when their levels are high. Polyamines such as Putrescine, Cadaverine, Spermidine and Spermine although not having a direct toxic effect, can enhance the toxic effects of Tyramine and Histamine by competing for the decarboxylase enzymes by human beings. Now a days, the regulatory limits are focused of the content of histamine for 50mg/kg (USFDA) while other biogenic amines have no accordant limits. Some biogenic amines can appear during food fermentation process or food storage under certain conditions, if amino acid decarboxylase positive microorganisms are present. These compounds are the chemical indicators of spoilage of fish and can be formed in relatively high concentration due to prolonged storage time. The high content of protein in meat results in an increased probability of fast decomposition processes. So biogenic amines irrespective of their important health significance can serve as alternative food quality markers, especially their changes in non fermented foods such as meat and fish during storage.

The present study confirms to the earlier published work, highlighting the strong antimicrobial activity of rosemary. Biogenic amines index as a sum of putriscine, cadaverine and histamine determined by a simple method carried out in this work could be effectively employed for the early detection of quality deterioration, as well as for the evaluation of acceptable stage for mackerel. Determination of biogenic amines using the methods developed in the study can be applied to other fish products as part of quality assurance methods.

CHAPTER 6

DRIED AND VALUE ADDED PRODUCTS OF MACKEREL

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Section (A): Salted and Dried mackerel

6.1 (A) Introduction

Mackerel is one of the most common food fishes in India and in other countries it is a potential raw material for canning, drying and smoking considering its abundances and nutritive value. In India, dried fish is acceptable to all income groups and it is considered as staple food item which provide good source of proteins. With a 200 km Exclusive Economic Zone (EEZ) around a coast line of more than 5600 km., India has vast marine fisheries resources. The hilly regions of India, still depends on dry fish sources. Since, a well organized cold chain is yet to be established, the interior regions still depend on cured fishery products for the their supply of fish. Depending on the regional variation in taste and consumer preference, different types of cured products are popular in different parts of the country. The southwest coast had always been the major fish landing areas in India. Sun drying had been a popular method of fish preservation along this coast. Oils sardines, mackerel sole, white bait etc., are traditionally sun dried on the sandy beach. This crude method naturally yielded poor products contaminated with sand and dirt. With the advent of improvised technological investigation, hygienic drying practices were accepted. The use of preservatives (Sodium propioniate) was induced as an effective and cheap method for producing good quality cured fish of longer shelf life. Even though the export of dry fish is very low, over the past decades improved methods for maintaining the quality of a cured product should not be over looked. The present study involves drying incorporated with spice oleoresin extracts as natural preservative on dry fish curing; which aims at deriving a new product that is free of chemical preservatives.

6.2 (A) Review of Literature

The most common method of utilization of fish in India is in fresh and cured from (drying, salting and smoking) Prasad and Panduranga, 1994). In India, 20% of fish catch is preserved by curing (Bindu, 2004). The low cost of production, transport and storage give the cured product a substantial market in India as well as in many tropical countries (Yean et al., 1998).

Salted fish processing started antiquity (Cutting, 1955, 1962; Kruezer, 1994). In Asia where consumption is highest, dried salted fish is also an important source of low-cost dietary protein (Poernomo et al., 1992)

Each country has its own standard as to the amount of salt and moisture desirable in their products (Tapiador & Carroz, 1963; Voskresensky, 1965) In Asian countries where most of the processing and trade in salted dried fish takes place the problem of incorrect moisture and salt content is widespread and accounts for heavy losses of products (Zain and Yusuf , 1983).

During brining salt penetrates the fish flesh with accompanying loss of moisture. Under ideal conditions salt uptake will continue until salt concentration in the aqueous phase of the tissue becomes equal to that in the brine.

6.2.1 (A) Effect of Sodium chloride

Sodium chloride, commonly known as salt, table salt, or rock salt, is a vital part of human life. Salt enhances the flavor of foods and plays a functional role in food processing. For instance, salt controls microbial growth and controls yeast activity; it enhances the texture, ripening and shelf life extension in cheese; it lowers water activity, strengthens gel structure and enhances color in processed meats (Ravishankar and Juneja, 2000).

In fish, salting has been one of the oldest methods of preservation. Fish is one of the commodities to which a large amount of salt is sometimes added and if less salt is added, it is usually combined with other methods of preservation. Dipping fish in sodium chloride solution preserves the texture and color combined with modified atmospheric packaging (MAP) and storage (Mitsuda et al., 1980). Hake slices were dipped in sodium chloride (5 min in 5% brine) and MAP stored and these were compared with MAP stored slices of hake without sodium chloride dipping (Pastoriza et al., 1998). In sodium chloride dipped slices, biochemical, microbial and sensory deterioration changes were inhibited, shelf life was extended and the total volatile bases and total viable microbial counts were ignificantly lower than those of non-dipped slices. The postmortem changes (rigor mortis) of Atlantic salmon influenced the salt uptake of the fish muscle (Wang et al., 1988). The equilibrium salt concentration of pre-rigor fillet was much lower (0.53 g/g salt-free solids) than that of in-rigor (0.66 g/g salt-free solids) and post-rigor mortis (0.75 g/g salt-free solids) salmon fillets in 20% (w/v) sodium chloride solution at 10^{0}C.

Dried fish having pH of 6.0-6.9 are considered to be of very good quality. The high drying periods required to achieve low moisture contents to ensure the keeping quality of product increases the tendency of the fat to become rancid (FAO, 1981).

6. 2. 2. (A) Spoilage Bacteria

Fish spoilage is a complex process involving both nonmicrobiological and microbiological processes. Nonmicrobiological deterioration is caused by endogenous proteolytic enzymes, which are concentrated in the head and viscera and attack these organs and surrounding tissues after death. Enzymatic spoilage is followed by the growth of microorganisms, which invade the fish flesh, causing breakdown of tissues and a general deterioration of the product. During processing of fish (e.g., deheading, eviscerating, cutting), the microorganisms present in the surface slime layer, the gills and the gut can be spread onto the processing equipment, the workers and the flesh of the fillet. Hence, the normal sterile flesh can be contaminated with millions of bacteria (Banwart, 1989; Bonnell, 1994; Garthwaite, 1997; Inglish et al., 1993).

6.3 (A) Materials and methods

6.3.1 (A) Preparation of fish for drying

Fresh mackerel was purchased from Munabam Harbour, Kochi. They were dressed as butterfly fillet with head on, washed thoroughly and soaked in 1:3 brine incorporated with 0.05% concentration of spice oleoresins of rosemary, ginger, pepper and clove and BHA. The duration of soaking was two hours. The soaked fish was arranged in trays and subjected to sun drying. It was cooled and packed in polythene covers (Fig: 6.1). A control was also prepared without incorporating any spice. The dried samples were stored at room temperature (28 $\pm$ 2°C) and at 15°C. Sensory, chemical and microbiological test were conducted in the products. The general acceptability score was evaluated. Samples were analysed for a period of eight weeks for the following parameters.

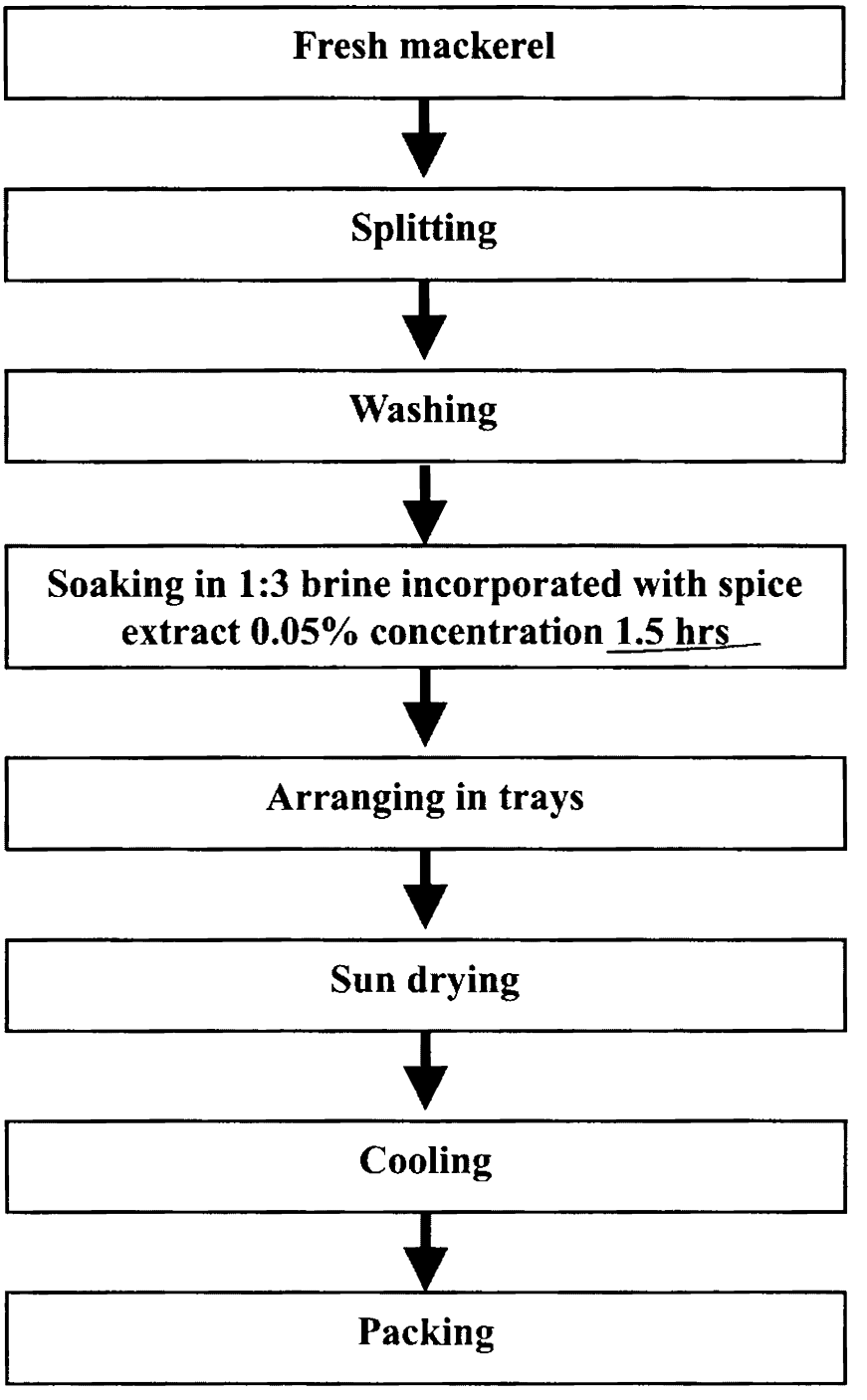


Figure 6.1 Flow chart showing procedure of mackerel drying

Plate 6.1 Treated dry fish samples

6.3.1.1 (A) Moisture content

Moisture content of the samples were analysed according to AOAC, (1995).

6.3.1.2 (A) Salt content

Weighed out 0.4 g of dried cured material into a 250 ml conical flask added 20 ml of dilute nitric acid (1:4). Heated to boil. Cooled and added 20 ml of silver nitrate (0.1 N), boiled again until all salts except silver chloride dissolved. Cooled and added 20 ml of distilled water and 2 ml of ferric alum indicator and titrated against standard ammonium thiocynate solution. The end point was determined by a permanent pink colour and a blank was also run simultaneously (FAO, 1981).

Calculation:

$$\text{Percentage of NaCl} = \frac{5.85 \times V_1N_1 - V_2N_2}{W}$$

Where, W = Weight of sample

$V_{1=\text{Volume of Silver nitrate}}$

N_1 = Normality of Silver nitrate

$V_{2=}$ Volume of ammonium thiocynate

$N_{2=}$ Normality of ammonium thiocynate

6.3.1.3(A) Peroxide value

Peroxide value was determined as detailed in chapter 3 (3.3.3.3) by the method of AOCS, (1999).

6.3.1.4 Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric acid reactive substances was analysed as given in Chapter 3 (3.3.3.4) by the method of Tarladigs et al., (1960)

6.3.1.5 (A) Microbiological Test

Enumeration of mold in the samples of dried fish was done by the procedure of Pitt et al., (1992).

i) Dichloran Rose Bengal Chloramphenicol (DRBC) agar (M183)

ii) Sample and media preparation (Plate 6.2)

Preparation of DRBC of agar Suspend 15.75 grams in 500 ml distilled water. Heated to boiling to dissolve the medium completely. Sterilized the medium completely. Sterilize by autoclaving at 15 lbs pressure (121° C) for 15 minutes. Cooled to 50 °C and aseptically add sterile reconstituted contents of 1 vial of Choramphenicol .Selective Supplement (FD033). Mixed well and pour into sterile petriplates. Media should be prepared no more than 24 hours prior to use. Before plating, held the sample at –20°C for 72 hours to kill mites and insects that might interfere with analysis

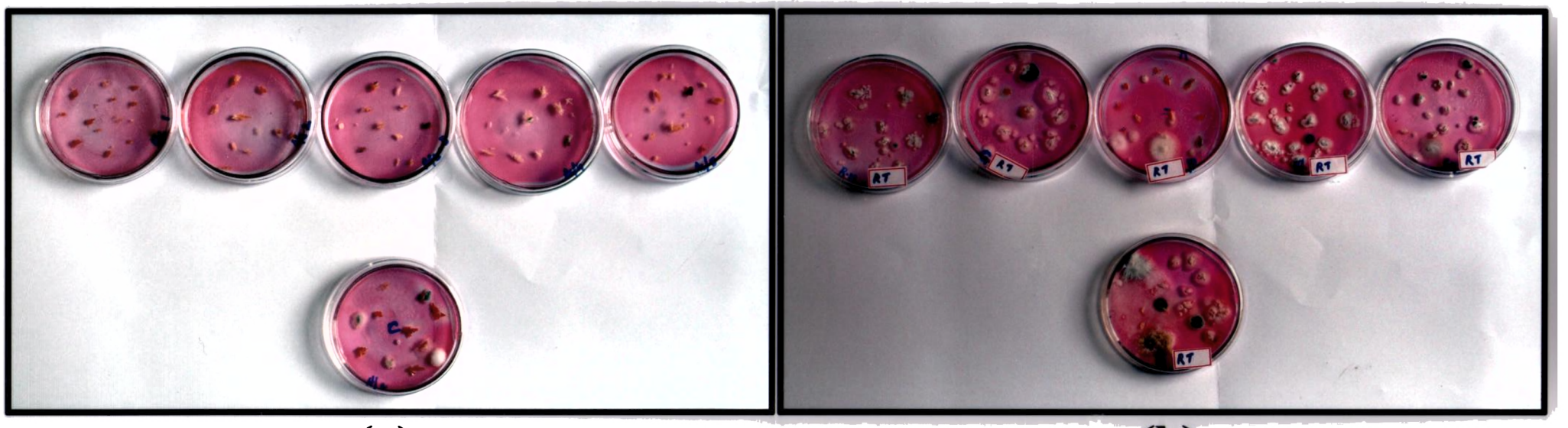
iii) Plating and incubation of sample (Plate 6.3)

From each sample, transferred about 50 g in to a sterile 300 ml beaker. Using 95% ethanol flamed forceps place intact food items on surface of solidified agar. 5-10 item per plate (depending on size of food item), 50 item total per sample. Flame forceps between plating of each item. Use several forceps alternatively to avoid overheating. Aligned 3-5 plates in stacks and identified with sample number plus date of plating. Incubated stacks, undisturbed in the dark at 25°C for 5 days. If there was no growth at 5 days of incubation, re-incubated for another 48 hour to allow heat or chemically – stressed cells and spores enough time to grow.

iv) Reading of plates

Determined the occurrence of mold in percentages. If mold emerged from all 50-food items, moldiness is 100%; if from 32 item, moldiness is 64%. Determined percent occurrence of individual mold on the above basis.

Plate 6.2 Media used for enumeration of mould
(Dichloran base /w rosebengal agar)



(a) (b)

Plate 6.3 Petri dishes showing mould growth

(a) Samples stored at 10˚c (b) Samples stored at 26˚c

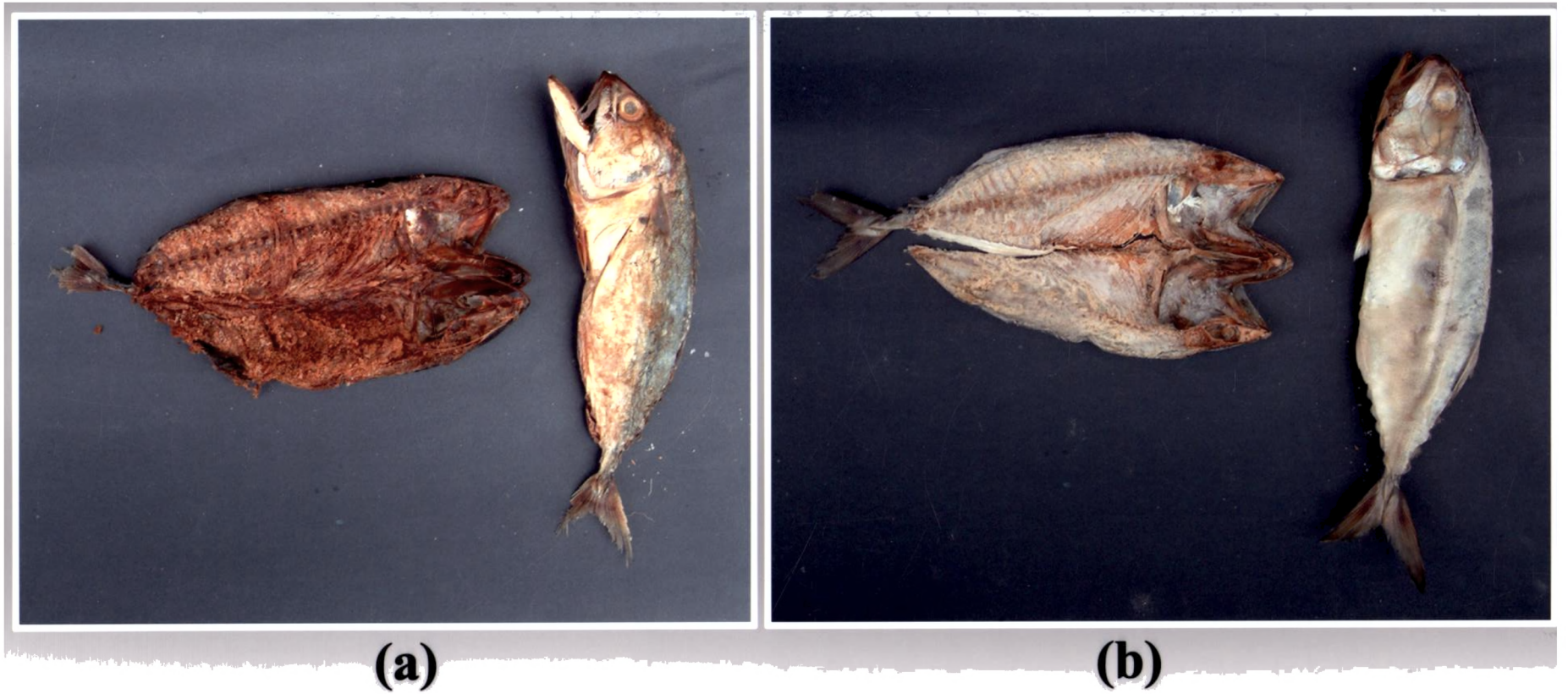


Plate 6.4 Dried samples (3 months)

(a) Control at 26˚c (b) Treated sample (Clove) at 26˚c

6. 4 (A) Results

6.4.1 (A) Moisture Content

Table 6.1 Moisture content of samples stored at 15°C

	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
CNT	31.50	42.33	42.03	42.73	42.18	44.20
RM	41.77	41.13	41.42	41.97	40.79	42.32
GIN	42.67	41.36	41.22	42.64	42.35	43.60
PEP	42.17	44.32	36.33	41.16	40.86	42.50
CLO	41.33	41.25	40.91	42.99	41.96	42.78
SYN	40.83	40.89	43.85	41.42	40.51	41.82

Table 6.1 shows the variation in moisture content of dried mackerel stored 15 ^{0}C for 6 weeks. There is significant difference between storage periods ($p<.05$). Six weeks stored samples gave significantly high moisture content than 4 weeks samples. There is significant difference between treatments. Control and ginger gave significantly higher values than others. Significantly lower values are obtained by pepper followed by rosemary and clove (Appendix 6.1 A).

In the comparison between control and pepper there is significant difference between storage period.($P<.05$). The percentage moisture content in 6 weeks is significantly higher than that in 4 weeks storage. [Appendix 6.1 (b)]

Table 6.2 Moisture content of samples stored at room temperature

	2 weeks	3 weeks	4 weeks	6 weeks
CNT	31.22	21.93	13.00	33.11
RM	31.63	32.92	32.22	42.82
GIN	31.28	31.95	39.22	39.61
PEP	30.60	30.32	32.85	40.53
CLO	31.32	30.98	40.22	39.22
SYN	20.74	22.75	24.17	40.19

Table 6.2 shows the moisture content of the sample stored at room temperature. Moisture content of 6 months storage is significantly higher that than of 4 months ($p<.05$) [Appendix 6.1 (c)].

6.4.2 (A) Peroxide Value (PV)

Table 6.3 Peroxide value of dried samples stored at 15°C

	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
CNT	30.14	46.02	50.59	50.51	47.59
RM	7.68	11.61	24.68	42.59	38.87
GIN	12.32	13.23	27.53	49.86	32.56
PEP	22.55	28.48	18.02	53.88	33.49
CLO	11.13	16.79	21.15	41.18	45.11
SYN	14.60	51.49	28.96	50.18	34.53

Table 6.4 Peroxide values of dried samples stored at Room temperature

	2 Weeks	4 Weeks	5 Weeks	6 Weeks	8 Weeks
CNT	28.9	32.96	54.63	68.25	52.46
RM	23	29	52	60.75	42.13
GIN	31.6	29.6	45.98	70.17	54.26
PEP	35	24.7	37.13	64.28	40.62
CLO	37.3	23.2	32.42	56.75	49.35
SYN	23	24.7	42.51	45.26	49.35

Table 6.3 gives the peroxide values samples stored at 15 °C .The comparison of peroxide value of dried samples stored at a15°C shows significant difference between storage ($p<.01$),. Ten weeks stored samples gave higher peroxide values [Appendix 6.2 (a)].

Table 6.4 gives the peroxide values samples stored room temperature. In the comparison of peroxide values for samples stored at room temperature there is significant difference between storage periods. 8 weeks stored period significantly higher than 4 weeks, Appendix 6.2(b)

6.4.3. (A) Thiobarbituric Acid Reactive Substances (TBARS)

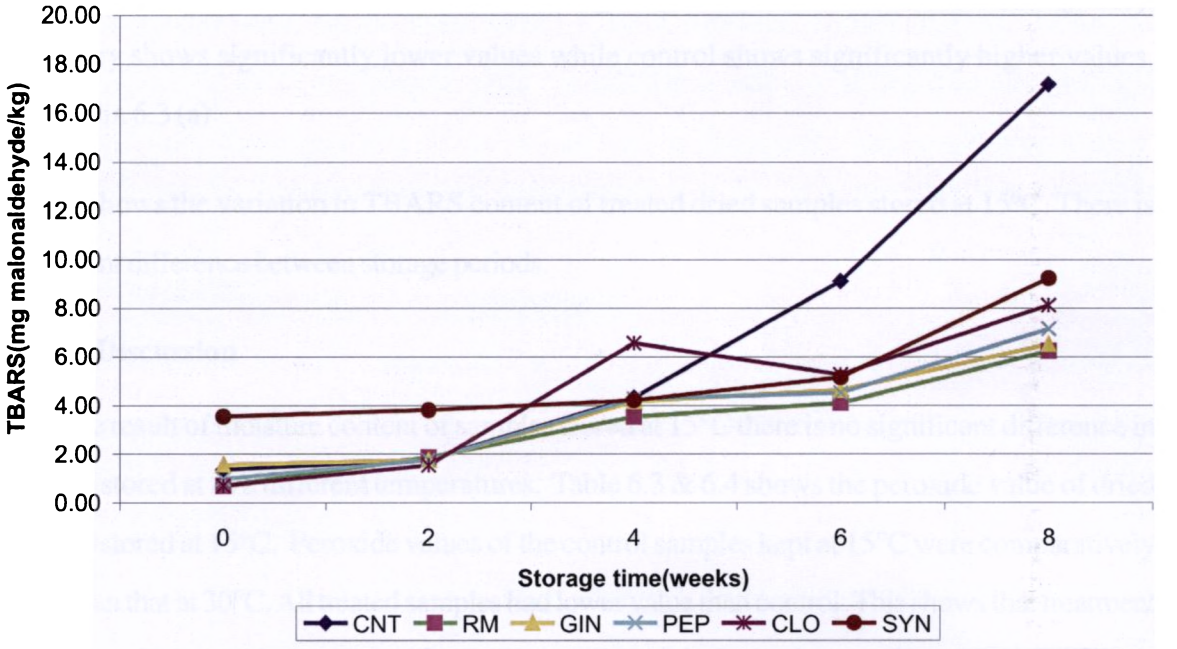


Figure 6.1 Variation in TBARS content of dried samples stored at room temperature

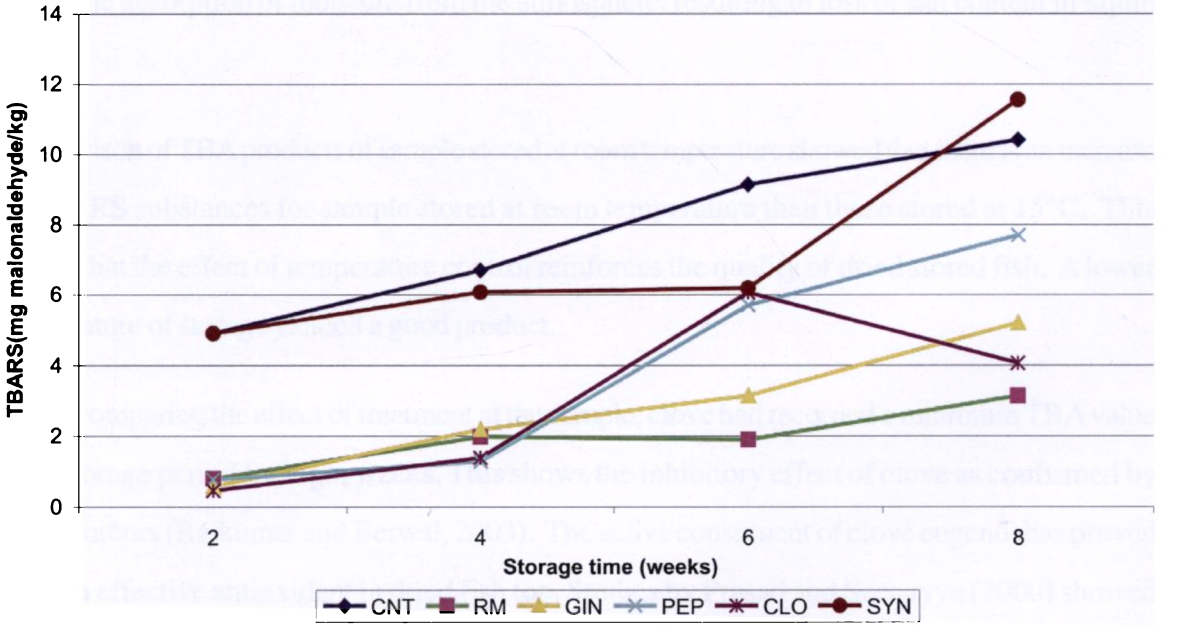


Figure 6.2 Variation in TBARS content of dried samples stored at 15°C

Fig 6.1 shows the variation of TBARS content of treated dried samples kept at room temperature. There is significant difference between storage period ($p<.05$). TBARS 8 weeks stored samples significantly higher than of 6 weeks. There is significant difference between treatment ($p<.05$). Rosemary shows significantly lower values while control shows significantly higher values. (Appendix 6.3 (a)

Fig 6.2 shows the variation in TBARS content of treated dried samples stored at 15°C. There is significant difference between storage periods.

6. 5 (A) Discussion

From the result of moisture content of samples stored at 15°C there is no significant difference in samples stored at two different temperatures. Table 6.3 & 6.4 shows the peroxide value of dried samples stored at 15°C. Peroxide values of the control samples kept at 15°C were comparatively lower than that at 30°C. All treated samples had lower value than control. This shows that treatment is effective. The samples treated with rosemary had lower values when compared to control. This again highlights the antioxidative role of the spice in dry products. Salt content of the cured sample stored at room showed a decrease in salt content after a period of 8 weeks. This probably might be due to the absorption of moisture from the atmosphere, resulting in loss of salt content in liquid forms.

Comparison of TBA products of sample stored at room temperature showed that there is an increase in TBARS substances for sample stored at room temperature than those stored at 15°C. This shows that the effect of temperature control reinforces the quality of dried stored fish. A lower temperature of storage yielded a good product.

While comparing the effect of treatment at the sample, clove had recorded a minimum TBA value after storage period for eight weeks. This shows the inhibitory effect of clove as confirmed by many authors (Rajkumar and Berwal, 2003). The active constituent of clove eugenol has proved to be an effective antioxidant in dried fish too. Studies by Prasad and Seenayya (2000) showed that cloves and clove oil were very effective on salt cured fish at 2 and 0.1% (v/v), respectively. Onion, coriander, garlic, asafoetida, mustard and spilanthes showed excellent growth control. Red chillies, turmeric, ginger, cumin seed and fenugreek were very good in inhibiting the growth. In

the present study rosemary has shown to be a good antioxidant as confirmed by the low values of PV and TBARS. Samples treated with BHA did not show any good values.

Yang et al., (1981) found that the added ascorbic acid had an antioxidative effect for 2 weeks of storage. They concluded that the organoleptic evaluation indicated no significant decrease in flavor until after 4 week of storage and statistical test showed no significant differences for appearance, odour, and texture among salted grayfish and salted cod fillets.

6.6 (A) Conclusion

Peroxide values of the control samples kept at 15°C were comparatively lower than that at 30°C. All treated samples had lower value than control. This shows that treatment is effective. The samples treated with rosemary had lower values when compared to control. This again highlights the antioxidative role of the spice in dry products. While comparing the effect of treatment at the sample, clove had recorded a minimum TBA value after storage period for eight weeks. This shows the inhibitory effect of clove as confirmed by many authors. Comparison of TBA products of sample stored at room temperature showed that there is an increase in TBARS substances for sample stored at room temperature than those stored at 15°C. This shows that the effect of temperature control reinforces the quality of dried stored fish. A lower temperature of storage yielded a good product. Salt content of the cured sample stored at room showed a decrease in salt content after a period of 8 weeks. This probably might be due to the absorption of moisture from the atmosphere, resulting in loss of salt content in liquid forms.

Section (B) Value Added Products

6. 1. (B) Introduction

With global rise in interests of fishery products there is need for parallel progress in technology for their processing and value addition in order to satisfy the consumer demand for convenience products and thereby to enhance the marketability (Venugopal, 1995). These products should assume economic viability and fatty fishes like mackerel could be converted to products with good organeoleptic qualities and storage stability. In this study attempt is made to formulate some products from Indian mackerel treated with rosemary oleoresin extract. Here, only rosemary is selected for this study taking into consideration the maximum antioxidant and antimicrobial effect

of rosemary compared to other spices as confirmed from the previous chapters. A control sample without spice treatment was also taken for comparison for all the following preparations.

6.2. (B) Recipe and methods of preparation

6.2.1 (B) Fish Cutlets: **(Plate 6.5)**

Ingredients:

Cooked Fish Meat	1000 g
Salt	25 g (approx – to taste)
Oil	125 ml
Green chilli	15 g
Ginger	25 g
Onion	250 g
Potato (cooked)	500 g
Pepper (powder)	3 g (to taste)
Clove (powdered)	3 g
Cinnamon(powered)	2 g (to taste)
Turmeric	2 g
Eggs	4 Nos.
Bread powder	200 g

Method of preparation:

Cook fish mince in boiling water for 20 min.

Drain off the water. (In cawe of whole fish, dress the fish and cook for 30 min. and drain)

Remove skin, scales and bones and separate the meat.

Add salt and turmeric to the cooked meat and mix well.

Fry chopped onions in oil till brown. Fry chilli and ginger. Mix these with the cooked meat.

(a) (b)

Plate 6.5 Fish Cutlet

(a) Battered and Breaded Cutlet (b) Fried Cutlet

Add mashed potato and spices and mix well with the meat.

Shape 40 g each of this in oval or round form, dip in beaten eggs, roll in bread powder and store in deep freezer.

Thaw and fry in oil before use.

6.2.2 (B) Fish Pickle: (Plate 6.6)

Ingredients:

1.	Fish (Dressed and cut into small pieces)	1 Kg
2.	Mustard	10 g
3.	Green Chilli (Cut into pieces)	50 g
4.	Garlic (peeled)	200 g
5.	Ginger (peeled and chopped)	150 g
6.	Chilli powder	50 g
7.	Turmeric powder	2 g
8.	Gingelly oil	200 g
9.	Vinegar (Acetic acid 1.5%)	400 ml
10.	Salt	60 g
11.	Pepper (powdered)	2.5 g
12.	Sugar	10 g
13.	Cardamoon, clove, cinnamon (powdered)	1.5 g

Method of preparation:

Mix the fish thoroughly with 3% of its weight of salt and keep for two hours. Light salted and partially dried fish also may be used. Fry the fish in minimum quantity of oil. Set apart the fried fish.

Plate 6.6 Fish Pickle

Fry the ingredients 2-5 in the remaining quantity of oil and then add chilli powder, pepper powder and turmeric powder and mix well over low flame for a few minutes. Remove from fire, add fried fish and mix well. When cooled, add vinegar, powdered cardamom, clove, cinnamon, sugar and remaining salt and mix thoroughly. Sufficient quantity of boiled and cooled water may be added to over the ingredients well. Transfer to clean, sterile glass bottles and seal with acid proof caps. Take care to see that there is a layer of oil over the contents in the bottle.

Flexible pouches made of 12 ì polyester lamined with 118 ì LD HD co- extruded film can also be used for packing the pickle.

6.2.3 (B) Fish Balls: (Plate 6.7)

Ingredients:

1.	Fish Mince (Mackerel)	1 Kg.
2.	Turmeric Powder	10 g.
3.	Pepper Powder	20 g.
4.	Salt	25 g.
5.	Cornstarch	50 g.

Method of preparation:

xed mince prepared from fish using a mechanical meat bone separator after heading, gutting and washing thoroughly with 1% salt and 5% corn starch. Prepared balls, 2-3 cm in diameter, from the resultant mass and cooked in boiling 1% brine for 5-10 minutes.

Cooled the cooked balls after which they are battered and breaded. Packed the balls preferably in thermoformed trays as such or after flash frying in hot vegetable oil. Preserved by freezing.

6.2.4 (B) FISH CURRY: (Plate 6.8)

Ingredients:

Dressed fish	1 Kg treated meat
Chilly Powder	100g
Turmeric	10 g
Tamarind	25 g
Salt	20 g
Ginger	50 g
Garlic	20g
Small onion	5 g
Curry leaves	5 g
Oil	5ml
Water	1 liter
Coconut	½ table spoon

Method of preparation:

Fish was cleaned and dressed, and cut into piece of desired length and kept aside. In a pan, added oil, and fried all green curry leaves, garlic, small onion, chilly powder, turmeric, salt, and tomato. Added tamarind puree in water and allowed to boil. Added fish pieces and cooked for 20 minutes.

6. 3 (B) Results

Sensory analysis of the products was done for the four products by a panelist of six members. In the present study oleoresin rosemary treated sample was used as the base material. The two products (cutlet and fish balls) from the minced sample, pickle and fish curry from whole treated samples were analysed for overall acceptability. Compared to control sample, rosemary treated samples gave a good score of overall acceptability. As these products are meant for human consumption, sensory evaluation of the product is very important. Rosemary treated sample did not have any rancid taste.

Plate 6.9 Fish Curry

Plate 6.7 Fish Balls

Table : 6.5 Mean sensory evaluation score for fish cutlet

Attributes	Attribute scores	
	Control	Rosemary treated
Colour	6	7
Odour	5	6
Taste	7	9
Texture	4	8
Overall score	5.5	7.5

Mean of 10X3 readings

Table 6.6 Mean sensory evaluation score for Fish Pickle

Attributes	Attribute scores	
	Control	Rosemary treated
Colour	6	7
Odour	6	8
Taste	5	7
Texture	6	8
Overall score	5.75	7.5

Mean of 10X3 readings

Table 6.7 Mean sensory evaluation score for Fish balls

Attributes	Attribute scores	
	Control	Rosemary treated
Colour	4	8
Odour	5	6
Taste	6	7
Texture	5	7
Overall score	5	7

Mean of 10X3 readings

Table 6.8 Mean sensory evaluation score for Fish curry

Attributes	Attribute scores	
	Control	Rosemary treated
Colour	6	7
Odour	5	6
Taste	4	7
Texture	6	9
Overall score	5.25	7.25

Mean of 10X3 readings

6.4. (B) Discussion

Moisture due to dehydration is a common problem in frozen food products. Functionally coating provides a moisture barrier to the product. It also helps in reduction of weight loss during frozen storage and also while reheating before consumption. A good consumer appeal is offered for the product by improving sensory value of the processed items. Coating also provides an opportunity to increase the nutritional value of the product through incorporation of nutrients in the coating. Breading is cereal based coating often as bread crumbs. Fish cutlets are battered and breaded products. To protect food item from oxidation addition of spices like rosemary has to be adopted which can provide a longer shelf life in the frozen stage. This is mainly due to its antioxidant constituents. Breaded and coated products have a strong consumable demands all over the world. The quality control of coated products is important for standardization of the process. With respect of fat oxidation studies from the previous chapters rosemary has proven to possess potent antioxidant property. Currently USA is the country which markets rosemary as a colourless, odourless compound. Taking into consideration health aspects involved by the use of synthetic antioxidant, the qualities of rosemary spice has to be popularized for preparing value added products.

6.5 (B) Conclusion

Of all the spices used in this study rosemary and clove have shown to be the most effective natural antioxidants on salt cured fish. As there is great demand for sea food and seafood based products, large number of diversified and ready to eat products can be prepared from low priced fish like mackerel by incorporating proper additives of natural origin. To popularize the products, this technology can be extended to society for gainful employment of women

CHAPTER 7

SUMMARY AND CONCLUSION

Summary and Conclusion

Seafood has gained popularity and market shares in most of the countries due to being exotic, tasty, light and healthy. This trend has been questioned by another trend as consumers are becoming more aware of safety and food poisoning. Quality has increasingly included concern on safety and this has highlighted the importance of temperature effects on bacteria and their activities in fish and shellfish. Production of biogenic amines especially histamine, is both a spoilage and a safety concern, and much progress has been made in identifying the factors controlling the process and the bacteria which may be involved.

Indian mackerel as a fatty fish has all necessary proteins, vitamins and minerals in the desired proportion along with the high content of PUFA like EPA and DHA. Like any other fatty fishes, the commercial use of mackerel has been limited by the susceptibility of the fish to oxidative reactions. The presence of many pro oxidants in the muscle tissue of mackerel, especially in the dark muscle, accelerates the lipid oxidation process.

The present study describes the inhibitory effect of the selected spices *viz* rosemary, ginger, pepper and clove on the biogenic amine production in mackerel and also their role as a potent antioxidant source in the preservation of mackerel and its products. The thesis contains seven chapters.

Chapter 1 is an introduction about the importance of Indian mackerel, its value as a whole fish item and its area of distribution in the Indian Exclusive Economic Zone. It also deals with the various aspects of mechanism of lipid oxidation and the ways and means to prevent oxidation of fish. It also gives an account of the role of spices as antioxidant during the post mortem changes and the inhibition of biogenic amine formation.

Chapter 2 deals with a review of the antioxidant components of the selected spice ingredients. The spices selected for the study are rosemary, ginger, pepper and clove. This chapter provides information regarding the geographical distribution and description of these spices and the extraction methods of their active constituents and structures of the active antioxidants. It also deals with the procedures for isolation and fractionation of the various active ingredients present in the spices. There are 9 active ingredients of rosemary, and of them carnosol and carnosic acid are found to possess the antioxidant properties. Ginger has the active constituents, gingerols and shogals. Pepper has the effective constituent piperine; and in clove, the effective antioxidant is eugenol.

Chapter 3 deals with the study to assess the role of spices on chilled and frozen storage. It also deals with the two kinds of treatment methods, commonly practiced in the seafood industry namely, dip treatment and glaze treatment, at two different spice oleoresin concentrations (0.02% and 0.05%). The study was conducted to assess the antioxidant effect of these spices on treated samples of whole fish and fish products a synthetic antioxidant, BHA (0.02%) treated sample along with a control without any antioxidant. The various parameters analyzed are the peroxide value (PV), thiobarbituric acid value, heme iron content, met myoglobin content, and total heme pigments as acid hematin. The peroxide values of treated, chilled samples at 0.02% concentration, there was significant difference between treatments. The studies on the various parameters of the fat oxidation indices revealed that the samples treated with rosemary oleoresin gave lower peroxide values on chilled storage condition upto 12 days, confirming its antioxidative property.

On the whole, from the results of various lipid oxidation parameters of samples given, different treatments with spice extracts in comparison with a synthetic antioxidant, BHA and control samples, the spices used could be graded as Rosemary > Clove > Ginger > Pepper, for their antioxidant properties. Among the various pretreatments given as glaze and dip of whole fish and frozen stored at –18°C, the glazed samples with 0.02% spice treatment is found to be most effective. Fillets dip treated with 0.005% concentration and frozen stored gave the optimum results. In this case also, the antioxidant properties was maximum for rosemary followed by clove, ginger, pepper and synthetic antioxidants as confirmed by the TBARS and Peroxide values. The retention of heme iron, metmyoglobin and acid pigments were also maximum in rosemary treated samples followed by clove explaining the protective effect of the treatment in maintaining the colour and other textural profiles of the samples. The synthetic antioxidant treated samples on frozen storage gave low values of pigments compared to spice treated samples, showing a bleaching action and hence loss of sensory qualities. The fact that the active constituents of rosemary and clove can be made use of for effectively preventing oxidation, thereby maintaining the original characteristics of mackerel has been confirmed.

Chapter 4 deals with the analysis of the various parameters of texture profile in treated samples of mackerel. The whole mackerel was dip treated with two different concentrations of spices (0.02% and 0.05%) with dip time of 5 minutes and 10 minutes. The dip treated samples were cooked for 3 minutes, at three different temperatures of 45°C, 70°C and 100°C and cook loss was assessed.

Samples with 0.02% concentration dip treatment for 5 minutes, gave better results of springiness parameter of texture. Among the four spices, pepper showed a good result for springiness. Cook loss studies revealed that in samples cooked at 100^{0}C, a higher cook loss than those cooked at 70^{0}C. Though rosemary and clove showed good antioxidant properties, their contribution to textural parameters seems to be limited. Further work in the case of prolonged frozen storage, needs to be done so as to assess the effect of spices on textural parameters during frozen storage.

Chapter 5 deals with the role of spices in preventing the formation of biogenic amines in mackerel. Biogenic amine content of 0.05% oleoresin treated samples and kept at ambient temperature were analysed at three different time intervals of 4 hours, 9 hours and 20 hours. The quantitative determination of biogenic amines was performed using a Waters HPLC system and data analysis was performed using EMPOWER 2 chromatography software. The chapter also deals with the antimicrobial activity of the spices. The bacteriological analysis for both total plate count and histidine decarboxylating bacteria reveals that rosemary had the maximum inhibitory effect on bacterial growth; confirming its antimicrobial activity. It was further observed that, after a certain period (9 hours), the amine level showed decrease in all the treated samples, which may be due to the amine oxidase activity of bacteria. The present study confirms to the earlier published work, highlighting the strong antimicrobial activity of rosemary. Biogenic amines index as a sum of putrescine, cadaverine and histamine determined by a simple method carried out in this work could be effectively employed for the early detection of quality deterioration, as well as for the evaluation of acceptable stage for mackerel. Determination of biogenic amines using the methods developed in the study can be applied to other fish products as part of quality assurance methods.

Chapter 6 deals with value added and dried products of mackerel incorporated with spice extract. The study revealed the strong antimycotic activity clove; while rosemary showed a strong antioxidant activity. The treated samples were stored at room temperature and at 15°C. The spice treated sample at 15°C showed better organoleptic qualities. Peroxide values of the control samples kept at 15°C were comparatively lower than that at room temperature. All treated samples had lower value than control. The samples treated with rosemary had lower values when compared to control. This again highlights the antioxidative role of the spices in dry products. While comparing the effect of treatment of the sample, clove had recorded a minimum TBA value after a storage period for eight weeks. Studies also showed that there is an increase in TBARS for samples stored

at room temperature than those stored at 15°C. This reinforces the effect of temperature control on the quality of dried stored fish. form.

Of all the spices used in this study rosemary and clove have shown to be the most effective natural antioxidants on salt cured fish. As there is great demand for seafood and seafood based products, large number of diversified and ready to eat products can be prepared from low priced fish like mackerel by incorporating proper additives of natural origin. To popularize the products, this technology can be extended to society for gainful employment of women. Taking into consideration health aspects involved by the use of synthetic antioxidant, the qualities of rosemary has to be popularized for preparing value added products.

Even though strong antioxidant activities of many plant extracts and spices have been reported, the need for novel natural antioxidants is obvious and food industries continue to look for them. It is possible that the more polar antioxidants to be more active in pure lipids, and non-polar antioxidants to be most active in a polar substrate like oil – in – water emulsions. This may partially explain the variation of antioxidative activity for different spices in different foods. It is also known that there is a reduced antioxidant activity in extracts prepared from an equivalent amount to spice as opposed to that prepared from the all spices, confirming that a wide range of compounds act together as antioxidants in the plant material, which may act synergistically. The stabilization effect of the spices depends strongly on the composition of the lipids present in fatty fishes like mackerel. Spices need to be evaluated at concentration accepted by the senses and with all interfering and synergistic compounds present. Modern fish processing technologies such as Modified Atmosphere Packaging, refrigerated/frozen storage, reduction in time between catch and consumption along with the use of natural antioxidants can promote the quality fatty fishes for human consumption. Also novel products from high fat fishes like mackerel with natural antioxidant content will probably help people suffering from arteriosclerosis or similar diseases which can also scavenge free radicals in blood plasma.

Some of the more popular synthetic antioxidants used are phenolic compounds such as Butylated Hydroxy Anisole (BHA), Butyled Hydroxy Toluene (BHT), Tertiary Butyl Hydroxy Quinone (TBHQ) and Propyl Gallate (PG). The synthetic antioxidants in use are subjected to a limit of 0.02% of the fat or oil content of the food. They have been very thoroughly tested for their

toxicological behaviors and still new toxicology data impose some caution in their use. In this context, natural products appear as healthier and safer than synthetic antioxidants.

The use of preservatives is therefore, an important factor of food product regulation and ensuring food safety. In recent times, as demand increased for a wide range of different, often processed fishery products and challenging lifestyles, require products with longer shelf lives and use of natural preservatives has become essential. However, the current trend is to use chemical preservatives in food in minute quantities, which has implications for the storage and safety of food products. Many countries have strict regulatory controls on use of chemical preservatives. In this context, the present study will be of immense significance.

Future Prospects

The future research and development in food preservatives should contribute to solving the food preservation and food safety problems in the areas of culture of fish, handling, processing, trade and distribution of fishery products. Risk analysis for food borne pathogens is a new emerging discipline and according to this the main objectives of food preservation are to prolong shelf life and to guarantee safety of the consumer. In this respect further study on the effect of natural antioxidants and antimicrobials from spices and herbs on the storage stability of fatty fishes like mackerel is needed to be emphasized with a risk analytical approach.

APPENDICES

APPENDIX A

3.3 (a) ANOVA TBARS measured in mg malonaldehyde per 1000 gm for glazed mackerel at (0.02 %) stored at -18^{0}C

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	249.82	5	49.96	8.92	0.02
b/n storage period	15.69	1	15.69	2.80	0.16
Error	28.01	5	5.60		
Total	293.52	11			

3.3 (b) ANOVA TBARS measured in mg malonaldehyde /1000 gm for glazed mackerel at (0.05 %) stored at -18^{0}C

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	198.5074	5	39.70147	7.869359	0.020449
b/n storage period	35.96326	1	35.96326	7.128395	0.044354
Error	25.22535	5	5.045071		
Total	259.696	11			

3.3 (c) ANOVA TBARS obtained from mackerel dip treated (0.02 %) stored at -18^0 C

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	5.47	5	1.09	10.23	0.01
b/n storage period	0.63	1	0.63	5.90	0.06
Error	0.53	5	0.11		
Total	6.63	11			

3.3 (d) ANOVA TBARS obtained from mackerel fillets (dip treated 0.005%) and stored at -18^0 C

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	31.112	5	6.222	6.029	0.035
b/n storage period	12.976	1	12.976	12.573	0.016
Error	5.160	5	1.032		
Total	49.249	11			

3.3 (e) ANOVA TBARS obtained from mackerel fillets (dip treated 0.005%) and stored at -18^0 C between control and pepper

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	1.385	1	1.385	55.533	0.085
b/n storage period	11.222	1	11.222	449.819	0.030
Error	0.025	1	0.025		
Total	12.633	3			

3.3 (f) ANOVA TBARS obtained from mackerel fillets (dip treated 0.005%) and stored at -18^0 C between control and pepper

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	1.693	1	1.693	82.836	0.070
b/n storage period	11.323	1	11.323	554.101	0.027
Error	0.020	1	0.020		
Total	13.036	3			

ANOVA 3.4(a) variation in Heme content samples dip treated and 0.02 concentration

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	13.821	5	2.764	14.753	0.005
b/n storage period	8.800	1	8.800	46.967	0.001
Error	0.937	5	0.187		
Total	23.557	11			

ANOVA 3.4(b) variation in Heme content samples dip treated and 0.02 concentration between control and synthetic

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	2.271	1	2.271	84.782	0.069
b/n storage period	5.734	1	5.734	214.077	0.043
Error	0.027	1	0.027		
Total	8.032	3			

3.5(a) ANOVA variation in Heme Iron content in frozen mackerel glazed at 0.02 % concentration

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	39.545	5	7.909	11.276	0.009
b/n storage period	0.367	1	0.367	0.524	0.502
Error	3.507	5	0.701		
Total	43.419	11			

3.5(b) ANOVA variation in Heme Iron content in frozen mackerel glazed at 0.02% concentration between control and rosemary

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	19.877	1	19.877	495541.897	0.001
b/n storage period	3.072	1	3.072	76583.280	0.002
Error	0.000	1	0.000		
Total	22.949	3			

Appendix B

4.1 Preforma 1 for hedonic scaling (Texture)

The response to the properties of the material on the first bite:

Initial characteristics:

1. Wateriness: The release of water on compression: this is the initial response and is to be distinguished from juiciness - Scale points: 1, much less water released: 5 neither much nor less: 10, much more water released.
2. Firmness: The force required to compress the material between the molars or between the tongue and palate – Scale points: 1, much softer and less consistent; 5, neither soft nor firm; 10, much firmer and more consistent.
3. Elasticity: The ability of the material to return to its original shape after deformation. It is judged by compressing the substance slightly between the molars or between the tongue and the palate and noting to what extent the material returns to its original shape- Scale points: 1,muchmore plastic;5,neither much nor less cohesive;10, much more cohesive.
4. Cohesiveness: The extent to which a material can be deformed before it rupture-Scale point: 1, much more cohesive; 5, neither much nor less cohesive; 10, much more cohesive.

The response to the properties of the material after chewing a few times:

Secondary characteristics:

1. Hardness: Resistance to breakdown on chewing to a state, suitable for swallowing-Scale points: 1, much more tender: 5, neither much nor less tender; 10, much tougher.
2. Juiciness: The sensation of a progressive increase of free fluids in the oral cavity during mastication-Scale points: 1, much drier; 4, neither much nor less juicier; 7, much juicier.

4.2 Preforma for hedonic scaling (sensory)

Date ..... Name.....

Sample code	Odour	Colour	Flavour	Touch	Overall acceptability scoring

Appendix C

5.1 ANOVA TPC between 4 hours and 9 hours

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	32.377	5	6.475	4.872	0.054
b/n storage period	33.267	1	33.267	25.030	0.004
Error	6.645	5	1.329		
Total	72.289	11			

5.2 ANOVA TPC between 0 hours and 9 hours

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	22.282	5	4.456	1.804	0.267
b/n storage period	58.935	1	58.935	23.853	0.005
Error	12.354	5	2.471		
Total	93.571	11			

5.3 ANOVA Histidine decarboxylating bacteria at ambient temperature 9 and 20 hours

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	261.0362	5	52.20724	5.535198	0.041847
b/n storage period	574.8213	1	574.8213	60.94461	0.000553
Error	47.15933	5	9.431866		
Total	883.0169	11			

5.4 ANOVA Histidine decarboxylating bacteria at ambient temperature 9 and 20 hours; rosemary and control

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	123.469	1	123.469	1632.650	0.016
b/n storage period	143.560	1	143.560	1898.318	0.015
Error	0.076	1	0.076		
Total	267.105	3			

5.5 ANOVA Histidine decarboxylating bacteria at ambient temperature 9 and 20 hours; control and ginger

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	47.08247	1	47.08247	4869.201	0.009123
b/n storage period	152.646	1	152.646	15786.43	0.005067
Error	0.009669	1	0.009669		
Total	199.7382	3			

5.6 ANOVA Biogenic amine index after 4hrs an d 20 hrs

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	332.150	5	66.430	0.736	0.628
b/n storage period	1034.473	1	1034.473	11.457	0.020
Error	451.466	5	90.293		
Total	1818.089	11			

Appendix D

6(a). ANOVA Mositure content of dried mackerel samples stored 15 0

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n treatments	5.623	5	1.125	5.430	0.043
b/n storage periods	1.550	1	1.550	7.486	0.041
Error	1.036	5	0.207		
Total	8.209	11			

6(b). ANOVA Mositure content of dried mackerel samples stored 15 0 between control and pepper

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n treatments	2.673	1	2.673	632.716	0.025
b/n storage periods	1.955	1	1.955	462.801	0.030
Error	0.004	1	0.004		
Total	4.633	3			

6.1 (c). ANOVA Mositure content of dried samples kept at ambiant temperature

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	402.726	5	80.545	2.295	0.192
b/n storage period	241.203	1	241.203	6.872	0.047
Error	175.495	5	35.099		
Total	819.424	11			

6. 2 (a) ANOVA Peroxide value to dried samples stored at 15⁰ C

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	363.691	5	72.738	1.924	0.245
b/n storage period	1490.384	1	1490.384	39.426	0.002
Error	189.010	5	37.802		
Total	2043.085	11			

6.2 b ANOVA Peroxide values samples stored at Room temperature

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	150.8777	5	30.17555	2.116485	0.215039
b/n storage period	1281.54	1	1281.54	89.88603	0.000221
Error	71.28694	5	14.25739		
Total	1503.705	11			

6.3(a) ANNOVA TBA content of dried sample stored at room temperature

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	88.394	5	17.679	6.634	0.029
b/n storage period	39.042	1	39.042	14.650	0.012
Error	13.325	5	2.665		
Total	140.761	11			

6.3 b ANOVA TBA content of dried treated samples stored 15 ^{0}C

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
b/n Treatments	76.0145	5	15.2029	9.637487	0.013265
b/n storage period	73.21821	1	73.21821	46.4148	0.001038
Error	7.887377	5	1.577475		
Total	157.1201	11			

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