FISH OIL: REFINING, STABILITY AND ITS USE IN CANNED FISH FOR THE INDONESIAN MARKET

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ABSTRACT

Fish oil has been proved to have health benefits for humans, but the utilization of fish oil for human consumption is very limited.

A survey of 19 Indonesian fish oil producers showed that fish oil was produced from fish meal processing and fish canning. Most Indonesian fish oils, especially fish meal oil, were chemically, physically and organoleptically unacceptable. But, as they contain high levels of omega-3 fatty acids, a refining process was required to improve the quality making the oil acceptable for human consumption.

The resin refining process, a no heat process, was used to refine the crude oil. Fish oil-resin volume ratio affected the refined fish oil quality and ratio 1:1 was recommended. The refined oil quality could be further improved by multiple refinings, and this method was successfully applied to Indonesian fish meal oil having a strong undesirable odour. The refining rate could be accelerated by application of vacuum pressure to the column. The height of the column showed a significant effect on the refined fish oil quality, but the column diameter had no effect. Resin refining reduced the quantity of natural antioxidants and changed the proportion of volatile flavour compounds. Most Indonesian fish oil producers intend to adopt the resin refining process.

Storage tests indicated that the refined oil deteriorated faster than unrefined oil. This trend was shown by both Indonesian and New Zealand oils. Ter-butylhydroquinone (TBHQ) proved as the most effective antioxidant for fish oil, but this antioxidant is not listed as a permitted antioxidants for use in Indonesian foods. Butylated hydroxyanisole (BHA), as the best alternative, is recommended. 0.01% BHA was sufficient to recover the loss of natural antioxidant during resin refining. Vacuum package was very effective in reducing the deterioration rate due to autoxidation.

Canned fish was used as a mean of delivering fish oil to Indonesian consumer. The proposed product type was generated through supermarket, consumer and canned fish processor surveys. The survey results suggested that the fish oil to be disguised in a canned fish product using sardine as raw material, tomato sauce as medium and 155g tall tube-can as the container.

The most acceptable tomato sauce formula developed using mixture design is 18.6% tomato paste, 28.0% fish oil, 46.6% water, 3.7% salt and 3.1% sugar. The canned fish should be sterilized using

vacuum head space-can at 121.1°C to obtain optimum protection of omega-3 fatty acids.

The experiment using the Plackett and Burman design showed that the canned fish product should involve pre-cooking, vacuum head space, garlic, shallots and vinegar additions. Sterilization time needed to be optimized. The optimization experiment indicated that 50 minutes was recommended to sterilize the canned fish with disguised fish oil. Sterility and incubation tests showed that sterilization at 121.1°C for 50 minutes was sufficient to produce safe product.

Consumer testing in five cities of Indonesia showed that only a minority of consumers did not like the developed product. Most of the consumers intend to buy the product, if the product is released to the market. A survey of medical doctors supported the proposed product, as over 90% of them were willing to suggest patients consume the product for nutritional purposes.

And if all the trees on earth were pens and the ocean (were ink), with seven oceans behind it to add to its (supply), yet would not the words of Allah be exhausted (in the writing): for Allah is Exalted in power, full of Wisdom.

(The Holly Qur'an 31: 27)

To my wife, Giyatmi, and my daughter,
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Chapter 1

INTRODUCTION

Indonesia is an archipelago consisting of approximately two million square kilometres of land and approximately 5.8 million square kilometre water including 2.7 million square kilometres of exclusive economic zone (Soekandar and Mihardjo, 1989). The potential production from marine resources is approximately 6.6 million tonnes per year, with a marine fishery production of 2.27 million tonnes. Total Indonesian fishery production in 1989 was 3.04 million tonnes (Directorate General Of Fishery, 1991). The export volume and value of fish products in 1990 were 0.3 million and one billion US dollars respectively (Directorate General Of Fishery, 1991). The average monthly spending for an Indonesian on fish was Rp.1,188 (Central Bureau Of Statistic, 1991). These statistics indicate that the fisheries sector is important in the Indonesian economy.

Fish oil consumption is still a new product for the Indonesian consumer, even though fish oil has been traditionally prescribed by medical practitioners because of its importance as a source of vitamin A. Fish oil consumption in healthy people is relatively uncommon. The benefits of fish oil as a health supplement has been widely reported both in the press and in scientific publications. Since the Indonesian fish oil industry is relatively underdeveloped, it is predicted that this market sector will further expand as abundant raw materials are efficiently utilized. For example, shrimp trawl by-catch, as reported, was approximately 0.9 million tonnes in 1984 (Directorate General of Fishery, 1985). If the fish oil industry can be developed properly, the industry will move from its current status as a by-product of the fish meal industry to the dominant processed fish product.

The exact total volume of fish oil production is unknown. However Indonesia currently exports approximately 1,170 tonnes of fish oil, with a value of $US 11 million in 1990 (Directorate General Of Fishery, 1991). The most common problem associated with fish oil consumption is its undesirable odour. Indeed, it is common practice in fish canning to intentionally remove the fish oil in order to improve product acceptability.

Refining is mostly used to improve fish oil quality chemically, physically and organoleptically. Refining does not only improve fish oil quality, but also adds value and utility. With improved quality, fish oil can be used to replace vegetable oils in some cases, but some technological considerations should be given special attention, particularly in relation to the high content of

unsaturated fatty acids.

Since fish oil is still a by-product of fish meal manufacture, the improvements in product quality are not yet receiving urgent attention by fish processing factories in Indonesia. Manufactures are satisfied with the present conditions, since they have had an established market for the oil. With this in mind, establishing a suitable method for improved fish oil refinement should be carefully selected to ensure rapid implementation of any new technology. For example, the refining technology should be relatively simple, have a low labour content, and have low installation and operation costs.

A new market for refined fish oil should be created, allowing fish oil producers to benefit from the introduction of a refining process. The food industry is the most likely area to consume large quantities of refined fish oil. The foods to be processed with fish oil must be selected carefully to avoid imparting negative effects on the food properties, such as texture and flavour.

To date, the published scientific studies on Indonesian fish oil and fish oil industries are very limited. Therefore, intensive studies are required to provide information for food industries, particularly about fish oil quality and quality improvement methods.

THE OBJECTIVES OF THIS STUDY

The four objectives of this study are

* to develop a relatively new and improved resin based fish oil refining technology, with a view to improving oil value and utility;

* to measure the chemical, physical and organoleptic improvements in fish oil quality and investigate the stability of the oil;

* to investigate the prospect of a resin refining method to be used by Indonesian fish oil manufactures; and

* to develop a canned fish product with fish oil disguised in it for the Indonesian market.

Chapter 2

GENERAL LITERATURE REVIEW

2.1. CHEMICAL PROPERTIES OF FISH OIL

Knowledge of the chemistry of fish oils is important for the development of high quality and high value fish products, and for evaluation of the nutritional importance of fish oils. Many studies have been reported on the chemical and nutritional properties of fish oil, highlighting the fact that fish oil is generally characterized as containing large groups of saturated and unsaturated fatty acids, mixed triglycerides (Gruger, 1967), fatty alcohol (wax ester), sterol (sterol ester), phosphoric acid and amines (phospholipids) (Morris and Culkin, 1989).

2.1.1. Triglycerides and fatty acids

According to Stansby (1979), fish oil differs from other natural oils in that the triglycerides have:

* a much greater number of different constituent fatty acids;
* a greater proportion of long chain fatty acids;
* a considerable proportion of fatty acids which are in a highly polyunsaturated form (up to six double bonds); and
* abundant qualities of long chain polyunsaturates of the omega-3 type.

The carbon chain length of fish oil fatty acids commonly exceeds C-18, and a considerable portion of the fatty acids contain 20, 22 and, to a limited extent, 24 carbon atoms. The total proportion of these long chain fatty acids usually amounts to 25-30% of the total, and sometimes approaches 50% of the total fatty acids (Stansby, 1969).

Gruger (1967)has reported that fatty acids derived from fish oil are of three principal types: saturated, monounsaturated and polyunsaturated.

Saturated fatty acids (SAFAs) normally constitute 20-30% of the total fatty acids of marine organisms and are thus readily available to fish from food chain organisms (Ackman, 1982). The

SAFAs have carbon chain lengths that generally range from C-12 (lauric acid) to C-24 (lignoceric acid). Traces of C-8 and C-10 acids may also be found in some fish oils. C-5 acids (isovaleric) have been identified in the jaw-bone oil of the dolphin and the porpoise (Gruger, 1967). In spite of the chemical variety of compounds only three saturated fatty acids are quantitatively important in fish oil: palmitic acid (C16:0) at 10-15%, myristic acid (C14:0) at 3-13% and stearic acid (C18:0) at 1-4% (Ackman, 1982).

The monounsaturated type is comprised of monoethenoic acids and the polyunsaturated type is comprised of polyethenoic acids which contain from 2 to 6 ethylenic bonds per acid. The carbon chain lengths of the unsaturated acids range, generally, from C-14 (9-tetradecenoic acid) to C-22 (4,7,10,13,16,19-docosahexaenoic acid). Small amounts of C-10 and C-12 monoenoic acids have been found in some fish oils. There are no naturally occurring acetylenic acids and hydroxy carboxylic acids presently known in fish oil (Gruger, 1967).

Most of the polyunsaturated fatty acids (PUFAs) in fish oils occur as the omega-3 type and are related to linolenic acid. In contrast omega-6 type fatty acids commonly predominate in most other oils. These compounds are biosynthetically related to linoleic acid (Stansby, 1982). The ω-3 PUFAs in marine lipids receiving the most interest are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Pigott and Tucker, 1987). EPA (C20:5ω-3) and DHA (C22:6ω-3) are regarded as either nutritional supplements, or as therapeutic agents inhibiting a variety of pathological conditions in man and are popularly known as the "omega-3" fatty acids. These naturally make up, at most, only about 25% of fish and fatty acids (Ackman, 1988a). In the human body, linolenic acid can be slowly converted too both EPA and DHA, especially in the presence of large quantities of linoleic acid which compete for the same enzyme system (Yongmanitchai and Ward, 1989). Fish heads and eyes are a rich source of DHA (Anonymous, 1991).

A number of unusual fatty acids have been found in marine fish. Odd carbon numbered fatty acids (C-15, C-17, C-19) have been identified in the body and gonad lipids of smelt. Baltic herring have been found to contain fatty acids of longer carbon chain length (C-24 to C-32) than in the usual marine acids (Moris and Culkin, 1989).

The proportionate distribution of fatty acids in fish oils can be influenced by fish diet. In addition, the proportion of fatty acids to fish oils is also affected by environmental factors such as geographic location of catch, and season of the year, which may be related to water temperature (Gruger, 1967). Triglycerides in fish oil from different species are normally characterized by different fatty acid compositions (Windsor and Barlow, 1981).

2.1.2. Wax esters

Wax esters are formed by esterification of long chain fatty acids with long-chain alcohols (Morris and Culkin, 1987). These compounds are found, often in abundance, in the oil fraction of a number of marine species, and presumably serve as an energy reserve (Malins, 1967).

The fatty alcohol fractions of all marine wax esters are either comprised of saturated fatty acid 16:0, for 20 - 80%, or monounsaturated, mainly 20:1 and 22:1. The fatty acid components of wax esters tend to be more varied than the long-chain alcohols, and generally typical of marine fatty acids contain carbon chains ranging from C-14 to C-22. Some of the fatty acids in the wax esters of a marine animal tend to reflect those of its diet (Morris and Culkin, 1989). Moreover, Ackman (1980) simply divided wax ester into two main classes, those rich in 16:0, and those rich in 22:1 fatty acids.

2.1.3. Phospholipids

Phospholipids are water-insoluble compounds similar to triglycerides, but with a phosphorus component substituted for one fatty acid (Pigott and Tucker, 1987). Fish flesh contains about 0.5% phospholipids (Sheppard et al, 1978). The main phospholipids of marine animals are lecithins (phosphatidyl cholines), phosphatidyl ethanolamines, phosphatidyl serine and phosphatidyl inositol with minute quantities of sphingomyelin, lysophosphatylcholine, and cardiolipin (Malin, 1967; Moris and Culkin, 1989). Lecithins are usually present in the highest concentration. A number of other components common to land animal are also present in lesser amounts (Malin, 1967).

2.1.4. Free fatty acids (FFAs)

The splitting of the attachment between the glycerol and the fatty acids through hydrolysis results in the release of free fatty acids, the presence of which, in an oil, is commercially undesirable. Usually fish oil is sold on the basis of 3% free fatty acids with a maximum allowable content, normally 7%. The proportion can be up to 20% in certain cases (Windsor and Barlow, 1981). A partial hydrolysis will generate mono- and di-glycerides. Some glycerol produced may ultimately be broken up to CO_2 and H_2O depending on the conditions (Patterson, 1989).

2.1.5. Ether groups

Evidence accumulated suggests that the ether-containing lipids of fish occur primarily as diacyl glyceryl ethers in which the 1- and 2-positions of glycerol are esterified with fatty acids. The presence of a high percentage of glyceryl ethers in the non-saponifiable fractions of liver and body oil of a number of marine fish and mammals is reported, and chimyl, batyl and salicyl alcohols are assumed to be the principal constituents (Malins, 1967). Structurally related materials such as hydroxyalkyl glycerols or methoxyglyceryl ether are usually minor components in fish lipids (Ackman, 1980).

2.1.6. Hydrocarbons

Naturally occurring hydrocarbons are not usually important constituents of fish oils. It is well known that the highly unsaturated hydrocarbon, squalene-$C_{30}H_{50}$, is present in certain shark liver oils. The saturated isoparaffine, pristane, and the unsaturated hydrocarbon, zamene, have been found to accompany squalene in minute quantities (Toyama and Kaneda, 1965). The isoprenoid alkanes, pristane and phytane, together with odd-numbered C-15 to C-33 n-alkanes are commonly found in a wide range of fish species including herring, sprat, mackerel, cod, eulachon, plaice, gurnard, salmon, bass, whiting, and sole. The wide structural variation in this chemical class could be a useful chemotaxonomic tool for isolating food chains because of their relative metabolic stability (Morris and Culkin, 1989).

Other unsaturated isoprenoid hydrocarbons such as C-10 and C-20 mono-, di- and tri-olefins have been found in some fish. Hydrocarbons usually constituent less than 1% of the total lipid fraction (Morris and Culkin, 1989).

2.1.7. Sterols

Sterols, together with vitamins A, D, and E, are the major components in the unsaponifiable portion of fish oils. Cholesterol itself is the most common sterol in most marine species (Kinsella, 1987), existing either in free form or as an ester. Typical examples of the range of cholesterol distribution include halibut liver oil 7.0%; Atlantic cod liver oil 0.3%; salmon egg oil 3.0%; commercial

pilchard oil 0.7%; and oil from shrimp waste 19.0% (Brody, 1965).

2.1.8. Heavy metals

Heavy metals which are frequently found in fish oils include mercury, lead, cadmium, arsenic and copper and zinc (Kinsella, 1988). The metal content of fish oil is clearly influenced by the metal content of fish tissue used as raw material and the type of processing and storage conditions of the oil. Phospholipid content of the oil increases on storage, and during production - this class of compounds carrying metals into the oil. Metals that are complexed by phospholipids are removed more easily by degumming and alkali refining (Kinsella, 1987).

2.1.9. Pigments

The variety of pigments which have been identified in fish oil arise from two sources: natural oil-soluble pigments found in fish, and colour changes occurring in fish oil either before, during or after processing. Carotenoids constitute the most common pigments in fish (Brody, 1965). These include:

* asatacin - principal red colour in salmon oil;
* fucoxantin - principal yellow pigment in pilchard oil;
* xanthophylls - pilchard oil;
* carotene - pilchard oil; and
* toraxanthin and zeaxanthin - found in the skin of a large number of species.

In conjugation with protein, chitin, calcium salts, or other lipids, carotenoids can yield blue, purple, green, orange, red, pink, and yellow colour (Pennock, 1977).

Chlorophylls occur in pilchard oil and arise from dietary microscopic green plants (Brody, 1965; Cutting, 1969).

2.2. NUTRITIONAL PROPERTIES

Nutritional factors affect health, quality of life, general well-being, and longevity in humans and are important criteria used by contemporary consumers to select food (Kinsella, 1988). Many authors have tried to show the nutritional superiority of fish oils as a human food.

2.2.1. Essential fatty acids

The human body can make all the fatty acids required, except for the essential fatty acids (EFA), linoleic acid (18:2ω-6) and α-linolenic acid (18:3ω-3) (Fogerty, 1989).

Most fish oils are a rich source of essential polyenoic fatty acids which are reported to have numerous health and nutritive benefits such as eliminating various skin related disorders, promoting physical growth and for the integrity of cellular endoplasmic reticulum and mitochondrial membranes (Stansby, 1982; Kinsella, 1986). One of the most fascinating aspects of the biological effects of the polyenoic acids is their in vivo biosynthesis and catabolism. These reactions control various biological feedback mechanisms which finally regulate the natural abundances of these acids, e.g. the effect of acids of the omega-3, linolenic acid family, on the essential fatty acid of the omega-6, linoleic acid family (Jorgenson, 1967). The amount of the linoleic acid family of fatty acids in body oils of marine fish is around 3 %, with some species as high as 6 %. The content of linoleic family of fatty acids in the oil of fresh water fish is somewhat higher, perhaps in the range of 5-9% (Stansby, 1969).

In EFA deficiency, the content of polyenoic acids of the oleic and palmitoleic acid families are significantly increased, because oleic and palmitoleic acids can be biosynthesized, while linoleic and linolenic can not (Jorgensen, 1967).

According to Kinsella (1986), it is generally believed that, in humans, 1-2% of caloric intake of linoleic acid and up to 0.5% of linolenic acid is sufficient to provide the recommended daily requirement for essential fatty acids. Currently, the intake ranges from 5 to 10% of dietary calories.

2.2.2. Vitamins

Fish flesh, visceral portion, and liver oils are rich in vitamin A and D (Pigott and Tucker, 1987). The amounts vary widely within and between fish species. In contrast, the vitamin content of liver oil varies with species, age, size, sex, nutritional conditions, and spawning stage of the fish, as well as the geographical locale and season of catch. Variations in feeding and the reproductive cycle are most likely responsible for seasonal differences (Kinsella, 1987). Cod liver oil is normally used as vitamins A and D supplements (Gauglitz et al, 1974).

The biologically active forms of vitamin A in fish oils are retinol (vitamin A_1) and 3-dehydroretinol (vitamin A_2). Vitamin A_1 is the predominant form, particularly in fish liver. Approximately 4-20% of the total vitamin A content may be 3-dehydroretinol (Brody, 1965 and Kinsella, 1987). Most of the vitamin A in fish liver oil occurs in the form of esters which are more resistant to oxidation than the free-alcohol form. Some antioxidants, such as tocopherol, prevent deterioration of vitamin A. Pure vitamin A, extracted from fish, has a biological activity of about 4,000,000 USP units per gram (Brody, 1965).

Vitamin D occurs naturally in at least two major forms that show antirachitic activity (Brody, 1965): vitamin D_2, also known as "calciferol", or activated ergosterol, and vitamin D_3 also known as "activated 7-dehydrocholesterol" and is the predominant vitamin D in fish oil. They differ in their physiological activity (Brody, 1965; Kinsella, 1987). Vitamin D is associated with calcium distribution and calcification of bone. In its active form, vitamin D promotes the synthesis protein involved in the distribution of calcium (Kinsella, 1988). Unlike vitamin A, vitamin D is not present in nonliver visceral oils in amounts exceeding liver oil content (Kinsella, 1987).

Vitamin E (tocopherol) is also found in fish oil of which α-tocopherol is the most abundant form. The content of tocopherol in fish body oil ranges from less than 1mg/100g oil to 75mg/100g oil (Kinsella, 1987).

2.3. FISH OIL AND DISEASES

Current interest in the biological effects of fish oil developed largely from studies on Greenland Eskimos. The main stimulus for such studies was the realisation that these people show little evidence of cardio vascular disease, although they eat a diet that is high in fat and animal protein.

This observation was linked to their high intake of seafoods, which are rich in omega-3 polyunsaturated fatty acids (Carroll, 1986; Burr, 1991).

Highly unsaturated omega-3 (n-3) fatty acids in marine lipids can be important in preventing or reducing certain diseases (Pigott and Tucker, 1987). Only animals that are part of the food chain from the sea have these long chain omega-3 fatty acids, because these substances are made in the first place, by phytoplankton - the tiny aquatic plants that serve as food for small fish. Actually a few plant oils have small amounts of linolenic acid, which is an omega-3 fatty acid. This particular omega-3 fatty acid has fewer double bonds and is shorter than the omega-3 fatty acids found in fish. Metabolically, it does not behave in the human body in the same way as fish oil omega-3 fatty acids do, but it can be converted to the type of omega-3s found in seafood (Nettleton, 1987). The best vegetable source of omega-3 fatty acids are linseed, rapeseed and soybean oils (Niazi, 1987).

Omega-3 fatty acids in marine lipids currently receiving the most interest are eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) (Pigott and Tucker, 1987). Although linoleic acid competes with linolenic acid for the same enzymes in the desaturation and elongation reactions, the formation of arachidonic acid from linoleic acid is favoured (Kinsella, 1987).

Unfortunately, the vital omega-3 fatty acids are susceptible to selective destruction during fish processing (Griggs, 1986). With this in mind, new processing methods designed for the production of high quality fish oils must be developed with care.

Human diseases, which have been studied and claimed can be prevented and/or cured with fish oil, are cardiovascular diseases, atherosclerosis, hypertension, immune system deficiency, rheumatoid, arthritis, psoriasis, asthma, cancer, skin diseases, lupus, diabetes, and stroke (Robinson et al, 1985; Niazi, 1987; Nettleton, 1987; Kinsella, 1987; Anonymous, 1987; Pigott and Tucker, 1987; Anonymous, 1988, Kinderlerer, 1989; Stansby, 1990; Simopoulos, 1991).

2.4. FISH OIL PRODUCTION

Fish, used in fish meal production, shows a wide variation in the oil content within and between species. Intra species variation occurs with the changing seasons and with the age of fish (Young, 1982). In general, the overall composition of fish oil in terms of its content of triglycerides, free

fatty acids, moisture , etc. is not species specific (Windsor and Barlow, 1981). Depending on the oil content, fish can be grouped into two categories: oily fish with a fat content of more than 2.5%, and non oily fish (lean fish or white fish) with a fat content of less than 2.5% (Barlow, 1977). The most important factor in the production of a high quality crude fish oil is the condition of the raw material available for processing. As far as possible, the fish should be undamaged and held under chilled conditions to effectively minimize the effect of microbial and enzymatic degradation of the fish tissue (Young, 1982).

In tropical and temperate areas of the world, special consideration must be paid to developing the most appropriate post-harvest method. According to Barlow (1977), in cases where fish are caught at high temperatures and far away from the plant, chilling appears to be the most effective method of preserving bulk stored fish. Two methods of chilling may be considered: refrigerated or ice chilled water method, or mixing of ice with the fish.

2.4.1. Extraction technology

Processing methods normally used for the manufacture of crude oil from whole fish or waste products from food fish processing are now discussed.

2.4.1.1. Wet rendering process

This process is used almost exclusively for processing oily fish, including menhaden, herring, pilchard, and tuna cannery waste (Lee, 1963). The principal steps of the wet rendering process are:

Cooking, where the oil and water in the fish can be easily separated from the solid protein. To obtain a satisfactory product, the cooking temperature and pressure must be tailored to the particular species of fish.

Pressing, where a screw-type press squeezes both oil and water from the fish.

Centrifugation is now used in preference to settling tanks for recovery of the oil from the aqueous liquid portion.

For maximum separation and recovery, two centrifugation steps are used (Considine and Considine,

1982). The additional centrifuge steps ensure complete removal of solids and aqueous fractions from the oil which could ultimately lead to rapid deterioration of the oil during storage. Normally, hot water is added to the oil to extract remaining impurities. Final separation and isolation of the oil is achieved by centrifugation with close control of temperature, since specific gravity and viscosity of the oil are temperature dependent (Windsor and Barlow, 1981).

2.4.1.1. Dry rendering process

This process is used primarily for raw material which is both relatively low in oil content and available in relatively small quantity (Lee, 1963; Pigott, 1967). The process is usually by batch, rather than continuous process, and involves a combined cooking and drying step. The raw material is normally loaded into a large, steam-jacked cylinder drier. Inside the drier is a rotating scraper which brings all material into quick contact with the hot inside wall, yet prevents the material from sticking. The drying is done either under vacuum, or at atmospheric pressure. The oil is separated from the dried scrap by batch pressing in a hydraulic press. Fish oil is the only product recovered from this pressing operation (Pigott, 1967; Lee, 1963; Considine and Considine, 1982).

2.4.1.3. Solvent process

Solvent extraction has been used in the past when fish oil was the product of choice such as in the preparation of vitamin oils from fish liver. Some technological variations of this process are available depending on whether raw fish, cooked or pressed fish, dried scrap or fish meal constitutes the starting material. Raw fish can be handled in two ways: by an azeotropic process using solvents that are non-miscible with water, or by direct extraction, in which case, solvents must remove both water and fat (Lee, 1963).

Typical of the azeotropic extraction technique is the two-step Vio Bin process which utilizes ethylene dichloride in the azeotropic distillation-liquid extraction. The first extraction takes place between 71°C, the azeotropic temperature, and 83°C, the normal boiling point of ethylene dichloride. The water is removed by distillation, thus preventing the extraction of any water-soluble nutrients from the fish. A second extraction with isopropanol removes any traces of

ethylene dichloride (Pigott, 1967).

Isopropanol has been used for direct extraction of fish oil. The process is basically simple. The coarsely ground fish is mixed with the hot solvent. Then the oil is removed by filtration and/or by centrifugation. The residue is then treated with successive batches of fresh solvent until water and oil are reduced to the desired point (Lee, 1963).

2.4.1.4. Enzymatic process

Despite the variety of techniques in this process, the basic approach is to remove or homogenize the fish and treat with approximately 0.25-0.50% enzymes based upon the weight of the whole fish. Following enzymatic digestion, the reactive material is pressed and filtered. Oil that is liberated by this type of process will float to the surface and can be skimmed or centrifuged in a manner similar to that used for reclaiming oil from stick water or press cakes in the conventional process (Pigott, 1967).

Shirakawa et al (1989) proposed an enzymatic technique by using a protease. Fish are incubated with protease at 0.001-1.0% by weight at 45-75°C for 40-50 minutes. The slurry formed is separated into solid and liquid fractions in a continuous decanter to produce fish meal and fish oil. This method yields comparable volumes of fish oil, as a conventional process, but with retention of valuable trace components which otherwise would be lost due to thermal decomposition.

2.4.1.5. Silage Process

Fish silage is liquified fish, stabilized against bacterial decomposition by an acid, such as formic, propionic, sulphuric or phosphoric acids. The process involves mincing the fish followed by the addition of the selected acid for preservation. The enzymes in the fish hydrolyse the endogenous proteins into smaller soluble units (peptide components). The acid provides the optimal hydrolysis conditions, while preventing bacterial spoilage. Fish oil is obtained by the centrifugation of the silage. It is noteworthy that silage made from white fish offal contains relatively little oil. However when made from fatty fish, such as herring, it is necessary to remove the oil (Bimbo, 1990).

2.4.2. Processing of fish oil

Processing fish oil for industrial products usually involves the application of winterization, refining and bleaching operations.

2.4.2.1. Winterization

Winterization is a thermomechanical separation process where component triglycerides of fats and oils are crystallized from a melt. The fraction is obtained via partial solidification of certain triglyceride components which are subsequently separated from the oil by filtration. Fat crystallization occurs in two steps: the first step involves a crystal formation process called nucleation, the second is crystal growth. Technically, the winterization process involves two component fractional crystallizations in terms of solid and liquid fractions. The fraction consists mainly of higher melting triglycerides, whereas the liquid fraction is dominant in low-melting components (Puri, 1980).

In the winterization process, the oil must be cooled slowly to produce readily filtered crystals, often a time consuming and difficult operation. The traditional crystallization process normally takes five days, after which the oil is filtered, when cold, either through plate and frame filter presses, or through a rotary vacuum filter (Bimbo, 1989). The winterization may be terminated when the temperature falls below the lowest temperature attained immediately preceding the rise in temperature (Chang, 1967).

2.4.2.2. Refining

There are some refining methods which can be applied to fish oils.

2.4.2.2.1. Degumming

Degumming is only a partial refining, since free fatty acid is not reduced and even the gums are not completely removed (Gunstone and Norris, 1983). Principally, degumming is the process by which phosphatides and certain other mucilaginous materials are removed from the fish oil by

treatment with 2-3% water, or with an aqueous solution of boric acid or salt such as sodium chloride or pyrophosphate at 30°-50°C. Phosphatides can also be insolubilized with 80% phosphoric acid. The sludge is then removed by centrifugation (Chang, 1967; Kinsella, 1987).

2.4.2.2.1. Alkali refining method

The alkali (caustic soda) refining method is carried out in batch, semi- continuous and fully continuous modes. Of these, the most favoured is the centrifugal, continuous, refining line. After the degumming treatment already described, the hot oil is treated with 4N caustic soda to neutralize the free fatty acid content and to solubilize phospholipids, nitrogen and sulphur containing compounds and some pigments. In this way a large part of these impurities can be removed in the aqueous phase resulting from the first centrifugation step. Oils of poor quality are then given a second caustic soda treatment primarily to remove more sulphur, phosphorus and colour followed by phase separation. The oil is then washed with water to remove soap, and centrifuged before drying. Average to good quality oils are given a water wash in place of the second caustic soda treatment. To ensure removal of the soap from the oil, phosphoric or citric acids are often added to the final wash water. The acids also act as trace metal deactivators either by chelation or by the formation of water soluble salts (Young, 1982).

The minimum amount of alkali required for neutralization can be calculated from the free fatty acids (FFA) of the oil to be refined, using the formula (Gunstone and Norris, 1983):

$$\% \text{ NaOH} = \% \text{ FFA} \times 0.142,$$

where FFA is expressed in terms of oleic acid. For any desired excess of NaOH, the calculation is

$$\% \text{ NaOH} = \% \text{ FFA} \times 0.142 + \% \text{ excess NaOH}$$

From an application view point, alkali refining or neutralization of the oil results in a product which, when heated, will not darken, foam or smoke, become cloudy and then form a precipitate. The product can also be readily bleached (Bimbo, 1989).

2.4.2.2.3. Physical refining

The physical refining method requires a thorough degumming of the oil prior to distillative removal of the fatty acids, heat degradable pigments and other impurities at temperatures in the order of 250°C at 2-5mmHg absolute pressure with open injection (Young, 1982).

Although this method produces higher yields, it has not yet proved practical due to variability in quality and quantity of impurities (especially sulphur) in the oil (Kinsella, 1987).

2.4.2.2.4. Sodium carbonate method

Sodium carbonate was once popular, because it neutralized free fatty acids without saponifying any oil. In a second step, stronger alkali could be used for colour reduction, etc. Foaming problems and the equipment required, have caused this method to be discontinued in most refineries (Gunstone and Norris, 1983).

2.4.2.3. Bleaching

The main objective of oil bleaching is to reduce coloured materials and natural pigments, and to absorb suspended mucilaginous, colloid-like matters and any traces of soap if still present (Chang, 1967). The process leads to a fish oil product with improved colour, flavour, and oxidative stability, free from impurities (Bimbo, 1989).

The process involves heating the oil to 90°-110°C, often under vacuum condition, in the presence of bleaching clay (0.2-0.3% by weight depending on oil quality), for the desired period of time and then removal of the spent clay by filtration (Bimbo, 1989; Young 1982). Semi-continuous processing plants are preferred for this treatment. Efficient filtration for the removal of the clay is essential because impurities adhering to the clay particles remaining in the oil act as autoxidation catalysts.

The activated clay, made by acid treatment of neutral clay, is more effective in bleaching. The activated clay may cause a rise in the free fatty acid content of bleached oil. Activated carbon is

normally used in combination with clays as an effective means of reducing the odour of fish oils (Chang, 1967).

2.4.2.4. Deodorization

The oxidation products of the highly unsaturated fatty acids of fish oils, whether free or bound in the triglycerides, are generally regarded as the causative agents of the characteristic fish odour of fish oils. One method to alleviate, but not entirely eliminate, this undesirable condition, involves vacuum dry oxygen free steam distillation of the oil at a relatively high temperature (Brody, 1965; Windsor and Barlow, 1981).

Hydrogenation of fish oils free from non-oil fishy material is another method for reducing fish odour. This is achieved by adding approximately 5% calcium hydroxide (slaked lime) and 5% calcium oxide (quicklime), to the oil and then agitating and filtering. Under these conditions the oil becomes simultaneously deodorized, decolourized, stabilized and also partially refined (Brody, 1965).

2.5. INDUSTRIAL APPLICATIONS OF FISH OIL

Numerous studies have reported on optimising the use of fish oils in food, pharmaceutical and animal feed industries. Further, the application of fish oils for non-edible uses have been identified.

2.5.1. Fish oil applications in foods

Over one million MT (metric tonnes) of marine oil are used annually in food products. Most of the world's marine oil production is consumed in Europe, South America and Japan, primarily in the forms of salad oils, frying fats, table margarines and other spreads, low caloric pastries, cakes, cookies, biscuits and synthetic creams. Other uses of fish oil in food include hard fats, shortening, pastry fats, bread fats, frying oils, cake shortenings, bread dough, biscuit fillings, canning oils, cooking oils, and emulsifier (Brody, 1965; Pillai, 1974; Young, 1982; Bimbo, 1987; Bimbo, 1989a;

Barlow et al, 1990). Crude fish oil used for these purposes must be winterized, alkali refined and bleached. In some cases, hydrogenation and possibly deodorization may be required (Bimbo and Crowther, 1990).

2.5.2. Fish oil applications in pharmaceuticals

Probably the most important pharmaceutical properties of fish oil is directly related to the relatively high polyunsaturated fatty acids (omega-3), with vitamin A and D content.

2.5.2.1. Concentrate of omega-3 fatty acid

Fish oils can be concentrated by several means, starting with the simple winterization or slow chilling of the oil (Ackman, 1988a) or by using urea complexation method (Haagsma et al, 1982). In an experiment conducted by Haagsma et al (1982), the urea complexation method was found to give better results with respect to yield and fatty acid composition in the concentrate. The urea fractionation of fatty acids is mainly based on the degree of unsaturation: the more unsaturated, the less they will be included in the urea crystals.

Tocher et al (1986) described a simple and rapid method for the preparation of a marine oil fraction highly enriched in (ω-3)-polyunsaturated fatty acids, using cod roe. Incubation of a concentrated aqueous extract of the roe with porcine pancreatic phospholipase A_2 (EC 3.1.1.4) took place followed by extraction of the freeze-dried reaction product with diethyl ether containing formic acid which produced an oil yield of 1.0g/100g wet weight. The oil contained over 95% of free fatty acids, with 20:5ω-3 and 22:6ω-3 accounting for up to 24% and 40%, respectively, of the total free fatty acids.

Ackman (1988a) noted that menhaden oil can be enriched without solvent from 13.9% EPA and 9.7% DHA to 15.3% and 10.9% respectively. With a nominal concentration of one EPA or one DHA per triglyceride molecule, enrichment of triglycerides without enzymatic inter-esterification or resynthesis is not very practical beyond the 300mg/g usually listed for these two fatty acids alone. By adding other omega-3 fatty acid such as 16:4ω-3, 20:4ω-3, and 22:5ω-3 it is possible to obtain a total of 500mg/g.

2.5.2.2. Capsule of omega-3 fatty acids

Commercially available omega-3 fatty acids from fish oil are primarily marketed in 1g gelatin capsules. High quality products contain only traces of organohalogens and less than 1mg/g of cholesterol, and are essentially free from vitamin A and D. Organohalogens are toxic to humans and can accumulate in fish due to environmental exposure of the fish to organohalogens. Vitamin E is usually added at 1IU/g as a stabilizer. The gelatin capsules of fish oil and/or commercially available concentrates usually contain a desirable clear yellow oil. Study on the storage of encapsulated fish oils showed that Max EPA capsules survived four years in the dark at ambient temperature with no obvious effect on the content (Ackman, 1988a and 1988).

Ackman et al (1989) analyzed seventeen brands of encapsulated fish oil or fish oil concentrate products consisting of two product types - triglyceride and alkyl ester oils. The alkyl ester and free fatty acid products showed very high levels of EPA (259-300mg) and DHA (172-254mg). In contrast triglyceride oils had relative low levels of EPA (80-250mg) and DHA (78-156mg) per gram of capsule content.

2.5.2.3. Vitamin A And D concentrates

Vitamin A and D from fish oils can be concentrated using commonly available methods such as saponification, short-path distillation and adsorption (Brody, 1965).

2.5.2.3.1. Saponification

Saponification effectively splits oil triglycerides into its component parts, glycerol and fatty acids, with the concomitant formation of soaps. Incomplete reaction leaves some unsaponified oil, because the presence of some unsaponified oil renders the process more efficient. After dilution of the soaps, vitamins can be readily extracted with a water immiscible solvent such as diethyl ether, or ethylene dichloride. The extract is separated from the aqueous portion, and the solvent is removed by distillation. In the saponification process both vitamins A and D are extracted simultaneously.

2.5.2.3.2. Short-path distillation

The efficiency of the equipment used in this method has been significantly improved with the development of the high vacuum pump operating in the region of one micron (0.001mmHg) pressure. Under high vacuum thin film of oil is distributed on a heated surface. Under these condition the vitamin concentrate distils and condenses on a nearby cooled surface. In this process, vitamin A and D can be removed from the oil separately.

2.5.2.3.3. Adsorption

Vitamin A in fish oil is concentrated using a two step process. Vitamin A is first converted to its alcohol form by methanolysis. This alcohol is then separated from the resulting fatty-acid methyl ester mixture by adsorption using alumina or silicic acid. Alternatively, a vitamin A concentrate can also be obtained either by alcoholysis followed by adsorption on alumina or by its adsorption on soap formed in situ.

2.5.3. Fish oil applications in animal and fish feeds

For many years fish oil has been added as a supplement to animal feeds. It is an economical source of calories, and stimulates growth. Growth enhancement results from the high concentration of linolenic acid homologies - omega-3 fatty acids. The fish oil used in animal rations must be fresh since autoxidized oils are known to be toxic to some domestic animals (Gauglitz et al, 1974). Animal feeds containing fish oil include poultry, pig, cattle, fish, mink and pet feeds (Karrick, 1967; Gauglitz et al, 1974; Barlow, 1986; Watanabe and Takeuchi, 1989).

2.5.4. Fish oil applications in non-edible uses

Major industrial uses take advantage either of the unique type and high degree of unsaturation of fish oil to produce elastic durable polymers or of the long and diverse mixture of chain length to add lubricity, detergency, and plasticity functions (Fineberg and Johanson, 1967). In addition, the

unique compositions of fish oils make them adaptable to a wide number of uses. The highly unsaturated fatty acids of fish oils allow competition with drying oils of vegetable origin. The high percentage of saturated fatty acids in fish oil allow competition with fats and oils such as tallow and vegetable oils of low iodine value (Dyer, 1967).

Non-edible uses of fish oil include soaps, detergents, leather tanning, protective coating, lubricants, plasticizers, pesticides, fungicides, polyurethane foams, buffing compounds, glazing compounds, caulking compounds, sealing compounds, ore floatation, air entraining agent for concentrate, water repellents, rubber compounds, synthetic waxes, corrosion inhibitor, automotive gaskets, core oils, tin plate oils, rust proofing agents, refractory compounds, linoleum, presswood fibre boards, oiled fabrics, ceramics deflocculans, fermentation substrates, illuminating oils, fuel oils, mushroom culture and fire retardants (Dyer, 1967; Fineberg and Johanson, 1967; Bimbo, 1989)

Chapter 3

MATERIAL AND ANALYSIS METHODS

3.1. MATERIALS

3.1.1. Fish oils

Fish oils were obtained from both Indonesia and New Zealand. The Indonesian oils were collected from several fish meal factories in Muncar, East Java and Negara, Bali. The New Zealand fish oils were supplied by Sealord Product Ltd., Nelson. Details of fish oils used will be given for each experiment in the appropriate chapters.

3.1.2. Resin

Macroporous strong acid cation resin used for fish oil refining was supplied by Dow Chemicals, USA. The resin, consisting of a styrene/divinyl benzene matrix structure had sphere form, sodium ionic, 1-7 meq/ml/min total exchange capacity, 150 Å pore size and 42-48% water retention capacity. Glass columns in which resin was packed were fabricated by the Massey University Glass Blower.

3.1.3. Fish

New Zealand sardine/pilchard (Sardinops neopilchardus) was used as raw material in the canning experiment. The fish was supplied by Star Fish Supply Ltd., Napier, New Zealand. The fish size was 17.5±0.5cm in length, by 1.8±0.1cm thick and 44.2±4.9g in weight. The proximate composition of the fish was 71.8% moisture, 22.2% protein, 2.4% fat and 3.1% ash. The pH of the fish was 6.3. The fish was received frozen, and was kept at -7.5°C until needed.

3.1.4. Can

Cans (4.6 cm in height, 6.6 cm in diameter) used in the fish oil experiment were obtained from J.Watties Foods Ltd., Hastings, New Zealand. The cans (8.2 cm height, 5.1 cm diameter) used in the fish canning experiment were imported from Nippon Suisan Kaisha, Ltd., Tokyo, Japan.

3.1.5. Tomato sauce materials

Tomato paste with 28-30% tomato soluble solids was supplied by J.Watties Foods Ltd, Hastings, New Zealand. Salt, sugar, shallot, garlic and white vinegar were purchased from supermarkets and retailers in Palmerston North.

3.2. FISH OIL EXTRACTION

Fish oil samples extracted from fish flesh and tomato sauce were used for fatty acid profiles, anisidine value and TBA value analysis. Modified Bligh and Dyer method was applied to extract the oil (Hanson and Olley, 1963).

A sample (15g) was placed into a Waring Blendor-jar and diluted with 30ml $CHCl_3$ and 60 ml CH_3OH. The mixture was then homogenized for two minutes. The sample was further diluted with 30ml $CHCl_3$ and rehomogenised for 30 seconds. This last step was repeated using 30 ml H_2O. The fat solution was separated by centrifuging the mixture at 9000 rpm for 10 minutes. The lower $CHCl_3$ layer containing the fish oil was separated off and evaporated using a rotary evaporator at 45°C.

3.3. METHODS OF ANALYSIS

3.3.1. Chemical analysis

3.3.1.1. Free fatty acid value

Analysis was carried out only on fish oil using the procedure described by Fernandez (1986). Samples (7g) were dissolved in 50ml ethyl alcohol and titrated with 0.1N NaOH using a phenolphthalein indicator. The blank determination was carried out using the same procedure, but in the absence of fish oil. The free fatty acid (FFA) values were computed by the formula:

$$\text{FFA value (as \% oleic acid)} = \frac{(S-B) \times N \times 28.2}{\text{weight of sample}}$$

Where, S = titration of sample (ml)
B = titration of blank (ml)
N = normality of NaOH

3.3.1.2. Fatty acid profile

Fish oil and the oils extracted from fresh fish, sterilized fish, canned fish and tomato sauce were esterified prior to being analyzed for fatty acid content according to the procedure described by van Wijngaarden (1966). One ml of 6% methanolic potassium hydroxide was added to 20-25 mg of fish oil in a screw cap vial and then heated for 10 minutes in a water bath at 70°C with stirring. Two ml of 14% BF_3-methanol was then added and the reaction mixture was heated for a further 10 minutes. Two ml hexane was added and shaken thoroughly. Six ml of distilled water was then added and the reaction mixture shaken again. The hexane layer was separated for methyl ester analysis.

The fatty acid methyl esters were analyzed by gas chromatography using a Hewlett-Packard Capillary gas chromatography Model 5890 Series II, fitted with a FID detector and equipped with DB-Wax capillary column (30 m x 0.25 mm ID). The carrier gas was nitrogen at 10psi. This equipment was connected to the Hewlett-Packard 3393 integrator. The detector was kept at 260°C and the injection port at 220°C. The column temperature was programmed from 160°C to 250°C

at the rate of 2°C per minute and held at the upper temperature for 25 minutes. The attenuation used was 4. The peaks were identified using the standards of PUFA-1 and PUFA-2 obtained from Supelco, Inc., Bellefonte, USA.

3.3.1.3. Tocopherol And Tocotrienols Analysis

Quantitative analysis of tocopherol and tocotrienol in fish oil was carried out before and after refining. Approximately 0.1g of fish oil was accurately weighed into a 5 ml volumetric flask and then made up with hexane containing 200 ppm BHT antioxidant. Constituents were separated by using Maxima 820 high performance liquid chromatography (HPLC) Model 510 (Waters Associates, Milford, MA, USA) equipped with a Hitachi fluorescence spectrophotometer Model F1000 (Hitachi Ltd., Tokyo, Japan). The excitation was set at 295nm and emission at 330nm. The mobile phase was HPLC grade hexane containing 7% diisopropyl ether and 0.05% acetic acid at the rate of 2ml per minute. A zorbax silica column (0.5μ, 30cm x 3.6mm) was used to provide the separation of α, β, γ and δ tocopherols, tocomonoenol and tocotrienol. The column temperature was maintained at ambient temperature ± 20°C. The volume injected was 50μl.

3.3.1.4. Volatile Flavour Analysis

An all glass-purging system for collection of volatile flavour compounds of fish oil was constructed as shown in Appendix 3.1. The size of the tube was 20cm in length and 2.3cm in diameter. The length of the purge tube was 15cm, and was terminated with fine nozzles at one end. A 15ml fish oil sample was sparged with Nitrogen (N_2) at 85ml/minute through the purging tube. The purging unit was placed in a constant temperature water bath held at 30°C. Samples were prepurged for two minutes to remove oxygen from the tube to avoid oxidation during sample extraction. The volatile components were entrained and concentrated onto a porous polymer Tenax TA trap (60/80 mesh size, Alltech Association, Illinois, USA) attached to the exit port of the purging unit. Purging time was four hours. To desorb the volatile flavour components, the tenax polymer was washed with 7.5 ml of triply glass distilled diethyl ether, and then concentrated by carefully passing a fine jet of N_2 over the surface of the solution to evaporate most of diethyl ether.

The volatile flavour concentrate was analysed using a gas chromatography-mass spectrometry (GC-

MS). Volatile compounds were separated on a Hewlett-Packard 5890 Series II equipped with a DB-wax capillary column (30 m x 0.25 mm ID). The GC condition was set the same as in the fatty acid profile analysis, except the column temperature was programmed from 40°C to 280°C at a rate of 5°C/minute. GC peak identification and quantification were carried out using a VG70-250S high resolution mass spectrometer.

3.3.1.5. Peroxide value

a. For fish oil

Peroxide value analysis for fish oil was based on the modified method described by Windsor and Barlow (1981). A sample (2.5 g) was weighed into a 250ml erlenmeyer flask and diluted with 15 ml of a acetic acid-chloroform (3:2) solution. The flask was then swirled until the sample dissolved. 0.25 ml of saturated potassium iodide solution was added. The solution was allowed to stand with occasional swirling for exactly 1 minute before diluting with 15 ml of distilled water.

The solution was titrated with 0.1N sodium thiosulphate and vigorously shaken until the yellow colour almost disappeared. Approximately 0.5 ml of starch indicator was added and then the titration continued. The flask was shaken vigorously near the end point to extract all the iodine from the chloroform layer until the blue colour disappeared.

A blank determination was carried out by omitting the oil sample, but using the same procedure and reagents. Peroxide value (PV) was calculated using the following equation:

$$\text{PV (meq/kg sample)} = \frac{(S-B)(N)(1000)}{\text{weight of sample (g)}}$$

where : S = titration of sample (ml)
B = titration of blank (ml)
N = normality of sodium thiosulphate solution

b. For fish flesh and tomato sauce

The method developed by Pearson (1973) was used to determine peroxide value in the tomato sauce. A sample (approximately 30 g) was placed in a Waring Blendor and diluted with 150ml of chloroform. The mixture was blended for 2 minutes before filtering through Whatman filter paper. The filtrate was centrifuged at 10,000rpm for 10 minutes. The clarified solution was used for PV analysis.

10 ml of solution was pipetted into a 150 ml erlenmeyer flask and diluted with 14.8 ml of glacial acetic acid and 0.4 ml of freshly prepared saturated potassium iodide solution. The solution was allowed to stand for exactly 1 minute with occasional swirling before further diluting with 12ml of distilled water. The solution was titrated with 0.01N sodium thiosulphate using a starch indicator solution. PV was calculated using following equation:

$$\text{PV (meq/kg sample)} = \frac{\text{titration x N x 1000}}{\text{weight of sample (g)}}$$

where: N = normality of sodium thiosulphate

The weight of the sample was determined by pipetting 10 ml of solution into a weighed aluminium dish. The solvent was evaporated at 102°C for 20 minutes and then reweighed, after cooling, in a desiccator.

3.3.1.6. Thiobarbituric acid (TBA) value

TBA values for fish oil and oil extracted from tomato sauce were determined using the method outlined by Fioriti et al (1974). Samples (0.12g) were dissolved in 2 ml 50%(v/v) absolute alcohol in 2,2,4-trimethylpentane (isooctane) to facilitate analysis. To the solution in the test tubes, stoppered with plastic caps, was added 5 ml isoOctane and 3 ml thiobarbituric acid solution (0.33 g TBA in 10 ml distilled water and 90 ml isopropyl alcohol). The capped tubes were shaken vigorously for approximately 30 seconds, using a Chiltern shaker, and then held in a temperature controlled water bath at 60°C for exactly 30 minutes. The solution was scanned against pure

isoOctane at 532 nm in a Shimadzu UV Spectrophotometer Model UV-110-02. Recorded absorbances were used to compute TBA values using the formula:

$$\text{TBA value } (\mu\text{mol/g sample}) = \frac{(\text{absorbance of sample} - \text{absorbance of blank})\ 10}{\text{weight of sample (g)}}$$

The absorbance for the blank was measured using the same procedure, but in the absence of the sample.

3.3.1.7. Anisidine value

The method described by Windsor and Barlow (1981) was used to determine anisidine value in the fish oil and the oil extracted from the tomato sauce.

A 0.5 g-sample was weighed into a 25 ml volumetric flask. The sample was dissolved and made up to volume (25 ml) with isooctane and then shaken. The absorbance for the oil solution was measured against pure isooctane at 350 nm in a Shimadzu-UV Spectrophotometer Model UV-110-02. In a 2 test tube assay system, 5 ml of the oil solution was pipetted into test tube "A", and 5 ml isooctane was pipetted into test tube "B". One ml of the anisidine reagent (0.25 g para-anisidine in 100 ml acetic acid) was added to both test tubes. The tubes were stoppered and shaken vigorously and left in the dark for exactly 10 minutes. The absorbance of sample and the blank were measured at 350 nm in the same spectrophotometer. Anisidine values (AV) were calculated using the formula:

$$AV = \frac{25(1.2E_b - E_a)}{\text{weight of sample (g)}}$$

where: E_a = the net absorbance of the oil solution
E_b = the net absorbance of fat-anisidine-solution

3.3.1.8. Total oxidation value (Totox value)

A combination of primary oxidation and secondary oxidation led to the formulation of an empirical known as the "Total Oxidation Value" (Totox Value). This gives a useful, if not a complete picture of the present state and past history of an oil in oxidation terms (Patterson, 1989). Since a peroxide group has twice the oxygen of an aldehyde group, the conversion equation states are as follows:

$$\text{Totox Value} = 2\,PV + An.V$$

Where : PV = peroxide value
An.V = Anisidine Value

3.3.1.9. Moisture content

Samples were prepared by chopping and grinding fresh fish and canned fish in a mortar until finely ground. The moisture content of 2 g samples contained in aluminium dishes was determined by drying in an air oven at 100 $\pm$ 2°C for approximately 16 hours. Water content was determined by comparison of weight difference before and after sample drying.

3.3.1.10. Protein Content

Protein content of the samples (fresh fish and canned fish product) was measured by the Kjeldahl method using a Kjeltec 1026 System Distillating Unit (Tecator, Sweden), which is a semi-automatic apparatus. The sample was weighed accurately and put into the digestion tube. Two Kjeltab 3.55 (containing 3.5 g K_2SO_4 and 0.0035 g Se) and 12 ml conc. H_2SO_4 were added. Digestion was then carried out using a Digestion System 1007 (Tecator, Sweden) at 420°C for 30 - 45 minutes. After cooling, the solution was diluted with 75 ml distilled water. Distillation was then applied using a distillation unit programmed to use 2 ml alkali with 0.2 minute delay time and 3.6 minute steaming time. The liberated ammonia was collected in 25 ml 4% Boric acid solution. When distillation was complete, the sample was titrated with 0.1M HCl to pink end point. In calculation,

1 ml 0.1M was defined as being equivalent to 1.4 mgN and a multiplication factor of 6.25 used to calculate crude protein from nitrogen content.

3.3.1.11. Fat content

The fat contents of the fresh fish and canned fish were determined using the official AOAC method (AOAC section 18.044, 1984). The sample (8g) was weighed into 50ml beaker and mixed with 8ml HCl. The mixture was then heated in a steam bath for 90 minutes with occasional stirring. After cooling, the solution was transferred to a mojonnier fat extraction flask. The beaker was rinsed with 7ml ethanol and then with 25ml diethyl ether. The extraction flask was subsequently shaken vigorously. 25ml of petroleum ether was added and the flask was again vigorously shaken. The Mojonier flask was centrifuged at 600rpm for 5 minutes and the ether - fat solution was then separated as much as possible. To optimize the fat extraction, the second extraction step was introduced using 15ml diethyl ether and 15ml petroleum ether. This time the centrifugation step was reduced to 3 minutes.

The ether-fat solution was dried using a two step process. Most of the solvent was removed using a rotary evaporator at 45°C and then the sample was completely dried by heating in an oven at 100±2°C for 40 minutes.

3.3.1.12. Ash content

The ash content of the fresh fish and canned fish samples was determined using the official AOAC method (AOAC section 18.025, 1984). A 2 g sample in silica dishes were burned and then heated in a furnace to approximately 600°C. Ashing took three hours.

3.3.1.13. Salt Content

The salt content of raw fish and canned fish were determined by the official AOAC method (AOAC section 18.035, 1984). The sample (5-10g) was placed in a 250ml erlenmeyer and diluted

with 50ml 0.1M $AgNO_3$ and 20ml HNO3. The mixture was boiled gently for 15minutes, cooled and further diluted with 50ml distilled water and 5ml of ferric indicator. The mixture was titrated with a 0.1N NH_4SCN solution until the mixture became permanently light brown. The salt content was calculated using the equation:

$$\text{Sodium Chloride (g/100g)} = \frac{(V_2 - V_1) \times N \times 5.85}{\text{Weight of sample}}$$

where, V_1 = volume (ml) of thiocyanate used in the sample
V_2 = volume (ml) of thiocyanate used in the blank
N = normality of thiocyanate

3.3.1.14. pH

The pH was determined using an Orion pH-meter Model 720 (Orion Research, Massachusette, USA). Fresh fish and canned fish samples (10 g) were prepared by mixing with distilled water in the ratio of 1:2. The mixtures were homogenized and the pH measured immediately by immersing. The pH of tomato sauce was measured directly without additional treatment.

3.3.2. Physical analysis

3.3.2.1. Colour

a. Fish oil colour

Two methods were used to measure the colour of the fish oil during experiments.

1. The method described by Fernandez (1986) was used to measure the absorbance of fish oil using a Shimadzu Spectrophotometer Model UV-110-02 at 490 nm. The results were corrected using a petroleum ether as blank. Samples were equilibrated to 30°C in a water bath prior to measurement.

2. The Photometric Method based on AOCS Official Method Cc 13c-50 (AOCS, 1973) was used only for the fish oil from the sterilisation experiment. Sample absorbance was measured at 460, 550, 620 and 670 nm using the same spectrophotometer as before. The photometric colour was calculated using the equation:

$$\text{Photometric Colour} = 1.29A_{460} + 69.7A_{550} + 41.2A_{620} - 56.4A_{670}$$

where, A = absorbance.

b. Fish flesh and tomato sauce colour

Hunter colour readings (L, a, b values) were determined using a Hunterlab Colourquest Spectrophotometer Model CQ1200k with version 2.33 software and calibrated with a white tile standard. For fish flesh, the sample (3.25 g) was placed in a metal container. Tomato sauce (22.5 ml) was placed in a glass cuvette. The dimensions of container used for fish flesh and a glass cuvette for tomato sauce are shown in Appendix 3.2.

3.3.2.2. Refractive Index

Refractive Index (RI) values were determined using Bellingham Stanley Refractometer (Bellingham + Stanley Limited, England) at 20°C for samples from the refining experiment (AOAC, 1984), and at 25°C for samples from the storage experiment (Arya, 1969). The refractometer RI range was 1.30 - 1.74.

3.3.3. Sensory Analysis

Sensory analysis was conducted on fish oil, tomato sauce and canned fish. Panellists were chosen from among Indonesians residing in Palmerston North, because of their familiarity with the fish taste and fish odour.

Panellists were trained prior to participation in sensory tests. They were experienced in detecting fishy odour and taste levels for the refining experiment, and some rancid odour and taste levels for the fish oil storage experiment. During panel selection, their interests, motivation, availability, and attitude towards the products were considered. During evaluation, more attention was paid to clarify confusion, and to monitor panellists.

The product attributes, evaluation, and sensory sheets used, will be discussed for each experiment in the appropriate chapters. Definitions of each attribute listed in the sensory sheets for each experiment are described as follows:.

A. For fish oil refining experiment:

1. **Odour** : the overall strength of fishy and undesirable odour detected in the sample.
2. **Taste** : the overall strength of fishy and undesirable taste detected in the sample.

B. For Fish Oil Storage Experiment:

1. **Odour** : the overall strength of rancid odour detected in the sample.
2. **Taste** : the overall strength of rancid taste detected in the sample.

C. For Fish Oil Sterilisation Experiment:

1. **Fishy Odour** : the overall strength of fishy odour detected in the sample.
2. **Fishy Taste** : the overall strength of fishy taste detected in the sample.
3. **Rancid Odour**: the overall strength of rancid odour detected in the sample.
4. **Rancid Taste** : the overall strength of rancid taste detected in the sample.

D. For Tomato Sauce Formulation Experiment:

1. **Consistency** : the overall impression of consistency when the product was stirred.
2. **Odour** : the overall impression of odour detected in the sample.
3. **Colour** : the overall impression of visual colour detected in the sample.

4. **Mouth Feel** : the overall impression of the sauce texture when touching all parts of the mouth, especially in terms of the effect of the presence of fish oil.
5. **Overall Acceptability**: evaluation of the overall acceptability of the sample, and rating acceptability score for the sample.

E. For Canned Fish Experiment:

I. Fish

1. **Fish Surface Appearance**: the smoothness, or damage to the fish surface in the canning process.
2. **Flesh Texture**: the overall tenderness of the sample, determined by the degree of force required to compress the sample between the molar teeth.
3. **Softness of Bone**: the overall softness of bone, determined by the degree of force required to compress the sample between the molar teeth.
4. **Sourness** : the overall strength of sourness taste from tomato paste, and vinegar, as detected in the fish flesh.
5. **Saltiness** : the overall strength of saltiness detected in the fish flesh.
6. **Overall Spiciness**: the overall strength of garlic, and shallot taste in the fish flesh.
7. **Fishiness** : the overall strength of the fish taste detected in the fish flesh.

II. Tomato Sauce

1. **Colour** : the intensity of red colour detected in the tomato sauce.
2. **Mouthfeel** : the oily impression of the sample in the mouth.
3. **Sourness** : the overall strength of sourness from tomato paste, and vinegar detected in the tomato sauce
4. **Saltiness** : the overall strength of saltiness detected in the tomato sauce.
5. **Overall Spiciness**: the overall strength of garlic, and shallot taste detected in the tomato sauce.
6. **Fishiness** : the overall strength of the fish taste of fish oil detected in the tomato sauce.

III. Overall Acceptability: evaluation of the overall texture, and odour of fish and tomato sauce, and rating acceptability for the canned fish product.

3.3.4. Canned fish analysis

3.3.4.3. Fo determination

Fo was used to measure total lethality of the thermal process. A wire thermocouple was mounted at the geometric centre of the can and injected into one of the fish pieces. The hole in the can was sealed with epoxy resin and hardened. The can was then filled with tomato sauce and seamed. During seaming, hot steam was passed over the surface of the product to facilitate the vacuum condition in the can. The head space in the can was about 1cm. The Fo determination was carried out in a pilot plant scale retort (90 cm long and 56 cm diameter). The thermocouple was connected to a Molytek Data Reader and computer, supported with a locally made Fo Calculation Program (Van Til, 1991). The Fo for the heating and cooling process was automatically calculated and displayed on the screen. The Fo determination was conducted at a retort temperature of 121.1°C.

3.3.4.2. Sterility test

Total plate count (TPC) analysis was used to determine the sterility of the canned fish. The analysis was carried by spreading 0.1 ml of diluted sample (10^{-1}) on the surface of nutrient agar plates. The plates were incubated aerobically and anaerobically at 30 and 55°C, respectively.

The dilution was prepared by weighing a portion of the fish flesh (25 g) and placing the portion in 225 ml 0.1% peptone water. The sample was obtained from two canned fish products.

3.3.4.3. Incubation test

The canned fish products were incubated at 30 and 55°C (5 cans at each temperature) for 14 days. Every seven days the cans were observed for swelling and one was opened for detection of undesirable odour.

3.4. DATA ANALYSIS

Analysis of variance, t-test and crosstab were performed using Stat-Packets 1.0 (Wallonick Associates, 1987). Regression analyses was carried out using both Stat-Packets 1.0 (Wallonick Associates, 1987) and MUTAB/PC-Version 3.01 (Boag, 1988). The Plackett and Burman Program (Van Til, 1991) was used to identify variables showing significant effects on the experimental canning process.

Chapter 4

FISH OIL PRODUCTION IN INDONESIA

4.1. BACKGROUND

Currently, fish oil is produced as a by-product of the fish meal industry. Fish oil is produced in floating meal factories, floating canneries or inland fish meal factories (Tanikawa, 1971). The technology involved is not very complex, but some technological aspects must be seriously considered in order to obtain a good quality fish oil.

According to the Food and Agriculture Organisation of the United Nations (FAO, 1986), raw materials used for fish meal and oil processing fall into several categories:

* fish caught specifically for processing into meal, and oils such as menhaden, anchovy and sardine;
* incidental or by-catch from another fishery, for example shrimp trawl by-catch; and
* fish offal or waste from edible fisheries such as cutting from filleting operations, fish cannery waste, roe fishery waste and surimi processing waste.

Several different processes have been evolved for the manufacture of meal and oil including wet and dry rendering, and solvent, enzymatic, and silage processes (Lee, 1963; Pigott, 1967; Considine and Considine, 1982; Bimbo, 1990). These processes have been reviewed previously in Chapter 2.

The quality of crude fish oil is dependent on the handling and the storage of raw fish prior to processing, on the type and operational efficiency of the fish processing plants, and on the handling and storage of the crude oil prior to refining (Young, 1982).

As information about Indonesian methods of production and quality of the product were unavailable, a survey and analysis of Indonesian fish oil samples were conducted.

4.2. OBJECTIVES

The survey of Indonesian fish meal factories was aimed at determining the present status of fish meal and fish oil in the fishing industry. The survey was also designed to obtain technical and financial information about present production.

Samples of unrefined and refined fish oil were collected from the producers and analyzed chemically, physically and organoleptically in order to determine quality. This information was used to decide whether Indonesian fish oil required further refinement or not, for human consumption.

4.3. METHODOLOGY

Survey of the Indonesian fish meal factories was limited to factories located around the Bali Straits, where most Indonesian fish meal and fish canning factories are concentrated. Since official information on the number of fish meal factories was unavailable, the survey was conducted of all fish meal processors under the guidance of local government fishery officers. Eighteen conventional fish meal producers and one traditional fish meal processor participated. A conventional fish meal producer is defined as one using a standard, modern fish meal processing unit. Labour costs here are relatively low. In comparison, the traditional fish meal producer is one using very simple processing equipment, where high labour costs are significant. Some factories refused to be interviewed. The survey was carried out by direct interview of factory managers and staff. A copy of the questionnaire used during the survey is shown in Appendix 4.1.

Fish oil samples, collected during the survey, were held in polypropylene bottles with 200 ppm BHT antioxidant. Samples were airfreighted to Massey University for free fatty acid value, refractive index (20°C), colour (absorbance at 490nm) and visual colour, sensory analysis of odour, and for fatty acid profiling. Visual colour description was given by a trained PhD student in the Consumer Technology Department, Massey University. Sensory analysis on odour was undertaken by seven trained Indonesian students. The panellists were asked to assess the intensity of fishy and undesirable odour using a nine point scale (1 = non-fishy/undesirable odour, 9 = extremely strong fishy/undesirable odour). The score sheet used to evaluate the odour of the oil is shown in Appendix 4.2.

New Zealand fish oils, crude (mainly hoki) and orange roughy oils, were used for comparative purposes. Both oils were processed using the wet rendering method.

4.4. RESULTS

4.4.1. Position of fish meal in Indonesian fishery industry

Of the 19 Indonesian fish meal processors surveyed, 6 factories produced fish meal as the only main product, while 9 factories produced 2 main products, fish meal and canned fish. Four factories stated that the fish meal was a by-product.

4.4.2. Raw fish used for fish meal/oil production in Indonesia

Raw fish used to produce fish meal in the surveyed factories is shown Table 4.1.

Table 4.1. Raw fish used for fish meal production (as number of factories)

Raw Fish	Fish Species	Product Status		
		Fish Meal as Only Main Product	Fish Meal as One of Main Products	Fish Meal as By-Product
Whole Fish	Sardine	6	9	
	Mackerel		1	
Mixture of fish species		1	4	
Fish Waste	Sardine		4	3
	Tuna			2

Note: A factory might use more than one raw material type

Generally, fish meal processors utilised whole oil sardine (Sardinella longiceps) and sardine waste from canneries as raw material. Other fish species used in fish meal production were mackerel and tuna. Where a mixture of fish species is reported these consisted primarily of sardine, scad, mackerel and red snapper. Only factories producing fish meal as one of two main products used both whole fish and fish waste as raw materials. Fish waste from canneries consisted of heads, tails and offal. Fish meal produced as a by-product was processed from sardine and tuna waste only.

4.4.3. Fish meal and fish oil processing in Indonesia

Three commercial processing methods were encountered during the survey, as shown in Table 4.2. The wet rendering process was used by 17 processors surveyed. One processor used the dry rendering process and one used a cooking process without pressing. In this latter process, the fish was boiled and then air dried, without pressing between these processing steps.

All fish meal factories using the wet and dry rendering processes were able to separate fish oil from the liquid phase freed during pressing. The factory using the cooking process without pressing was separating fish oil from water used for cooking. In this process, the water and oil were allowed to separate for sometime and then the water fraction was removed. Production of fish oil from all types of raw materials was conducted by all factories except in 1 case where oil was not separated from tuna offal.

Table 4.2. Fish oil production information obtained during the survey

	Number of Factories
a. Fish meal production method:	
1. Wet rendering process	17
2. Dry rendering process	1
3. Cooking process without pressing	1
	Total 19
b. Oil Separation after pressing/cooking	
1. Factories separating the oil	19
2. Factories not separating the oil	0
	Total 19
c. Relationship between oil separation and raw material:	
1. Separation conducted to all raw materials	18
2. Separation not conducted to all raw materials	1
	Total 19
d. Refinement application to fish oil:	
1. Factories refining the oil	3
2. Factories not refining the oil	16
	Total 19

The alkali refining process was identified in three surveyed factories, representing each of the three factory types, as shown in Table 4.1.

4.4.4. Prices and buyers of fish oil

Unfortunately, some factories surveyed did not answer the questions relating to product prices, and buyers of their fish oil. Therefore, the results presented in Table 4.3 are limited to those factories which provided requested information in sufficient detail.

Table 4.3. Price and buyers of fish oil (by number of factories)

	Status of fish meal production			
	The Only Main Product	One Of main products	By-product	Total
a. Price*)(Rp./l):				
100 - 200	2	1		3
201 - 300	1	9	2	12
301 - 400		1	1	2
401 - 500		1		1
b Buyers:				
1. Fish oil traders	3	3	2	8
2. Feed companies	2	5	1	8

Note: *) A factory could give more than one price

The price of fish oil varied from Rp.100.-/l to Rp.500.-/l. In the main, fish oil was sold at a price between Rp.201.-/l-Rp.300.-/l. One factory sold fish oil according to its free fatty acid (FFA) value: Rp.425.-/l for fish oil having FFA value less than 5% and Rp.300.-/l for fish oil having FFA value more than 7%. Another factory reported that the price of canning waste oil was higher than the price of oil obtained from fish meal processing. The types of fish meal produced did not show a correlation with the fish oil price.

Results showed that fish oil buyers were mainly local fish oil traders and animal feed companies.

4.4.5. Chemical, physical and sensory analysis of Indonesian fish oil

Results of chemical, physical and sensory analysis of fish oil collected from factories/processors during the survey is presented in Table 4.4. As a requirement of the survey conducted by an Indonesian Fishery Officer, the names of the factories participating in the survey need to be kept confidential. Thus the alphabetic codes used below do not relate in the same sequential order of the participating factories identified in Appendix 4.3. In general, the results showed that Indonesian

fish oil varied chemically, physically and organoleptically. All fish oil samples from either fish meal processing or canning operations were obtained from oil sardine and in one case, from tuna waste. The oil separation from fish meal production has been described previously in Section 4.4.3. Factories having more than one main product did not separate the oil processed from the whole fish and fish waste. Fish oil obtained from the fish canning operation was collected during the pre-cooking operation.

Factories A and E produced fish oil from fish meal processing with extremely low FFA content, 0.08 and 0.09% respectively. However fish oil produced in other factories showed FFA value ranging from 1.70 - 18.77%. The Refractive Index (RI) also varied from 1.4767-1.4785. Absorbance values of colour showed a relatively high value in the range, 1.45-2.55. This corresponded to a colour change ranging from yellowish orange to brownish black. Panellists observed that the odour score was in the range, 4.57-7.86, indicating that a fishy or undesirable odour was detected.

Factories processing fish meal as the main product, together with other main products, produced fish oil from canning waste during the pre-cooking stage and from the pressing stage in fish meal processing. Generally, the fish oil collected from canning waste had a lower FFA value than the oil separated from fish meal production, except the canning waste oil produced by Factory M had a relatively high FFA value, but the value was still lower than the FFA values analyzed in the oil from fish meal processing. The RI values of the oils also varied and the source of the oil did not give any specific indication on RI value. The colour performance and absorbance value of the oils were significantly influenced by the source of the oils. The canning waste fish oil had a much lower absorbance value than the oil from fish meal processing. The canning waste oil showed a yellow colour while the colour of the oils from the fish meal operation were darker, ranging from yellowish brown to very dark reddish brown. According to panellists, the fish oil odours were also correlated to the source of the oils. A low odour score was obtained for canning waste oil. The oil obtained from fish meal processing generally had a high score reflecting a relatively high degree of off-odour development. Refined oil from fish meal produced by Factory L demonstrated an improved quality. The quality classification given by factory H did not exactly reflect the quality of the oil produced in terms of FFA value.

Table 4.4. Chemical, physical and sensory analysis of Indonesian fish oil

Product type of fish meal/factories	Oil Sources	Fish oil quality	FFA (% oleic acid)	Refractive Index (20°C)	Colour (absorbance at 490 nm)	Visual colour	Odour score
Main Product:							
A	S*/FM**	-	0.08	1.4785	1.45	yellowish orange	7.86
B	S/FM	-	5.34	1.4780	1.81	brown	7 14
C (trad.)	S/FM	-	5.42	1.4780	2.34	dark reddish brown	4.57
D	S/FM	-	18.77	1.4767	2.39	very dark reddish brown	7.57
E	S/FM	-	0.09	1.4800	2.55	brownish black	7.64
F	S/FM	-	8.78	1.4772	2.43	dark brown	7.00
One of Main Products:							
G	S/CW***	-	0.15	1.4790	0.22	yellow	2.86
H	S/FM	I	4.18	1.4789	1.70	reddish brown	6.29
H	S/FM	II	21.76	1.4761	2.55	dark brown	8.50
H	S/FM	Ill	10.61	1.4771	2.51	brownish black	7.29
H	S/CW	-	0.56	1.4786	0.34	yellow	3.86
I	S/FM	-	8.47	1.4775	2.56	very dark reddish brown	7.14
I	S/CW	-	0.06	1.4784	0.48	yellow	3.71
J	S/FM	-	4.49	1.4785	2.52	greenish brown	8.79
K	S/FM	-	55.69	1.4750	2.52	brownish black	8.10
L	S/FM	Refined	0.15	1.4790	0.96	yellowish brown	3.64
M	S/FM	A	0.24	1.4800	2.10	reddish brown	5.64
M	S/FM	B	14.06	1.4770	2.45	brownish black	7.21
M	S/CW	-	1.15	1.4780	0.34	yellow	2.43
By-Product:							
N	S/FWM+	-	25.72	1.4760	1.34	reddish brown	4.29
O	S/CW	I	0.23	1.4785	0.27	yellow	2.86
O	S/FWM	II	19.58	1.4760	2.43	dark brown	7.86
P	T++/FWM	-	15.21	1.4800	1.85	dark reddish brown	5.71
Q	S/FWM	-	6.99	1.4780	2.29	reddish brown	7.64

Note: * S = Sardine *** CW = Canning waste ++ T = Tuna
** FM = Fish meal + FWM = Fish waste meal

Producers who considered fish meal as a by-product, used fish cannery waste as the raw material. Only one factory produced fish oil collected from the pre-cooking stage in canning operation. High FFA values, 6.99-25.72%, for fish oils separated from fish waste meal, and a low FFA value, 0.23%, for canning waste oil were noted. The RI values varied from 1.4760 to 1.4785. All fish oils obtained from fish waste meal processing tended to have a brown colour with absorbance value ranging from 1.34 to 2.43; while the canning waste oil was yellow with absorbance value of 0.27. Panellists gave a relatively high odour score, 4.29 - 7.86, to fish oil from fish waste meal production. In contrast, the canning waste oil was given a low odour score, 2.86.

4.4.6. Fatty acid profiles of fish oil

Fatty acid profiles of fish oils collected from the fish meal factories during the survey are shown in Table 4.5.

Fish oil from the factories processing fish meal as the only main product produced from the whole fish, primarily sardine, had 36.2-40.8% saturated fatty acids (SAFA), 28.4-33.8% monounsaturated fatty acids (MUFA), and 28.6-34.1% polyunsaturated fatty acids (PUFA). The relative amounts of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were 24.1-29.5%, 9.2-20.1% and 3.5-12.5% respectively. The single traditional processor also produced fish oil having a high omega-3 fatty acid content, 25.6%.

Canning waste oils and fish oils from fish meal operation produced by factories processing fish meal as the main product, together with other main products, did not show any pattern in fatty acid profiles reflecting the effects of fish oil source. Relative amounts of SAFA, MUFA and PUFA of canning waste oils were 38.4-40.0%, 28.8-32.1% and 27.9-31.6% respectively. The fish oils from fish meal processing had 37.5-41.2% SAFA, 28.4-35.1% MUFA, and 24.9-32.8% PUFA. The omega-3 fatty acids, EPA and DHA of canning waste oil were 23.7-27.2%, 15.4-17.6% and 4.9-6% respectively. The relative amounts of these acids in the oil from fish meal production were 18.5-28.0%, 20.6-28.2%, 11.5-18.6% and 5-12.2% respectively.

Table 4.5. Fatty acid profiles of Indonesian fish oils (%)

Product Type of Fish Meal/ Factory	Oil Source	Quality	SAFA	MUFA	PUFA	ω3FA	EPA	DHA
Main Product:								
A	S/FM	-	30.7	29.1	34.1	29.5	20.1	5.8
B	S/FM	-	40.5	28.4	31.1	26.8	17.1	6.1
C (trad)	S/FM	-	40.8	29.1	30.1	25.8	17.1	5.0
D	S/FM	-	38.8	31.3	30.0	25.7	16.4	5.8
E	S/FM	-	40.6	30.8	28.6	24.1	9.2	12.5
F	S/FM	-	38.2	33.2	28.7	24.1	17.4	3.5
One Of Main Products:								
G	S/CW	-	39.6	28.8	31.6	27.2	17.6	5.7
H	S/FM	I	41.1	28.4	30.5	26.0	16.0	6.2
H	S/FM	II	41.2	28.9	30.0	25.5	15.2	6.4
H	S/FM	III	41.1	32.7	26.3	22.2	13.9	5.0
H	S/CW	-	38.4	30.2	31.3	26.7	16.7	6.0
I	S/FM	-	38.9	29.1	32.0	27.6	18.6	5.3
I	S/CW	-	39.6	29.1	31.3	26.8	17.2	5.8
J	S/FM	-	37.8	31.2	31.0	26.3	17.4	5.2
K	S/FM	-	40.0	36.1	24.8	20.6	11.5	5.3
L	S/FM	ref.*	39.6	29.4	31.0	26.4	16.6	6.1
M	S/FM	A	37.5	29.6	32.8	28.2	12.9	12.2
M	S/FM	B	38.3	30.0	31.8	27.1	16.1	7.4
M	S/CW	-	40.0	32.1	28.0	23.7	15.4	4.9
By-Product								
N	S/FWM	-	39.1	30.0	30.9	26.5	17.2	5.8
O	S/CW	I	39.8	28.5	31.8	27.4	17.6	6.0
O	S/FWM	II	41.3	28.8	20.0	25.1	15.2	6.4
P	T/FWM	-	38.6	27.4	34.0	29.5	5.0	22.2
Q	S/FWM	-	38.5	30.6	31.0	26.1	16.0	6.0

Note : Fatty acids : SAFA = saturated fatty acids
MUFA = monounsaturated fatty acids
PUFA = polyunsaturated fatty acids
ω-3 FA = omega-3 fatty acids
EPA = eicosapentaenoic acid
DHA = docosahexaenoic acid

Oils : *) ref. = Refined oil

Raw materials:
S = Sardine
FM = Fish meal
CW = Canning waste
FWM = Fish waste meal
T = Tuna

In terms of fish species used to extract fish oil, two kinds of fish oils, sardine and tuna, were obtained from fish meal processors who considered fish meal as a by-product. Tuna oil contained 38.6% SAFA, 27.4% MUFA and 34.0% PUFA; while the sardine oil contained 38.5-41.3% SAFA, 28.5-30.6% MUFA and 30.0-31.8% PUFA. The relative quantities of omega-3 fatty acids, EPA and DHA in tuna oil were 29.5%, 5.0% and 22.2%, while the quantities of those acids in sardine oil were 26.2-27.4%, 15.2-17.6% and 5.8-6.4% respectively.

The quality classification made by the above fish meal factories showed a relationship with the relative quantity of omega-3 fatty acids. The better the quality, the higher the omega-3 fatty acid content.

4.4.7. New Zealand fish oil used as comparison with Indonesian fish oil

Results of chemical, physical, sensory and fatty acid profiles of two New Zealand fish oils are shown in Table 4.6.

Table 4.6. Chemical, physical, sensory and fatty acid profiles of New Zealand fish oils

Parameters	Crude oil	Orange roughy oil
FFA (% oleic acid)	0.67	0.40
RI (20°C)	1.4730	1.4652
Colour (absorbance at 490 nm)	0.90	1.96
Visual colour	yellowish orange	reddish brown
Odour score	6.38	4.06
Fatty acid profiles (%):		
SAFA	25.3	6.0
MUFA	60.3	87.7
PUFA	14.4	6.2
HUFA	10.1	2.7
Omega-3 FA	11.6	3.5
EPA	4.2	1.3
DHA	5.1	1.2

Both crude, mainly hoki, and orange roughy oils had lower FFA values compared to most Indonesian fish oils, but those values were higher than the FFA values of canning waste oils. Further, the RI values of both New Zealand oils were lower than those of Indonesian oils. The colour absorbance values of New Zealand oils were in the range of absorbance values analyzed in Indonesian fish oils. The odour score of crude oils was relatively high. On the other hand, the orange roughy oil had a odour score considered low.

In terms of fatty acid profiles, Indonesian and New Zealand oils showed differences. Indonesian oils were significantly richer in polyunsaturated fatty acid content than New Zealand oils, particularly in omega-3 fatty acids content. The New Zealand oils had a higher content of MUFA. However, orange roughy oil is not eatable, since the oil is predominantly wax esters (Buisson, et al, 1982)

4.5. DISCUSSION

4.5.1. Fish oil production

The survey of Indonesian fish meal producers and samples collection from Indonesian fish oil producers indicated that the fish oil could be obtained from fish meal processing and fish canning.

Although fish meal processing factories were found to use various processing technologies, including wet rendering, dry rendering and cooking without pressing, most processors favoured the wet rendering method. It is noted that all processing methods use heat treatment as an integral part of fish meal production. In this case, heating or cooking is used to coagulate or denaturate fish protein to facilitate mechanical separation of liquids from solids. Under these conditions, fat cells are also ruptured, releasing the oil into the liquid phase (Bimbo, 1990; Kinsella, 1987; Ilyas et al, 1985). The efficient liberation of water and oil by cooking and pressing is an important aspect in producing high quality fish meal (Beraquet et al, 1984). In both wet and dry rendering methods, the liquid phase was released during the pressing step. In the cooking method without pressing, the liquids are released into cooking water from which fish oil can be subsequently separated.

Fish oil is also found produced from pre-cooking steps during the canning operation. Pre-cooking

is normally done by steaming the fish for approximately 20 minutes. Indeed, one of the purposes of the pre-cooking step during fish canning is to release body lipids if the fish are excessively oily or if the oil has a very strong flavour (Warne, 1988; Codex Alimentarius Commission, 1976). It is known that when fish flesh is heated, a significant proportion of water is released from the protein. The amount varies, approximately 17.5% for tuna, 19-34% for sardines, depending on the endogenous fat content (Van Den Broek, 1965).

4.5.2. Fish oil quality

Indonesian fish oil quality varied by factory and by oil source. Even in one factory, the fish oil produced could vary in quality. Three factories surveyed have classified their fish oil in terms of quality, using different parameters: free fatty acid content, fish oil colour and fish oil source. One factory classified fish oil quality in terms of FFA content, in which Grade I oil had a FFA value of less than 5%, Grade II oil, 5-7% and Grade III oil, more than 7%. This quality grade classification is similar to that reported by Windsor and Barlow (1981), who indicate that, usually, fish oil is sold on the basis of 3% FFA with a maximum allowable content, normally of 7%, which can, in certain cases, be up to 20%. On this basis, the Indonesian fish oil samples analysed in this study are classified as predominantly Grade I, 48%, but with some samples falling into Grade II, 20%, and Grade III, 32%, as shown in Table 4.7. All canning waste oils were classified as Grade I. The two New Zealand oils were grouped as Grade 1.

Table 4.7. Classification of Indonesian Fish Oil Quality in Terms FFA Value

Grade	Factory Producer	Fish Oil Source	Factory Grade	FFA Value (% oleic acid)
GRADE - I (FFA value: < 5 %)	A	S/FM	-	0.08
	E	S/FM	-	0.09
	G	S/CW	-	0.15
	H	S/FM	I	4.18
	H	S/CW	-	0.56
	I	S/CW	-	0.06
	J	S/FM	-	4.49
	L	S/FM	Refined	0.15
	M	S/FM	A	0.24
	M	S/CW	-	1.15
	O	S/CW	I	0.23
GRADE - II (FFA value: 5 - 7 %)	B	S/FM	-	5.34
	C (Trad.)	S/FM	-	5.42
	Q	S/FWM	-	6.99
GRADE - III (FFA value: > 7 %)	D	S/FM	-	18.77
	F	S/FM	-	8.78
	H	S/FM	II	21.76
	H	S/FM	III	10.61
	I	S/FM	-	8.47
	K	S/FM	-	55.69
	M	S/FM	B	14.06
	N	S/FWM	-	25.72
	O	S/FWM	II	19.58
	P	T/FWM	I	15.21

Note: S = Sardine
FM = Fish meal
CW = Canning waste
FWM = Fish waste meal
T = Tuna

The FFA value of canning waste oils were generally lower than the value measured in the oil from fish meal production. It is worth re-emphasising that the source of FFA is from hydrolysis of triglycerides (Windsor and Barlow, 1981). In this regard, different methods of oil extraction are likely to play a major role in determining the final oil quality. A relatively short heating time of 20 minutes at $\pm$ 100°C (Directorate General Of Fishery, 1984), and the use of the whole fish in the fish canning process are likely to contribute to minimal triglyceride hydrolysis. Fish oil produced from a fish meal processing involves more heating of the fish pulp. For example, in fish

meal processing, the fish pulp is cooked at 90°C for 30 minutes, and then dewatered. The liquid phase containing the oily emulsion is then reheated to 90°C prior to oil separation (Hoffmann, 1989). In this process the opportunity for accelerated triglyceride hydrolysis is relatively high. The other major factor affecting fish oil quality is undoubtedly raw material quality. Low quality fish, normally processed into fish meal, may have also caused the fish oil produced to have a high FFA value, since, according to Young (1982), spoilage in fish is responsible for increased free fatty acid content by lipases/enzymatic activity.

Canning waste oil consistently appeared lighter in colour than the oil recovered during fish meal processing. The difference in the colour is probably due to the method of oil extraction. It has been reported by Brody (1965) that excessive heat applied to fish for prolonged periods results in the production of darker oil. Chemically, these darker oils probably arise from heat-induced protein breakdown products acting as catalysts to accelerate autoxidation of the endogenous oil. This situation is exacerbated by the use of improperly cleaned containers contaminating the oil. For example, it is known that the oxides of metals such as iron, lead, and copper, when dissolved in oil containing water and free fatty acids, can accelerate the oxidation and darkening of fish oil (Brody, 1965). The raw material, oil sardine, may have affected the colour of the oil, the third grade oil sardine darkening the fish meal oil significantly more than the oil produced from canning waste, where the first grade oil sardine was used (Irianto and Fawzya, 1987). The raw material for fish production should be as fresh as possible in order to yield light coloured oil. Oil extracted from deteriorated raw material yields dark oil (Brody, 1965). In fact, the oil sardine used to produce fish meal is the sardine which does not meet required quality for processing into other products: canned fish, boiled salted-fish, and dried salted fish.

The raw material quality used to produce these fish oils may have affected the differences in odour as observed by panellists. The sardine, which is normally used as the raw material in fish meal production, is bought from the boat without an insulated fish hold (Irianto and Fawzya, 1987), thus the fish receives improper handling to keep its freshness. The improper handling continues when the fish arrives in the factory by without refrigeration storage. For example, Total Volatile Base (TVB) values and Total Plate Counts (TPC) of sardine, normally processed into fish meal, were found to be 27.4-35.1mgN% and (60.5-88.5)10^3 respectively. In contrast sardine used by canneries usually have a TVB of 20.7-22.6mgN% and a TPC of (12.1-22.1)10^3 (Irianto and Fawzya, 1987). Decomposition products which accumulate during fish spoilage are nitrogenous and sulphur compounds. Both compounds, if present in the fish at the time of processing, will pass over in part into the oil and occur in trace amounts sufficient to alter odour and flavour. The sulphur compounds possessing the more obnoxious odour cause more serious alterations in the quality of

the oil, but in well handled fish their presence is minimal (Stansby, 1990). This indicates that the odour of canning waste oil was better than the odour of oil obtained from fish meal processing. According to the panellists, the odour of canning waste oils was more uniform than the oil from fish meal processing. This was possibly affected by the quality of the raw fish. The results also showed a tendency for the oil with a darker colour to have a high odour score. From the above results, it can be concluded that there was a close relationship between raw material quality and both fish oil odour and colour.

Fish oil produced by Indonesian factories had relatively higher quantities of PUFA and omega-3 fatty acids than New Zealand crude oil from, mainly, hoki. The Indonesian oil had 20.6-29.5% of omega-3 fatty acids and New Zealand crude oil had 11.6% of omega-3 fatty acids. Analysis of sardine oil conducted by Setiabudy (1990) indicated that this oil contained 25.2% omega-3 fatty acids, which falls into the range found in this study for Indonesian oils. The level of omega-3 fatty acids in fish is known to be a function of fish species, age, sex and season (Moeljanto, 1982). The relatively high levels of omega-3 fatty acids in Indonesian fish oils suggests that the industry should seriously evaluate oil usage as a high quality product suitable for human consumption, rather than for non-human food purposes, such as animal feeds, as the survey results reveal.

To this end, fish oil quality should be improved chemically, physically and organoleptically by application of a suitable and properly evaluated refining process. As the fish oil was a by-product from fish canning and fish meal processing, most processors did not give, proper attention to fish oil quality improvement. Thus, the refining method to be introduced to Indonesian fish oil producers should be simple, low cost and labour efficient.

4.6. CONCLUSIONS

Fish oils in Indonesia were found to be produced from two fish processes: canning and fish meal processing. Chemically, physically and organoleptically, the canning waste oils were of a higher quality than oil recovered during fish meal processing. The levels of omega-3 fatty acids found in Indonesian oils is significantly higher than measured in New Zealand crude oil. However, it was found that for both Indonesian and New Zealand oils to be of acceptable quality for human consumption there was a need for an ultimate refining step.

Chapter 5

OPTIMIZATION OF THE RESIN REFINING PROCESS OF FISH OIL

5.1. BACKGROUND

The commercial process of fish oil refining, such as degumming, alkali refinement, bleaching and deodorization, involves heat. Since fish oil is the most polyunsaturated of all the oils, application of these operations would be detrimental to fish oils, due, mainly, to the susceptibility of polyunsaturated fatty acids to heat and consequent oxidation (Banks, 1967). This would probably affect the fatty acid quality of fish oil, especially ω-3 fatty acids, because the ω-3 fatty acids are susceptible to selective destruction during the process (Griggs, 1986).

Fernandez (1986) introduced the use of macroporous resin for fish oil refining, a method without heat involvement, based on the fact that resins are sorbent materials. The most known sorption operation where resins are involved is ion exchange. An ion exchanger is basically an electrolyte solution containing cations, anions and water, differing, however, in that one or other ion is bound to an insoluble microporous matrix (Patterson, 1970). The typical reaction occurring in the ion exchange reaction is as follows:

$$AR + B_{(aq)} \text{ ------> } BR + A_{(aq)}$$

B ions, initially distributed throughout the solution, must first find their way to the surface of the resin grains, because A ions can not leave the resin until B ions enter, as electroneutrality must be preserved. This first transport step takes place partly by the flow of solution, and partly by diffusion of the ions in solution. Next, when some B ions wander into the pores of the resin, an equivalent number of A ions can wander out, and so the exchange proceeds by diffusion throughout the interior of the resin until, eventually, an equilibrium distribution of A and B ions is reached. Meanwhile A ions entering the solution are also being distributed through the whole volume by diffusion and mixing (Kitchener, 1957).

During the ion-exchange reaction, the ion-exchange resins are concentrated into soluble acids, bases, or salts. Cation-exchange resins contain fixed electronegative charges which interact with mobile counter-ions having the opposite, or positive charge. Anion-exchange resins have fixed electropositive charges and exchange negatively charged anions (Considine, 1974).

Early ion exchangers were zeolites consisting of aluminium silicates (Samuelson, 1953). However most ion exchange resins used by industry today are manufactured from uniform spheres of styrene-divinylbenzene (DVB) copolymers having diameters 0.3 - 1.0 mm (20 - 50 mesh). The copolymer beads are formed by pearl polymerization and converted to ion-exchange resins by a second processing step. Sulfonic-type cation-exchange resins are made by sulfonation of the polymer beads at elevated temperatures. Strong base anion-exchange resins are produced by means of chloromethylation and amination of the copolymer spheres (Considine, 1974).

Overall exchange rate may be influenced by a change in solvent nature and content, particle size, temperature, and the functional group. Exchange rates are most rapid in water system, and become increasingly slower with less polar solvents. This is true because the solutes are more highly ionized in the more polar solvents as are the ion exchange resins. Similarly, exchange rates are more rapid in low cross-linked resins of the same inherent dry-basis capacity because of their higher moisture content. The ion exchange process involves diffusion through the film of solution in close contact with the resins and diffusion within the resin particle. Film diffusion is rate-controlling at low concentrations and particle diffusion is rate-controlling at high concentrations. Whether film diffusion or particle diffusion is the rate-controlling mechanism, the particle size of the resin is also a determining factor. Elevated temperatures increase exchange rates. The kind of functional group and its degree of dissociation under a given set of conditions greatly affects exchange rates where particle diffusion is controlling, but has no effect on film diffusion. Furthermore, a high degree of substitution of the functional groups on the inert polymer matrix directly enhances overall reaction rates (Wheaton and Lefevre, 1981).

Ion exchange between strong electrolytes can usually be carried out until most of the stoichiometric capacity of the exchanger has been used. Consequently, the total sorbent capacity is practically constant regardless of the composition of the solution being treated. An apparent exception arises if a weak acid or base is involved, either in the resin or in solution (or in both), when the apparent capacity of the resin may be much less than its

stoichiometric value (Pery et al, 1973).

Another operation where resins or resin-like sorbents are involved are adsorption, molecular sieving, and gel permeation (Perry et al, 1973)

Fernandez (1986) found that the macroporous resin process, which requires no application of temperature over 65°C during refinement, was the only technology in her experiment which improved fish oil sensory characteristics and maintained fish oil chemical characteristics. The technology has proved to be superior over molecular distillation and freezing fractionation. However Fernandez (1986) did not optimize the process as carried out in this experiment.

5.2. OBJECTIVES

The experiment was intended to optimize the refining process of fish oil using a macroporous resin packed column in order to obtain good quality fish oil for human consumption. The experiment also aimed to reveal changes in fish oil, before and after refining.

5.3. METHODOLOGY

5.3.1. Materials

Fish oils used for the investigation were New Zealand crude, mainly hoki, and orange roughy oils.

5.3.2. Experimental Methods

Four aspects were investigated to determine the possible ways to optimize the resin refining process and fish oil quality:

* fish oil-resin volume ratio;
* multiple refining;
* use of vacuum pressure during refining; and
* height and diameter ratio of column.

5.3.2.1. Experiment I: Effect of fish oil - resin volume ratio

A column 1.15cm in diameter and 53cm long was packed with 55cc of resin. The fish oil volume passed through depended on the fish oil-resin volume ratio tested, at 1, 2, 3 and 4 times resin volume. The column was undisturbed until all freely flowing oil passed through the column. The oil fraction obtained by oil free flowing from the column was called fraction-1 or refmed oil. Fraction-2 was defined as the amount of oil retained in the column and flushed out with one equivalent volume packed column (55cc) of petroleum ether. The column was washed with methanol (55cc) in order to clean any polar compounds that might be attached to the resin. Then, petroleum ether (55cc) was used to flush the packed column. In order to ensure that the column was completely clean, 27.5cc methanol and 27.5 cc petroleum ether were passed through the column in sequence. The solvents were evaporated using a rotary vacuum evaporator at 65°C to obtain fraction-2 oils.

The flow rate of fish oil passing through the column was 0.42-0.65ml/min. depending on the fish oil type. The flow rate decreased with the increase of fish oil volume. Refining was conducted at ambient temperature 18-23°C.

5.3.2.2. Experiment II: Effect of multiple refining on fish oil quality

The procedure was the same as used in Experiment I, with the addition of multiple refining where the oil was passed through the clean column one, two, three and four times. The experiment was carried out at ambient temperature 18 - 23°C.

5.3.2.3. Experiment III: Use of vacuum pressure in resin refining process

A column of 1.65cm in diameter and 39cm long was used. The column was cleaned using the method outlined in the experiment I, but modified. 40cc of petroleum ether was passed into the column. Then the column was washed with methanol, 40cc. The same volumes of petroleum ether and methanol were used to wash the column a second time. Finally, 40cc of petroleum ether was passed through the column in order to prepare the column for reused.

The fish oil-resin volume was at ratio 1:1. The vacuum pressure applied consisted of two steps: vacuum pressure at 74kpa was applied to guide the oil through the column; when the oil reached the end of column, the vacuum pressure was reduced to 60kpa. The time required for the first and the second pressure was 18-22 minutes and 5-7 minutes respectively. The time taken in the refining without pressure application was 80-91 minutes. The study was performed at ambient temperature 18 - 23°C.

5.3.2.4. Experiment IV: Effect of column size on fish oil quality

Two separate experiments were performed. The first experiment varied the height of the column with diameter size constant, the second experiment was performed by varying the column diameter size with height size constant. In the first experiment, the column diameter was 2.6cm. The diameter-height ratios investigated were 1:1, 1:2, 1:3 and 1:4. In the second experiment, the column height was 39 cm and various column diameter sizes observed were 1.65, 2.60 and 3.20 cm. The fish oil-resin volume ratio used was 1:1. The washing method of the column was the same as in the experiment III.

5.3.2.5. Experiment V: Investigation of natural antioxidant and volatile flavour compounds quantity changes during refining

The oils obtained from Experiment I, with fish oil-resin volume ratio of 1:1, were used in this experiment. To investigate the changes in the quantity of natural antioxidant, the tocopherol level of unrefined and refined oil was measured using HPLC. The unrefined and refined oils were also identified for changes in volatile flavour compounds using gas chromatography/mass spectrometer.

5.4. RESULTS

5.4.1. Effects of fish oil-resin volume ratio on fish oil quality

5.4.1.1 Effect on free fatty acid value

Results clearly indicate that the fish oil volume passed through the resin column affected the free fatty acid (FFA) value of fraction-1 and fraction-2 oils of both crude and orange roughy as shown in Figure 5.1. The FFA values for crude and orange roughy oils decreased significantly at the fish oil-resin volume ratio 1:1. Increasing the fish oil-resin volume ratio to 4:1 did not show any further significant reduction in FFA values for crude and orange roughy oils.

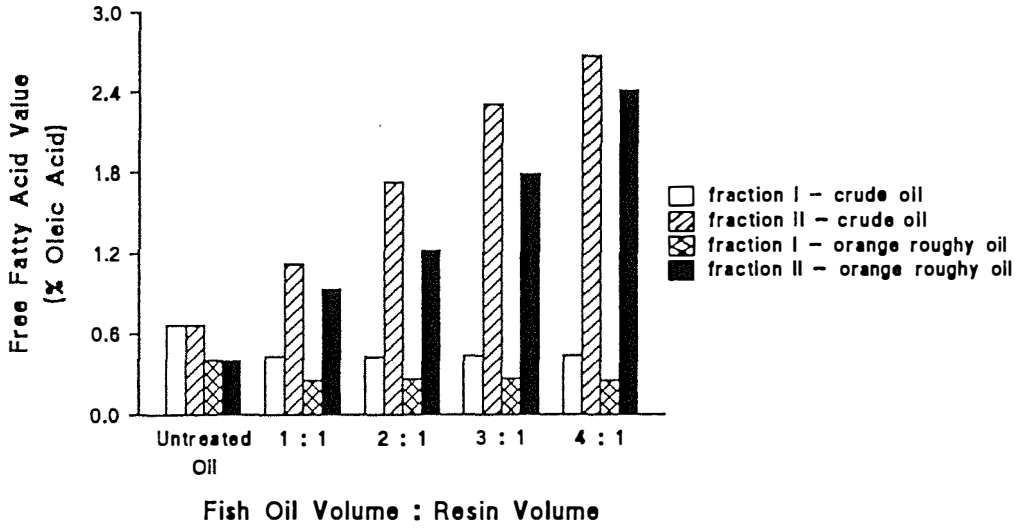


Figure 5.1. Effects of fish oil-resin volume ratio on free fatty acid value of fish oil

A different behaviour of FFA content was observed in the fraction-2 of crude and orange roughy oils, where the FFA value of the oils tended to increase with increasing fish oil volume. The FFA value changes in fraction-1 and fraction-2 of crude and orange roughy oils as a result of various fish oil volumes refined showed a similar pattern.

5.4.1.2. Effects on refractive index

Statistical analysis indicates that the fish oil volume passed through the resin column significantly influenced the refractive index (RI) values of both fraction-1 and fraction-2 obtained from crude and orange roughy oils. The changes in RI value of fraction-1 and fraction-2 for both oils during refining is shown in Figure 5.2.

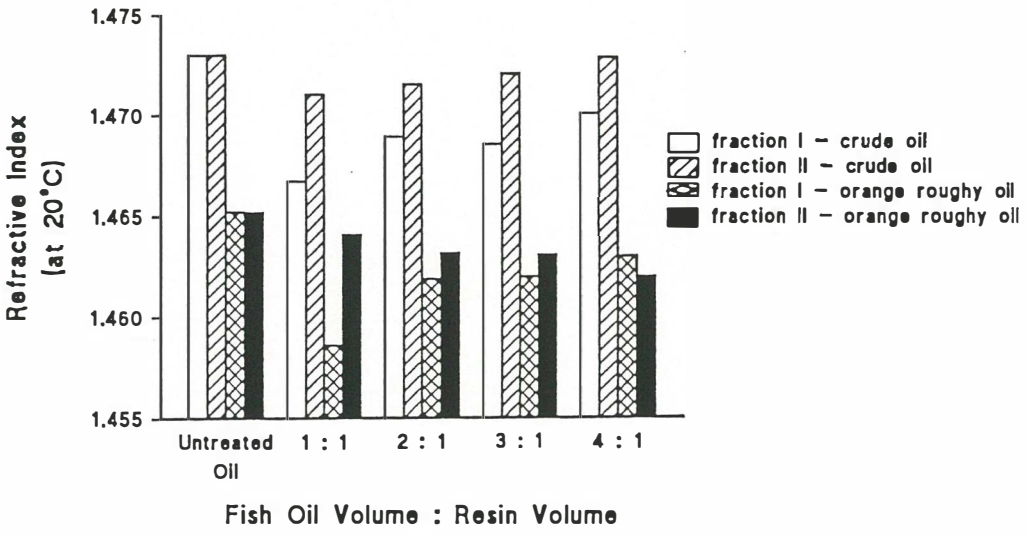


Figure 5.2. Effects of fish oil-resin volume ratio on refractive index value of fish oil

Resin refinement of the oils at ratio 1:1 decreased the RI values of both. However as the fish oil volume was increased to 2, 3 and 4 times the resin volume, the RI value of the fraction-1 oils increased accordingly.

Fraction-2 for both oils obtained from refining at ratio 1:1 had a lower RI value than the raw oils. When the fish oil volume was increased to 2, 3 and 4 times that of the resin volume, the fraction-2 oils obtained from crude oil showed a significant increase in RI value. However the fraction-2 orange roughy oil showed a significant reduction in the RI value. These significancies were shown using t-test at 95% significant level with 4 degree of freedom.

5.4.1.3. Effects on colour absorbance

Analysis was performed only for fraction-1 oils. Absorbance values of crude and orange roughy oils were significantly decreased with refining at fish oil-resin volume ratio 1:1. However, increasing the fish oil volume to 2, 3 and 4 times that of the resin volume resulted in a gradual increase in absorbance of both crude and orange roughy oils as shown

in Figure 5.3. The increase was more pronounced in orange roughy oil.

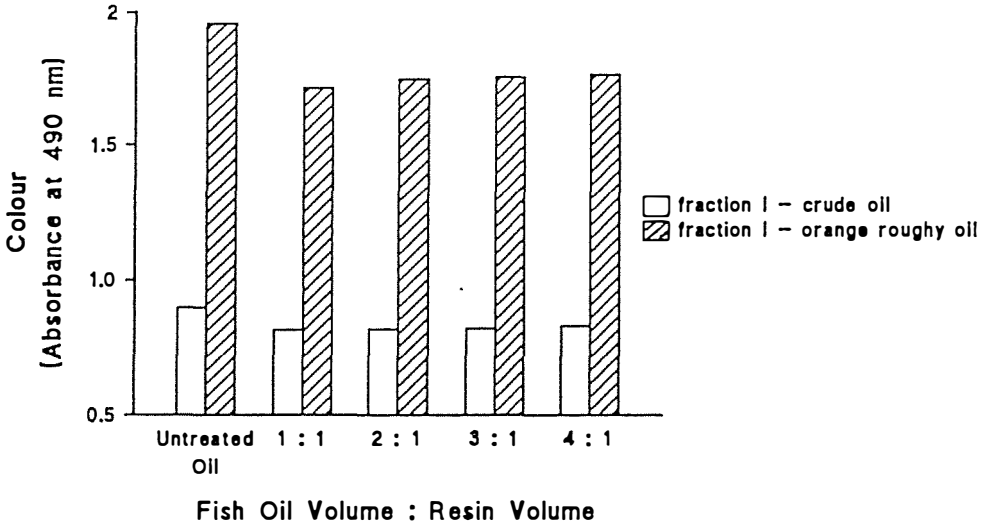


Figure 5.3. Effects of fish oil-resin volume ratio of colour absorbance value of fish oil

5.4.1.4. Effects on fatty acid profiles

Fatty acid profile behaviour of crude and orange roughy oils from fraction-1 and fraction-2 before and after resin refining can be seen in Tables 5.1 and 5.2.

In terms of saturated fatty acids (SAFA), the relative quantities in fraction-1 and fraction-2 of crude and orange roughy oils showed a similar change trend. Fraction-1 oils had a slightly lower SAFA value in comparison to the unrefined oils. The SAFA value found in fraction-2 oils tended to be higher than the values analyzed in the unrefined oils and fraction-1 oils.

The monounsaturated fatty acid (MUFA) levels observed in the fraction-1 oils from crude and orange roughy oils were higher than the amounts measured in the unrefined oils, while the quantity of MUFA analyzed in the fraction-2 oils tended to be lower than in the unrefined oils and fraction-1 oils.

Table 5.1. Effects of fish oil:resin volume ratio on fatty acid profile of crude fish oil (% fatty acid)

Fraction	Oil : resin	SAFA	MUFA	PUFA	EPA	DHA	Omega-3 f.a.
Untreated oil		25.3	60.4	14.4	4.2	5.1	11.6
I	1 : 1	24.5	60.7	14.8	4.3	5.2	12.0
	2 : 1	25.0	60.2	14.9	4.4	5.3	12.1
	3 : 1	24.7	60.5	14.7	4.4	5.1	11.9
	4 : 1	24.2	60.9	14.9	4.4	5.3	12.1
II	1 : 1	26.0	58.9	15.1	4.4	5.4	12.2
	2 : 1	26.4	58.7	14.9	4.3	5.6	12.3
	3 : 1	26.1	58.2	15.3	4.7	5.5	12.5
	4 : 1	26.4	58.3	15.3	4.7	5.5	12.5

Statistically, the polyunsaturated fatty acid (PUFA) value of crude oil was relatively constant during the refining process and this occurrence was revealed in both fraction-1 and fraction-2 oils. Fraction-1 orange roughy oil showed the same response as in fraction-1 crude oil. Fraction-2 orange roughy oil indicated a different pattern, where the PUFA value was relatively constant when refined with fish oil-resin volume ratios 1:1 and 2:1. This value was significantly higher when the fish oil volume was elevated to three and four times resin volume. The PUFA value in fraction-2 orange roughy oil was significantly higher than the value in fraction-1. This occurrence was not noted in the crude oil.

Table 5.2. Effect of fish oil-resin volume ratio on fatty acid profile of orange roughy oil (% fatty acid)

Fraction	Oil : Resin	SAFA	MUFA	PUFA	EPA	DHA	Omega-3 f.a.
Untreated oil		6.2	87.6	6.3	1.5	1.1	3.5
I	1 : 1	6.1	87.9	6.0	1.4	1.0	3.3
	2 : 1	5.7	88.2	6.0	1.3	1.0	3.3
	3 : 1	5.7	88.2	6.1	1.4	1.0	3.3
	4 : 1	5.7	88.1	6.2	1.5	1.0	3.3
II	1 : 1	6.7	87.1	6.2	1.4	1.1	3.7
	2 : 1	7.0	86.8	6.1	1.4	1.2	3.7
	3 : 1	7.1	86.4	6.5	1.5	1.3	3.9
	4 : 1	7.4	85.6	7.0	1.6	1.5	4.1

The resin refining process did not significantly change the omega-3 fatty acid value of crude oil, where unrefined, fraction-1 and fraction-2 oils from all tested fish oil-resin volume ratios did not show any pronounced different in omega-3 fatty acid value. The refining process also seemed to keep the omega-3 fatty acid value in fraction-1 orange roughy oil constant. The omega-3 fatty acid value in fraction-2 orange roughy oil indicated a significant increase when the fish oil ratio was increased to 4:1. In both oils the omega-3 fatty acid value in fraction-2 oils tended to be markedly higher than the value in fraction-1 oils.

Eicosapentaenoic acid (EPA) values of orange roughy oil was insignificantly affected by the resin refining process, since the values in both fraction-1 and fraction-2 oils were relatively similar to the value analyzed in unrefined oil. EPA value in fraction-1 crude oil was also found relatively constant. The significant increase of EPA value was found in fraction-2 crude oil when the fish oil volume ratio was elevated to three and four time that of resin volume according to t-test value at 95% significant level with 4 degree of freedom.

Docosahexaenoic acid (DHA) value of the crude oil did not indicate any significant change due to the resin refining process. Also fraction-1 orange roughy oil did not show an

obvious change, even though the fish oil volume was raised to ratio 4:1. A constant DHA value in fraction-2 orange roughy oil was encountered until the fish oil-resin volume ratio was 3:1. However that value significantly increased when the fish oil volume ratio was increased to 4:1 according to t-test value at 95% significant level with 4 degree of freedom.

5.4.1.5. Effects on sensory properties

The resin refining process demonstrated a significant effect on the odour and taste scores of fraction-1 and fraction-2 crude and orange roughy oils as shown in Figures 5.4 and 5.5.

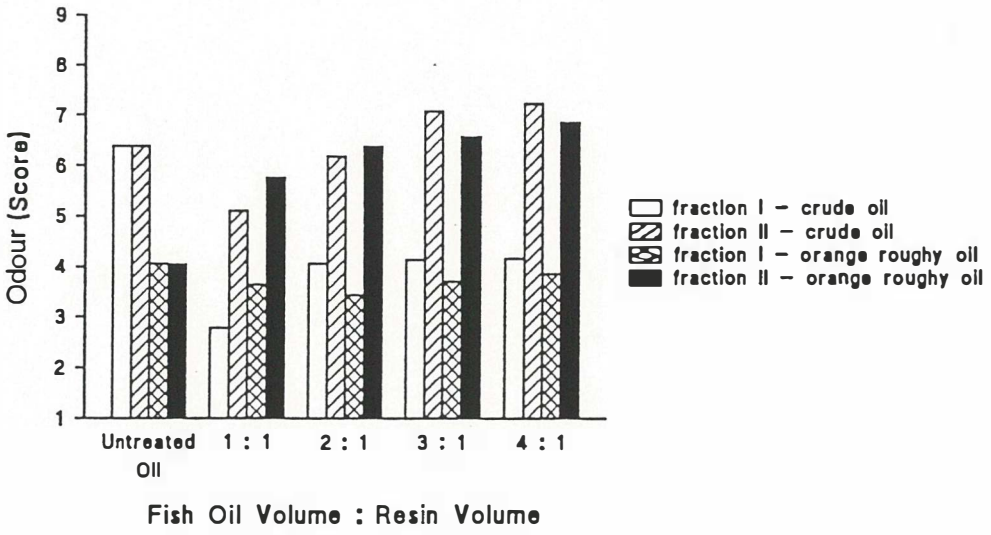


Figure 5.4. Effects of fish oil-resin volume ratio on odour score of fish oil

Odour and taste scores of both oils were apparently improved by the resin refining process. In the crude oil, as shown by odour and taste scores of fraction-1 oils, the best quality oil was obtained at fish oil-resin volume ratio 1:1. When the fish oil volume ratio increased, the odour and taste scores became higher, reflecting an inferior quality oil. In the refinement of orange roughy oil at fish oil-resin volume ratio 1:1, the fraction-1 oil obtained also improved its quality in terms of odour and taste. Increasing fish oil volume to ratio 4:1 did not, significantly, affect the odour and taste scores of fraction-1, refined oils.

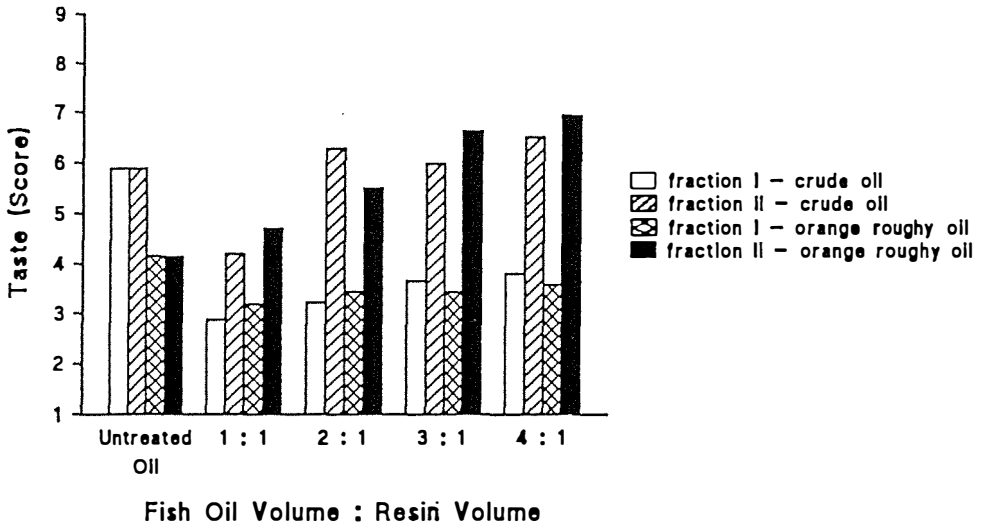


Figure 5.5. Effects of fish oil-resin volume ratio on taste score of fish oil

Odour and taste scores of fraction-2 crude oil produced from refining with fish oil-resin volume ratio 1:1, was lower than the unrefined oil. When the fish oil volume ratio increased to 4:1, the odour and taste scores of fraction-2 oils tended to be relatively equal, or higher, than the scores for fraction-2 oils from previous refining. However the odour and taste scores of fraction-2 orange roughy oil, from all tested fish oil-resin volume ratios, were higher than the scores for unrefined oil.

In general, fraction-2 oils from both crude and orange roughy oil had higher odour and taste scores, compared to the fraction-1 oils.

5.4.2. Effects of multiple refining on fish oil quality

5.4.2.1. Effects on free fatty acid value

Both crude and orange roughy oils showed a similar response in terms of free fatty acid value to multiple refining treatment, as shown in Figure 5.6.

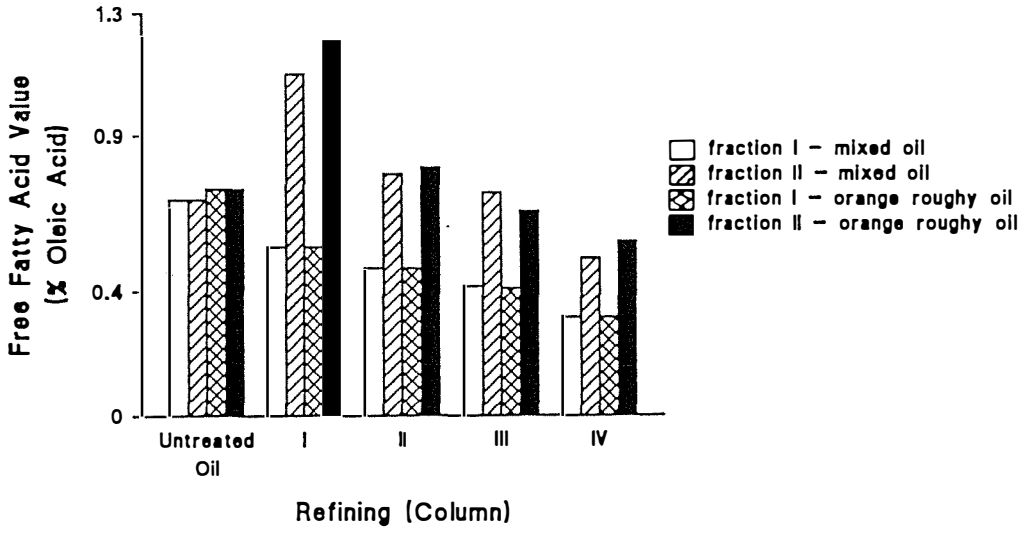


Figure 5.6. Effects of multiple refining on free fatty acid value of fish oil

Fraction-1 oils from the first, second, third and fourth refinings indicated significantly lower free fatty acid (FFA) values than unrefined oils. Fraction-1 crude oils from the first, second and third refining showed an insignificant different in FFA values. The FFA values of fraction-1 oil from the first and second refinings indicated a higher value compared to the value of fraction-1 oil from the fourth refining. The fraction-1 orange roughy oil collected from the first and second refinings indicated an insignificant different in FFA values. However the FFA values of the fraction-1 oil from the first refining was apparently higher than the values of fraction-1 from the third and fourth refinings. The values of fraction-1 oils from the second and third refinings showed a significant increase over the value of the fraction-1 oil from the fourth refining.

Each refining consistently produced fraction-1 oils with a lower FFA value in comparison to fraction-2 oils. The FFA value of fraction-2 for both oils from the first refining was markedly higher than the value of unrefined oil. That value significantly decreased in the second, third and fourth refinings.

5.4.2.2. Effects on refractive index

Multiple refining showed a significant effect on the refractive index (RI) changes in fraction-1 and fraction-2 for crude and orange roughy oils as shown in Figure 5.7.

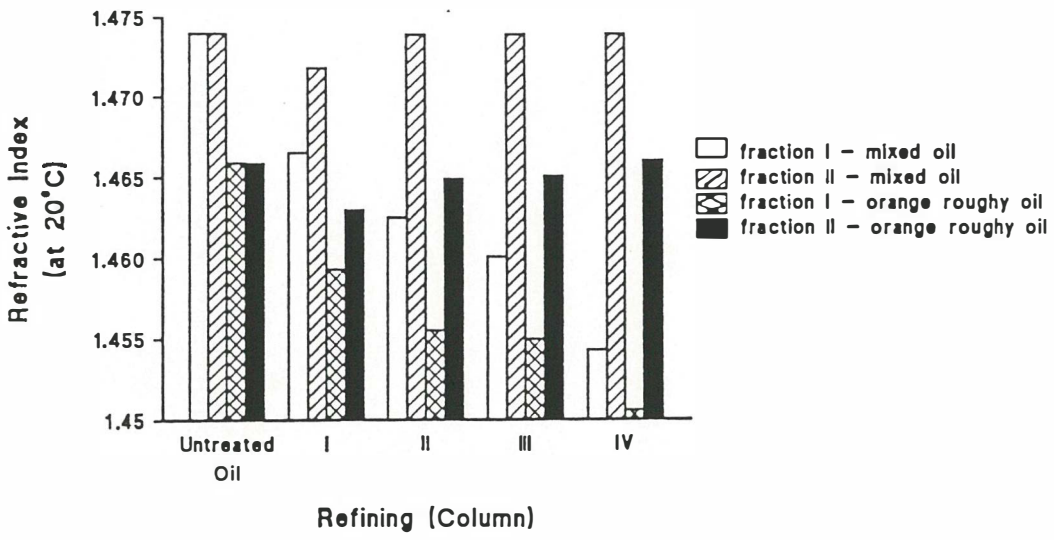


Figure 5.7. Effects of multiple refining on refractive index of fish oil

RI values of fraction-1 oils from first, second, third and fourth refinings were significantly lower compared to the value observed in unrefined oils. The RI values of fraction-1 oils from the first refining could be reduced by passing the oil through the column a second time. The third refining could be used to lessen the RI value of fraction-1 oils from the second refining, except for the crude oil. Further decreasing values occurred when the fraction-1 oils from the third refining were passed through the resin packed column for the fourth time.

The RI values of fraction-2 for both oils showed a similar pattern. The RI values of fraction-2 oils from the first refining were lower than the values in unrefined oils, but the second, third and fourth refining resulted in fraction-2 oils having RI values which were relatively the same value as untreated oils. In general, the RI values of fraction-2 oils were obviously higher than the values of fraction-2 oils.

5.4.2.3. Effects on colour absorbance

Analysis of variance indicated that the fish oil colour could be improved by application of multiple resin refining as shown in Figure 5.8.

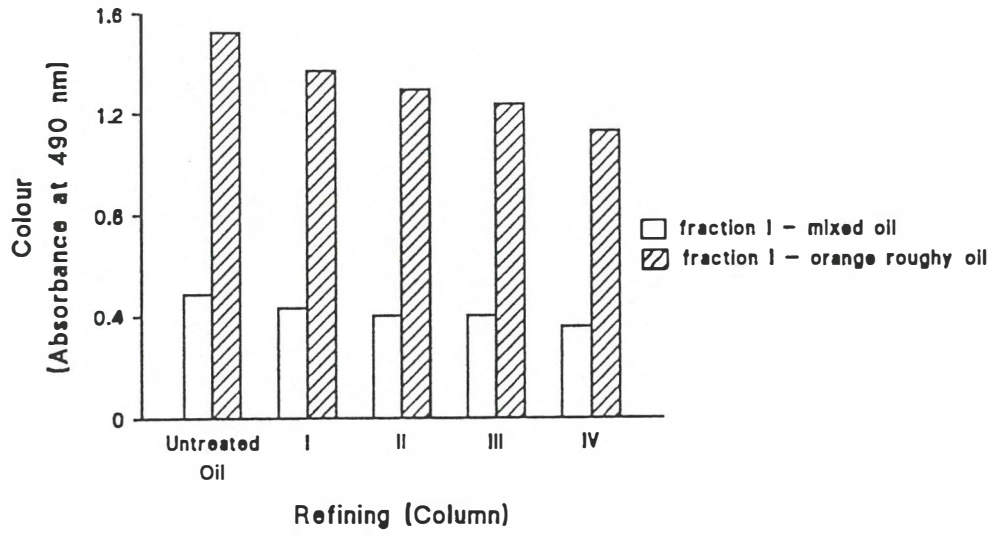


Figure 5.8. Effects of multiple refining on colour absorbance value of fish oil

The colour absorbance values of unrefined oils was reduced during the first refining. Those values were reduced again, when the fraction-1 oils were passed through the column for the second time. The third refining decreased the colour absorbance value of fraction-1 orange

roughy oil from the second refining. This did not occur in the crude oil. However the fourth refining significantly further reduced the absorbance values of both fraction-1 oils from the third refining.

5.4.2.4. Effects on fatty acid profiles

Fatty acid profile changes in fraction-1 and fraction-2 crude and orange roughy oils, as the result of the effect of multiple refining application, can be seen in Tables 5.3 and 5.4.

Multiple refining did not result in any pronounced differences in the relative quantities of saturated fatty acids (SAFA) in fraction-1 and fraction-2 crude and orange roughy oils from the first, second, third and fourth refinings. In addition, these values were relatively the same as the relative value analyzed in unrefined oil.

The relative quantities of monounsaturated fatty acids (MUFA) of fraction-1 and fraction-2 of both oils from the first, second, third and fourth refinings did not indicate any significant different from the quantity analyzed in the unrefined oil. The multiple refining treatments did not produce any significant MUFA difference between fraction-1 and fraction-2 oils.

Statistically, the multiple refining treatments did not result in any pronounced effect on the relative quantities of polyunsaturated fatty acids (PUFA) for both oils.

Table 5.3. Effect of multiple refining on fatty acid profile of crude fish oil (% fatty acid)

Fraction	Refining	SAFA	MUFA	PUFA	EPA	DHA	Omega-3 f.a.
Untreated oil		27.3	59.2	13.5	3.9	5.0	11.1
I	I	26.1	59.3	14.6	4.2	5.7	12.2
	II	27.1	58.9	14.0	4.0	5.3	11.6
	III	26.7	59.0	14.3	3.9	5.6	11.8
	IV	27.0	59.1	13.9	3.9	5.4	11.5
II	I	27.4	58.5	14.2	3.9	5.6	11.6
	II	26.9	59.3	13.8	3.8	5.2	11.2
	III	27.5	59.2	13.3	3.8	4.9	10.8
	IV	27.3	59.2	13.4	3.8	5.0	10.9

Table 5.4. Effect of multiple refining on fatty acid profile of orange roughy oil (% fatty acid)

Fraction	Refining	SAFA	MUFA	PUFA	EPA	DHA	Omega-3 f.a.
Untreated oil		8.4	86.3	5.4	1.1	1.2	3.3
I	I	8.3	85.3	5.5	1.2	1.1	3.4
	II	8.0	86.8	5.2	1.2	1.2	3.3
	III	8.8	85.9	5.3	1.3	1.2	3.4
	IV	8.7	85.9	5.3	1.3	1.1	3.3
II	I	9.3	85.3	5.4	1.1	1.2	3.4
	II	8.3	85.8	5.4	1.3	1.2	3.4
	III	8.2	85.6	6.2	1.6	1.2	4.0
	IV	8.1	86.2	5.7	1.8	1.1	3.7

The total omega-3 fatty acid values of crude and orange roughy oils were not significantly affected by multiple refining treatments. This result occurred in both fraction-1 and fraction-2 oils. The significant changes in eicosapentaenoic acid (EPA) values were observed only in fraction-1 crude oil, where an increased EPA value was noted in fraction - 1 from the first refinings, but was followed by a decreasing pattern with further refining. Obvious docosahexaenoic acid (DHA) changes occurred in orange roughy oil. The increase in DHA values in fraction-1 oil was observed in the oil obtained from the second refining. The DHA value in fraction-2 orange roughy oil significantly decreased at the fourth refining. These significant changes were shown by t-test at 95% significant level with 4 degree of freedom.

5.4.2.5. Effects on sensory properties

In general, odour and taste scores for crude and orange roughy oils showed a similar response to the multiple refining treatment, as shown in Figures 5.9 and 5.10.

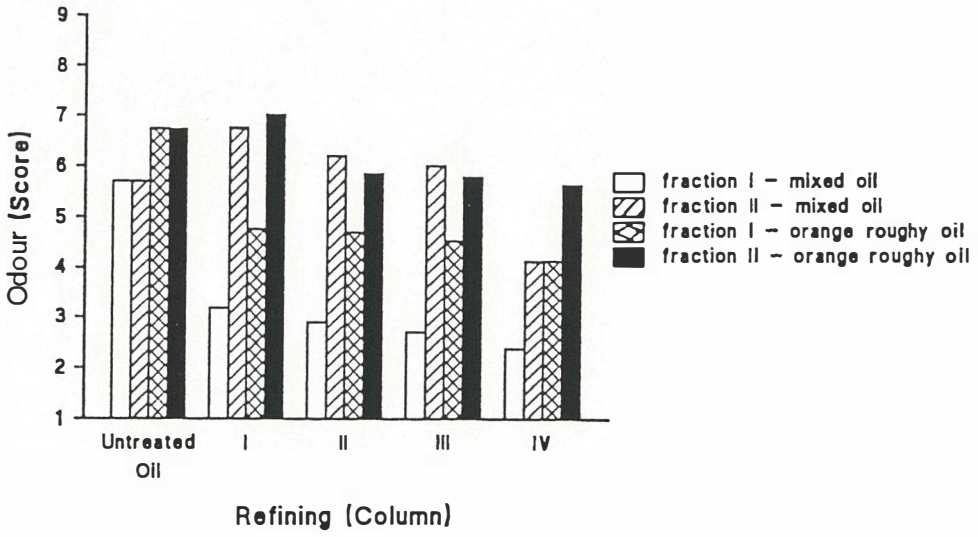


Figure 5.9. Effects of multiple refining on odour score of fish oil

The first refining resulted in fraction-1 oils having significantly lower odour and taste scores, reflecting a sensory improvement. Further refining tended to give lower scores,

indicating a better quality, but the changes were statistically insignificant.

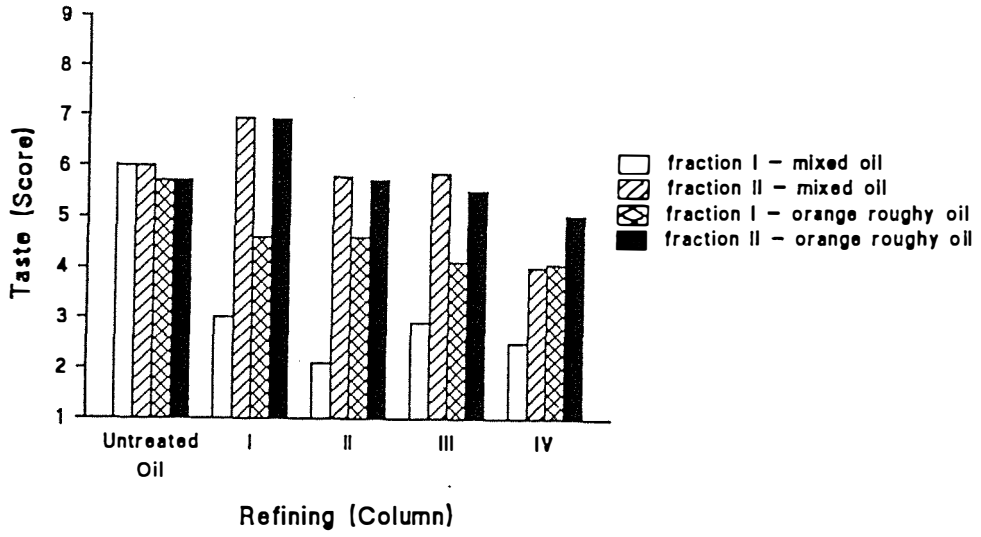


Figure 5.10. Effects of multiple refining on taste score of fish oil

The first refining produced fraction-2 oils with odour and taste scores significantly higher than unrefined oils. These scores tended to be lower compared to the fraction-2 oils obtained from further refining. In general, multiple refining resulted in the fraction-2 oils acquiring a more unpleasant odour and taste quality in comparison to fraction-1 oils.

5.4.3. Effects of vacuum pressure application on fish oil quality

Application of vacuum pressure at one end of the column was aimed at speeding the refining process.

5.4.3.1. Effects on free fatty acid value

Free fatty acid (FFA) values of fraction-1 crude and orange roughy oils showed a different response to vacuum pressure treatment as shown in Figure 5.11. However the FFA values

of both fraction-1 oils yielded from refining with and without vacuum pressure had a significantly lower FFA value than unrefined oils.

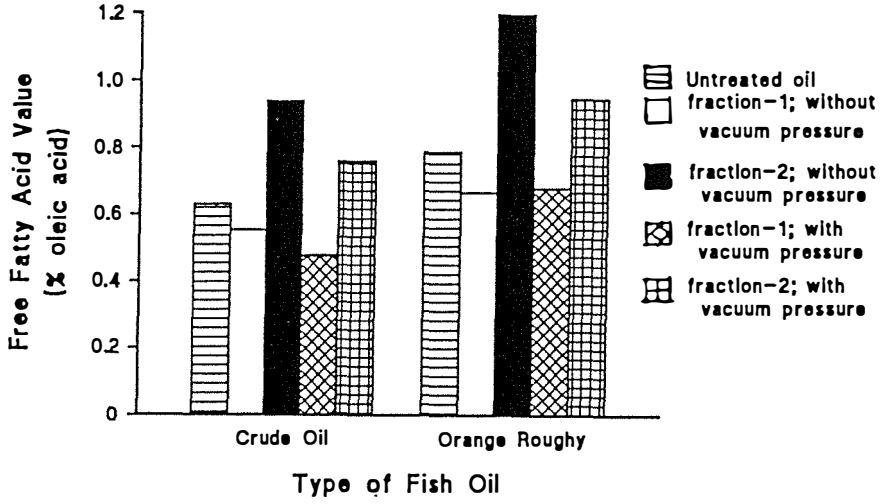


Figure 5.11. Effects of vacuum pressure during refining on free fatty acid value of fish oil

The fraction-1 crude oil obtained from the refining with vacuum pressure application exhibited a lower FFA value than the fraction-1 crude oil from refining without vacuum pressure. The FFA values of fraction-1 orange roughy oil obtained from refining with and without pressure did not show any significant different.

Fraction-2 crude and orange roughy oils exhibited the same response to the vacuum pressure treatment in terms of FFA value. The FFA values of fraction-2 oils were significantly higher than the value in unrefined oils. The FFA value of fraction-2 oils from refining without pressure was higher than the value analyzed in fraction-2 oils from refining with vacuum pressure.

In general, both refining with and without vacuum pressure application resulted in fraction-2 oils having a higher FFA values than fraction-1 oils.

5.4.3.2. Effects on refractive index

Refractive index (RI) values of fraction-1 crude and orange roughy oils from refining with and without vacuum pressure was markedly lower than the RI values of unrefined and fraction-2 oils as shown Figure 5.12. Crude oil refined using vacuum pressure yielded fraction-1 oil having a lower RI value than fraction-1 oil obtained from refining without pressure. However the fraction-1 orange roughy oil refined with and without vacuum pressure showed no significant differences in the RI value.

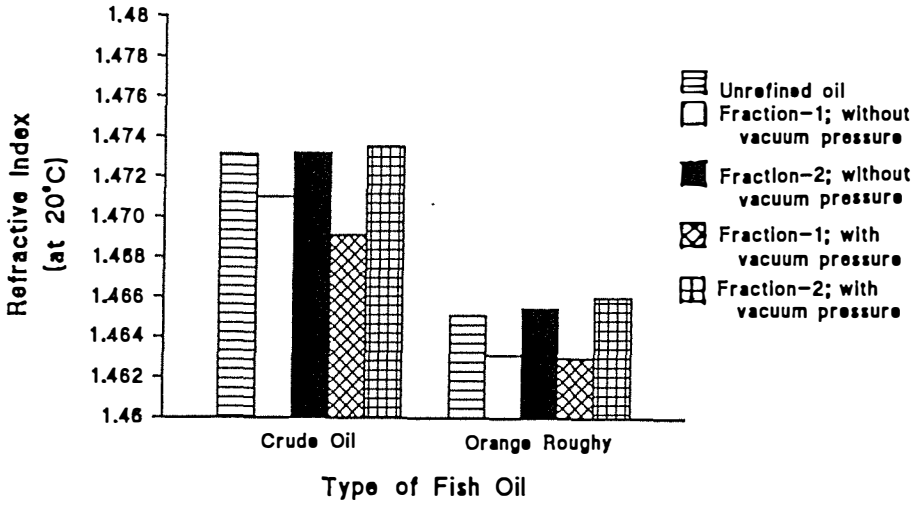


Figure 5.12. Effects of vacuum pressure during refining on refractive index value of fish oil

The RI values of fraction-2 oils obtained from refining with and without vacuum pressure were similar, and these values were relatively the same as measured in unrefined oils.

5.4.3.3. Effects on colour absorbance

In terms of colour absorbance value, crude and orange roughy oils showed a different response to vacuum pressure treatment during refining as shown in Figure 5.13.

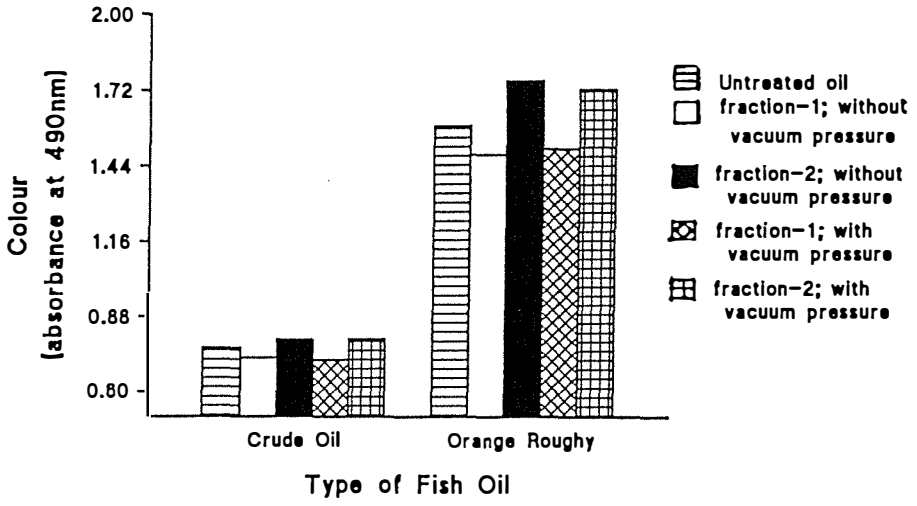


Figure 5.13. Effects of vacuum pressure during refining on colour absorbance value of fish oil

Fraction-1 oils from refining with and without pressure had a lower colour absorbance value compared to unrefined and fraction-2 oils, and the values of fraction-2 oils obtained from both refining methods were relatively similar.

The colour absorbance values of fraction-2 oils were markedly higher than the values analyzed in unrefined oils. Vacuum pressure treatment resulted in fraction-2 oils having a colour absorbance which was relatively similar to the value observed in fraction-2 oil from refining without pressure application.

5.4.3.4. Effects on fatty acid profile

Fatty acid profile changes during refining with and without pressure are shown in Tables 5.5 and 5.6.

Table 5.5. Effect of vacuum pressure during resin refining on fatty acid profile of crude fish oil (% fatty acid)

Fraction	Vacuum treatment	SAFA	MUFA	PUFA	EPA	DHA	Omega-3 f.a.
Untreated oil		19.4	62.6	18.0	4.6	7.7	14.9
I	Without Vacuum	20.0	62.2	17.8	4.4	7.7	14.7
	With Vacuum	20.0	62.0	18.0	4.4	7.7	14.8
II	Without Vacuum	20.0	62.5	17.4	4.4	7.7	14.6
	With Vacuum	19.8	62.6	17.6	4.6	7.3	14.4

Table 5.6. Effect of vacuum pressure during resin refining on fatty acid profile of orange roughy oil (% fatty acid)

Fraction	Vacuum Treatment	SAFA	MUFA	PUFA	EPA	DHA	Omega-3 f.a.
Untreated oil		6.2	85.3	8.4	2.1	1.7	5.5
I	Without Vacuum	6.3	85.6	8.0	1.9	1.9	5.1
	With Vacuum	6.2	85.9	7.8	1.8	1.9	5.0
II	Without Vacuum	6.8	84.7	8.5	2.1	2.1	5.4
	With Vacuum	6.7	85.6	7.7	1.8	2.1	5.0

Vacuum pressure treatment did not induce any significant effect on the fatty acid profiles of crude oil. Relative quantities of MUFA, PUFA, omega-3 fatty acids and EPA in orange

roughy oil relatively unchanged during refining. However relative amounts of SAFA in fraction-2 orange roughy oil was higher than the amount in fraction-1 oil. Analysis of variance indicated that DHA value of both fraction-1 and fraction-2 orange roughy oils from refining with and without vacuum pressure was relatively higher than the value in unrefined oil.

5.4.3.5. Effects on sensory properties

Fraction-1 crude and orange roughy oils obtained from refining with and without vacuum pressure had better odour and taste properties compared to the unrefined oils as shown in Figures 5.14 and 5.15. Vacuum pressure application did not result in any differences in odour and taste scores between fraction-1 oil from refining with and without vacuum pressure. However the odour and taste scores of fraction-2 oils were higher than the scores of fraction-1 oils, indicating that the odour and taste of fraction-2 oils were more unpleasant than those of fraction-1 oils.

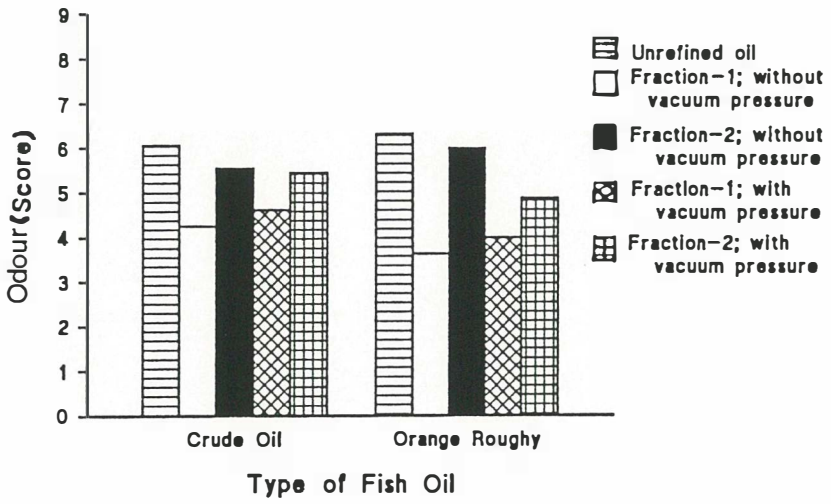


Figure 5.14. Effects of vacuum pressure during refining on odour score of fish oil

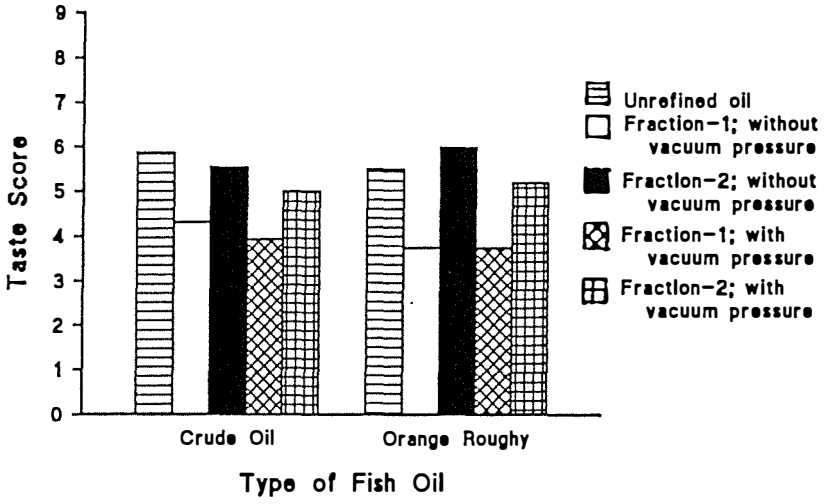


Figure 5.15. Effects of vacuum pressure during refining on taste score of fish oil

5.4.4. Effects of column size on fish oil quality

5.4.4.1. Effects of various height sizes of resin packed column

The diameter of the column was kept constant at 2.60cm while the column height was varied to 5, 10, 15 and 20 times diameter size.

Fraction-1 oils obtained from all diameter-height ratios had a significant lower free fatty acid (FFA) value compared to the unrefined oil, as shown in Figure 5.16. This trend showed that the higher the height the lower the FFA value, but varying height size in this

study did not induce any significant statistical different in the FFA value among fraction-1 oils. However treatment of various height column sizes affected the FFA value of fraction-2 oils: the higher the height the higher the FFA value. In general, the results indicated that the FFA value of fraction-2 oils was higher than the value analyzed in unrefined and fraction-1 oils.

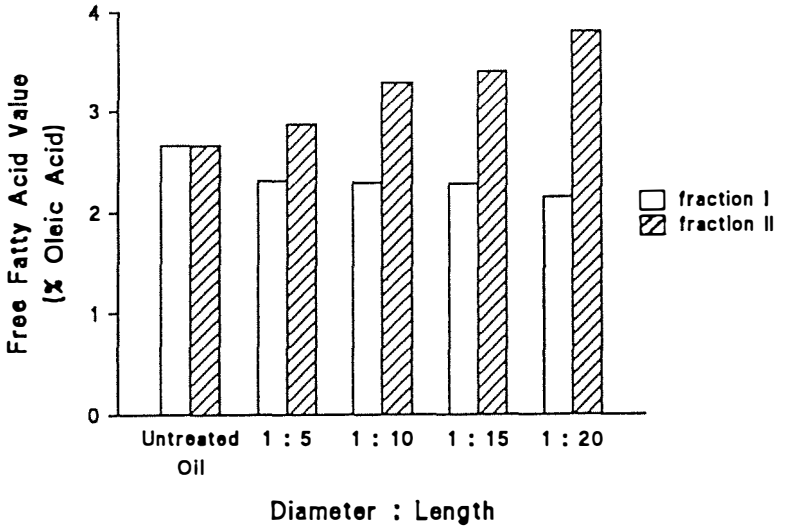


Figure 5.16. Effects of various height-diameter ratios of column on free fatty acid value of fish oil

As occurred in the FFA analysis, the RI values of fraction-1 oils were lower than the value analyzed in fraction-2 and unrefined oils as shown in Figure 5.17. The various height sizes did not affect the RI values of fraction-1 oils. The RI values of fraction-2 oils from refining with diameter and height ratio of 1:5 was lower than the value analyzed in fraction-2 oil from refining with the height size 10, 15 and 20 times diameter size.

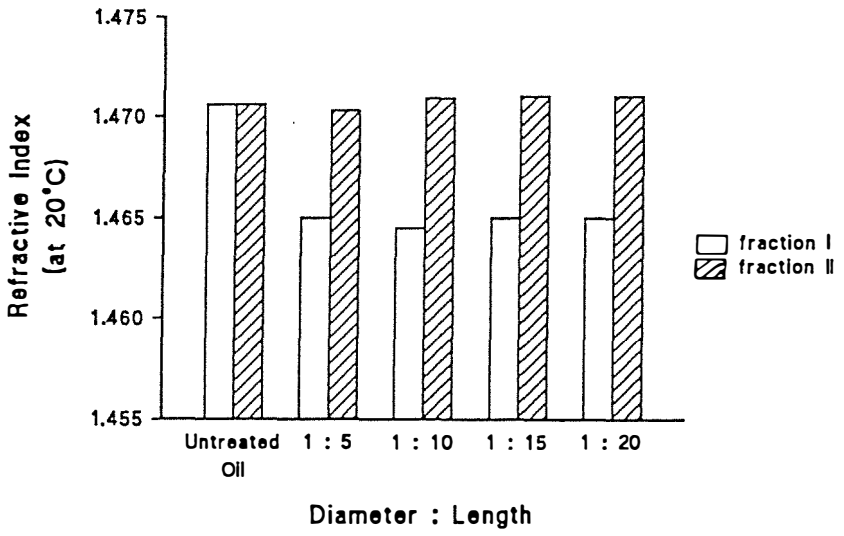


Figure 5.17. Effects of various height-diameter ratios of column on refractive index value of fish oil

As shown in Figure 5.18, the colour absorbance of crude oil could be reduced by passing the oil through the resin column with diameter-height ratio 1:5. When the height size of the column was extended to 10 times diameter size, the colour absorbance value of fraction-1 oil was lower than the value of fraction-1 oil from refining with column diameter-height ratio 5:1. The colour absorbance value would be relatively unchange, even though the column height size was extended to 15 and 20 times diameter size.

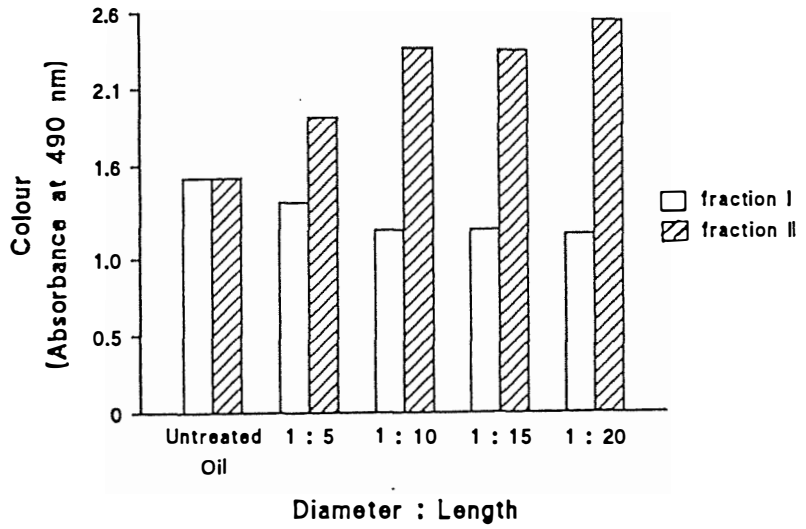


Figure 5.18. Effects of various height-diameter ratios of column on colour absorbance value of fish oil.

The increasing height size resulted in an increase in absorbance value of fraction-2 oils. The colour absorbance values of fraction-2 oil from refining with column diameter-height ratios 1:10 and 1:15 were insignificantly different. Those values were higher than the colour absorbance value of fraction-2 oils from refining with column diameter-height ratio 1:5, but lower than the value of fraction-2 oil from refining with column diameter-height ratio 1:20.

Results of fatty acid profile analysis are shown in Table 5.7. Analysis of variance showed that treatment of various height size did not produce any significant changes in relative quantities of saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acids in unrefined, fraction-1 and fraction-2 oils.

Table 5.7. Effects of height size of resin packed column on fatty acid profile of crude oil (% fatty acid)

Fraction	diameter: height	SAFA	MUFA	PUFA	EPA	DHA	Omega-3 f.a.
Untreated oil		21.0	59.3	19.7	5.5	7.8	17.0
I	1 : 5	22.8	58.2	18.9	5.1	7.5	16.2
	1 : 10	21.7	58.5	19.8	5.4	8.2	17.1
	1 : 15	18.8	60.5	20.7	6.1	8.2	17.7
	1 : 20	19.5	60.1	20.4	5.9	7.8	17.5
II	1 : 5	20.3	58.8	20.9	5.8	8.1	18.1
	1 : 10	17.1	60.4	22.5	6.2	9.3	19.6
	1 : 15	17.4	60.7	21.9	5.7	8.9	18.9
	1 : 20	19.4	59.4	21.2	5.9	8.2	18.1

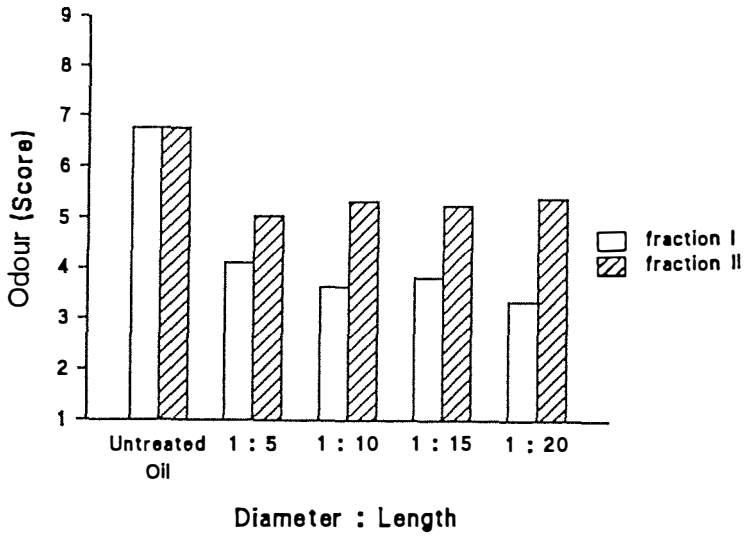


Figure 5.19. Effects of various height-diameter ratios of column on odour score of fish oil

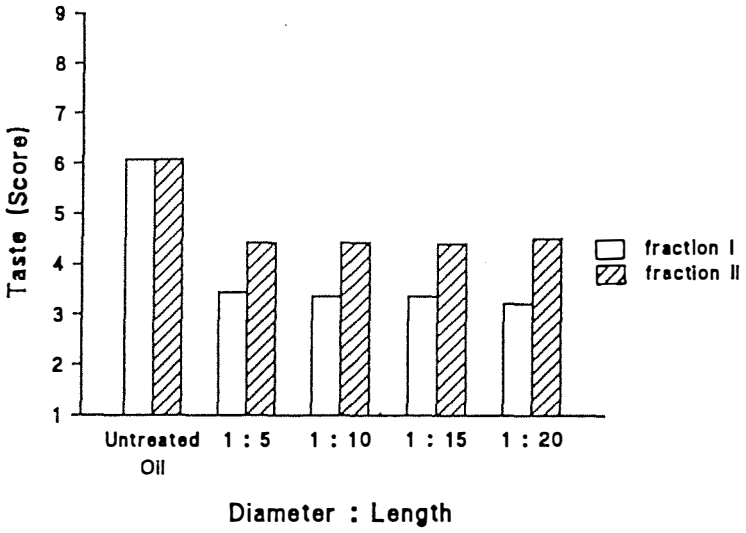


Figure 5.20. Effects of various height-diameter ratios of column on taste score of fish oil

Odour and taste scores of fraction-1 and fraction-2 oils from refining with all diameter-height ratios were lower than the scores observed in unrefined oil. However the odour and taste scores of fraction-1 oils were significantly lower than the scores of fraction-2 oils as shown Figure 5.19 and 5.20. The diameter-height ratio treatment did not result in any significant difference in odour and taste scores in fraction-1 or fraction-2 oils.

5.4.4.2. Effects of various diameter sizes of resin packed column

In this experiment, the column height was kept constant at 39cm. The column diameter sizes investigated were 1.65, 2.60 and 3.60cm.

Figure 5.21 shows the changes of FFA values in fraction-1 and fraction-2 oils obtained from

refining with various column diameter sizes. FFA values of fraction-1 oils obtained from refining with all column diameter sizes were significantly lower than the value of unrefined oils, while the FFA values of fraction-2 oils were significantly higher than the values of unrefined oil. Refining with various diameter sizes did not result in any significant differences in FFA values of fraction-1 oils or fraction-2 oils. FFA values for fraction-2 oils obtained from refining with column diameter sizes 2.6 and 3.2 cm were significantly higher than the values for unrefined oil.

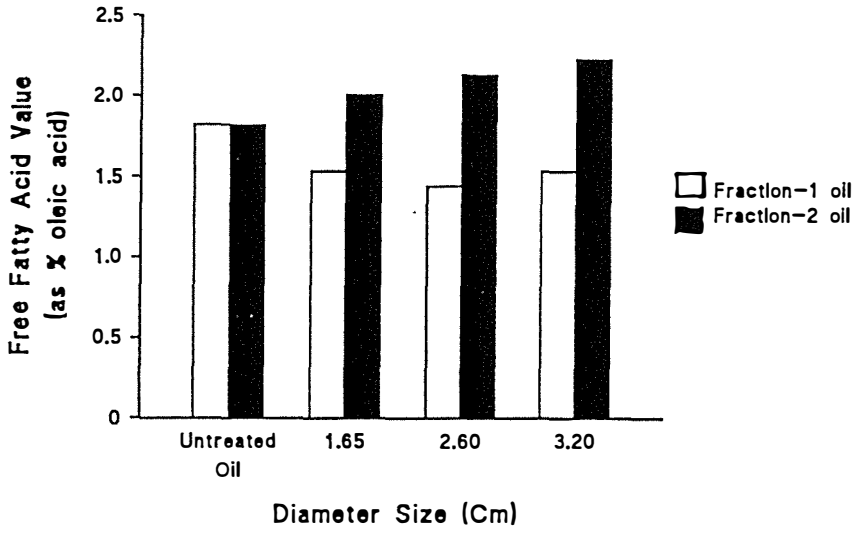


Figure 5.21. Effects of various diameter sizes of column on free fatty acid value of fish oil

Refractive index values of fraction-1 oils from refining with all column diameter sizes were significantly lower than the value measured in unrefined and fraction-2 oils as shown in Figure 5.22. The values analyzed in fraction-1 oil from refining with column diameters 2.6 and 3.2cm were markedly lower than the value observed in fraction-1 oil from refining with column diameter size 1.65cm, while the RI values of fraction-2 oils from refining with all column diameter sizes were similar to the value of unrefined oils.

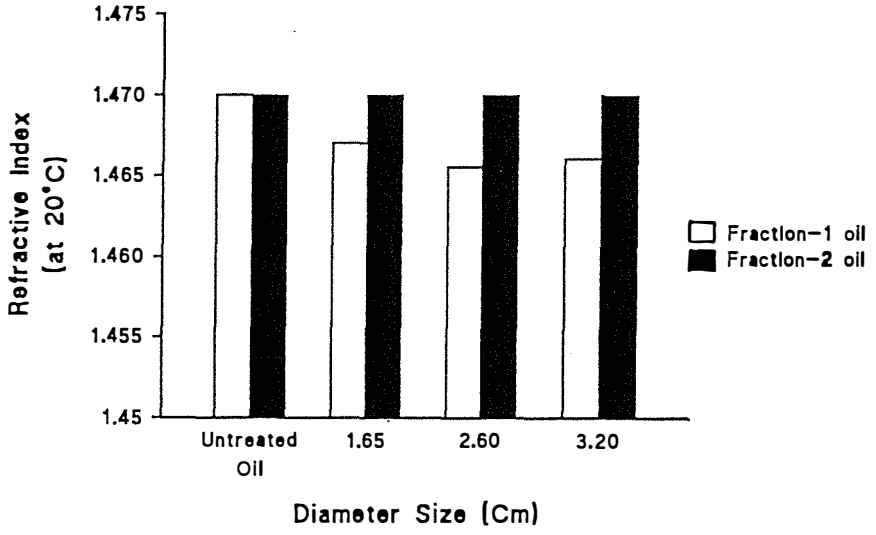


Figure 5.22. Effects of various diameter sizes of column on refractive index value of fish oil

Results of colour absorbance value of untreated, fraction-1 and fraction-2 oils are shown in figure 5.23. All column diameter sizes resulted in fraction-1 oils with obviously lower absorbance values than unrefined oils. The bigger diameter sizes tended to give a lower colour absorbance value in fraction-1 oils, but this was statistically insignificant. Colour absorbance value of fraction-2 oils from all refining was significantly higher than the value of unrefined oil. The colour absorbance values of fraction-2 oil from refining with column diameters 2.6 and 3.2cm were significantly higher than the value observed in fraction-2 oils from refining with a column diameter of 1.65cm.

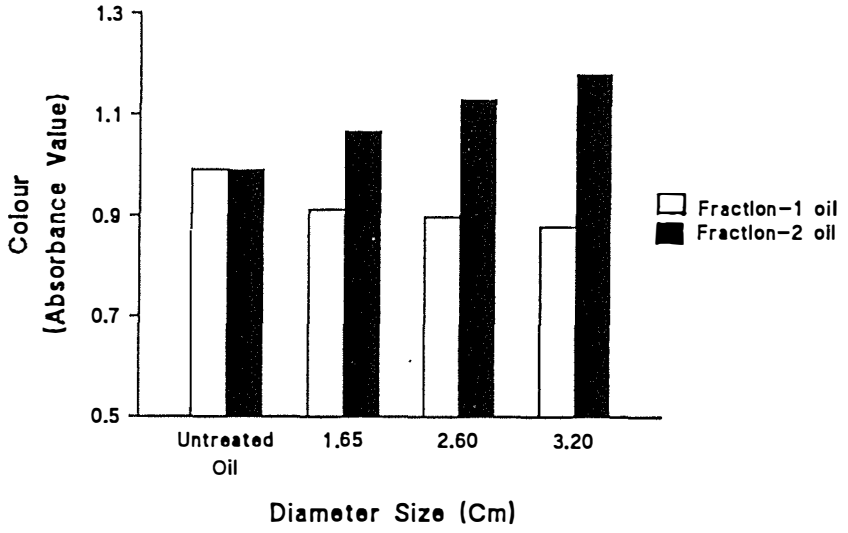


Figure 5.23. Effects of various diameter sizes of column on colour absorbance value of fish oil

Sensory evaluation results for odour and taste performed by eight Indonesian trained panellists are shown in Figures 5.24 and 5.25. Both odour and taste scores for fraction-1 oils, from all refinings, were significantly lower than the scores for unrefined oil. A lower score reflected a better acceptance by panellists. The odour and taste scores of fraction-1 oils obtained from refining using resin column with all diameter sizes, were insignificantly different. In general, the fraction-1 oils showed a better performance in odour and taste compared to the fraction-2 oils. However the odour and taste scores for fraction-2 oils from all refining was insignificantly different from the scores for unrefined oil.

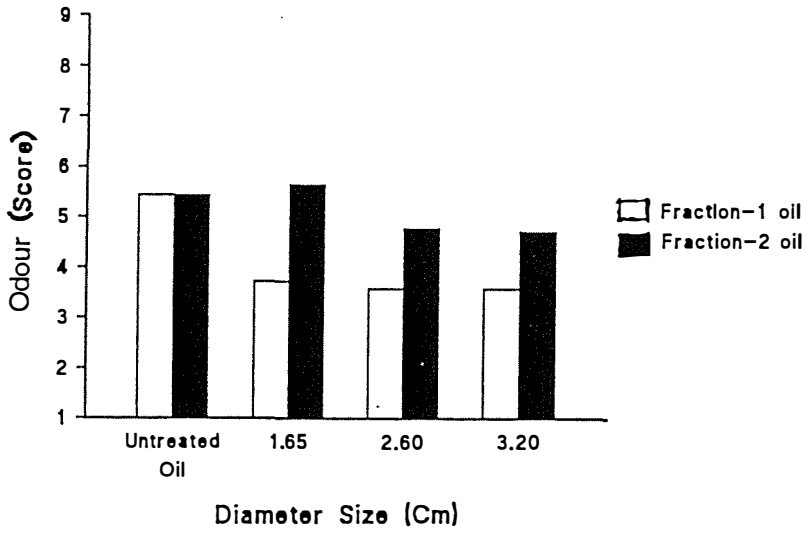


Figure 5.24. Effects of various diameter sizes of column on odour score of fish oil

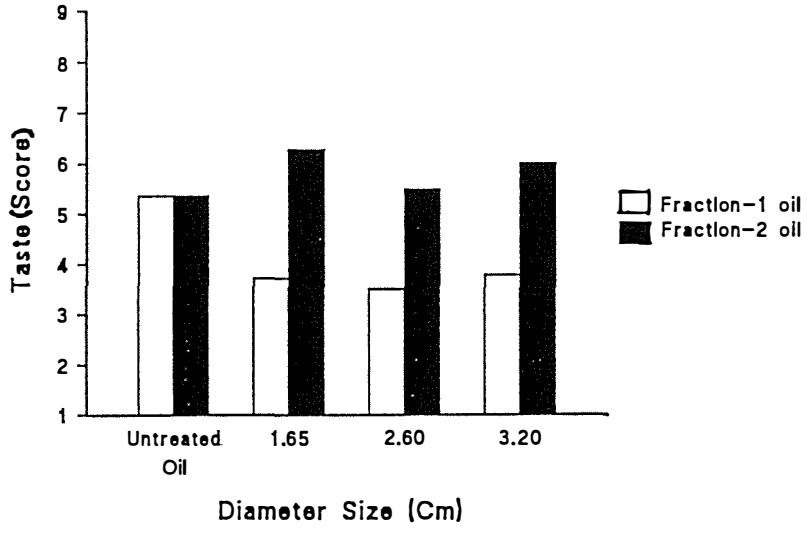


Figure 5.25. Effects of various diameter sizes of column on taste score of fish oil

5.4.5. Effects of resin refining on natural antioxidant contents of fish oil

As shown in Table 5.8, the natural antioxidants traced in crude and orange roughy oils were α-tocopherol and γ-tocopherol accompanied by α-tocomonoenol. Both oils consisted of mainly α-tocopherol. The natural antioxidant content (tocopherol group) decreased significantly during the resin refining process.

Table 5.8. Changes of natural antioxidant content of fish oil during resin refining process (ppm) (detection limit = 2-4% of value)

Fish oil	Treatment	α-tocopherol	γ-tocopherol	α-tocomonoenol
Crude oil	Unrefined	210.3	5.1	18.4
	Refined	179.8	4.5	13.1
Orange roughy	Unrefined	122.3	4.2	6.3
	Refined	106.8	3.6	5.0

5.4.6. Effects of resin refining on volatile flavour compounds of fish oil

Traces of volatile flavour compounds before and after refining treatment are shown in Figures 5.26, 5.27, 5.28, and 5.29. The relative quantity changes of volatile flavour compounds during refining are shown in Tables 5.9 and 5.10. Most of those compounds have been identified in fish and fish oil by other researchers as cited in footnotes to each Table.

The results showed that the volatile flavour compounds identified in crude oil consisted of 19 hydrocarbons, 1 alcohol, 5 esters, 2 aldehydes and 1 acid. Before refining treatment, methyl ethyl benzoate was the most abundant compound in the volatile flavour of unrefined crude oil followed by 1,1-dimethylethyl-2-methyl propionic acid and ethyl benzoate. After refining treatment, the relative amounts of most compounds decreased. However the relative amounts of methyl ethyl benzoate, 1,1-dimethylethyl-2-methyl propionic acid and ethyl benzoate were still high, while toluene increased significantly which indicates that this might be one of the most responsible compound of volatile flavour in refined crude oil.

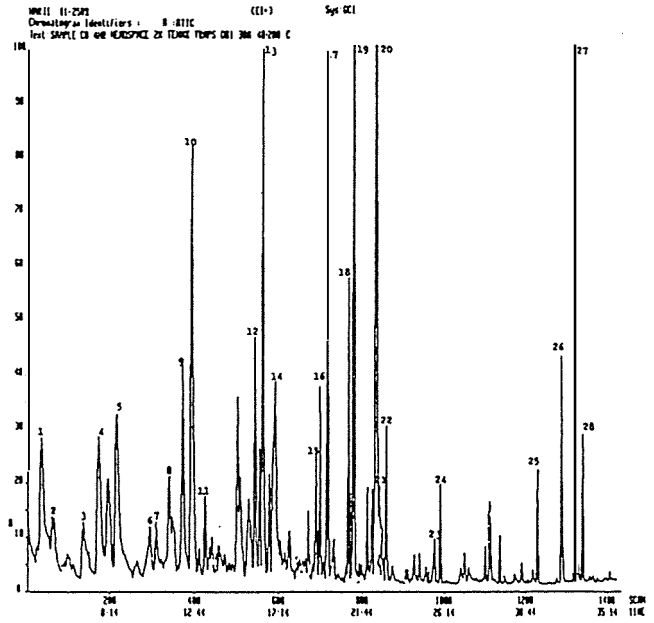


Figure 5.26. Traces of volatile flavour compounds of unrefined crude oil

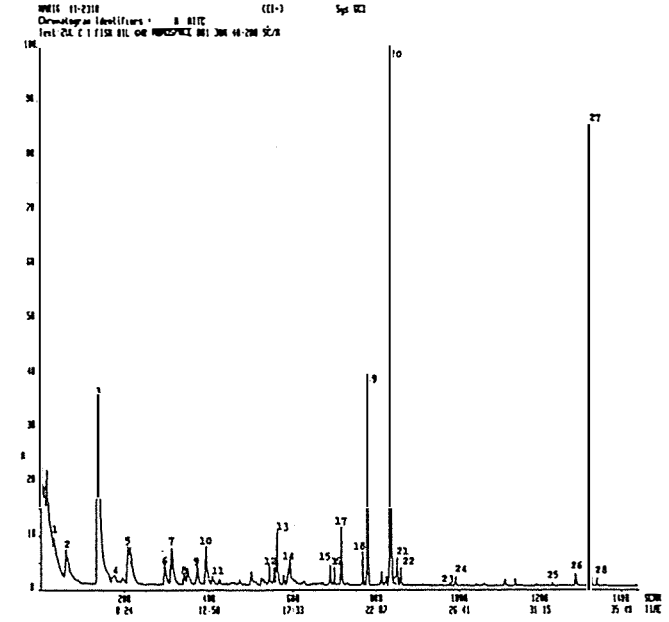


Figure 5.27. Traces of volatile flavour compounds of refined crude oil

Table 5.9. Relative amounts of volatile flavour compounds of crude oil during refining

Peak No.	Volatile flavour compounds	Relative amount (%)	
		Before refining	After refining
	Hydrocarbons		
2	heptane **b)**	1.0	3.4
3	toluene (methyl benzene) **a) b) h)**	1.7	23.2
5	octane **a) b) c) d)**	3.1	3.6
6	ethyl benzene **c) d)**	0.4	1.8
7	1,3-dimethyl benzene (m-xylene) **i)**	0.9	3.0
8	ethynyl benzene **f)**	1.8	1.0
9	1-nonene	2.9	1.6
10	nonane **i)**	4.7	2.0
12	1-decene **h) i)**	2.2	1.1
13	decane **e) g) i)**	3.8	1.7
14	limonene **a) c) d)**	3.8	1.5
16	1-undecene	1.5	0.7
17	undecane **e)**	3.8	1.7
21	1-dodecene	0.6	0.7
22	dodecane **e) f) i)**	1.4	0.6
23	1-tridecene **i)**	0.4	0.2
24	tridecane **f)**	0.7	0.3
25	pentadecane **e) h)**	0.8	0.2
28	hexadecane **c) d) e)**	1.1	4.9
	Alcohols		
1	1-penten-3-ol **a) d) f) g) i)**	2.5	1.8
	Esters		
11	butyl ester-2-hydroxy propionate	0.8	0.3
18	ethyl hexyl acetate	1.8	0.9
19	ethyl benzoate	10.4	7.3
20	methyl ethyl benzoate	29.5	19.4
26	diethylphtalate	2.4	2.2
	Carbonyls/aldehydes		
4	2-methyl-4-pentanal	2.4	0.9
15	1-nonanal **e) f) g) i)**	0.8	0.3
	Acids		
27	1,1-dimethylethyl-2-methyl propionic acid	12.4	13.4

Note: a) identified in crayfish waste by Tanchotikul and Hsieh (1989)
b) identified in haddock flesh by Angelini and Merritt (1975)
c) identified in crayfish tail meat by Vejaphan et al (1988)
d) identified in marine green algae by Sugisawa et al (1990)
e) identified in menhaden fish oil by Hsieh et al (1989)
f) identified in whitefish by Josephson et al (1983)
g) identified in cod liver oil by Karahadian and Lindsay (1989)
h) identified in salmon by Josephson et al (1991)
i) identified in tuna oil by Crawford et al (1976)

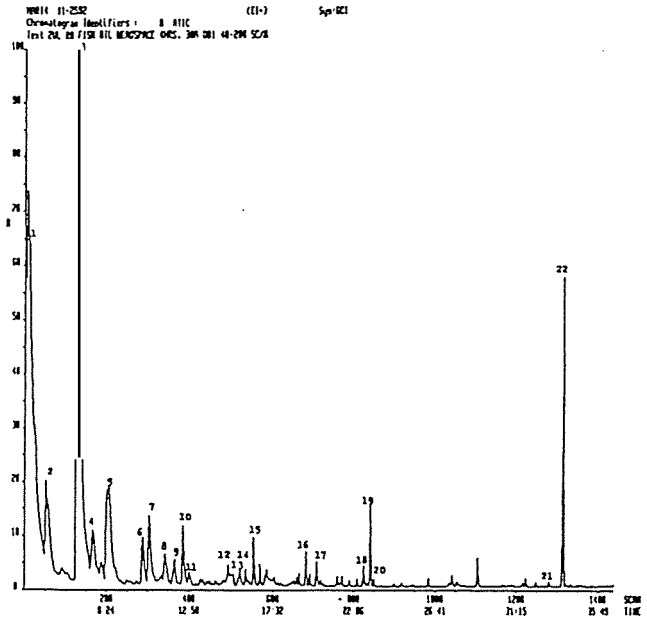


Figure 5.28. Traces of volatile flavour compounds of unrefined orange roughy oil

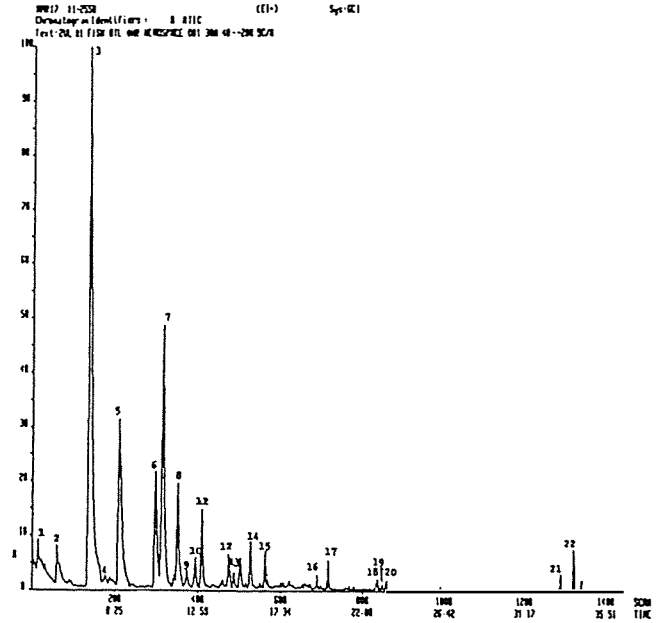


Figure 5.29. Traces of volatile flavour compounds of refined orange roughy oil

Table 5.10. Relative amounts of volatile flavour compounds of orange roughy oil during refining

Peak No.	Volatile flavour compounds	Relative amounts (%)	
		Before Refining	After Refining
	Hydrocarbons		
1	cyclohexane **i)**	6.4	3.6
2	3-methyl hexane	1.9	0.9
3	toluene (methyl benzene) **a) b) h)**	51.5	42.3
4	heptane **b)**	4.0	0.9
6	ethyl benzene **c) d)**	3.3	8.3
7	1,3-dimethyl benzene (m-xylene) **i)**	5.2	16.4
8	1,4-dimethyl benzene (p-xylene) **i)**	2.2	6.1
10	nonane **i)**	2.2	1.1
11	1-methylethyl benzene **i)**	0.8	3.8
12	1-ethyl-3-methyl benzene	0.2	1.2
13	1,3,5-trimethyl benzene **a) c) e)**	1.4	1.0
14	1,2,4-trimethyl benzene **a) c) e)**	1.0	1.8
15	decane **e) i)**	1.2	1.0
17	undecane **e) i)**	0.5	0.6
20	dodecane **e) f) i)**	0.3	0.2
	Alcohols		
9	2-butoxy ethanol **c)**	2.0	1.0
	Esters		
19	octyl acetate **i)**	1.6	0.1
21	diethyl phtalate	0.3	0.5
	Carbonyls/aldehydes		
16	1-nonanal **e) f) g) i)**	0.9	0.2
	Acids		
18	1-methylethyl ester benzoic acid	0.7	0.3
22	1,1-dimethylethyl-2-methyl propionic acid	5.7	0.9
	Halogens		
5	tetrachloroethene	6.8	7.5

Note: a) identified in crayfish waste by Tanchotikul and Hsieh (1989)
b) identified in haddock flesh by Angelini and Merritt (1975)
c) identified in crayfish tail meat by Vejaphan et al (1988)
d) identified in marine green algae by Sugisawa et al (1990)
e) identified in menhaden fish oil by Hsieh et al (1989)
f) identified in whitefish by Josephson et al (1983)
g) identified in cod liver oil by Karahadian and Lindsay (1989)
h) identified in salmon by Josephson et al (1991)
i) identified in tuna oil by Crawford et al (1976)

The volatile flavour compounds identified in orange roughy oil were 15 hydrocarbons, 1 alcohol, 2 esters, 1 aldehyde, 2 acids and 1 halogen. The volatile flavour compounds encountered in high proportion were toluene (51.5%), cyclohexane (6.4%), 1,1-dimethylethyl-2-methyl propionic acid (5.7%) and tetrachloroethene (6.8%). These results indicate that toluene seemed to be responsible for flavour performance. After refining, most volatile flavour compounds showed a decrease in relative quantities. Volatile flavour compounds exhibiting a significant increase in the relative quantities were m-xylene, p-xylene and 1-methylethyl benzene. The relative quantity of toluene decreased, due to refining treatment, but was still the most abundant compound (42.3%) in refined orange roughy oil.

5.5. DISCUSSION

Refining aims to improve fish oil quality by removing impurities. These impurities can be broadly subdivided into three types: insoluble, colloidal and soluble. The insoluble impurities are moisture, rust, dirt and protein. Protein can also be present in colloidal suspension as phosphatides and carbohydrates. The soluble compounds are pigments, oxidation products, trace metals, phosphatides, sulphur and nitrogenous chemicals, free fatty acids, mono and di-glycerides and unsaponifiable matter which is principally wax (Young, 1982; Windsor and Barlow, 1981). Some of the above impurities are used as parameters to determine the quality of refined fish oil in this study.

5.5.1. Effects of resin refining on chemical properties of fish oil

Free fatty acid (FFA) content of crude and orange roughy oils was reduced significantly by refining using a resin packed column. FFA content reduction occurred at all fish oil-resin volume ratios, and even more reduction was achieved with multiple refining by passing the oil through a clean column more than once. Increasing the refining rate by application of vacuum pressure at one end of the column had the same effect on FFA content reduction as shown in the refining without vacuum pressure application. Fraction-2 oils always had a higher FFA content than the fraction-1 oils. This indication demonstrated that free fatty acids might be binding with the resin, rather than being trapped by resin pores.

In terms of the column size, the height of the column showed more effect on FFA content of the oil than the diameter size. The higher the column size, the lower the FFA content of the refined oil obtained. Various diameter sizes tested in this study tended to produce refined oil with similar FFA content. The higher column size may have given more opportunity for the oil to contact with the resin, since the oil had to pass through more resin.

The reduction of the FFA content of fish oil due to the refining process was also noted by Koning and Milkovitch (1984a; 1984) using ethanolamine and glycerol. The same occurrence was also registered in refining vegetable oils using ordinary refining procedures, such as in coconut oil (Gordon and Rahman, 1991); soya bean oil (Sleeter, 1981); sunflower oil and rapeseed oil (Balicer et al, 1983).

As expected, resin refining did not induce any significant change in polyunsaturated fatty acid (PUFA), particularly omega-3 fatty acid, in the refined oil as compared to the unrefined oil. The same tendency was noted by Fernandez (1986) in pink salmon oil, using the same refining procedure. This result demonstrated the superiority of the use of cation-strong acid-resin packed column for fish oil refining over molecular distillation, in which ω-3 fatty acids were chemically modified by molecular distillation process (Fernandez, 1986). The PUFA and ω-3 fatty acid of fraction-2 oil were insignificantly different from the value in unrefined and fraction-1 oil. This indicated that the triglyceride might not bind with the resin, but were, rather, caught by the resin pores.

Natural antioxidants α-tocopherol and γ-tocopherol in the oils reduced in quantity as a result of the resin refining process. The decreased levels of both antioxidants were approximately 12 - 14%. α-tocomonoenol content of the oil decreased after refining, but the ability of α-tocomonoenol to inhibit oxidation is still unknown. The decrease of tocopherol in refined fish oil was also noted in cod liver oil and sprat oil refining using soda lye (Brzeska and Salmonowicz, 1973), and menhaden fish oil refining using different methods (Scott and Catshaw, 1991). The refining process also reduced tocopherol content in vegetable oils such as soya oil processed using degumming, neutralization, bleaching and deodorisation (Gutfinger and Letan, 1974, Sleeter, 1981); combined sunflower oil and rapeseed oil refined using acidification/neutralization, washing, drying, decolouration and deodorization (Ludwiki et al, 1986); soybean oil refined with activated carbons (Boki et al, 1991) and coconut oil refined using alkali refining, degumming, bleaching and deodorization (Gordon and Rahman, 1991).

If the storage treatment must be carried out the loss of tocopherol should be noted as a precaution. Brzeska and Salmonowicz (1973) reported that refined fish oil having a lower natural tocopherol content than unrefined oil showed less resistance to oxidation than unrefined oil.

5.5.2. Effects of resin refining on physical properties of fish oil

The refractive index (RI) of fish oil decreased after refining with a cation-strong acid-resin packed column. The RI of an oil is characteristic within certain limits for each type of oil. The RI is also used as a measure of purity of the oil (Rossel, 1986). The lower the RI value, the higher the purity level of the oil, a purity which can be further improved by multiple refining. The height and diameter sizes of the column did not affect the RI value of refined oil, since the values obtained from various height and diameter ratios were practically similar. Both refining with and without vacuum pressure applications showed the similar effects on the RI value of refined oils.

Since the impurities in fish oil are very complex, the all resin functions probably acted to trap the impurities. Operations which may be performed by resin are ion exchange, adsorption, molecular sieving and gel permeation (Pery et al, 1973). Meanwhile strong acid cation exchange might separate peptides, amino acids, cations and metals (Bio Rad Laboratories Pty Ltd, 1990).

The colour of fish oil was significantly improved by the resin refining process. The colour of fraction-2 oil was always worse than the colour of refined and unrefined oils. Further improvement in oil colour could be obtained by using the multiple refining method. Vacuum pressure application resulted in refined oil with comparable colour quality to the refined oil obtained from resin refining without vacuum. The effect of height-diameter size ratio was noted until the ratio of 10:1. Further height size enlargement produced oil without further pronounced colour improvement. Diameter size did not markedly affect the colour quality of the oil. Carotenoids contributing mainly to fish oil pigment have unsaturated linkages occurring between alternative pairs of carbon atoms in a long multiple branched chain (Fox, 1957). The larger number of double bonds within the molecules resulted in a

higher probability of weak bonds forming at the intramolecular level. These intramolecular bonds are hydrogen bonds and/or Van der Walls force, and/or polar bonds (Bottino et al, 1967). These have a greater tendency to form bonds making oil more likely to interact with a macroporous resin backbone (Fernandez, 1986). This indication was shown in this study. The fraction-2 oils had a much darker colour than fraction-1 oils and unrefined oils, in which fraction-2 oils seemed to have a higher concentration of carotenoids separated by resin.

5.5.3. Effects of resin refining process on volatile flavour compounds

5.5.3.1. Volatile flavour compounds in fish oil

The two unrefined New Zealand oils studied had different compounds responsible for their volatile flavour. As shown in Tables 5.9 and 5.10 the compounds identified in both oils have been identified in fish, fish oils and marine green algae by Tanchotikul and Hsieh (1989), Angelini and Merritt (1975), Vejaphan et al (1988), Sugisawa et al (1990), Hsieh et al (1989), Josephson et al (1983), Karahadian and Lindsay (1989), Josephson et al (1991) and Crawford et al (1976). The compounds contributing to the volatile flavour of crude oil were methyl ethyl benzoate (29.5%), ethyl benzoate (10.4%) and 1,1-dimethylethyl-2-methyl propionic acid (12.4%). These compounds were probably responsible for the strong fishy odour and taste in unrefined oil as evaluated by trained Indonesian panellists. Ethyl benzoate was also found as a volatile flavour in plums (Dirninger, 1989), providing aromatic odour (Stecher et al, 1968). Alkane compounds of heptane, undecane and dodecane found in the unrefined oil were encountered in vanilla aroma (Vidal et al, 1989). Nonane, tridecane, pentadecane and hexadecane detected in this fish oil were found in plums (Etievant et al, 1986; Dirninger et al, 1989). Octane and dodecane traced in crude oil also contributed to the aroma of strawberries (Belitz and Grosch, 1987). Limonene, as analyzed in crude oil, was also detected in plums (Dirninger, 1989).

Toluene (51.5%) was noted as the most abundant volatile flavour compounds in unrefined orange roughy. Other compounds encountered at a significant level were cyclohexane (6.4%), 1,1-dimethylethyl-2-methyl propionic acid (5.7%) and tetrachloroethane (6.8%). According to panellists, orange roughy oil, having these as major volatile flavour compounds, was detected as having a significantly less fishy odour and taste than crude oil.

Toluene was traced in the aroma of vanilla as well (Vidal et al, 1989) imparting a benzene like odour (Stecher, 1968) or a plastic like odour (Tanchotikul and Hsieh, 1989). Both m-xylene and p-xylene detected in orange roughy were also found in plums (Dirninger et al, 1989). Octyl acetate in volatile flavour of orange roughy also contributed to strawberry aroma (Belitz and Grosch, 1987). Tetrachloroethene, giving a chloroform like odour (Stecher et al, 1968), was encountered in the orange roughy volatile flavour compounds while other halogen compounds such as dichloromethane and trichloromethane are also reported to contribute to the volatile flavour of fish (Van Straten and Maarse, 1983).

Toluene, limonene, xylene and benzene derivatives in crude oil and orange roughy oil were probably degradation products of carotenoids (Tanchotikul and Hsieh, 1989; Belitz and Grosch, 1987; Josephson et al, 1991). This degradation process may have occurred during the heat cooking stage of fish meal production. Diethylphtalate was traced in crude and orange roughy oils. This compound was also detected in the volatile flavour compound of plums (Dirninger et al, 1989). However diethylphtalate is odourless, as reported by Stecher et al (1968). Some of these volatile flavour compounds were also detected in marine green algae (Sugisawa et al, 1990), indicating that these compounds were probably obtained by fish during feeding.

5.5.3.1. Effects of resin refining on volatile flavour compounds

Significant changes in relative quantities of volatile flavour compounds of crude and orange roughy oils during resin refming were observed as shown in Tables 5.9 and 5.10.

The relative quantities of some volatile flavour compounds in refmed crude oil showed a marked change in comparison to unrefined oil. The relative quantities of toluene increased greatly from 1.7% to 23.2%. Ethyl benzoate and methyl ethyl benzoate indicated a reduction in their relative quantities, but still showed relatively high percentages, 7.3% and 19.4% respectively. 1,1-dimethylethyl-2-methyl propionic acid also showed a significant contribution to the volatile flavour performance of refined oil. These compounds, together with other minor compounds, gave a better volatile flavour perception to refined crude oil. This was reflected by the results of sensory evaluation, where, according to panellists as discussed in Section 5.5.4, refined crude oil had just a slightly fishy odour and taste.

A different occurrence was encountered in the orange roughy oil. Toluene showed a reduced relative quantity compared to the unrefined oil, but it was still the compound which contributed the most to the refined orange roughy oil at 42.3%. Fish oil type might show a different effect in terms of volatile flavour compounds when passed through the resin packed column.

M-xylene, p-xylene, ethyl benzene and tetrachloroethane contributed at 16.4%, 6.1%, 8.3% and 7.5% respectively to volatile flavour of refined orange roughy. With these compounds as the main volatile flavour compounds, the improvement in odour and taste was obtained, as indicated by the results of sensory evaluation in Section 5.5.4.

The above results indicated that toluene, providing a benzene like odour (Stecher, 1968) or a plastic like odour (Tanchotikul and Hsieh, 1989) as the important compound in the improvement of fish oil odour and taste, since this compound was present at the highest relative amount in refined crude and orange roughy oils.

5.5.4. Effect of resin refining on sensory properties of fish oil

Fish oil quality was improved in terms of sensory properties of both odour and taste. The changes in relative quantity of volatile flavour compounds were detected as discussed in the previous section, where it states that toluene tended to appear as the highest relative compound in the refined oil. The odour and taste qualities of fraction-2 oil was always inferior to the odour and taste of fraction-1. Fernandez (1986) proved that most of undesirable compounds were bonding or interacting with the resins, rather than being simply caught in the resin pores.

Further improvement of odour and taste could be achieved by application of multiple refining. This finding provides very significant information for the Indonesian fish oil industry, especially as fish meal oil has a very unpleasant odour, as discussed in Chapter 4. The odour of this oil may not have been significantly improved by refining the oil once. This will be discussed in detail in Chapter 8.

The odour and taste of refined fish oil became less acceptable, when the fish oil-resin volume ratio increased more than 1:1. This indicated that the binding capacity of resin to

undesirable compounds decreased with increasing fish oil volumes, and consequently resulted in the refined fish oil having more undesirable compounds than refined oil obtained from refining with fish oil-resin volume ratio of 1:1. This finding suggested the use of fish oil-resin volume ratio of 1:1 to guarantee the organoleptical fish oil quality.

Height and diameter sizes of the column did not apparently give any odour and taste differences in refined oils. All refined oils from various height and diameter sizes of column had significantly better odour and taste properties compared to the fraction-2 oils. This revealed that in terms of odour and taste properties the effectiveness of macroporous strong acid cation resin did not change with the changes in height and diameter sizes of the column when the fish oil-resin volume ratio of 1:1 was used.

Differences in odour and taste properties of refined fish oils obtained from the refining with and without vacuum pressure application were insignificant. Thus this study proves that the application of vacuum pressure in the resin refining process as an effective method of increasing the refining rate, where the refining process rate could be increased to more than 300%.

5.6. CONCLUSIONS

The above results indicate that the use of the resin packed column to improve the chemical, physical and organoleptical qualities of fish oil, to meet required human consumption standard, is valid. The most important finding is that the ω-3 fatty acids could be retained in the refined oil.

In order to guarantee the quality of the refined fish oil product, the fish oil-resin volume ratio of 1:1 is recommended. Multiple refining is suggested for fish oil of inferior quality, particularly in terms of odour and taste quality. In general terms, the height and diameter sizes of column did not show any significant different in the quality of refined fish oil obtained. In addition, the study showed that more attention needs to be paid to the fish oil-resin volume ratio, rather than height-diameter ratio in installing the resin refining unit. Vacuum pressure application is recommended to accelerate the refining rate.

Toluene was noted as the most abundant volatile flavour compound in refined fish oil.

Since the natural tocopherol antioxidant value of fish oil is reduced during resin refining, a further study on the stability of refined and unrefined oils during storage was conducted and is discussed in Chapter 6.

Chapter 6

STORAGE TEST OF REFINED AND UNREFINED FISH OILS

6.1. BACKGROUND

Long term stability of fish oil during storage and transport is one of the most important food quality and safety issues to be considered, when designing suitable storage conditions including temperature limits and control, container type and antioxidant use.

Autoxidation is commonly suspected as the main chemical process in reducing fish oil quality. The principle sites of attack by oxygen during the oxidative process are the unsaturated portions of the fatty acid moieties within triglycerides. Oxidation of saturated fatty acyl groups occurs too slowly to have any significant effect on fish oil quality. In general, fish oil is highly susceptible to autoxidation because of the high proportion of unsaturated fatty acids, especially those with five and six double bonds, such as eicosapentaenoic acid (20: 5ω3) and docosahexaenoic acid (22: 6ω3) which are very labile to oxidation (Lundberg, 1965; Kinsella, 1987; Li and Regenstein, 1990; Fujimoto et al, 1990).

The autoxidation of oils causes rancidity and the development of unpleasant flavours and odours in food. Rancidity can not be detected at an early stage of oxidation, because the small molecular weight compounds formed provides very little off-odour and off-flavour (Labuza, 1971).

Among the established diagnostic procedures, determination of the peroxide value (PV) is commonly used to monitor the extent of oxidative rancidity (Quast and Karel, 1971; Mc Water, 1971; St. Angelo, 1977). The index provides an early warning of staleness and rancidity development as a result of peroxidation during storage (Read et al, 1988; Robert et al, 1988; Wallerstein et al, 1989). Hydroperoxides are generally known as primary products of lipid oxidation. Therefore, it seems reasonable to determine the concentration of peroxide as a measure of the extent of oxidation (Grey, 1978; Jackson, 1981).

6.2. OBJECTIVES

The objectives of this experiment were:

* to evaluate the relative stability level of resin refined fish oil in comparison with unrefined oil during storage; and
* to develop a predictive shelf life equation as the function of storage temperature.

6.3. METHODOLOGY

6.3.1. Materials

Crude oil was used in this study. The refined oil was obtained by passing the oil through a resin column 2.6 cm in diameter and 39 cm in height, with the fish oil-resin volume ratio 1:1. The flow rate of the oil was 2.2 ml/minute and refining was conducted at ambient temperature (18-23°C).

6.3.2. Methods

To test the stability of refined and unrefined oils during storage, 22 ml samples were held in 30 ml polypropylene vials fitted with air tight lids.

To investigate the fish oil stability at several temperatures, the oils were stored in temperature controlled rooms at 2, 20, 30 and 40°C. Samples were kept in the dark to minimize light inducing oxidation. The relative humidity of the storage rooms was not controlled. Samples were withdrawn after 0, 2, 5, 10 and 15 weeks of storage. Chemical, physical and sensory measurements for the samples stored at 40°C were performed until week 15. The experiment was conducted with two replications, as was the analysis. Seven trained Indonesian panellists participated in the sensory analysis. The samples were served in two ways: one at ambient temperature ("cold") and the other warmed to 55°C. The sensory sheet used is shown in Appendix 6.1.

6.3.3. Determination of the deterioration rate of fish oil during storage

The rate of reaction causing quality deterioration in fish oil, was determined by applying zero and first-order mathematical models. The rational behind this determination is based on reports by Sagui and Karel (1980) and Labuza (1982) who noted that quality deterioration of foods generally follow either zero- or first-order models depending on the mode of deterioration involved.

A zero order reaction is described as :

$$C = C_0 \pm k_0 t \quad \text{..... 6.1.}$$

and, for a first-order reaction:

$$\ln C = \ln C_0 \pm k_1 t \quad \text{..... 6.2.}$$

Where: C_0 = concentration of the quality factor at zero time
C = concentration of quality factor at time t
t = time (weeks)
k_0 = zero-order rate constant (concentration/time)
k_1 = first-order rate constant ($time^{-1}$)

The order of reaction was selected based on the goodness of fit of data as measured by the coefficient of determination (r^2).

6.4. RESULTS

6.4.1. Effects of storage on peroxide value (PV) of fish oil

The PV of the refined and unrefined fish oils during storage at various tested temperatures are shown in Figure 6.1. In general, the PV registered a progressive increase during storage at all temperatures.

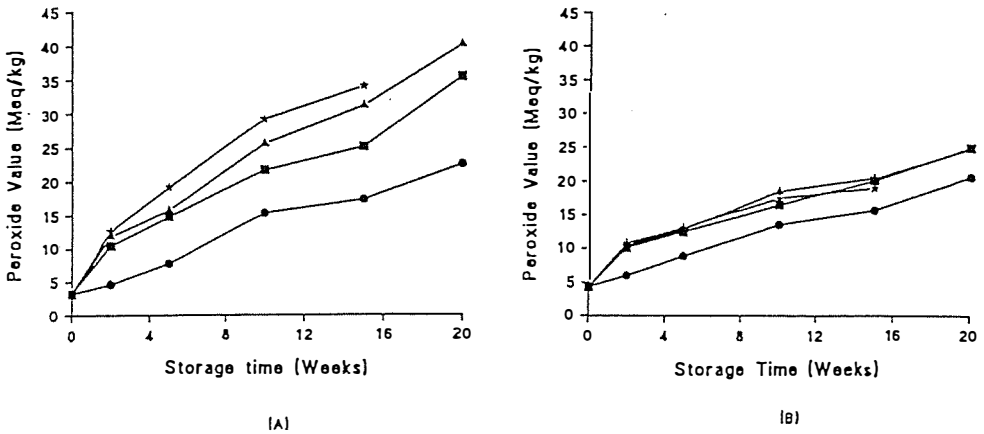


Figure 6.1. Peroxide value changes in fish oil during storage at various temperature (A = refined fish oil; B = unrefined fish oil; ● = 2°C storage; ■ = 20°C storage; ▲ = 30 °C storage; ✱ = 40°C storage).

Analysis of variance shows a significant difference in the PV due to the storage temperature. The PV increases with increasing temperature. This trend was most obvious for the refined oil, where 40°C of storage temperature gave a significantly fastest rate of PV increase which declined under 30, 20 and 2°C storage temperatures. The storage temperatures of 20, 30, and 40°C applied to the unrefined oil did not show any pronounced difference in the rate of PV increase, but the rate was apparently faster than in the unrefined oil stored at 2°C. A very sharp increase in PV, after two weeks, was noted in samples from all storage temperatures, except for the oils stored at 2°C, where the PV increase was gradual.

6.4.2. Effects of storage on refractive index value of fish oil

Figure 6.2. shows the RI value changes found for refined and unrefined fish oils during storage at several storage temperatures.

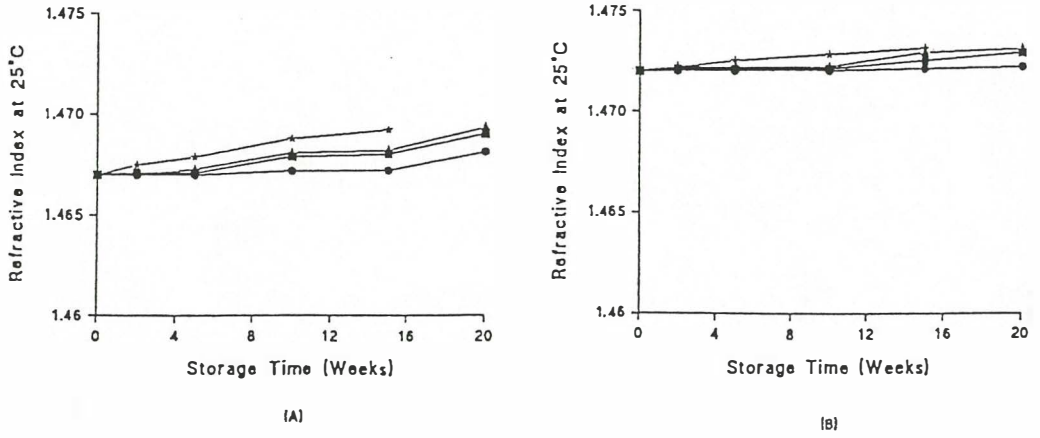


Figure 6.2. Refractive index changes in fish oil during storage at various temperatures (A = refined fish oil; B = unrefined fish oil; ●= 2°C storage; ■= 20°C storage; ▲= 30°C storage; ★= 40°C storage)

The most important observation was that RI values for the refined oil at all temperatures did not exceed the values observed for the unrefined oils. During the first five weeks, the RI values showed a small increase, except for the oil stored at 40°C. Changes in RI due to the various storage temperatures could be distinguished, especially in the refined fish oil. At a storage temperature of 2°C, minimal changes in RI were recorded over the trial. In contrast, the RI increased in the oils stored at 40°C.

6.4.3. Effects of storage on colour of fish oil

Colour intensity changes in both refined and unrefined fish oils are shown in Figure 6.3.

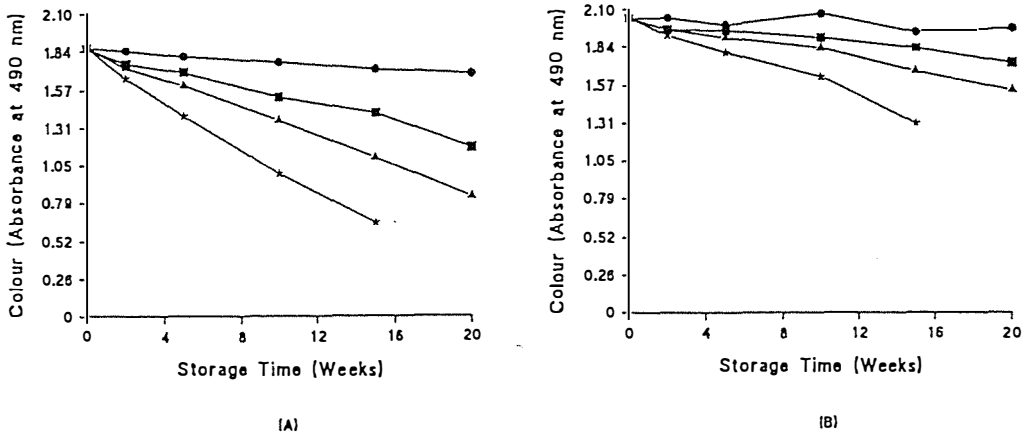


Figure 6.3. Colour absorbance value changes in fish oil during storage at various temperature (A= refined oil; B = unrefined oil; ● = 2°C storage; ■= 20°C storage; ▲ = 30°C storage; ★ = 40°C storage)

The results indicated that colour intensity reflected by absorbance values measured at 490nm was significantly affected by storage temperatures. The sharpest reduction in absorbance was observed in the oils stored at 40°C, followed by the oils stored at 30°C and 20°C. Again the lowest storage temperature (2°C) effectively minimized changes in oil colour over 20 weeks.

The rate of absorbance decrease in the refined oil was significantly higher than found for the unrefined oil.

6.4.4. Effects of storage on sensory properties of fish oil

The panel data obtained from the sensory evaluation of oil odour and taste of refined and unrefined oils are shown in Figures 6.4 and 6.5. The mean score for all the sensory attributes tested increased with time. The increase was more noticeable in samples stored at 40°C.

Results showed that changes in the odour and taste scores for cold and warm samples exhibited a similar trend. However the sensory scores of cold samples tended to be higher than the scores of warm samples.

The effects of storage temperatures on the development of rancid odour and taste as perceived by panellists were more significant at higher temperatures. The panellists observed that during the first five weeks of storage odour and taste scores of oils stored at all tested temperatures increased sharply. After this period each storage temperature exhibited a different pattern. The scores for oils stored at 2°C and 20°C increased gradually with five weeks of storage. The odour and taste scores of the oils stored at 30°C and 40°C increase sharply at the tenth week. After that period there was a slow increase. The increase in odour and taste scores for both the refined and the unrefined oils stored at 40°C was significantly more rapid in comparison oils stored at other temperatures.

The development pattern of rancid odour and taste scores showed similar trends for both oils. However the refined oil generally showed a slightly higher increase rate in odour and taste scores. Results also indicated that the increase in odour score was at a higher rate than the increase of taste. This trend was observed at all storage temperatures.

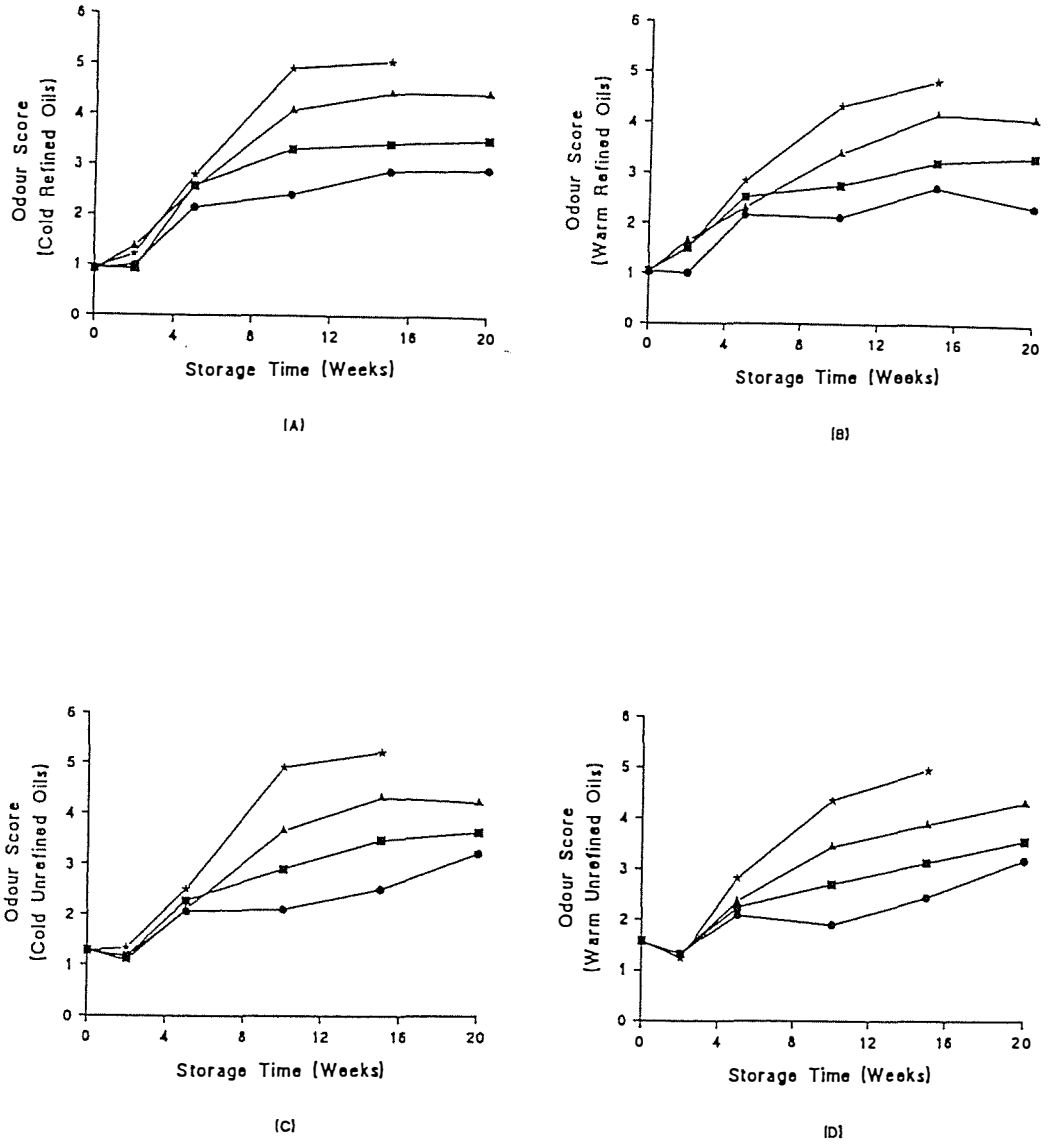


Figure 6.4. Odour score changes in fish oil during storage at various temperatures (A= cold refined oil; B= warm refined oil; C= cold unrefined oil; D= warm refined oil; ● = 2°C storage; ■= 20°C storage; ▲= 30°C storage; ✶= 40°C storage)

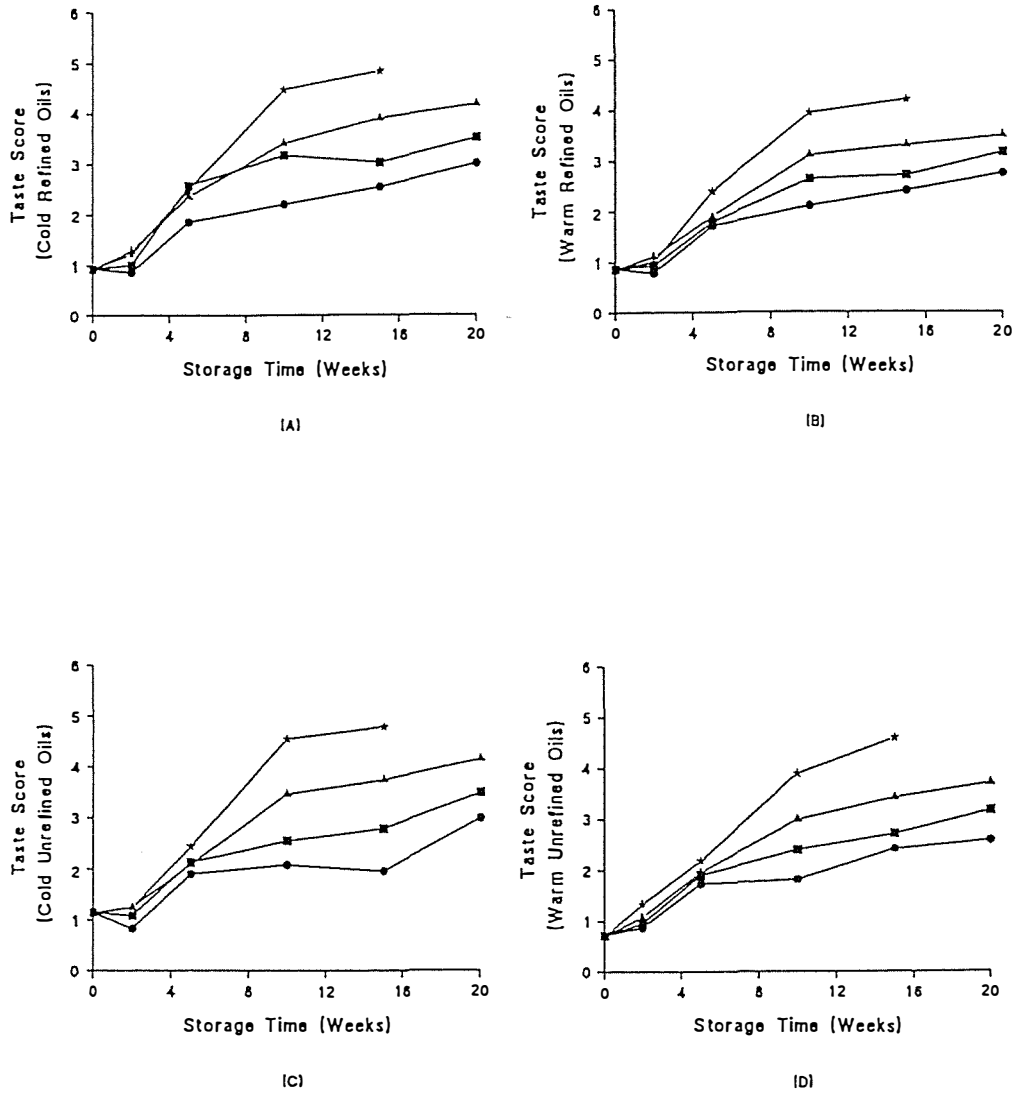


Figure 6.5. Taste score changes in fish oil during storage at various temperatures (A = cold refined oil; B = warm refined oil; C = cold unrefined oil; D = warm unrefined oil; ● = 2°C storage; ■= 20°C storage; ▲ = 30°C storage; ★= 40°C storage)

6.5. DETERMINATION OF RATE CONSTANTS AND ORDER REACTION MODEL

Two models were evaluated, one for a zero-order reaction and one for first-order reaction. The rate constant for refractive index changes were not determined, since the changes were practically insignificant. Calculated rate constants for both zero- and first-order reactions are shown in Table 6.1. These results show that in all cases, the rate constant is a function of temperature.

The rates of peroxide value, odour and taste scores changes in both oils were found to follow a zero-order reaction rather than the first-order reaction. The extent of colour loss in both oils followed both zero- and first-order reaction models, in that both models had high R square values.

To predict the quality losses in fish oils at various temperatures, plots of the natural logarithm of rate constant versus reciprocal of absolute temperature (°K) for each quality parameter were calculated. These are shown in Appendix 6.3. The trend of actual value was calculated using linear regression.

Table 6.1. Rate constant of zero- and first-order reactions of each parameter during storage of fish oil at various storage temperatures

Parameter	Fish Oil Sample	Storage Temp.(°C)	Zero Order		First Order	
			k	r^2 (%)	k	r^2(%)
Peroxide Value	Refined Oil	2	0.94	98.05	0.14	91.58
		20	2.15	96.82	0.27	78.09
		30	2.54	97.90	0.29	77.40
		40	3.13	94.34	0.35	72.83
	Unrefined Oil	2	0.84	98.95	0.12	93.41
		20	1.56	96.34	0.19	80.73
		30	1.65	93.89	0.20	77.36
		40	1.82	86.46	0.23	70.42
Colour Absorbance Value	Refined Oil	2	0.010	99.00	0.005	99.58
		20	0.037	98.60	0.022	97.73
		30	0.054	99.83	0.032	98.00
		40	0.093	99.54	0.062	99.14
	Unrefined Oil	2	0.010	68.28	0.003	62.64
		20	0.020	96.22	0.010	96.41
		30	0.028	98.65	0.014	98.37
		40	0.050	98.51	0.026	96.82
Odour Score from Cold Sample	Refined Oil	2	0.131	85.49	0.086	78.03
		20	0.216	78.69	0.121	71.70
		30	0.251	86.95	0.144	79.97
		40	0.296	91.94	0.159	86.93
	Unrefined Oil	2	0.095	92.12	0.056	87.38
		20	0.136	91.72	0.071	84.14
		30	0.169	89.03	0.087	83.90
		40	0.252	93.23	0.095	91.08
Odour Score from Warm Sample	Refined Oil	2	0.126	69.33	0.068	67.17
		20	0.191	86.25	0.118	77.24
		30	0.229	92.17	0.134	84.73
		40	0.292	95.36	0.157	88.40
	Unrefined Oil	2	0.079	85.94	0.044	84.17
		20	0.114	94.25	0.059	87.36
		30	0.150	93.78	0.070	86.97
		40	0.228	93.37	0.102	85.65
Taste Score from Cold Sample	Refined Oil	2	0.112	92.75	0.088	83.75
		20	0.172	78.74	0.118	71.91
		30	0.214	92.58	0.130	83.84
		40	0.268	94.58	0.150	89.74
	Unrefined Oil	2	0.098	80.70	0.062	73.31
		20	0.119	93.28	0.070	85.68
		30	0.160	93.33	0.084	89.97
		40	0.225	93.20	0.108	90.33
Taste Score from Warm Sample	Refined Oil	2	0.106	92.01	0.082	82.86
		20	0.128	91.59	0.088	84.45
		30	0.170	90.09	0.115	84.12
		40	0.228	92.88	0.129	86.11
	Unrefined Oil	2	0.100	91.15	0.104	83.09
		20	0.157	93.05	0.126	84.18
		30	0.198	93.42	0.148	83.91
		40	0.295	97.93	0.208	89.78

Note : Units used for each rate constant are as follows:

A. Peroxide Value: 1. zero order reaction: (meq/kg)/week
2. first order reaction : $week^{-1}$

B. Colour absorbance value:
1. zero order reaction: abs/week
2. first order reaction: $week^{-1}$

C. Sensory scores: 1. zero order reaction: score/week
2. first order reaction: $week^{-1}$

6.6. ESTIMATION OF SHELF LIFE OF FISH OIL

For information about the shelf life of a product, especially for a product developed using a new process, it is necessary to demonstrate the strengths and the weaknesses of the process. The information obtained will be used for further required action such as the need of antioxidant addition, and the use of a special packaging.

Sensory parameters can be regarded as the primary determinant of fish oil shelf life, since these parameters have a direct relationship to consumer acceptability of the product. Two sensory parameters, odour and taste, observed from cold and warm samples, were used to estimate, and to establish a model for predicting shelf life.

The shelf life of both oils at each storage temperature was determined using odour and taste sensory parameters calculated using the equation: $C = C_0 \pm k_0 t$. Since not all samples reached a quality reject point, the reject point was set when odour and taste scores reached 5. The results of this calculation are shown in Table 6.2.

Table 6.2. Calculated shelf life of refined and unrefined fish oil based on the odour and taste parameters from various storage temperatures (weeks)

Fish Oil Sample	Storage Temp. (°C)	Odour		Taste	
		Cold*)	Warm**)	Cold	Warm
Refined	2	31	31	36	39
	20	19	21	24	32
	30	16	17	19	24
	40	14	14	15	18
Unrefined	2	39	43	39	38
	20	27	30	32	27
	30	22	23	24	22
	40	15	15	17	15

Note: *) sample was evaluated at ambient temperature
**) sample was warmed up to 55°C

Calculation shows that storage temperature is the primary determinant of shelf life for both oils: the higher the storage temperature, the shorter the oil shelf life. In general, the shelf life of unrefined oils was longer than the shelf life of refined oil, except where the shelf life was calculated from taste scores for warm samples. Shelf life calculated from the odour scores for cold samples gave the lowest calculated shelf life. This suggests that the odour evaluated from cold samples should be used as the parameter determining the shelf life of fish oil and in establishing the equation for shelf life prediction.

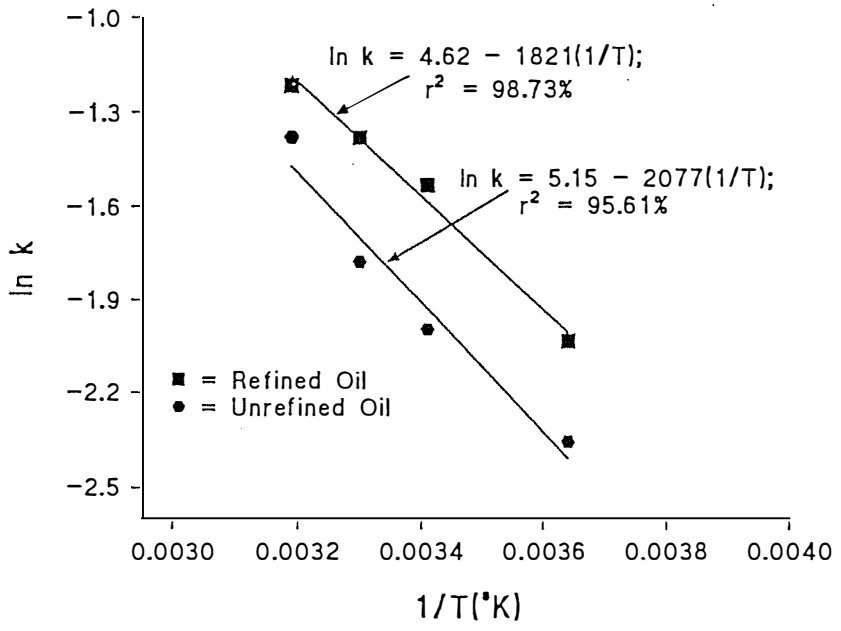


Figure 6.6. Linear relationship between the natural logarithm of rate constant of fish oil and the reciprocal of absolute temperature

Plots for the natural logarithm of rate constant for odour score changes observed for the cold oil samples versus the reciprocal of absolute temperature (°K) are shown in Figure 6.6. The linear equation used to estimate the rate constant at various storage temperatures obtained from these plots for the refined oil samples is:

$$\ln k = 4.62 - 1821\ 1/T\ ;\ r^2 = 98.77\%$$

and, for unrefined oil samples:

$$\ln k = 5.15 - 2077\ 1/T\ ;\ r^2 = 95.61\%$$

The estimated shelf life calculated from the above equations is shown in Table 6.3.

Table 6.3. Estimated shelf life of fish oil at various storage temperatures (weeks)

Fish Oil Sample	Storage Temperature (°C)	Shelf Life (weeks)
Refined Oil	2	30
	20	20
	30	16
	40	13
Unrefined Oil	2	41
	20	26
	30	20
	40	16

By plotting the natural logarithm of shelf life (θ) versus the reciprocal of absolute temperature as shown in Figure 6.7, the linear equation for the prediction of shelf life for refined oil is:

$$\ln \theta = -3.36 + 1860\ 1/T\ ;\ r^2 = 99.91\%$$

And the unrefined oil:

$$\ln \theta = -3.93 + 2102\ 1/T\ ;\ r^2 = 99.88\%$$

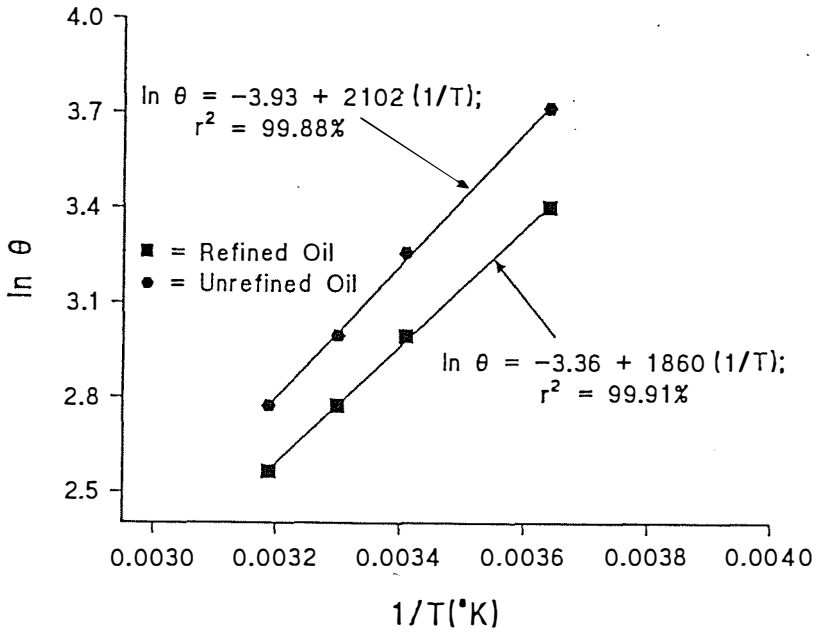


Figure 6.7. Linear relationship between the natural logarithm of estimated shelf life and the reciprocal of absolute temperature

6.7. CORRELATION BETWEEN SENSORY RESULTS AND OTHER PARAMETERS

Investigating the correlation between sensory results and other parameters was designed to show whether the changes in sensory properties correlate with the changes in peroxide, colour absorbance and refractive index values.

Odour scores observed from cold samples were selected to represent sensory results, because this evaluation was identified as the most important parameter in determining fish oil shelf life. A regression analysis was used to reveal the relationship between odour score and other parameters. The results, as shown in Table 6.4., indicate that relations between odour and peroxide value, as well as colour absorbance value, were found to be linear, but, in general, peroxide value showed a better correlation. Not all results at different storage temperatures indicated a linear relationship between odour score and refractive index value. In fact, a linear relationship was only found from data in which the oil was stored at 40°C

Table 6.4. Regression analysis between odour score and other parameters (peroxide value, colour absorbance value and refractive index value)

Linear Equation For:	Fish Oil Sample	Storage Temp.(°C)	a	b	$r^2_{(\%)}$
Odour Score (OS) Versus Peroxide Value(PV)	Refined Oil	2	0.79	0.11	86.26
		20	0.70	0.09	78.98
		30	0.63	0.11	89.41
		40	-0.03	0.15	93.77
OS = a + b(PV)	Unrefined Oil	2	0.37	0.14	92.73
		20	0.46	0.14	67.88
		30	-0.004	0.18	86.28
		40	-0.77	0.30	82.22
Odour Score (OS) Versus Colour Absorbance Value (CAV)	Refined Oil	2	23.06	-11.84	91.28
		20	8.91	-4.13	73.55
		30	8.17	-3.69	85.09
		40	8.03	-3.86	93.94
OS = a + b (CAV)	Unrefined Oil	2	31.35	-14.72	65.48
		20	19.07	-8.74	80.38
		30	15.67	-7.07	82.08
		40	13.81	-6.19	85.90
Odour Score (OS) Versus Refractive Index (RI)	Refined Oil	2	-1918.53	1308.96	40.00
		20	-1306.28	891.78	35.61
		30	-2262.85	1543.66	77.79
		40	-3088.31	2105.67	95.88
OS = a + b (RI)	Unrefined Oil	2	-11593.51	7877.17	74.59
		20	-3479.98	2365.30	65.36
		30	-3862.68	2625.21	65.39
		40	-6055.96	4114.72	92.63

6.8. DISCUSSION

6.8.1. Chemical and physical changes in fish oil during storage

The progressive formation of peroxides in fish oil during storage was evident from the increase in peroxide value (PV) in refined and unrefined oils. This phenomenon in the oxidation process has also been observed in the storage of some edible oils (Arumugham et al, 1984), in groundnut oil (Narasimhan et al,1986), in canola oil (Hawrysh et al, 1989) and in menhaden/soybean oil blends (Schnepf et al, 1991). In the production of hydroperoxides, the free radicals formed in the

initiation stage of oxidation react with oxygen to give a peroxy radical, which in turns react with the substrate to give a hydroperoxide (Hardy, 1980).

Natural colour loss in fish oil during storage resulted in oil with pale and clear colour. Carotenoids are the most common pigment composing fish oil colour. The carotenoids, embracing the red, orange and yellow oil soluble pigments occur naturally in a number of different plant and animal fats (Brody, 1965; Clydesdale and Francis, 1976). The decomposition of carotenoids would result in loss of colour and form a more weakly coloured product (Chou and Breane, 1972; Emods, 1978). The unsaturated portion of carotenoids is easily affected by oxygen (Stuckey, 1972). Thus, the main cause of carotenoids degradation in foods is oxidation by reaction with atmospheric oxygen at rates dependent on light, heat and the presence of pro- and antioxidant (Clydesdale and Francis, 1976). Enzymatic degradation of carotenoids was also noted (Simpson et al, 1981; Baumfeind, 1981).

An increasing pattern in refractive index (RI) was observed in fish oil during storage, and a similar occurrence is recorded by Janick and Pokorny (1960) and Arya et al (1969) in edible oils and fats. The increase in RI with autoxidation is possibly attributable to conjugation known to precede hydroperoxide formation in the secondary stage and polymerization of partially oxidized fats in the tertiary stage of autoxidation (Arya et al, 1969; Grey, 1978).

The changes in refined oil tended to occur at higher rate than in unrefined oil. The natural antioxidant lost during refining process, as mentioned in Chapter 5, was suspected as the main reason for this. Lower antioxidant content would lead to lesser protection capacity against oxidation attack. Therefore, peroxide formation, colour loss and RI increase were encountered faster in refined oil than in unrefined oil. Brezka and Salmonowicz (1973) report a similar result when investigating the stability of soda lye refined cod liver oil and sprat oils during incubation at 95 - 100°C, in which the refined oil had a lesser resistance to oxidation compared to unrefined oil. The tocopherol content of these refined oils was also found to be lower than unrefined oil.

The storage temperature affected peroxide formation, where the higher temperature tended to give a higher rate of peroxide formation. Unrefined oils stored at 20, 30 and 40°C showed an insignificant difference in peroxide value during storage period. Decomposition of peroxides into secondary products may have affected the total peroxide value. Previous studies have shown that the conversion rate of peroxides into secondary products increased with increasing temperature (Lea, 1962; Erickson and Bower, 1976; Karel, 1985). Possibly, under these conditions the peroxide formation rate in unrefined oil was nearly the same as the conversion rate into the secondary

products. The higher storage temperature also accelerated the colour loss and RI increase. Those facts are also supported by the increasing pattern of rate constant with increasing storage temperature.

As described above, the changes in fish oil properties still occurred even though the oils were stored at a low temperature (2°C). This means that oxidation still occurred at low temperatures. According to Gray (1978) lowering the temperature is not particularly helpful in preventing oxidation, since a low energy threshold is involved. The results indicated that the oxidation process, especially peroxide formation, could still occur with the oxygen available even at low temperature. However the low temperature storage was still very effective in slowing down the peroxide formation, colour loss and RI increase. This experiment also proved that enzymatic degradation on carotenoids had a lesser role in the decolouration in fish oil, since colour change still occurred at low temperature (2°C).

6.8.2. Sensory changes in fish oil during storage

Sensory evaluation was an important means in determining the acceptability of fish oil during storage, since, according to Gray (1978), the consumer uses an organoleptic evaluation to judge the quality of fats and oils.

Odour and taste scores observed from both cold and warm fish oil samples tended to increase during the storage testing period. This means that more rancid odour and taste was detected by panellists with increasing storage time. Similar results were obtained by Pohle et al (1964) in fats and shortening, Cowan et al (1971) in blends of soybean and peanut oils, and Narasimhan et al (1986) in groundnut oil. The objectionable flavour resulted from the accumulation of decomposition products of the oxidation reaction (Gray, 1978), including carbonyl compounds, alcohols, semi-aldehydes, acids, hydrocarbons, lactones and esters (Lilliard, 1978). Most important are the aldehydes, several unsaturated ketones and from derivates which exhibit a wide variety of aroma properties (Grosch, 1987). The odour in rancid sardine oil was found to be mainly due to low molecular weight volatile compounds, predominantly ethanol, propionic acid, pent-1-en-3-ol, ethanol, acetic acid, butanol, pentanal and similar substances, (Nakamura et al, 1980). Moreover, Karahadian and Lindsay (1989) found that rancid and "paint" flavour in fish oils were due to hexanal, t,c- and t,t-2,4-heptadienals and t,c-2,4-decadienal.

The increase of sensory scores was affected by storage temperatures, the higher the storage temperatures the faster the score increased. This means that a high temperature accelerated the formation of rancid odour and taste compounds. According to panell sts, the development of rancid odour and taste was effectively inhibited when the oil was stored at a low temperature (2°C). This indicated that the high temperature would accelerate the rancid odour and taste compounds. These results were supported by the rate constant, in wh ch the rate constant for these parameters increased by elevating storage temperatures.

In general, the results showed that the increase of odour and taste scores in refined fish oil was faster than in unrefined fish oil. The formation of rancid odour and taste compounds in refined oil may be affected by the decrease in quantity of natural antioxidants amount. As discussed in Chapter 5, the refined fish oil had a lower content of tocopherol which probably led to less protection against oxidation process compared to unrefined oil.

6.8.3. Shelf life of fish oil

In this study, refined and unrefined o ls investigated for their shelf life were contained in polypropylene vials. Sensory results were used to predict the shelf life of fish oil since, according to Jackson (1981) and Erickson and Bower (1976), whatever objective method was chosen it must be realized that the method must be correlated with some type of organoleptic evaluation of the food. Odour score observed from cold samples was chosen to predict the shelf life of both refined and unrefined oils, because in general, the score resulted in shortest estimated shelf life.

The average temperature of Indonesia is approximately 30°C (Pearce and Smith, 1984). If the oil stored in polypropylene containers at 30°C or below, it can be kept in acceptable quality for 16 - 30 weeks for refined oil and 20 - 41 weeks for unrefined oil. Shorter shelf life of refined oil was due to a higher rate of deterioration, especially in the oxidation process.

6.9. CONCLUSIONS

In conclusion, the results of this study show that the prediction of fish oil shelf life could be reasonably obtained from the changes in observed parameters which could be collected from a planned storage test. The shelf life of fish oil was limited by the sensory parameter, but not by chemical and physical parameters. The rate of fish oil deterioration was slowed by keeping the oil at a low temperature (2°C). At the storage temperature of 30°C in Indonesia, refined and unrefined fish oils had a shelf life up to 30 and 41 weeks respectively.

The results indicated that treatments were needed to extent the shelf life of refined fish oil. The treatments recommended are antioxidant addition to replace lost natural antioxidant during refining, and oxygen removal.

Chapter 7

STABILITY IMPROVEMENT OF RESIN REFINED FISH OIL

7.1. BACKGROUND

The resin refining process did not produce refined oil with either equal or better stability than unrefined oil as discussed in Chapter 6. Lower natural antioxidant in refined fish oil was suspected as the main reason of this occurrence. Chapter 5 illustrated how refining had decreased α-tocopherol, which is known as a predominant antioxidant in fish oil (Karahadian and Lindsay, 1986; Brody, 1965).

7.1.1. Antioxidant and oxidation

The addition of antioxidants is necessary to improve the stability of refined oil, since antioxidants are able to retard deterioration, rancidity, and discolouration due to oxidation (Dziezak, 1986). More specifically, the antioxidants are used to protect fats and oils from developing rancid flavour and odour (Haumann, 1990). Many studies, including Pokorny (1987), provide a detailed description of the inhibiting mechanism of oxidation by antioxidant. The most widely used antioxidants interfere with lipid autoxidation, chiefly by competing with free radicals. By reaction with an alkyl peroxyl (hydroperoxide) radical, antioxidants form a lipid hydroperoxide, and a less active inhibitor radical:

$$ROO^{\bullet} + InH \text{ ----> } ROOH + In^{\bullet}$$

Alkoxyl radicals can participate in an analogous way:

$$RO^{\bullet} + InH \text{ ----> } ROH + In^{\bullet}$$

Various side reactions may proceed simultaneously, resulting in chain initiation and thus increasing the reaction rate. For example:

$$In^{\bullet} + O_2 \text{ ----> } InOO^{\bullet}$$

$$InOO^{\bullet} + R\text{-}H \text{ ----> } InOOH + R^{\bullet}$$

Where In· radicals are very unstable, the following reaction is possible:

$$InH + O_2 \text{ ----> } In + HOO^{\bullet}$$

Reaction becomes important only in high concentrations of antioxidants. In rare cases the antioxidant molecule can react with lipid hydroperoxides forming two free radicals:

$$InH + ROOH \text{ ----> } RO^{\bullet} + H_2O + In^{\bullet}$$

$$ROO\text{-}In \text{ ----> } RO^{\bullet} + In^{\bullet}$$

These reactions are unimportant unless antioxidant concentration is very high. In some cases the antioxidant may react with R· radicals as well:

$$InH + R^{\bullet} \text{ ----> } In^{\bullet} + RH$$

With phenolic antioxidants, this reaction is insignificant only when oxygen is present in traces and hydroperoxide concentration is also negligible (Pokorny, 1987).

Antioxidants also minimize the oxidative destruction of certain vitamins and essential amino acids. Most useful are antioxidants that are soluble in fats and oils, odourless, tasteless, nontoxic at approved levels and effective in low concentration (Haumann, 1990).

Antioxidants are usually aromatic compounds which are phenolic in character. Phenolic antioxidants permitted in edible fats and oils in many countries include tocopherol, propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ter-butylhydroquinone (TBHQ). Permitted levels vary from country to country. The US Food and Drug Administration (FDA) regulations generally allow PG, BHA, BHT and TBHQ, or a combination, to be used at a level not exceeding 0.02% based on the weight of the fat and oil. Tocopherol and ascorbyl palmitate, which are used under good manufacturing practices (GMP), do not have a regulated limit (Haumann, 1990). BHA is the most common antioxidant used in the food industry in Indonesia (Sherwin, 1990).

Very few research papers have been published regarding antioxidants used in fish oil, but intensive experiments have been conducted in vegetable oils. However some experiments on the use of

antioxidants in fish oil have been carried out by Jurewicz and Salmonowicz (1973), Brzeska and Salmonowicz (1973), Ke et al (1977) Zama et al (1979), Karahadian and Lindsay (1988) and Taguchi et al (1988).

7.1.2. Oxygen removal and oxidation

Oxidation of olefinic compounds by atmospheric oxygen is important in the development of rancidity, in the production of desirable and undesirable flavours, in the polymerization of highly unsaturated oils, and in the production of compounds of significant physiological activity (Gunstone and Norris, 1983). When the effect of oxygen on oxidation of lipids is considered, it is often simply a question of total amount available for reaction with food components. If this amount is limited to a level which causes no significant effect on the food, and there is no potential for additional oxygen coming into contact with food, then the rate of reaction is irrelevant Karel, 1985). Thus, the most obvious precaution to take against oxidation deterioration is to remove the oxygen (Smouse, 1978; Belitz and Grosch, 1987; Ranken, 1989; Ranken, 1990; Hardy, 1980; Berger, 1989; Brookman, 1991).

Recently, vacuum packaging has been used as a method of oxidation control. From mechanistic considerations based on the rancidity of the polyenoic acids present in the oil, the oxidation rate will be dependent on oxygen concentrations. Even if oxygen diffuses through into the pack the rate will be reduced compared to non-vacuum packed oil (Hardy, 1980).

7.2. OBJECTIVES

1. The antioxidant experiments performed were intended to determine the best antioxidant to inhibit the oxidation process in fish oil, and to optimize its level of use.

2. This study also aimed to investigate an alternative way of vacuum packaging for stability improvement instead of the use of an antioxidant. Consumers are increasingly reluctant to accept synthetically derived additives in foods because of concerns about health (Clark et al, 1990; Han et al, 1991).

7.3. METHODOLOGY

7.3.1. Materials

Crude fish oil was used as the object of the study. Antioxidants used were BHA, TBHQ, Grindox 117 (propyl gallate) and D*l*-α-tocopherol. BHA was supplied by Serva Feinbiochemica, Heidelberg, for Experiment 1 and by Bronson and Jacobs, New Zealand, for Experiment 2. TBHQ was obtained from Aldrich Chemical Company, Inc., USA. Grindox 117 containing 7.5% ascorbyl palmitate (E304), 17.5% propyl gallate (E310), 10% citric acid (E330), 30% propylene glycol and 35% emulsifier (E471) was supplied by Bronson and Jacobs (New Zealand). D*l*-α-tocopherol was donated by Roche, New Zealand.

7.3.2. Methods

7.3.2.1. Experiment 1: Antioxidant selection

Antioxidant solutions were made by heating 20ml of refined crude oil to 80°C and then appropriate amounts of antioxidant were incorporated (Hawrysh et al, 1990). The amount of each antioxidant dissolved was 20% (w/w) of stock solution, and mixed for 30 minutes at 80°C.

Four antioxidant agents were tested in this experiment: three of them, BHA, propyl gallate/Grindox 117 and α-tocopherol, are permitted in the manufacture of Indonesian foods (Indonesian Health Ministry, 1974), while TBHQ is recognized as highly effective antioxidant. 0.02% of BHA and 0.02% of TBHQ, the highest levels permitted in oils by the Indonesian Health Ministry (1974) and US-FDA (Haumann, 1990), were added. Grindox 117 used in the experiment was 0.1% which is the highest level use suggested by the manufacturer. 0.1% of tocopherol was added to refined oil, as that level was suggested by Body (1991). Tocopherol was added in two ways: by direct addition of tocopherol solution, and by heating preparation as performed in other antioxidants.

The oil samples were prepared by mixing 150ml fish oil with appropriate amounts of antioxidant in a 500 ml bottle, and then shaken vigorously. For the stability test, 140 ml oil sample was placed in 250 ml beakers and covered loosely with aluminium foil lids. The stability test was carried out using the Schaal oven method (Thompson, 1966; Stuckey, 1972; Kochhar, 1988; Buck, 1991), by

placing the samples in a forced air oven at 63±2°C for up to 16 days. The temperature used in this method is much lower than in most other accelerated stability testing procedures. This method can therefore be recommended as the one having the fewest possible problems (Ragnarsson and Labuza, 1977). Oil samples were removed for analysis after 0, 1, 2, 4, 8, 12 and 16 days of storage. Immediately after each storage period, 20 ml samples were taken and placed in 35 ml polypropylene bottles for analysis. Each sample was made with two replications and each analysis was conducted in duplicate.

7.3.2.2. Experiment 2: Optimisation of antioxidant dosage level

BHA antioxidant from Experiment 1 was investigated to determine its optimum level. As discussed in Section 7.5, BHA was the antioxidant chosen. Unrefined oil was used as a control in order to compare the effectiveness of the standard level to be achieved by the refined oils containing specific quantities of the antioxidant, BHA. The BHA levels used were 0.005%, 0.01%, 0.015% and 0.02%.

The fish oil samples were prepared as in Experiment 1. The Schaal oven method was used to observe fish oil stability, where the incubation was conducted at 63±2°C. The samples were withdrawn for analysis after 0, 2, 5, 9, 13 and 17 weeks of storage. The experiment was performed with two replications, and each analysis was conducted in duplicate.

7.3.2.3. Experiment 3: Use of vacuum package for fish oil stability improvement

Both refined and unrefined oils (20 ml) were placed in 6.6 cm diameter and 4.6 cm high cans and sealed with 75 bar pressure for vacuum package, and without pressure for non-vacuum package. In the first investigation, the samples were observed for stability using the Schaal oven method by placing them in the forced air oven at 63±2°C for up to 16 days, and withdrawn for analysis after 0, 1, 2, 4, 8, 12, and 16 days of storage. In the second investigation, the samples were stored at 30°C, the average daily temperature in Indonesia. The sampling was performed after 0, 1, 2, 4, 8, 12 and 16 weeks of storage. Both investigations were run with two replications and each analysis was conducted in duplicate. Sensory analysis for the second investigation was undertaken by eight trained Indonesian panellists.

7.4. RESULTS

7.4.1. Selection of antioxidant

Six parameters of value, peroxide, thiobarbituric acid, anisidine, totox, colour absorbance and refractive index, were used to select the best antioxidant to improve stability against oxidation, in refined fish oil.

7.4.1.1. Effects of various antioxidants on peroxide value

Changes in peroxide value (PV) during storage is shown in Figure 7.1. The addition of antioxidant induced changes in the initial PV of the oil. Addition of BHA, TBHQ and Grindox 117 resulted in oils with lower initial PVs than the untreated oil used as the control. However the fish oil containing α-tocopherol gave a higher initial PV than the control. Direct addition of tocopherol gave a higher initial PV than the addition of tocopherol prepared the same way as other antioxidants.

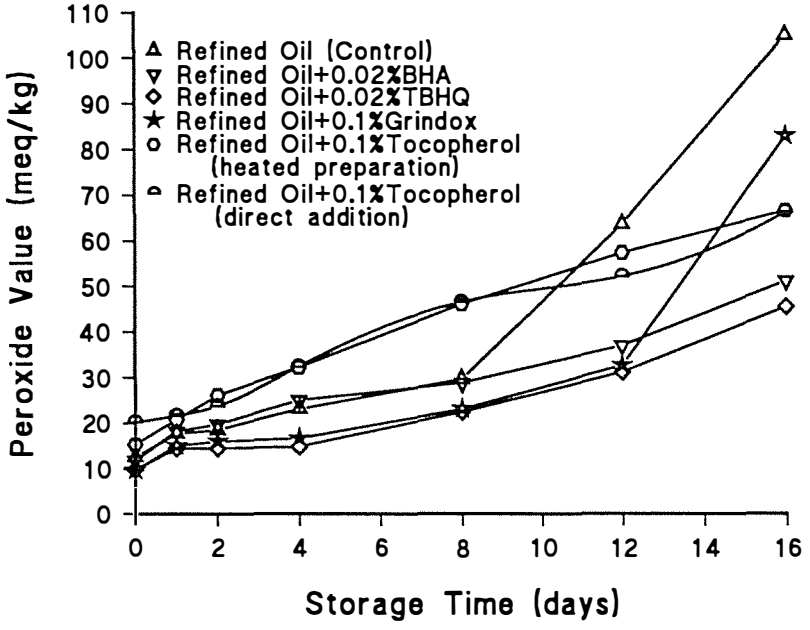


Figure 7.1. Effects of various antioxidants on peroxide value changes in fish oil during storage at 63±2°C

The PV of all oil samples increased significantly after one day's storage, except for the sample with a direct addition of tocopherol. On the second day of storage, the oils to which tocopherol was added displayed an increase in PV, but other samples, including the control, had a practically constant PV. TBHQ and Grindox still showed a relatively constant PV on the fourth day. Conversely, the PV in the oils containing BHA and tocopherol, increased markedly. Extension of the storage period until 16 days revealed that all samples indicated an increasing PV content, but each exhibited a different increase rate. The PV for untreated oil increased sharply on the twelfth day and even exceeded the PV in the other oil samples treated with antioxidants. After four days, the oil containing tocopherol, using both addition methods, had approximately the same rate of PV increase. In general, both methods of tocopherol addition did not induce any significant different in PV during storage. The PV in the oil containing tocopherol was always higher in comparison with the PV analyzed in the oils containing other antioxidants, except at the end of storage, where the PV in that oil was lower than in the oil containing Grindox. The PV in the oils containing TBHQ and Grindox increased at a comparable rate until the twelfth day, where values were lower than the values in other samples. After that period, the PV in the oil containing Grindox increased sharply and exceeded the PV in the oils containing other antioxidants. TBHQ and BHA showed as the most effective antioxidants to inhibit PV increase.

7.4.1.2. Effects of various antioxidants on thiobarbituric acid (TBA) value

The TBA test was used to measure malonaldehyde formation as a secondary product of the oxidation process. TBA changes in all samples during storage are shown in Figure 7.2. The addition of an antioxidant did not significantly affect the initial TBA values.

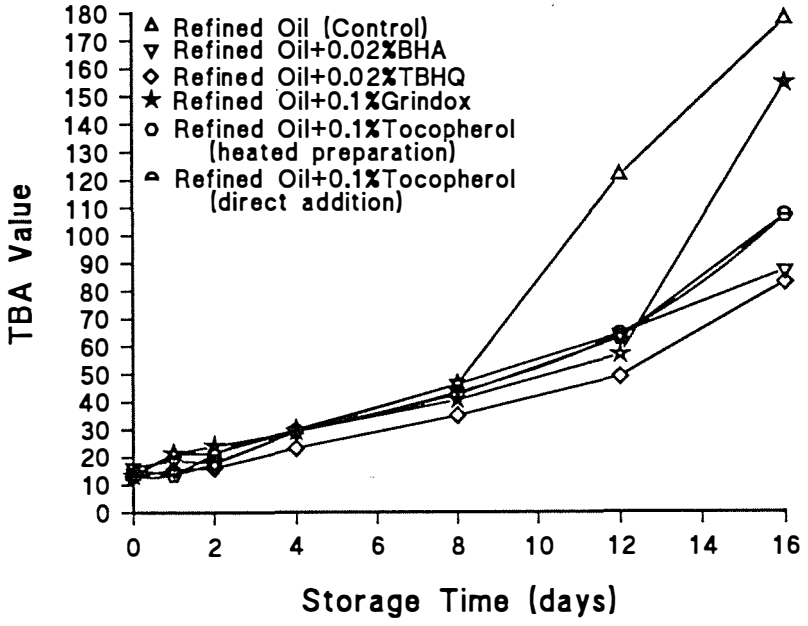


Figure 7.2. Effects of various antioxidants on TBA value changes in fish oil during storage at 63±2°C

TBA value of the control sample and the oil containing Grindox increased significantly after one day of storage, but the value remained virtually constant on the second day. The TBA value of the oils containing BHA, TBHQ and tocopherol (heated preparation) were statistically constant during the first two days. The oil containing direct addition of tocopherol had a relatively constant value of TBA after one day, but the value increased on the second day. On the fourth day, all oil samples displayed a significant increase in TBA value, and those values were relatively similar, except for the oil containing TBHQ which had an apparently lower TBA value.

The control sample revealed less stability compared to treated oils on the twelfth day, where its TBA value significantly exceeded the value of treated oils. This occurrence was evident until the end of storage, but the increased rate shown by each antioxidant was different. At the end of storage, TBA value in the oils containing TBHQ and BHA were significantly lower than the value in the oils containing other antioxidants. Moreover, the oil treated with TBHQ tended to show the

lowest TBA value during investigation. The two different tocopherol additions did not exhibit any significant difference in TBA value.

7.4.1.3. Effects of various antioxidants on anisidine value

Anisidine value analysis was used to determine alpha-beta aldehyde formation as a secondary product of oxidation. Anisidine value changes in the fish oil treated with antioxidants are shown in Figure 7.3. The antioxidant addition did not cause any significant difference in the initial anisidine values among the samples.

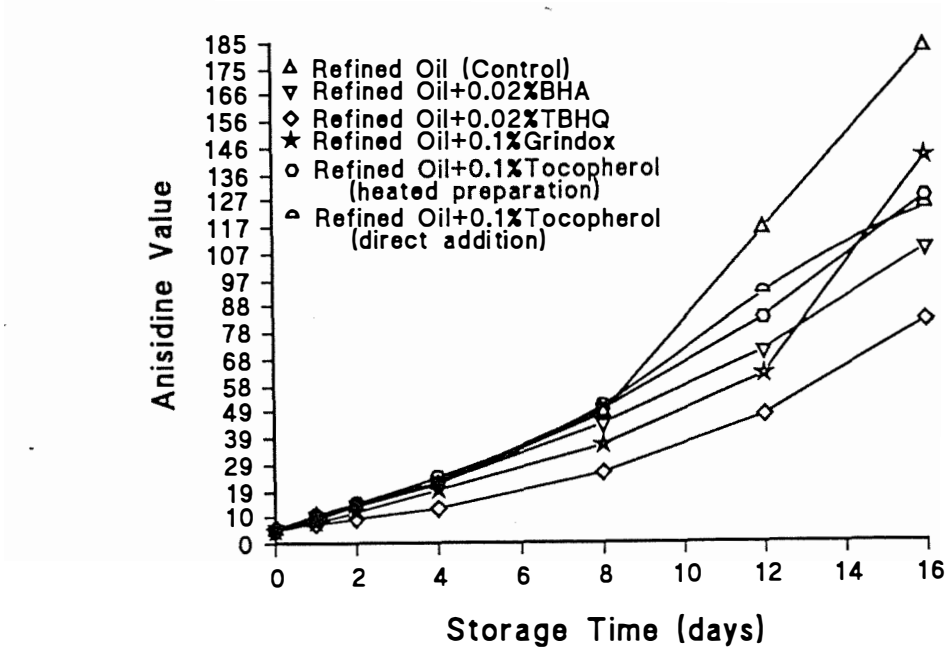


Figure 7.3. Effects of various antioxidants on anisidine value changes in fish oil during storage at 63±2°C

All oils did not exhibited any significant increase in anisidine value after one day of storage. The increase of anisidine value began on the second day, except for the oil containing TBHQ which still showed insignificant change. The increase of anisidine value for all samples was at

approximately the same rate until the fourth day, except for the oil treated with TBHQ. A significant effect of antioxidant use in terms of anisidine value was observed on the twelfth day, where the anisidine value in the control sample was much higher than the value in the treated samples. This occurrence was also found at the end of storage, where the oils containing TBHQ and BHA had a lower anisidine value than oils containing other antioxidants. Significantly, the value of the oil containing TBHQ was the lowest. The two methods of tocopherol addition did not exhibit any significant difference in the anisidine value.

7.4.1.4. Effects of various antioxidants on totox value

Totox value changes in all samples are shown in Figure 7.4. After one day of storage, the totox value increase could be detected only in the control sample and in the oils containing BHA, or tocopherol prepared by heating. TBHQ, Grindox and tocopherol added directly kept the totox value of oils nearly constant on the first day. After this period, totox values in all samples increased steadily, but at different rates, depending on the antioxidant.

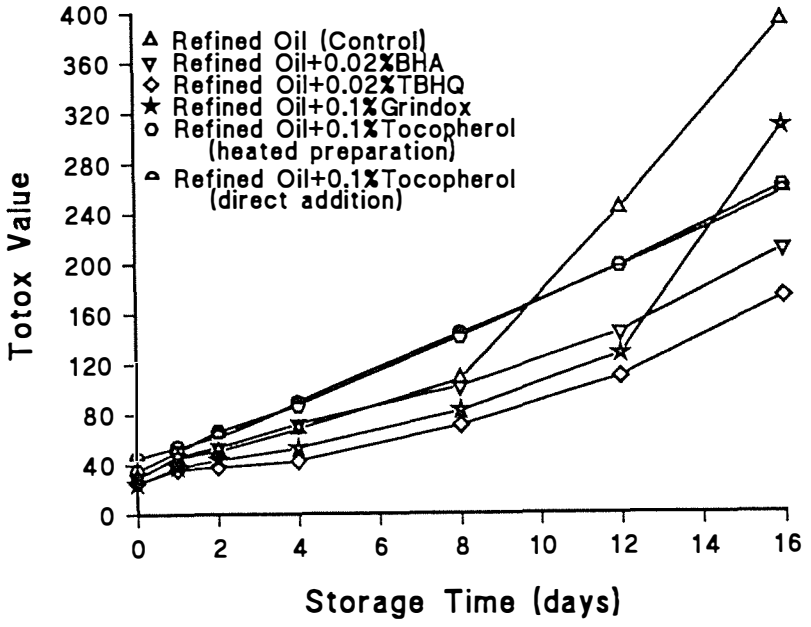


Figure 7.4. Effects of various antioxidants on totox value changes in fish oil during storage at 63±2°C

Effect of the use of antioxidant on totox value was significant on the twelfth day. The totox value for the control significantly exceeded the values in treated sample. The totox value in the oil containing TBHQ indicated the lowest totox value at all times during the period of observation. At the end of storage, oil treated with BHA showed a lower totox value than the oil treated with Grindox and tocopherol. The two different tocopherol additions did not exhibit any significant different in terms of totox value.

7.4.1.5. Effects of various antioxidants on colour absorbance value

The colour absorbance value of all oil samples did not display significant change after one day of storage. However after day one, the colour absorbance value of all oils decreased significantly as shown in Figure 7.5.

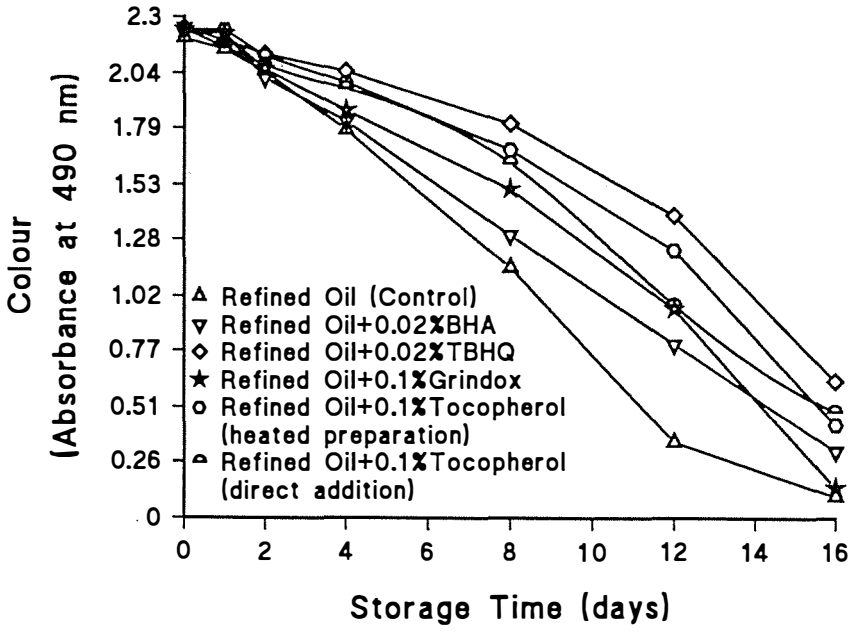


Figure 7.5. Effects of various antioxidants on colour absorbance value changes in fish oil during storage at 63±2°C

The oil containing antioxidants showed a marked lower decrease rate of colour absorbance in comparison to the control. This effect was apparent after eight days in which the absorbance value of the control sample was significantly lower than the value scanned in the treated oils. The oil containing TBHQ had the slowest decrease rate of colour absorbance value followed by the oils containing tocopherol. The two different tocopherol additions did not exhibit any significant difference in colour absorbance change.

7.4.1.6. Effects of various antioxidants on refractive index (RI) value

The antioxidant affected the initial RI value of the oils as shown in Figure 7.6.

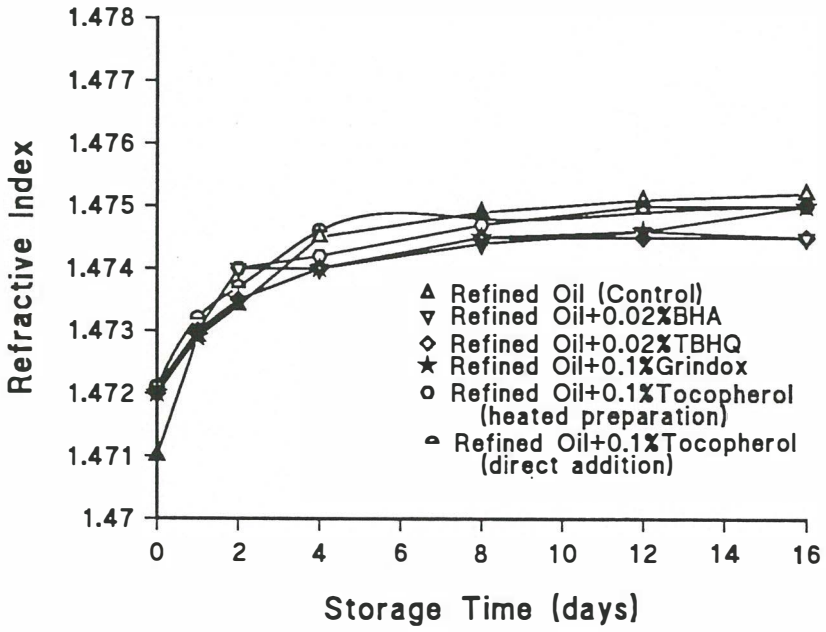


Figure 7.6. Effects of various antioxidants on refractive index changes in fish oil during storage at 63±2°C

The RI values of all oil samples increased with storage time. During the first day the control showed the highest increase rate. Up to the eighth day, all samples indicated an increasing trend

in RI value. After day eight, the RI value of the control sample tended to be higher than treated samples.

The oils treated with TBHQ, BHA and Grindox showed an insignificant difference in RI value until the twelfth day, but the RI value of the oil conta ning Grindox exceeded the value in the oils containing TBHQ and BHA at the end of storage trial. The two different tocopherol additions did not exhibit any effect on the changes in RI value.

7.3.2. Optimisation of antioxidant level

Even though TBHQ, as described in the discussion section of this chapter, was shown to be the best antioxidant in the refined fish o l during storage, this antioxidant could not be used in Indonesian oils and foods. TBHQ is not on the list of permitted antioxidants for Indonesian oils and foods (Indonesian Health Ministry, 1974). BHA as the best alternative was used for the further experiment in the optimisation of antioxidant levels in refined fish oil.

7.4.2.1. Effects of BHA levels on peroxide value (PV)

The PV changes in refined oil containing BHA at several levels and unrefined oil during storage at 63±2°C are shown in Figure 7.7.

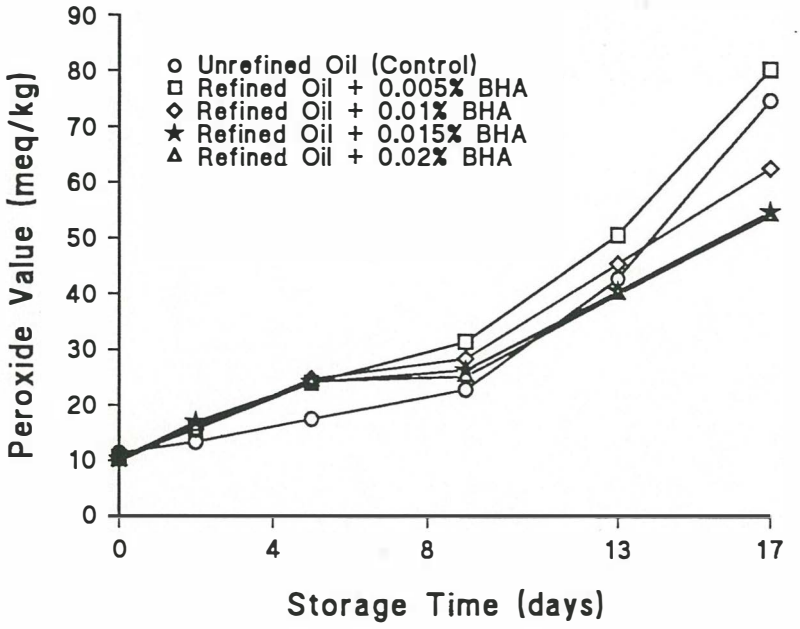


Figure 7.7. Effects of various BHA levels on peroxide value changes in fish oil during storage at 63±2°C

On the second day of storage, the unrefined oil did not reveal any significant PV increase, but other samples treated with antioxidants showed a pronounced PV increase. A significant PV increase for all samples was noted on the fifth day, and the PV of the unrefined oil was markedly lower than in all refined oils treated with BHA at several levels. On the ninth day, the PV increase in the refined oils treated with 0.015% and 0.02% BHA was insignificant, while the increase in other samples was significant. Unrefined oil and refined oil treated with 0.02% BHA had no significant PV difference. The storage extension to 17 days resulted in a PV increase in all oil samples. The refined oils treated with 0.01%, 0.015% and 0.02% BHA showed an insignificant PV difference over untreated oil on the thirteenth day. The refined oil treated with 0.015 and 0.02% BHA had no meaningful variance in PV at the end of storage, but this value was lower in comparison to other oils. The PV in the refined oil treated with 0.005% BHA was the only treated refined oil having a higher PV than unrefined oil at the end of storage.

7.4.2.2. Effects of BHA levels on thiobarbituric acid (TBA) value

All oil samples had an increased TBA value during storage as shown in Figure 7.8.

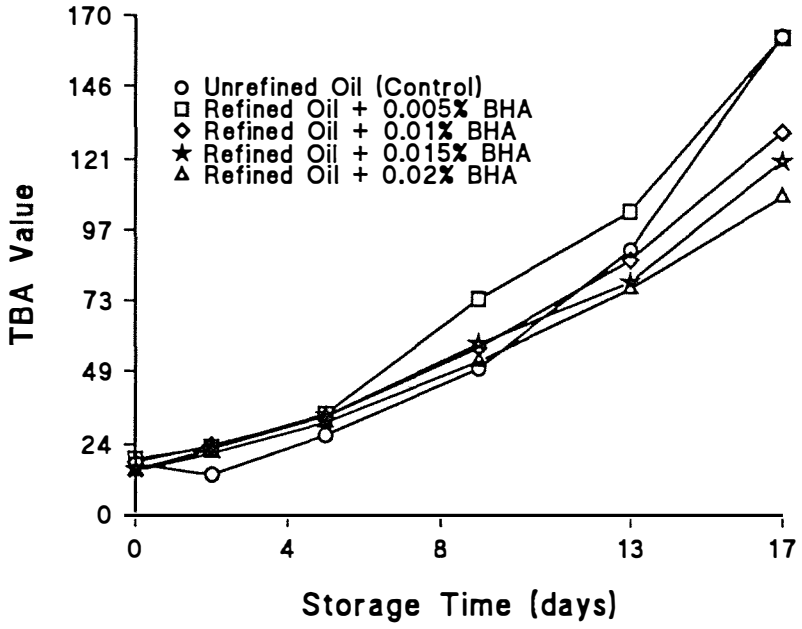


Figure 7.8. Effects of various BHA levels on TBA value changes in fish oil during storage at 63±2°C

On the fifth day of storage, the TBA value of unrefined oil was comparable to the value of the refined oil containing 0.02% BHA, but lower than the value in the refined oils containing 0.005%, 0.01% and 0.015% BHA. The TBA values of the refined oil treated with 0.015% and 0.02% BHA were significantly lower in comparison to the values measured in other oil samples on the thirteenth day. At this time, the refined oil containing 0.005% BHA was the only treated sample having a higher TBA value than the unrefined oil. Finally, the unrefined oil and refined oil containing 0.005% BHA exhibited a markedly higher TBA value than other treated samples at the end of storage, with similar TBA values in both oils.

7.4.2.3. Effects of BHA levels on anisidine value

Figure 7.9 shows the anisidine value changes in unrefined oil and refined oils treated with BHA at several levels during storage.

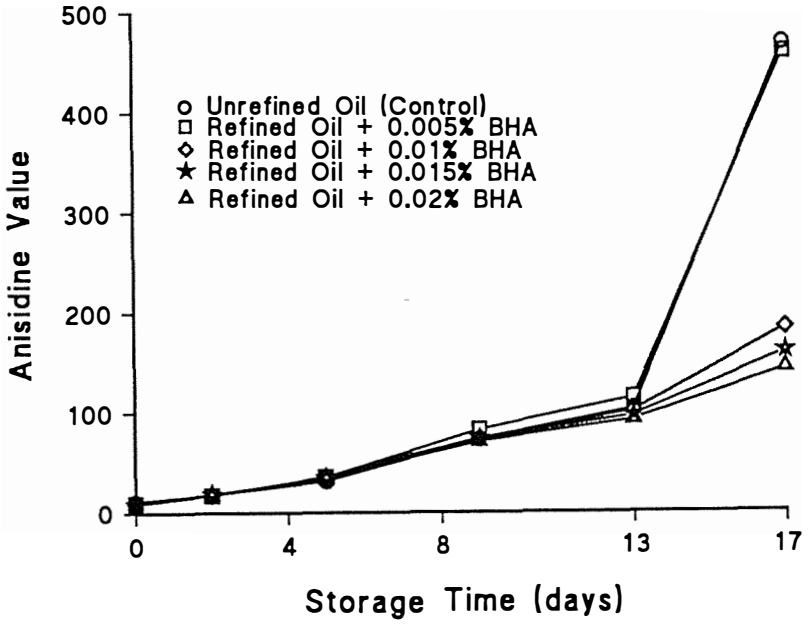


Figure 7.9. Effects of various BHA levels on anisidine value changes in fish oil during storage at 63±2°C

All investigated oils showed a significant increase of anisidine value until the end of storage. Anisidine value in all oils increased at the same rate until the fifth day, and these values were statistically similar. However on the ninth day, the anisidine value of refined oil treated with 0.005% BHA was markedly higher compared to the values of other samples which had a statistically insignificant different in anisidine value. On the thirteenth day, the refined oils containing 0.015% and 0.02% BHA exhibited a lower anisidine value than the unrefined oil and refined oils containing 0.005% and 0.01% BHA. At the end of storage period, the anisidine values of unrefined and refined oil containing 0.05% BHA increased sharply and both values were

obviously higher in comparison to the value analyzed in the refined oils containing 0.01%, 0.015% and 0.02%. In general, the results indicated that the higher the BHA levels, the lower the anisidine value of the oil.

7.4.2.4. Effects of BHA levels on totox value

Totox values of unrefined oil and refined oils containing BHA at several levels increased during storage as shown in Figure 7.10.

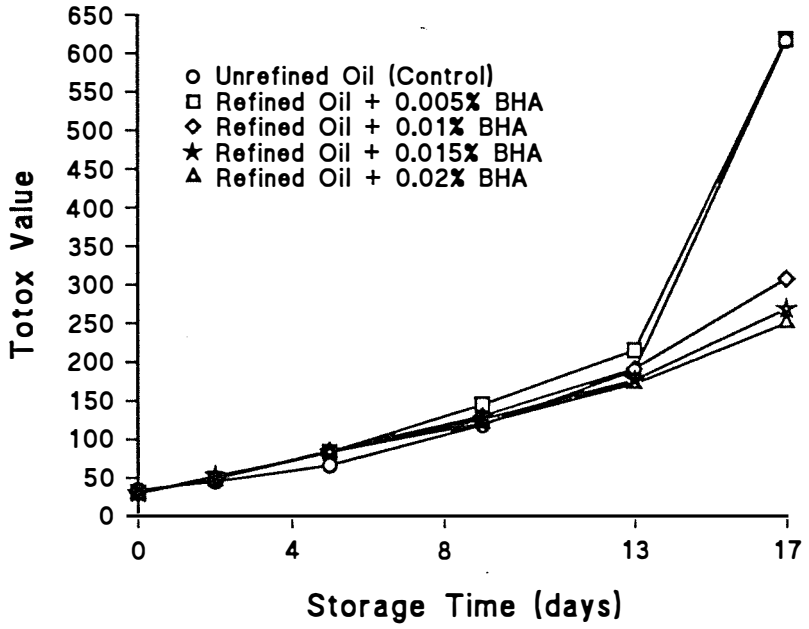


Figure 7.10. Effects of various BHA levels on totox value changes in fish oil during storage at 63±2°C

Until the fifth day of storage, the unrefined oil tended to show the lowest totox value, but at the ninth day that value was insignificantly different from the totox value in the refined oils containing 0.015% and 0.02% BHA. The totox value of unrefined oil was markedly higher than the values

in the refined oil containing 0.015% and 0.02% on the thirteenth day, and that value was lower than the value in the refined oils containing 0.005% and 0.01% BHA. At the end of storage, the totox values of unrefined oil and refined oil containing 0.005% BHA showed an insignificant difference, but those values were significantly higher than the values in the refined oils containing 0.01%, 0.015% and 0.02% BHA. The refined oil containing a higher level of BHA showed the lower totox value.

7.4.2.5. Effects of BHA levels on colour absorbance value

The colour absorbance value in all oils tended to decrease during storage as shown in Figure 7.11.

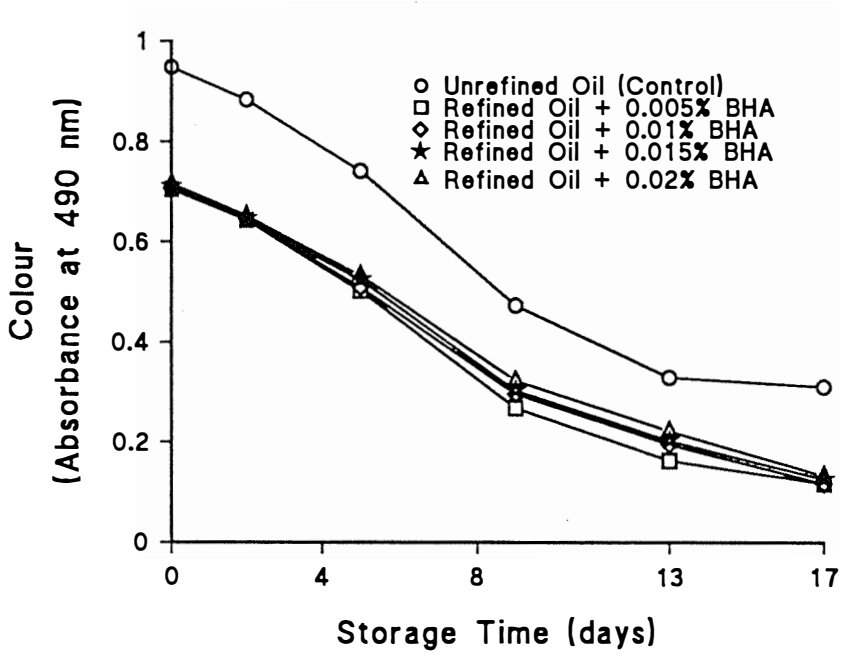


Figure 7.11. Effects of various BHA levels on colour absorbance value changes in fish oil during storage at 63±2°C

The higher the BHA level added to the oils, the higher the initial colour absorbance value will be. The colour absorbance value decrease in unrefined oils occurred at a higher rate than in the refined oils containing BHA. The colour absorbance value reduction was found to be faster in the refined

oil containing a lower BHA level than in the oil containing higher BHA levels. The end of storage showed that the colour absorbance value in unrefined oil decreased 0.64, while in the refined oils containing 0.005%, 0.01%, 0.015% and 0.02% BHA decreased 0.59, 0.59, 0.58 and 0.58 respectively.

7.4.2.6. Effects of BHA levels on refractive index (RI) value

All oils exhibited an increased pattern of RI value during storage as shown in Figure 7.12.

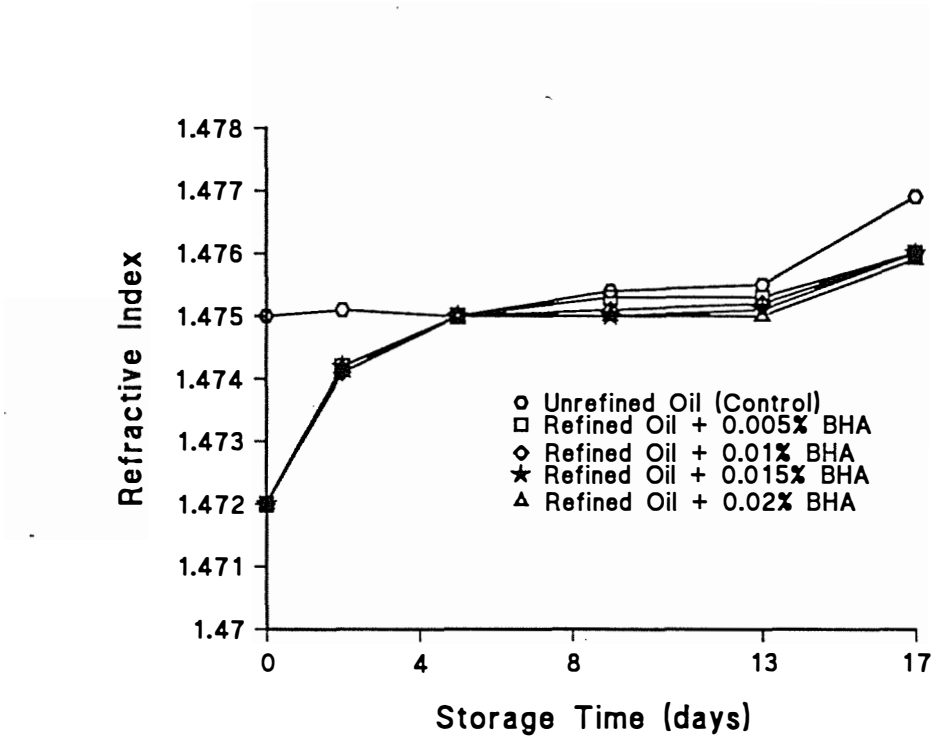


Figure 7.12. Effects of various BHA levels on refractive index changes in fish oil during storage at 63±2°C

Unrefined oil did not show an increase in RI value until the fifth day of storage. A significant increase was noted on the ninth day. All the refined oils containing BHA had a sharp RI increase during the first five days, an increase which continued at a slower rate until the thirteenth day. At

the end of storage, all oils exhibited a relatively sharp increase in RI value.

7.4.3. Use of vacuum package for fish oil stability improvement

7.4.3.1. Effects of vacuum package on peroxide value (PV)

Both refined and unrefined oils stored at 63°C and 30°C showed the same response to the vacuum treatment in terms of PV, as shown in Figure 7.13. The patterns of PV change at storage temperatures of 63°C and 30°C were similar, as the PV of oils increased sharply and then gradually decreased.

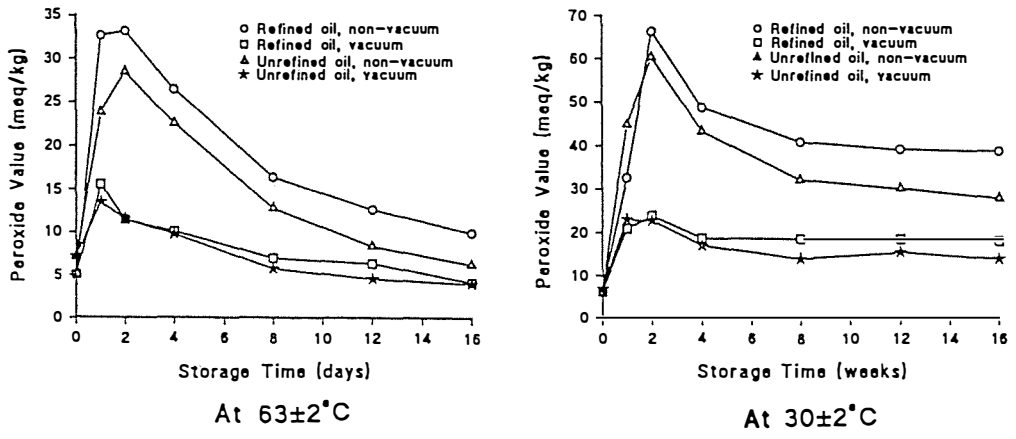


Figure 7.13. Effects of vacuum package on peroxide value changes in fish oil during storage at 63±2°C and 30±2°C

In the non-vacuum packages, the PV increased rapidly during the first two days at 63°C and after two weeks storage at 30°C. Then, the PV of oils stored at 63°C decreased until the end of storage. Until the eighth week there was a significant PV decrease in all oils stored at 30°C. From weeks 8 to 17, all PVs were relatively constant.

In the vacuum packages, sharp increases in the PV of the oils was noted on the first day at 63°C and at week one for storage at 30°C. After day one, the PV of the oils stored at 63°C decreased during further storage, while changes in the PV of the oils stored at 30°C was insignificant on the second week. A decreasing pattern was registered at the fourth week. Further storage for the oil at 30°C resulted in an insignificant change in PV.

The PV increase of the oils stored in non-vacuum packages occurred at a markedly higher rate than in the oils stored in vacuum packages. The refined oils tended to have a higher PV than the unrefined oils, except during the first week of storage for the oil stored at 30°C where the PV measured in unrefined oil was higher than in refined oil.

7.4.3.2. Effects of vacuum package on TBA value

The pronounced increase in TBA value of oils in non-vacuum packages at 63°C storage occurred on the first day as shown in Figure 7.14, but insignificant change occurred in the TBA value on the second day. Both refined and unrefined oils stored in non-vacuum packages at 30°C exhibited an increased TBA value during the first two weeks. After these periods the TBA values of oils decreased until the eighth day for oils stored at 63°C and the eighth week for the oils stored at 30°, followed by a relatively constant TBA value, to end of storage.

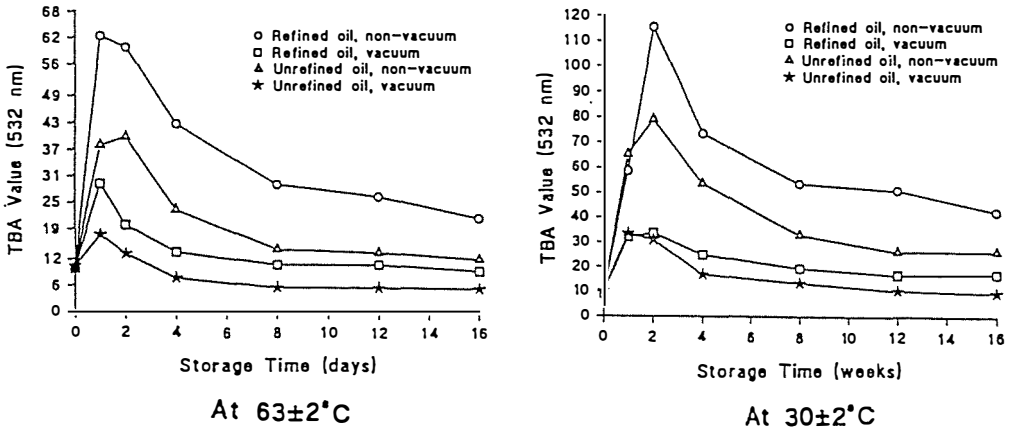


Figure 7.14. Effects of vacuum package on TBA value changes in fish oil during storage at 63±2°C and 30±2°C

Refined and unrefined oils stored in vacuum packages at 63°C had an increase in TBA values until the first day of storage. Then, values decreased until the fourth day. The extension of the storage period resulted in relatively constant TBA values. The TBA values of the oil stored in vacuum packages at 30°C increased significantly until the first week. Insignificant changes in the TBA values are noted in the second week. The sharp decrease in TBA value is then exhibited at the fourth week. Further storage gave insignificant changes in the TBA values.

The increase in TBA values in the oil stored in the vacuum package occurred at significantly lower rates than in the oil packed in non-vacuum conditions. In general, the refined oil had a higher TBA value than unrefined oils during storage.

7.4.3.3. Effects of vacuum package on anisidine value

The anisidine value changes in refined and unrefined oils during storage at 60°C and 30°C as shown in Figure 7.15. Both storage temperatures gave a similar response to anisidine value changes.

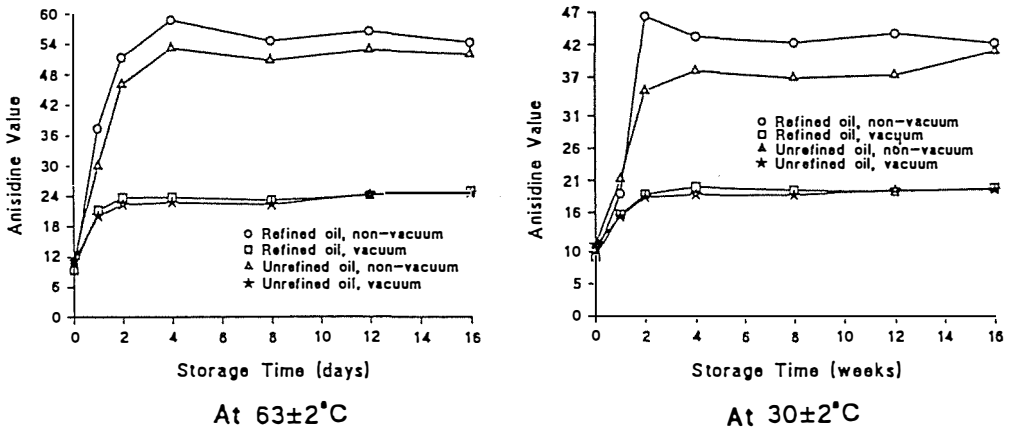


Figure 7.15. Effects of vacuum package on anisidine value changes in fish oil during storage at 63±2°C and 30±2°C

Non-vacuum packaged oils showed a rapid anisidine value increase during the first four days and two weeks at storage temperature 63°C and 30°C respectively. The extension of the storage period resulted in a relatively constant anisidine value. Vacuum packaged oils had a sharp increase in anisidine value during the first two days for oil stored at 63°C and during the first two weeks for the oils stored at 30°C. After these periods, the vacuum packaged oils showed an insignificant change in anisidine value during a further storage period.

The increase in anisidine value in the oils stored in non-vacuum packages was at a faster rate. The refined oils showed a higher increase in anisidine value than that seen in unrefined oils, and this characteristic was shown clearly in the oil packed under non-vacuum conditions.

7.4.3.4. Effects of vacuum package on totox value

Totox value changes of refined and unrefined oils stored in vacuum and non-vacuum packages at 63°C and 30°C are shown in Figure 7.16.

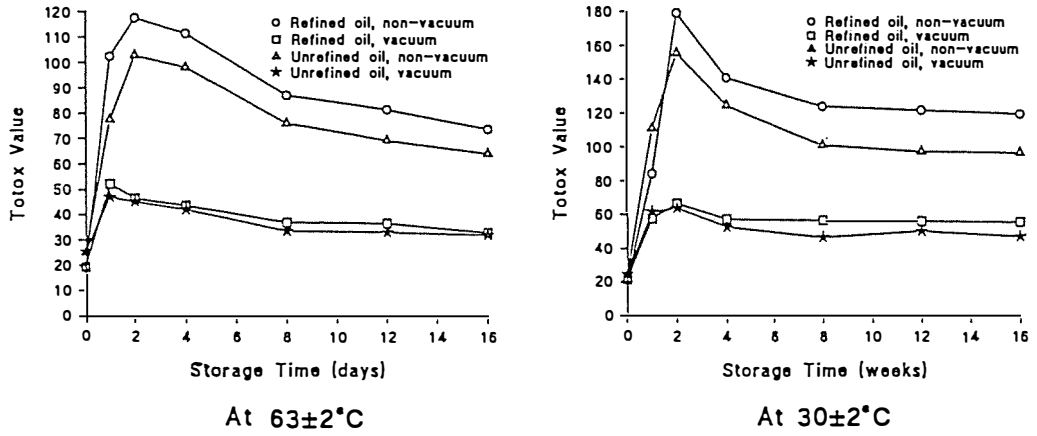


Figure 7.16. Effects of vacuum package on totox value changes in fish oil during storage at 63±2°C and 30±2°C

Refined and unrefined oils stored in non-vacuum packages at 63°C showed a marked increase in totox value until the second day. The significant increase in totox values for refined and unrefined oils stored in vacuum packages at 63°C occurred during the first day. Further storage of these oils resulted in a decrease in the totox value until the eighth day, which was followed by a relatively constant totox value.

An increase in totox value in the oils stored in vacuum and non-vacuum packages at 30°C was shown until the second week. After that period, the totox value of oils stored in non-vacuum packages decreased until the eighth day of storage, and the totox value showed insignificant change during further storage. While the totox value in the oils stored in vacuum packages reduced significantly on the fourth day, changes in value were subsequently insignificant.

The increase in totox value of the oil stored in non-vacuum packages occurred at a higher rate than in the oils stored in vacuum packages. In addition, the refined oil showed a higher increase in totox value compared to the unrefined oil.

7.4.3.5. Effects of vacuum package on colour absorbance value

The changes in colour absorbance value of oils stored in vacuum and non-vacuum packages are shown in Figure 7.17.

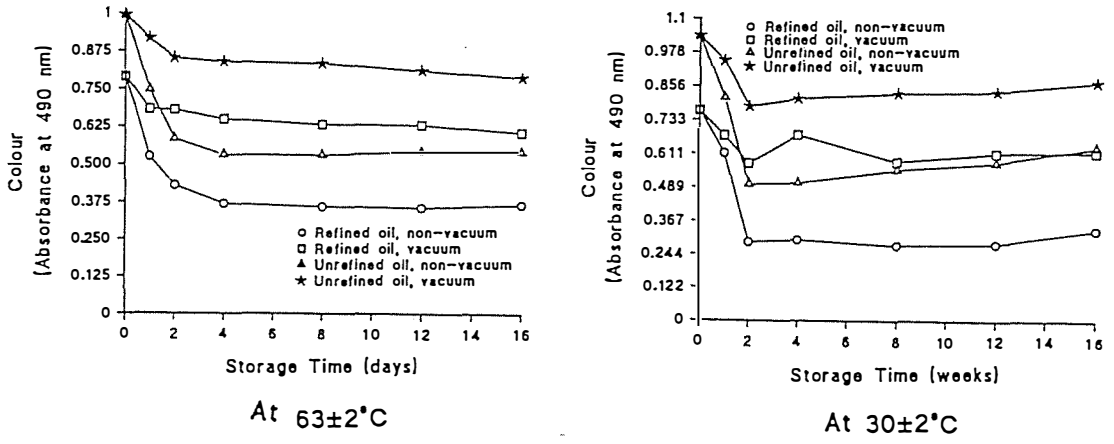


Figure 7.17. Effects of vacuum package on colour absorbance value changes in fish oil during storage at 63±2°C and 30±2°C

The significant decrease of colour absorbance of oils stored in vacuum and non-vacuum packages at 63°C was found during the first two days, after which the values tended to be relatively constant.

The colour absorbance changes in the oils stored at 30°C showed a different pattern. The decreasing pattern of the colour absorbance value in all oils stored at 30°C was noted during the first two weeks. Further storage for the unrefined oil in vacuum and non-vacuum packages indicated an increasing pattern at a slower rate than in the decreasing pattern. The refined oil stored in non-vacuum packages exhibited a significant increase in colour absorbance value on the fourth day. Then the value decreased again on the eighth day. A further storage resulted in an insignificant change in colour absorbance value. The colour absorbance value of refined oil stored in a vacuum package was relatively constant after two weeks.

The decrease in colour absorbance value in the oils stored in non-vacuum packages occurred at a higher level than in the oils stored in vacuum packages.

7.4.3.6. Effects of vacuum package on refractive index (RI) value

In general, the pattern of refractive index (RI) changes of the oils at storage temperature of 63°C and 30°C was relatively similar as shown in Figure 7.18.

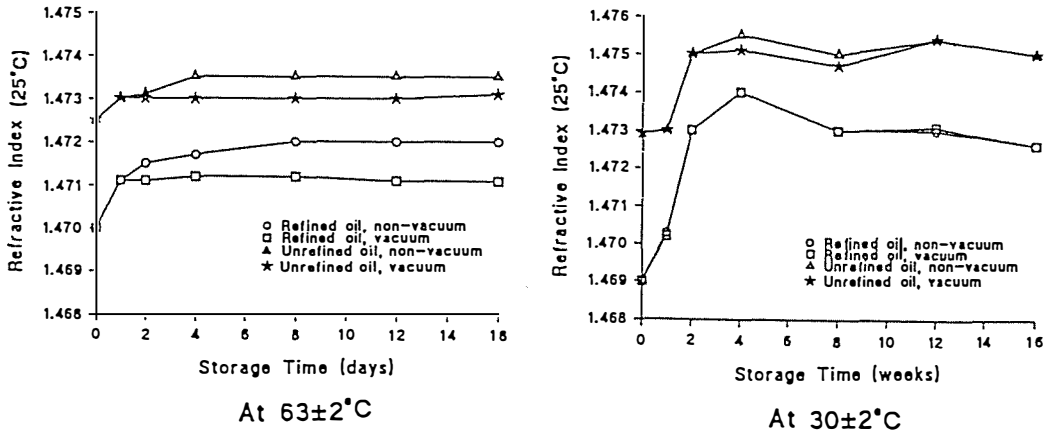


Figure 7.18. Effects of vacuum package on refractive index value changes in fish oil during storage at 63±2°C and 30±2°C

RI value of the oils stored in non-vacuum package at 63°C increased until the fourth day for unrefined oil and the eighth day for refined oil. The RI increase in the oils stored in vacuum packages was significant during the first day. After these periods, the change in the RI value for all oils was insignificant until the end of storage. The RI changes in the oil stored in non-vacuum packages indicated a higher rate than in the oil stored in non-vacuum packages.

The unrefined oil, stored at the 30°C, showed a relatively constant RI value during the first day, but an increase in RI was noted from the second day until the fourth day. The refined oil exhibited an increase in RI during the first two days. The reduction of RI values for both oils was observed on the eighth day. Storage extension to 12 days resulted in an increase in the RI value in both oils, but the increase in refined oil was registered as insignificant. In addition, the RI changes shown at the end of storage were insignificantly. The RI changes of the oil stored in vacuum and non-

vacuum packages were found to be nearly the same.

7.4.3.7. Effects of vacuum package on odour

Sensory observation tests for odour was performed only for the oils stored at 30°C. Eight trained Indonesian panellists performed the evaluation.

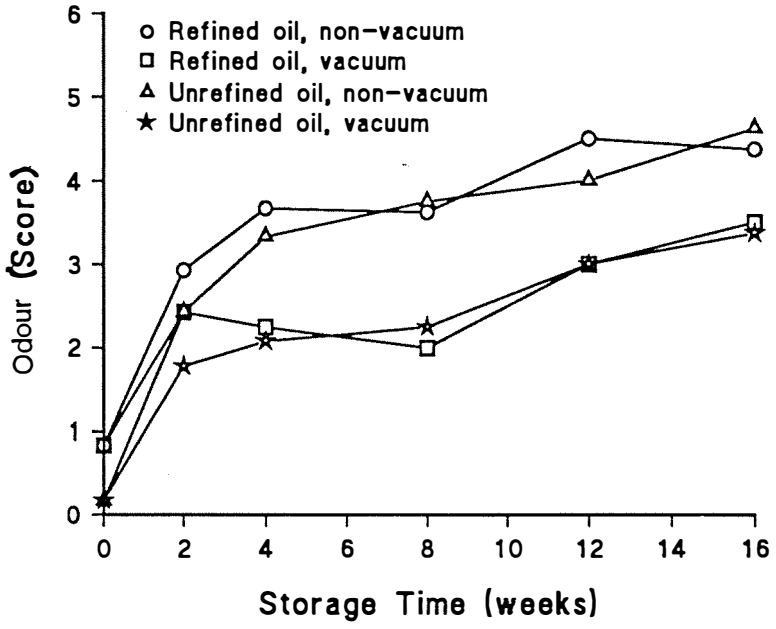


Figure 7.19. Effects of vacuum package on odour changes in fish oil during storage at 30±2°C

The odour scores of both refined and unrefined oils stored in vacuum and non-vacuum packages tended to increase during storage as shown in Figure 7.19. The increase odour score rate for the oils stored in vacuum packages occurred at a lower rate than in the oils stored in non-vacuum packages. This tendency was noted in both oils

7.5. DISCUSSION

7.5.1. Use of antioxidants for fish oil stability improvement

The results of this investigation indicate that the antioxidant induced changes in the initial peroxide value of fish oil. A reduction in the PV in the oils as the result of BHA, TBHQ and Grindox-117 revealed that these antioxidants may have accelerated the conversion of hydroperoxide into the secondary products of oxidation during sample preparation. In contrast, the α-tocopherol resulted in an increase in peroxide value. Possibly the α-tocopherol itself was the main cause of that increase, since the α-tocopherol solution had a peroxide value of 1.92 meq/kg. However antioxidants did not cause any significant changes for the initial values of other observed parameters, such as TBA, anisidine, colour absorbance and refractive index.

All antioxidants used in this experiment showed their expected role: to reduce the incident of undesirable chemical and physical changes in fish oil. TBHQ and BHA demonstrated a very powerful antioxidant effect to inhibit hydroperoxide, malonaldehyde and α/β-aldehydes formation as shown by peroxide, TBA and anisidine value analyses respectively. TBHQ was also found superior in the protection of the oil from colour loss, while BHA had very poor ability to protect fish oil colour. The superior effectiveness of TBHQ in the protection of this food product against oxidation was also noted by Ke et al (1977) in mackerel skin lipid, and Tokarska et al (1986) and Hawrysh et al (1989) in canola oil. Sherwin (1990) states that TBHQ could interact with free amines to give a red colouration, and this may have given TBHQ more ability to keep the yellow-reddish colour of fish oil.

Propyl gallate is also an effective antioxidant in fish oil. However this antioxidant was not effective over a long period of storage, exhibiting only short term capability over oxidation. After a period of storage there was a sharp increase in peroxide, malonaldehyde and α/β-aldehyde formation in the oil. A similar occurrence was also encountered in oil colour loss where sharp decrease of colour absorbance value was noted after a period of storage. Propyl gallate has low oil solubility and losses its effectiveness under heat conditions (Sherwin, 1990; Dziezak, 1986; Coppen, 1989). These characteristics may have affected the effectiveness of this antioxidant in fish oil during this stability study. Propyl gallate chelates iron ions to form an aesthetically unappealing blue-black complex (Dziezak, 1986). However this complex did not occur in this study, as Grindox-117 was incorporated with the chelating agent, citric acid.

D*l*-α-tocopherol was not an effective antioxidant in inhibiting peroxide, malonaldehyde and α/β-aldehyde formation as the primary and secondary products of oxidation in fish oil. However tocopherol showed as an effective protection to colour, and this indication was supported by results of an experiment conducted by Cort (1974) where tocopherol had the greatest effect in protection of carotenoids and vitamin A. Carotenoids are the main pigment forming fish oil colour. The protection of vitamin A was also important, because fish oil is rich in vitamin A (Brody, 1966). The use of tocopherol as an antioxidant would fail to provide effective protection against oxidation, when the oil was contaminated with trace amounts of metals, such as Fe^{2+} or Cu^{2+} (Valenzuela et al, 1991).

The results suggest that the order of antioxidant effectiveness was found to be TBHQ > BHA > Grindox-117 (propyl gallate) > tocopherol. Unfortunately, TBHQ is not mentioned in the list of antioxidants permitted in food and drink in Indonesia (Indonesian Health Ministry, 1974). Thus, BHA, an alternative, was used in further experiments to establish the optimum levels. In the optimisation experiment, the results revealed that the higher the BHA level, the more effective the antioxidant to protect the oil against oxidation. At high antioxidant levels, the antioxidant might not only react with alkyl peroxy and alkyl radicals, but also react directly with oxygen which would give an overall protection to the oil against oxidation. The use of unrefined oil as control was to show the optimum antioxidant level necessary to recover the loss of natural antioxidant during the refining process. In the case of New Zealand crude oil refined using macroporous strong acid cation resin, 0.01% of BHA was sufficient to recover the loss of natural antioxidants.

7.5.2. Use of vacuum package for fish oil stability improvement

The results of the study of the vacuum effect on fish oil stability improvement at storage temperatures of 30°C and 63°C showed a similar pattern, but the peak values of PV, TBA and totox for oil stored at 30°C were higher than the values for oil stored at 63°C. The similar PV, TBA and totox values analyzed in oil stored at 30°C would probably be obtained in the oil stored at 63°C, if the analyses were carried in the period between 1 and 2 days storage.

In the oil stored in vacuum packages, where the oxygen level was limited, the peroxide formation was dependent on residual oxygen, and probably on oxygen in the sample itself. The recorded headspace vacuum in the can was approximately 17.7 - 19.2 inHg. Based on the relationship between the headspace vacuum and residual oxygen content, published by the CIG Gases Division (1989), the approximate residual oxygen content inside the cans was approximately 7.5 - 8.7%v/v.

Oxygen, or gas, can be present in liquid samples in three forms: physically entrapped, dissolved or chemically bound (Fletcher, 1987). The presence of oxygen in the oil stored in vacuum packages was shown by the increase of peroxide value, indicating the formation of the primary product of oxidation, hydroperoxide. The sensory evaluation also detected the odour changes in the oils stored in vacuum packages, although the changes were slower than those in the oil stored in non-vacuum packages.

The peroxide formation showed an increasing tendency, but a decreasing pattern was also noted. A similar pattern was obtained by Nair et al (1979) in skin and muscle lipids of oil sardine. This indicated that the decreasing pattern reflected the conversion state of peroxide into the secondary products of oxidation. TBA value showing malonaldehyde forming as a secondary product, exhibited a similar pattern as the peroxide value, where after having an increase pattern, the value decreased supporting the finding of Nair et al (1979). This decrease could be due to reaction malonaldehyde-type compounds, with amino acids, peptides and other compounds released from decomposition of protein (Kwon et al, 1965; Finley, 1985). Malonaldehyde can cross link protein through a *Schiff* base reaction with the ε-NH_2 group of lysine (Belitz and Grosch, 1987; Finley, 1985; Gillatt et al, 1988). Malonaldehyde can also undergo aldol-type reactions to produce a mixture of polymers (O'Brien, 1987). Anisidine values measuring secondary products of α/β-aldehydes increased at first and then showed a relatively constant value during a further storage period. According to Dugan (1976) aldehydes are presumable converted to acids. If the aldehydes are enals or dienals, these may oxidize further to provide yet shorter chain compounds. Thus, the constant anisidine value was probably due to the conversion rate from peroxide into α/β- aldehydes and the conversion rate from aldehydes to other compounds, as mentioned above which were the same.

Colour absorbance value of the oils stored at 30°C decreased at first and then tended to increase. This tendency was shown clearly in unrefined oil and refined oil stored in vacuum packages. The increase of colour absorbance value might be due to the result of the reaction between hydroperoxides and their degradation products with protein inducing browning (Belitz and Grosch, 1987). The rate of the discolouration of carotenoids was probably slower than the browning reaction rate which finally resulted in an oil of darker colour. At first, the oils stored at 63°C showed a decreasing pattern of colour absorbance value, and then a relatively constant value was noted. The constant value was probably due to the browning reaction rate, and the discolouration rate of carotenoid which occurred at the same rate.

This study revealed that vacuum package indicated a better protection for the oils compared to the

non-vacuum package by exhibiting slower changes chemically, physically and organoleptically.

7.6. CONCLUSIONS

The above results prove that antioxidants and vacuum packages could be used to improve fish oil stability during storage, especially for resin refined fish oil. Among the tested antioxidants, TBHQ was the best antioxidant to use in resin refined fish oil. However BHA as the second best alternative was used in the optimisation experiment, because TBHQ is not classified as a permitted antioxidant in Indonesia. 0.01% BHA was actually sufficient to recover the loss of a natural antioxidant due to the resin refining process.

Chapter 8

APPLICATION OF RESIN REFINING TO INDONESIAN FISH OILS

8.1. BACKGROUND

As discussed in Chapter 4, most Indonesian fish oil is not suitable for human consumption, especially the oil obtained from fish meal processing. A further selective refinement process is necessary to make the oil acceptable for human consumption.

Chapter 5 proved that resin refining could improve fish oil quality in New Zealand fish oils. In another experiment using salmon oil, Fernandez (1986) proved that resin refining could improve fish oil quality chemically, physically and organoleptically. However the use of the resin refining process for Indonesian fish oils has not been investigated.

In order to introduce the resin refining method to Indonesian fish oil industries, the superiority of this method should be studied using Indonesian fish oils. The stability of refined Indonesian fish oils should also be investigated. Information about the potential value of this method to the Indonesian fish industry is best obtained by conducting a survey.

8.2. OBJECTIVES

The experiment and survey were aimed to show the possibility of the application of the resin refining method to Indonesian fish oil, and the prospect of this method being adopted by Indonesian fish oil producers. The experiment was also designed to obtain information about the stability of refined Indonesian oils compared to unrefined oils.

8.3. METHODOLOGY

8.3.1. Materials

Two kinds of Indonesian fish oils, from fish meal processing and canning waste, were used in this study. Both oils were obtained from sardines. Fish meal oil was supplied by P.T.Sumber Rejeki, Muncar (East Java). The canning waste oil came from P.T.Karya Manunggal Tri Sukses, Muncar (East Java).

8.3.2. Methods

8.3.2.1. Fish oil refining

The oils were refined using a resin packed column 39 cm in length and 1.65 cm in diameter. The refining was performed with a fish oil-resin volume ratio of 1:1. Canning waste oil was refined by passing the oil through the column once. The fish meal oil was passed through the column twice, since the oil had a very undesirable odour. Seven Indonesian panellists participated in the sensory evaluation using the same format to evaluate fish oil samples as used in Chapter 4, and as shown in Appendix 4.2.

8.3.2.1. Fish oil stability

Fish oil stability was investigated using the Schaal oven method, also used in the experiment discussed in Chapter 7. The fish oil samples, each of 80 ml were contained in 100 ml beakers, and covered loosely with aluminium foil. The samples were stored in an oven at 63$\pm$2°C and withdrawn after 0, 2, 4, 7, and 11 days.

8.3.2.1. Survey on the response of Indonesian fish oil producers to resin refining method

A survey was carried out with the 19 Indonesian fish meal factories discussed in Chapter 4. The survey obtained information about their interest in adopting the resin refining process. The questionnaires used for the survey is shown in Appendix 8.1.

8.4. RESULTS

8.4.1. Effects of resin refining process on Indonesian fish oil

The fish oil quality was analyzed chemically, physically and organoleptically, using the same methods applied to resin refining optimisation in Chapter 5.

Free fatty acid (FFA) values of both fish meal and canning waste oils were reduced by the resin refining process as shown in Figure 8.1. The FFA values of fraction-2 for both oils were higher than the values measured in untreated and fraction-1 oils.

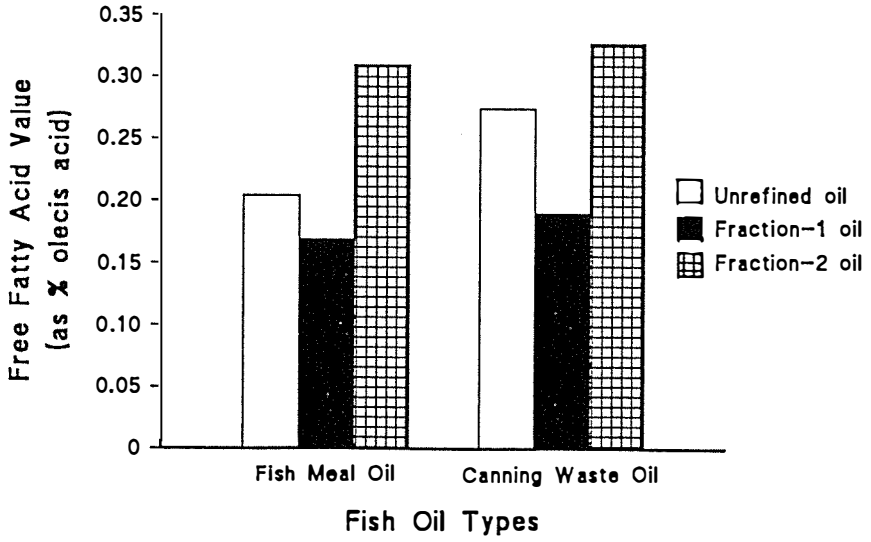


Figure 8.1. Free fatty acid value changes in fish meal and canning waste oils during resin refining process

Refractive index (RI) values of both oils also decreased after refining process as shown in Figure 8.2. The effect of two refinings on the fish meal oil was significant, exhibiting a higher decrease in RI value compared to canning waste oil which was passed through the column only once. The RI values of fraction-2 for both oils were higher than RI values for fraction-1 oils. However the value measured in fraction-2 fish meal oil was equal to the RI value of untreated oil, and the value of fraction-2 canning waste oil was slightly lower than the value in untreated oil.

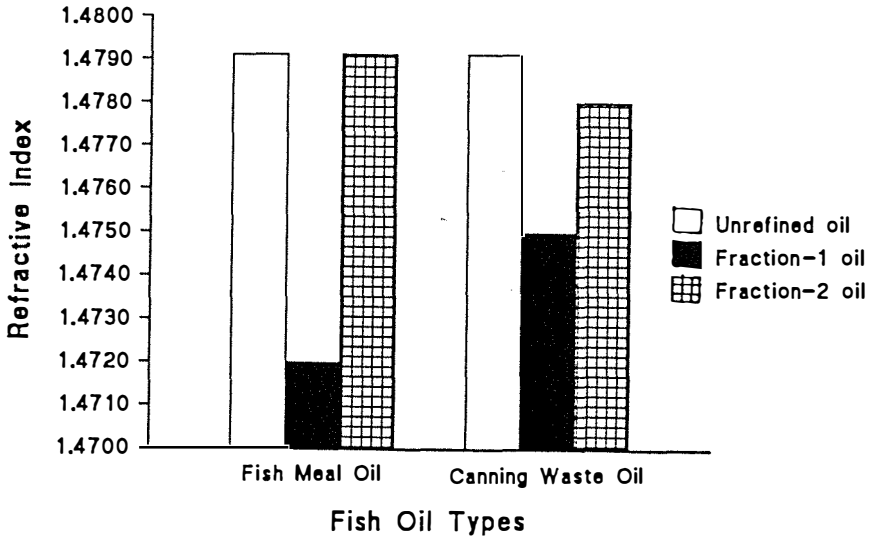


Figure 8.2. Refractive index value changes in fish meal and canning waste oils during resin refining process

The colour of both oils was also improved by resin refining. Oils with clearer colour were obtained, as shown in Figure 8.3. The colour of fraction-2 for both oils was darker compared to the colour of untreated oils and fraction-1 oils.

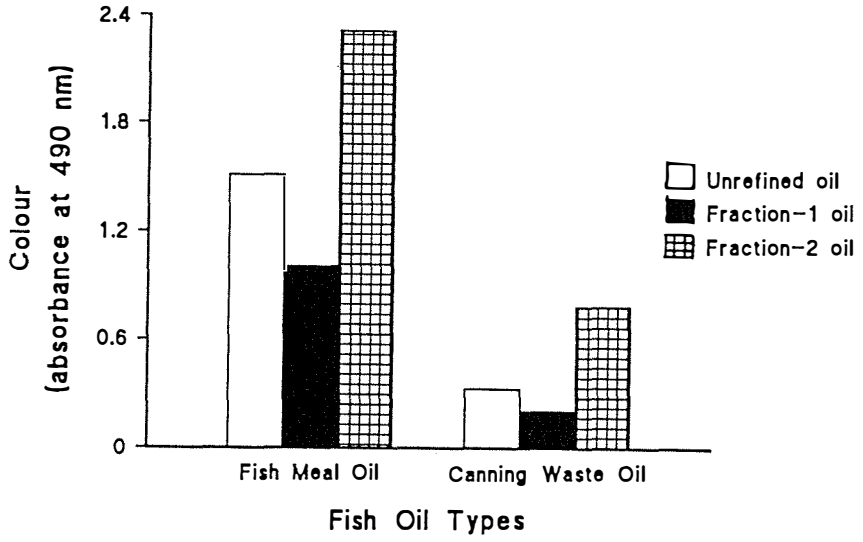


Figure 8.3. Colour absorbance value changes in fish meal and canning waste oils during resin refining process

The fatty acid profile changes in both Indonesian fish oils during resin refining are shown in Table 8.1.

Table 8.1. Fatty acid profiles changes in fish meal and canning waste oils during resin refining process

Fatty Acids	Fish meal oil			Canning waste oil		
	U.T.*)	Frac-1	Frac-2	U.T.*)	Frac-1	Frac-2
SAFA	37.7	38.5	41.9	39.2	38.7	39.4
MUFA	29.5	29.7	29.5	29.0	29.6	29.3
PUFA	32.6	31.6	28.5	31.8	31.7	31.0
ω-3FA	28.1	26.9	24.1	26.7	27.2	26.5
EPA	19.0	17.8	16.0	17.2	16.9	16.5
DHA	5.8	5.5	4.8	6.1	6.3	6.0

Note: *) U.T. = untreated oil

Saturated fatty acid (SAFA) value of fraction-2 fish meal oil was significantly higher than the values in untreated and fraction-1 oils. The SAFA values of untreated and fraction-1 oils did not show any significant different. However the SAFA values in untreated, fraction-1 and fraction-2 canning waste oils did not exhibit any pronounced difference.

Monounsaturated fatty acid (MUFA) values of untreated, fraction-1 and fraction-2 for both oils were relatively the same.

Polyunsaturated fatty acids (PUFA), ω-3 fatty acids and eicosapentaenoic acid (EPA) values of fraction-2 fish meal oil tended to be lower than the values analysed in untreated and fraction-1 oils. The untreated, fraction-1 and fraction-2 of canning waste oil showed insignificant differences in PUFA, ω-3FA and EPA values. Docosahexaenoic acid (DHA) values of untreated and fraction-1 fish meal oils were markedly higher compared to the value measured in fraction-2 oil. Untreated, fraction-1 and fraction-2 canning waste oils showed an insignificant difference in DHA value.

Refined oils showed a lower natural tocopherol antioxidant content than in unrefined oils, especially in terms of α, γ, δ-tocopherol and α-tocotrienol. Fish meal oil was rich in α-tocotrienol, which was not traced in canning waste oil. The only natural tocopherol antioxidant detected in canning waste oil was α-tocopherol, as shown in Table 8.2.

Table 8.2. Tocopherol content of fish meal and canning waste oils during refining process (ppm)

Tocopherols	Fish meal oil		Canning waste oil	
	Untreated oil	Refined oil	Untreated oil	Refined oil
α-tocopherol	25.3	14.3	7.1	4.6
α-tocotrienol	297.3	116.4	n.d	n.d
γ-tocopherol	4.6	1.8	n.d	n.d
δ-tocopherol	3.2	n.d	n.d	n.d

Note: n.d. = not detected

Panel results indicate that the undesirable odour in Indonesian fish oils could be reduced by using the resin refining method, as the odour score of fraction-1 oil was lower than the odour score of unrefined oil as shown in Figure 8.4. The odour score for fraction-2 fish meal oil was higher than

the odour score for fraction-1 oil and comparable to the odour score of untreated oil. However the odour score for fraction-2 canning waste oil was higher than both fraction-1 and untreated oils.

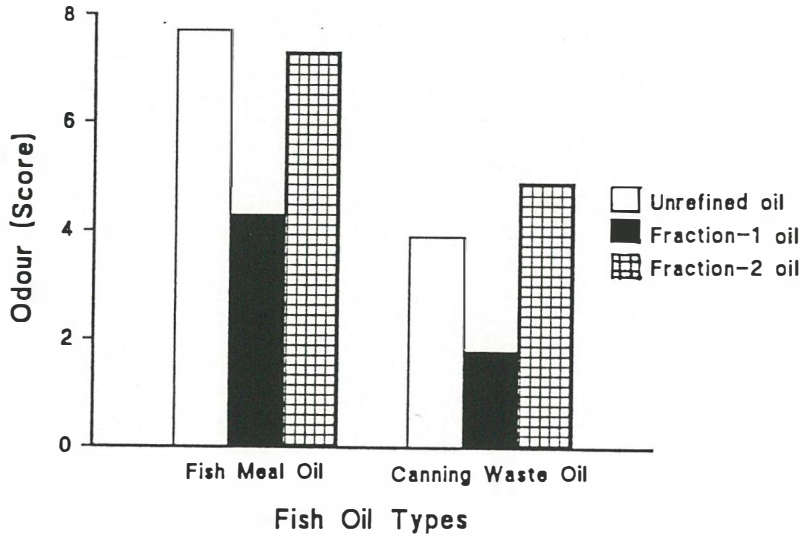


Figure 8.4. Odour score changes in fish meal and canning waste oils during resin refining process

8.4.2. Stability of refined Indonesian fish oil

8.4.2.1. Chemical changes

The changes of peroxide, TBA, anisidine and totox values of both fish meal and canning waste oils are shown in Figures 8.5, 8.6, 8.7 and 8.8.

Peroxide values of both oils were relatively constant during the first two days of storage but values increased on the fourth day, except for the untreated fish meal oil. These PV values still showed an increase until the end of storage. The fastest PV increase was noted in the refined canning waste oil. The PV increase in the refined fish meal oil and unrefined canning waste oil occurred at approximately the same rate. The PV changes in unrefined fish meal oil were very slow compared to other tested oils. The fastest PV increase was found between the seventh and eleventh days for all oils, except for the refined canning waste oil showing the highest PV increase rate between the fourth and the seventh day.

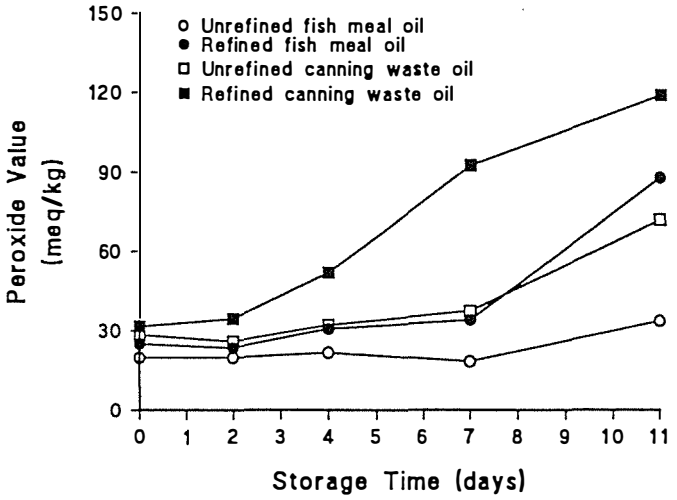


Figure 8.5. Peroxide value changes in both refined and unrefined fish meal and canning waste oils during storage at 63±2°C

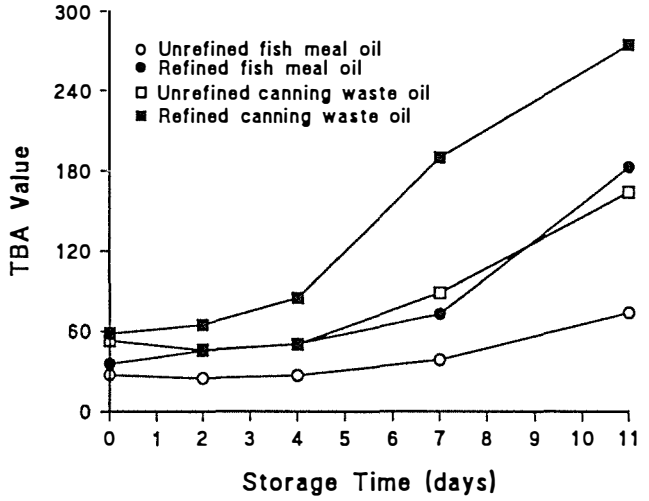


Figure 8.6. TBA value changes in both refined and unrefined fish meal and canning waste oils during storage at 63±2°C

All oils exhibited a very slow change in TBA values until the fourth day, except for refined canning waste oil where the TBA value increased significantly. Sharp increases in TBA values was observed starting on the fourth day for all oils. Refined canning waste oil showed the fastest TBA increase, while unrefined fish meal oil had the slowest increase in TBA value. Unrefined canning waste oil and refined fish meal oil displayed a similar increase rate in TBA value.

The linear increase in anisidine value was noted until the fourth day for refined canning waste oil, the seventh day for both unrefined canning waste oil and refined fish meal oils, and the end of storage for unrefined fish meal oil. After these periods, unrefined and refined canning waste oils, as well as refined fish meal oil, had a sharp increase in anisidine value. At the end of storage those oils had insignificant anisidine value differences. Unrefined fish meal oil showed the slowest anisidine value increase.

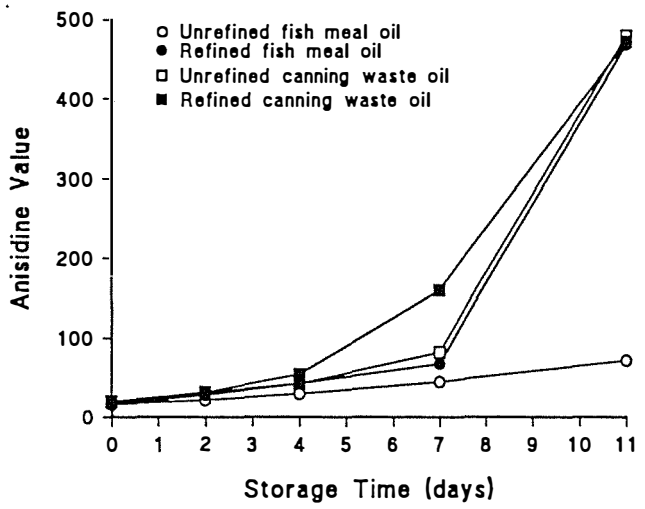


Figure 8.7. Anisidine value changes in both refined and unrefined fish meal and canning waste oils during storage at 63±2°C

The pattern of totox value changes was similar to the pattern of anisidine value changes. However at the end of storage the totox value of refined canning waste oil was significantly higher in comparison with the values of unrefined canning waste oil, unrefined fish meal oil and refined fish meal oil. The unrefined fish meal oil exhibited the lowest totox value increase at all times during storage.

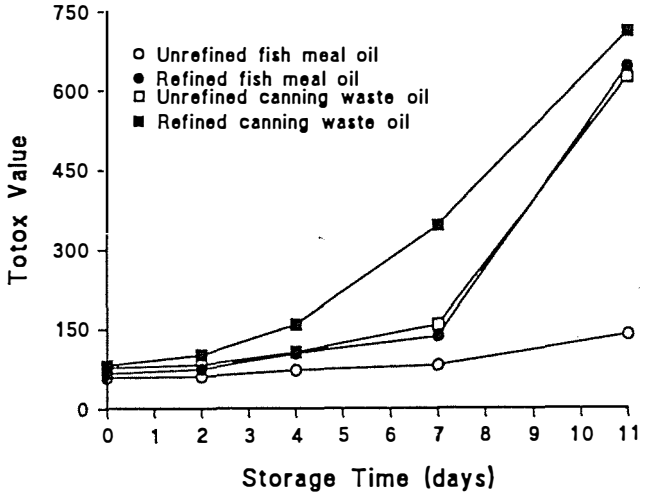


Figure 8.8. Totox value changes in both refined and unrefined fish meal and canning waste oils during storage at 63±2°C

8.4.2.2. Physical changes

The colour absorbance and refractive index (RI) values changes in both fish meal and canning waste oils during stability study are shown in Figures 8.9 and 8.10.

The colour absorbance value changes in fish meal oil showed a decreasing trend during storage. No significant change in absorbance value of unrefined oil was noted during the first two days of storage. The reduction of colour absorbance in refined oil occurred at a higher rate than in unrefined oil.

Canning waste oil exhibited a different trend in colour absorbance change when compared to the fish meal oil. Unrefined canning waste oil had a linear colour absorbance value increase until the fourth day. Then, colour absorbance value decreased gradually. Refined canning waste oil exhibited a gradual reduction pattern of colour absorbance value until the seventh day. Then, a relatively constant value was observed.

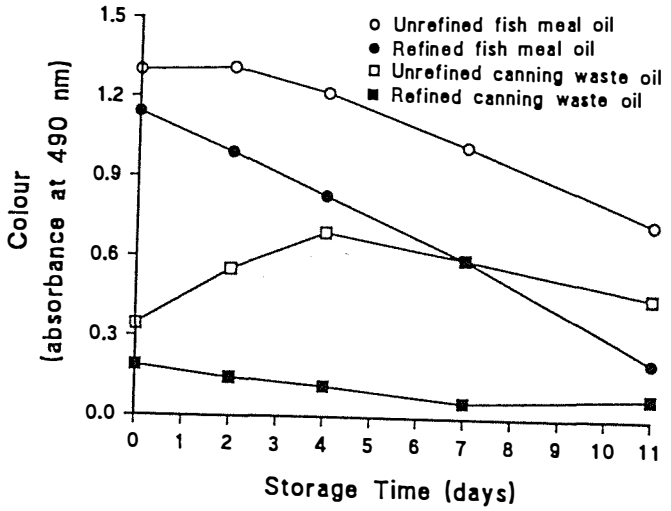


Figure 8.9. Colour absorbance value changes in fish meal and canning waste oils during storage 63±2°C

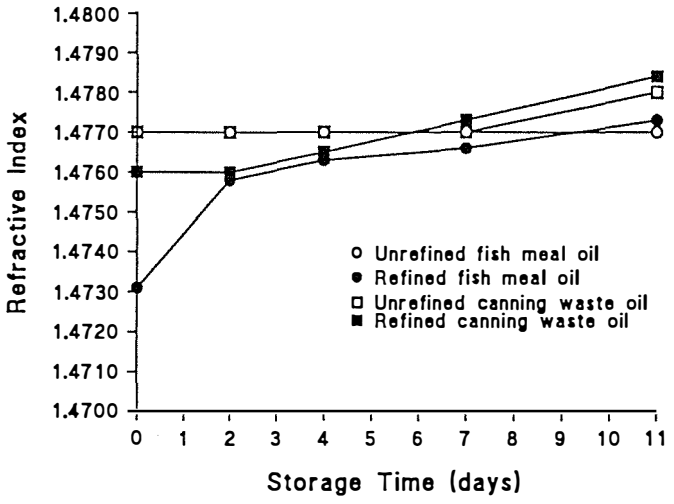


Figure 8.10. Refractive index value changes in fish meal and canning waste oils during storage 63±2°C

The refractive index (RI) values of unrefined fish meal oil was relatively unchange, but the refined fish meal oil showed an RI increase trend during storage. Sharp RI increases in fish meal oil occurred during the first two days but after that period, the RI value decrease gradually. At the end of storage, the RI value of refined oil was higher than unrefined oil.

The RI value of unrefined canning waste oil was relatively constant until the seventh day, but increased by the end of storage. The refined canning waste oil showed an increase trend of RI value starting at the second day. The RI value of this oil exceeded the RI value of unrefined canning waste oil starting at the seventh day.

8.4.3. Response of Indonesian fish oil producers to resin refining process

From the 19 factories participating in the survey, 17 factories commented that the resin refining process was an interesting idea. The other two factories were not interested at all. Only one of the interested factories did not intent to apply the refining method. This factory was the traditional fish meal processor.

All factories with refining units were interested in the new refining method being developed in this study. Two of them intended to replace their existing refining facilities if the method proved more effective. Another factory intended to operate the new refining process together with existing method. All factories would apply the new refining method for all fish oil types produced.

Moreover, 13 of the factories surveyed which do not have a refining unit intend to adopt this proposed technology for all fish oil types produced.

Table 8.3. Results of fish meal factory survey about the response to resin refining process (number of factories)

1. Factories comments about resin refining method being developed:	
a. an interesting idea	17
b. not an interesting idea	2
	19
2. Factories commenting that the resin refining method was an interesting idea intended to adopt the technology in the factory:	
a. intending to adopt	16
b. not intending to adopt	1
	17
3. The way to adopt resin refining method among factories which have been facilitated with refining unit:	
a. replace the existing method	2
b. operated together with existing method	1
	3
4. Fish oil types which would be refined using resin refining method among the factories which have been facilitated with refining unit:	
a. all fish oil types produced	3
b. certain fish oil types produced	0
	3
5. Fish oil type which would be refined using resin refining method among factories which have not been facilitated with refining unit:	
a. all fish oil types produced	13
b. certain fish oil types produced	0
	13

8.5. DISCUSSION

8.5.1. Effects of resin refining on chemical, physical and organoleptic properties of Indonesian fish oil

The above results proved that Indonesian fish meal and canning waste oils could improve their quality chemically, physically and organoleptically by application of the resin refining process. The same conclusion were reached using New Zealand fish oils as discussed in Chapter 5.

Canning waste oil, which required only one refining, was easier to refine than fish meal oil. This seemed that the original fish oil properties may have affected the refining process. As mentioned in Chapter 4, the oil produced from fish meal processing had a very undesirable odour, and this caused difficulties during refining, as one refining was not enough to reduce the undesirable strength of the odour. The application of a second refining significantly decreased the undesirable odour. The purity of the oil was also indicated by the significant decrease in RI value, a greater RI value reduction than occurred in canning waste oil. The colour absorbance value reduction in fish meal oil was also greater in value than the reduction in canning waste oil. This appeared to be the result of two refinings. This indicated that the multiple refining method, as investigated in the experiment discussed as in Chapter 5, provided important and valuable information for Indonesian fish oils which were mostly obtained from fish meal processing.

In terms of fatty acid profiles, both oils exhibited a different behaviour. The fatty acid profiles of canning waste oil were relatively unchanged. Changes in the fatty acid profile were noted in fish meal oil. Saturated fatty acid (SAFA) value in fraction-2 oil was higher than in fraction-1 oil, while polyunsaturated fatty acid (PUFA) and ω-3 fatty acids values in fraction-2 oil was lower than in fraction-1 oil. The results of fatty acid profiles in fish meal oil contradicted the results discussed in Chapter 5 and the results from experiments conducted by Fernandez (1986). Probably the natural properties of the oils affected the changing trend in fatty acid profile, but the exact reasons for these differences are unknown.

8.5.2. Stability of refined Indonesian fish oils

While fish oil quality could be improved using the resin refining process, fish oil stability during storage must be considered in determining overall quality.

Similar results, as shown in the experiment discussed in Chapter 6, are also demonstrated in this experiment. Refined oils have less stability against oxidation indicating a faster formation of primary and secondary products of oxidation. Generally, peroxide, malonaldehyde and α/β-aldehyde formation were faster in refined oil than in unrefined oil, displaying a faster increase in peroxide, TBA and anisidine values. The TBA and anisidine values of fish meal oil were always lower than the values in canning waste oil. One reason for this is the higher free fatty acid value in fish meal oil, as shown in the experiment in Chapter 4, suppressed the formation of aldehydes (Nair, 1979). RI value changes also supported the above statement, where the increase level of RI value in refined oil was faster than in unrefined oil.

Loos of colour in refined oils occurred at faster rates than in unrefined oils and this result was also the same as observed in the experiment detailed in Chapter 6. Unrefined canning waste oil showed unusual colour absorbance changes compared to the results from the previous fish oil stability study. The first four days of storage resulted in the increase of colour absorbance value in unrefined canning waste oil, but a further storage period exhibited a decreasing trend. As mentioned before, the darkening process might be due to the reaction between proteins with hydroperoxides and their degradation products producing browning process (Belitz and Grosch, 1987). The increasing colour absorbance value in the canning waste oil during the first four days might be due to the darkening process occurred at a higher rate than the carotenoids decolouration. In the unrefined oils, the natural antioxidant of tocopherol showed a higher protection of colour (carotenoids) against oxidation than in refined oils having a lower tocopherol content. After the darkening process achieved its peak, carotenoids decolouration process became more obvious showing a reduction in colour absorbance value.

In general, the stability experiment revealed that canning waste oil had less ability to protect itself against oxidation attack in comparison to fish meal oil. The presence of a natural antioxidant, particularly tocopherols, was an important factor in protecting fish oil from oxidation process, as mentioned in Chapter 6, and proved in Chapter 7. According to the results of analysis, the tocopherols obtained from fish meal oil were α-tocopherol, γ-tocopherol and δ-tocopherol accompanied by α-tocotrienol, while α-tocopherol was the only natural tocopherol traced in canning waste oil. The presence of α-tocopherol in canning waste oil was at a lower level than

in fish meal oil. Pokorny (1987) stated that α-tocotrienol possessed a slightly higher antioxidant activity than the corresponding tocopherol and this α-tocotrienol was present in fish meal oil at a higher level than other tocopherols. The above indications give a reasonable explanation for why fish meal oil has more protection against oxidation than canning waste oil.

8.5.3. Prospect of introduction of the resin refining process for Indonesian fish oil

The prospects of the introduction of the resin refining process to Indonesian fish oil industry appears to be very promising. It was determined that 84% of fish meal factories surveyed were interested in installing this refining process. All factories already having refining facilities considered adopting this process to replace, or to operate along side of the existing refining process. All factories intended to use this method to refine all fish oil types.

The factories would be more interested in this process if the method was profitable. Fernandez (1986) has carried out economic evaluation for both pilot plant and semi-commercial scale plant, both proving profitable. The larger the production scale or capacity the larger the profit. The findings in the study of resin refining optimisation as discussed in Chapter 5 indicate that the application of vacuum pressure treatment to resin column could accelerate the refining process. This could increase the effectiveness and profitability of the process. The refining process could be increased to 300% without vacuum pressure application. Consequently, the flow rate was increased as well. Thus production capacity was increased and the opportunity to increase profits is possible.

This study does not discuss the design of a refining unit using this technology. However, from the survey, some considerations could be suggested when designing the resin refining unit for Indonesian fish oil producers, especially for the producers located in Muncar (East Java) and Negara (Bali). Since the fish oil was just a by-product from fish meal and canned fish processing, the refining unit should be designed with automatic processes, thus more efficiently operated and with low labour costs. The materials used in constructing the refining unit should be available locally for easy of maintenance. The materials must be anticorrosive, since the factories are located close to the sea. All of these are expected to make the refining unit more attractive to the fish oil producers.

As discussed in the following chapter most canneries were interested in the production canned fish

enriched with fish oil. This could expand the market for refined fish oil. As discussed in Chapter 9, more than 30% of respondents to the consumer survey answered that they were willing to consume fish oil in capsule form. This means that the pharmaceutical market will also expand. Promotion to make the public aware of the health benefits of fish oil are very important to realize, and to open markets for fish oil. These would encourage producers to refine their oils to meet consumable standards making the prospect of the resin refining process adoption more feasible.

8.6. CONCLUSION

The above results indicate that Indonesian fish oil could be refined successfully using the resin packed column, where the oil qualities were improved chemically, physically and organoleptically. Treatments were required for refined oils to improve their stability during storage as suggested for New Zealand fish oils. Since the canning waste oil had a low natural antioxidant content, the application of the stability improvement method should be used on the unrefined oil as well. In this case, antioxidant additions, such as BHA, and vacuum package are advised. The prospect of adoption of the resin refining technology by the Indonesian industry is promising, and the prospects for new markets for refined oil appears to be an important factor in attracting producers.

Chapter 9

DETERMINATION OF CANNED FISH PRODUCT TYPE CONTAINING REFINING FISH OIL AS A MAJOR INGREDIENT

9.1. BACKGROUND

Most companies now recognize that the key to their future survival and growth rests in a continuous flow of new and improved products due to the dynamic changes in market needs. The development of new products remains an exceedingly difficult and challenging undertaking. It is difficult because the process of innovation is inherently complex, requiring the close coordination and control of a multitude of vastly different tasks. It is challenging because important decisions, often involving the very survival of the enterprise, must be made on the basis of very limited information. Product innovation has become a vital element in corporate strategy and planning for a number of reasons outside the control of any single company. These include changes in consumer and competitor behaviour, technology and government policy (Rothberg, 1981).

There are many ways in which a company may add products to its production for local and foreign markets. The most economic way, and a common practice, is to acquire a firm, or some operations of a firm which produces products with a potential market. A company can also add products to its own, by copying products developed successfully by others. Finally, a company can obtain new products by internal product development (Albaum et al, 1989). However commercial success is dependent on the following (Anderson, 1985):

* selection of a product with a high level of consumer demand;
* selection and definition of a product with the minimum of potential opposition from competitors; and
* development of a product which ultimately embodies those characteristics which are required by and are acceptable to the consumer.

A product has two key dimensions: technology and market. Technology is the fund of knowledge enabling economic production. Market relates to the "who" and "how" of product sale, enabling profitable distribution (Booz, Allen and Hamilton Inc, 1981). In product development terms, the food industry is adept at identifying popular preferences, and is a considerable force in persuading

consumers to try new taste sensations, thereby creating new preferences and expectations (Conning, 1990).

This study was conducted to obtain a new product nutritionally better than at present, by fish oil addition to improve ω-3 fatty acid content. As previously mentioned, health benefits of fish oil, particularly those provided by ω-3 fatty acids, are indisputable. In this study, product competitor, technological information and market preferences were identified using supermarket, canned fish producer and consumer surveys.

9.2. OBJECTIVES

The supermarket, cannery and consumer surveys were aimed at obtaining information regarding the following:

* existing canned fish products on the Indonesian market;
* technological aspects of canned fish production in Indonesian canneries;
* recent consumer behaviour relating to canned fish product; and
* consumer acceptability about the proposed product type, using fish oil as the main ingredient.

9.3. METHODOLOGY

9.3.1. Supermarket survey

The supermarket survey of canned fish product availability was carried out in 3 cities: Jakarta, Bogor (West Java) and Semarang (Central Java). Four supermarkets in Jakarta, 6 supermarkets in Bogor and 10 supermarkets in Semarang were surveyed. The questionnaire used is shown in Appendix 9.1.

9.3.2. Cannery survey

Sixteen canneries participated in the survey: 7 in Muncar (East Java), 8 factories in Bali and 1 factory on Bitung Island. Direct interview survey was undertaken for factories in Muncar and Bali. A survey was mailed to the factory on Bitung Island. The questionnaire used for the survey is shown in Appendix 9.2.

9.3.3. Consumer survey

A mail interview survey was used in this study. The questionnaire, as shown in Appendix 9.3., was distributed in two large cities, Jakarta and Semarang. Sixty-five questionnaires were distributed in Jakarta and 160 in Semarang. Classification of high, medium and low incomes were set at more than Rp.500,000, between Rp.150,000-Rp.499,999, and less than Rp.149,999 respectively (Heruwati, 1990).

Before the questionnaire was distributed in Indonesia, it was tested on Indonesians residing in New Zealand. The questionnaires were distributed to 35 people, with a 60% return. The questionnaire testing was expected to give provisional information on Indonesian consumer behaviour. Required changes in the questionnaire were made after this questionnaire testing.

9.4. RESULTS

9.4.1. Existing canned fish product in the market

9.4.1.1. Origin of the canned fish product

Of the 83 types of canned fish product on the Indonesian market in terms of trade mark, can size, fish species and medium, 43 (52%) were produced locally with the reminder (48%) imported. Thirty-four trade marks of canned fish are marketed consisting of 18 local and 16 imported brands. Countries exporting canned fish to Indonesia are Korea, People Republic of China, Mexico,

Australia, USA, Canada, Chili, Thailand, Portugal, Denmark and Norway.

9.4.1.2. Fish species used in canned fish

Three main fish species are processed internally for the Indonesian market: sardine, mackerel, and tuna, as shown in Table 9.1. Other fish species available are imported canned salmon, herring, skipper and dace.

Table 9.1. Percentage of canned fish product type on the Indonesian market according to fish species

Fish species	Local product		Imported product		Total product	
	number	%	number	%	number	%
Sardine	26	60.5	14	35.0	40	48.2
Mackerel	11	25.6	2	5.0	13	15.7
Tuna	6	13.9	11	27.5	17	20.5
Salmon	-	-	9	22.5	9	10.8
Herring	-	-	1	2.5	1	1.2
Skipper	-	-	2	5.0	2	2.4
Dace	-	-	1	2.5	1	1.2

Approximately 60% of the local products is processed using sardine as raw material. Imported canned sardine products constitute 35% of imported product type. Canned mackerel contributed to approximately 26% of local product type, but only 5% of imported product type. Canned tuna was approximately 14% of local product type and 28% of imported product type. Canned salmon accounts for approximately 23% of the total imported product.

In terms of the total number of canned fish products on the market, canned sardine, mackerel, tuna and salmon contributed to 48%, 16%, 20% and 11% of total number of product type respectively.

9.4.1.3. Medium used in canned fish

As shown in Table 9.2, 86% of locally canned fish is produced using tomato sauce as a medium, while, 32.5% of all import varieties are canned in tomato sauce, and 71% of import canned sardine uses tomato sauce as a medium. In total, 60% of all canned product types in Indonesia (imported and locally produced) use tomato sauce.

Table 9.2. Distribution of canned fish product in the market according to medium used

Type of medium	Local product		Imported product		Total product	
	number	%	number	%	number	%
Tomato sauce	37	86.0	13	32.5	50	60.2
Vegetable oil	3	7.0	9	22.5	12	14.5
Brine	2	4.7	12	30.0	14	16.9
Veg.oil and brine mixture	-	-	2	5.0	2	2.4
Fish oil	-	-	2	5.0	2	2.4
Others	1	2.3	2	5.0	3	3.6

Other mediums used in canned fish are vegetable oil and brine. These mediums are insignificant importance, contributing only 7% and 5% for the local product respectively. However these mediums made a 23% and 30% contribution to imported product type. The canned fish products of both these mediums are approximately 31% of total product type. Other mediums rarely used in canned fish are fish oil, a mixture of vegetable oil and brine, and sambal goreng (fried chili sauce). Two products having fish oil as a medium are imported from Norway, thus showing consumer acceptability.

9.4.1.4. Type of can used in canned fish

Four types of can were encountered in the market: tall tube, short tube, oval and rectangular. The size of these cans is shown in Table 9.3.

Table 9.3. Distribution of canned fish product in the market according to can type used

Type of can	Local product		Imported product		Total product	
	number	%	number	%	number	%
Tall tube can:						
155	16	36.4	2	5.1	18	21.7
230	-	-	1	2.6	1	1.2
425	12	27.3	2	5.1	14	16.9
440	-	-	2	5.1	2	2.4
Short tube can:						
105	-	-	2	5.1	2	2.4
141	-	-	1	2.6	1	1.2
170	-	-	2	5.1	2	2.4
180	-	-	2	5.1	2	2.4
184	1	2.3	3	7.7	4	4.8
185	4	9.1	4	10.3	8	9.6
200	1	2.3	-	-	1	1.2
210	-	-	2	5.1	2	2.4
220	-	-	2	5.1	2	2.4
Oval can:						
106	-	-	1	2.6	1	1.2
125	-	-	2	5.1	2	2.4
200	-	-	2	5.1	2	2.4
213	-	-	1	2.6	1	1.2
215	1	2.3	1	2.6	2	2.4
227	-	-	1	2.6	1	1.2
400	5	11.4	-	-	5	6.0
425	3	9.1	3	7.7	6	7.2
Rectangular can:						
106	-	-	2	5.1	2	2.4
125	-	-	2	5.1	2	2.4

Approximately 64% of the local product is produced in tube cans. More than 55% of the cans are 155g in size. However tall tube can only contributes approximately 18% of the imported product. In terms of total canned fish product, the tall tube can is used for approximately 42%, while 50% is produced in the 155 g can.

The short tube can is used for approximately 14% of the local product, and approximately 46% of imported product. In terms of total canned fish product type, this can is utilized for approximately 29%. Both local and imported product types showed that the 185 g can is the most commonly used.

Oval can type is used equally for both local and imported products: 22.7% for local product and 25.6% for imported product. Those product types account for approximately 24.1% of total product on the market. The rectangular can is used only in the imported product.

The cross-tabulation between the can type and fish species is shown in Table 9.4. Both sardine and mackerel are mostly canned in tall tube and oval cans. Sardine is also canned in the rectangular can. Tuna is canned only in the short tube can, while salmon is canned in both tall and short tube cans. Herring, skipper and dace are marketed in oval cans.

Table 9.4. Distribution of canned fish product based on the relation between fish species and can type

Fish species	Tall tube can		Short tube can		Oval can		Rectangular can	
	number	%	number	%	number	%	number	%
Sardine	25	30.1	-	-	11	13.2	4	4.8
Mackerel	8	9.6	-	-	5	6.0	-	-
Tuna	-	-	17	20.5	-	-	-	-
Salmon	2	2.4	7	8.4	-	-	-	-
Skipper	-	-	-	-	2	2.4	-	-
Herring	-	-	-	-	1	1.2	-	-
Dace	-	-	-	-	1	1.2	-	-

9.4.1.5. Price of canned fish

The price of canned fish on Indonesian market varies with fish species, can size and product origin as shown in Appendix 9.4.

Canned sardine is determined as the cheapest product. This is clearly evident in the local product. Can size affected the price of canned fish from the same product type: the bigger the can size, the more expensive the product. This occurrence can be seen clearly in the local product, where the price of canned sardine with tomato sauce medium in 155 g and 425 g tall tube cans is Rp.395 - 615. and Rp.960 - 1920. respectively. However this occurrence is inconsistent for imported products.

In general, the price of the local product is cheaper compared to the imported product. For example, the differences in the price of imported canned tuna is doubled: 185g can is Rp.975 - 1915. for the local product and Rp.3100 - 5280. for the imported products.

9.4.2. Production information for canned fish

9.4.2.1. Fish species used for canned fish production

Fish species and total volume for each species used for canned fish production in all canneries surveyed are shown in Table 9.6.

Table 9.6. Fish species used for canned fish production

Fish species	Fish weight (ton/year) to be processed into:			Number of factory using*)
	Product for local market	Product for export	TOTAL	
Sardine	24,210	600	24,810	13
Mackerel	550	-	550	3
Tuna	2,350	61,200	63,550	5
Skipjack	600	46,350	46,950	3
Scad	100	-	100	1

Note: one factory could use more than one fish species

Most canneries use sardines as raw material for production of canned fish for the local market. In terms of quantity, tuna and skipjack are consumed in greater quantity than other fish species. However most of the tuna and skipjack are canned for export purposes. Mackerels and scads are also canned, but in small quantities only.

9.4.2.2. Medium used for canned fish production

It was found that six types of mediums are used in the production of canned fish by Indonesian canneries as shown in Table 9.7. Tomato sauce is the common medium, and is used by 14 canneries. Vegetable oil and brine are used as a medium by 7 and 5 canneries respectively. Other mediums reported are brine and vegetable oil mixture, vegetable broth and sambal goreng (fried chili sauce).

Table 9.7. Medium used for canned fish production

Medium	Number of factory using*)
Tomato sauce	14
Vegetable oil	7
Brine	5
Vegetable oil and brine mixture	1
Vegetable broth	1
"Sambal goreng" (fried chili sauce)	1

Note: *) one factory could use more than one medium

9.4.2.3. Canned fish marketing by canneries

Five canneries export canned tuna and skipjack. Four factories export almost all their production of canned tuna and skipjack. One factory exported 25% of its canned sardine product.

Referring to the products marketed locally, fish species and medium used the most are sardines and tomato sauce respectively, as shown in Table 9.8.

Table 9.8. Fish species used for canned fish production for local market

	Number of factories using*)
A. Fish species:	
Sardine	13
Mackerel	3
Tuna	2
Skipjack	1
Scad	1
B. Medium:	
Tomato sauce	14
Vegetable oil	2
"Sambal goreng" (fried chili sauce)	1

Note: *) one factory could use more than one fish species and medium

9.4.2.4. Canneries opinion to the idea "fish oil disguised in canned fish"

Thirteen producers commented that the idea of disguising fish oil in canned sardine is an interesting one. Three canneries are not interested, because they produce mostly canned tuna for export. Among 13 canneries producing canned sardine, 10 are interested in the proposed product. Of the 14 canneries using tomato sauce as medium, 11 are interested. In general, most of the canneries (10 canneries) indicated that disguising fish oil in the tomato sauce medium is an interesting idea as shown in Table 9.9.

Fourteen canneries requested information about the technology for production, if the technology can be developed. Some of these canneries stated they would produce this product on condition that the product was acceptable to the market and had low production costs.

As shown in Table 9.9, 10 canneries are interested in using the technology for 1-10% of their total present production. Although, one factory did not market its product locally, it planned to enter the market if the use of fish oil addition created a promising market. One cannery intends to produce this product as 21-30% of total production. Two canneries plan to produce the canned fish

with disguised fish oil up to 40% of total production. One producer requested technological and product information before any decision is taken.

Table 9.9. Response of canneries to the idea "canned fish with disguised fish oil"

	Number of factory
1. Comment of canneries to the product idea "canned fish with disguised fish oil:	
a. interested	13
b. not interested	3
	16
2. Comment of canned sardine producers to the product idea "canned fish with disguised fish oil":	
a. interested	10
b. not interested	3
	13
3. Comment of canneries producing canned fish using tomato sauce medium to the idea "canned fish with disguised fish oil:	
a. interested	11
b. not interested	3
	14
4. Medium suggested by canneries to be disguised with fish oil:	
a. tomato sauce	10
b. vegetable oil	4
c. vegetable oil and brine mixture	2
	16
5. Canneries requesting to be informed with the technology to produce this product	
a. YES	14
b. NO	2
	16
6. Percentage of the product which was going to be produced (based on the percentage of total production):	
a. 1 - 10%	10
b. 11 - 20%	-
c. 21 - 30%	1
d. 31 - 40%	-
e. > 40%	2
	13

9.5.3. Consumer behaviour towards canned fish product

9.5.3.1. Demographic characteristics of respondent

Of the 225 questionnaires distributed 50 questionnaires (38%) were returned from Jakarta, and 80 questionnaires (62%) from Semarang. Demographic characteristic of respondents is shown in Table 9.10.

Approximately 60% of respondents participating in the survey are aged 20-29 years. Most respondents are in the middle income bracket. More than 79% of respondents worked in the private sector.

Table 9.10. Demographic characteristics of respondents

	Number	%
Income brackets:		
high income (>Rp. 500,000/month)	40	30.8
middle income (Rp. 150,000 - 500,000/month)	56	42.1
low income (<Rp. 150,000/month)	34	26.2
	130	100.0
Ages (years):		
20 - 29	79	60.8
30 - 39	30	23.0
40 - 49	14	10.8
> 50	7	5.4
	130	100.0
Occupations:		
private sector	103	79.2
civil servants	27	20.8
	130	100.0

9.5.3.2. Fish and fish product consumption

Consumption frequency of fish and fish products is shown in Table 9.11. By assuming that a month consists of four weeks. For the respondents consuming fish and fish products more than twice per week, an average of three times per week was taken. Then the monthly total consumption frequency was calculated as shown in Table 9.11.

Table 9.11. Consumption frequency of fish and fish product

Products	% respondent					Total consumption frequency/ month
	once/ week	twice/ week	>twice/ week	twice/ month	once/ month	
1. Fresh fish, including frozen and chilled fish	33.1	17.7	25.4	3.8	3.8	767
2. Processed products:						
- dried salted fish	16.1	10.8	6.1	9.2	22.3	345
- boiled salted fish	16.1	3.1	3.8	13.1	12.3	226
- fermented fish/shrimp paste	10.8	8.5	26.1	1.5	4.6	562
- pedah (moist fermented fish)	2.8	0.8	3.1	4.6	16.1	101
- jambal (spongy fermented fish)	6.1	3.1	1.5	0.8	8.5	101
- fish sauce	2.3	1.5	4.6	2.3	6.9	115
- canned fish	7.7	3.8	1.5	6.9	22.3	159
- smoked fish	3.8	1.5	2.3	7.7	10.8	106
- softened bone fish	10.0	5.4	3.1	5.4	26.1	204
- fish ball	7.7	3.8	6.1	3.8	9.2	200

The survey results showed that the respondents consumed fresh fish more often than processed fish products. Approximately 75% of respondents consumed fresh fish at least once a week. Among processed fish products, fermented fish/shrimp paste is consumed the most, but normally this product is consumed in small quantity as an appetizer. Other processed fish products consumed frequently are dried salted fish, boiled salted fish, softened bone fish and fish balls. Canned fish is consumed moderately,but more often when compared to moist fermented fish, spongy fermented fish, fish sauce and smoked fish.

9.5.3.3. Fish oil consumption

More than 73% of respondents were willing to consume refined fish oil as shown in Table 9.12. This indication was found for all income brackets, and age levels and occupation types. Respondents from medium and low income brackets showed more willingness to consume fish oil than respondents from the high income bracket.

Table 9.12. Preference of respondents to consume refined fish oil

	Willing to consume		Not willing to consume	
	Number	%	Number	%
Income brackets:				
high income	26	65.0	14	35.0
medium income	44	78.6	12	21.4
low income	25	73.5	9	26.5
Age (years):				
20 - 29	57	72.2	22	27.8
30 - 39	22	73.3	8	26.7
40 - 49	10	71.4	4	28.6
> 50	6	85.7	1	14.3
Occupation:				
private sector	74	71.8	29	28.2
civil servant	21	77.8	6	22.2

The respondents who preferred to consume fish oil in capsule, salad oil, food with disguised fish oil and direct fish oil consumption were 33.8, 11.5, 28.5 and 6.9% respectively as shown in Table 9.13.

Table 9.13. Fish oil consumption suggested by respondent

	Number of respondents*)	% respondent
Capsule	44	33.8
Salad oil	15	11.5
Direct consumption of fish oil	9	6.9
Food products with disguised fish oil	37	28.5

Note: *) respondent could choose more than one product

9.5.3.4. Respondent preference in buying canned fish

Most of the respondents stated that they selected a certain fish species and medium in buying canned fish. This indication is supported by the same tendency in all income brackets, age levels and occupation types, as shown in Table 9.14.

As shown in Table 9.15 the fish species and mediums which respondents are most like to purchase are sardine (59.2%) and tomato sauce (51.5%) respectively. Few respondents chose canned fish having other fish species (mackerel, tuna, squid and others) and other medium types (vegetable oil, brine, mixture of vegetable oil and brine and others).

Table 9.14. Respondent preference for a certain fish species and medium in buying canned fish

	Choose a certain				Not to choose a certain			
	fish species		medium		fish species		medium	
	No.	%	No.	%	No.	%	No.	%
Income brackets:								
high income	30	75.0	32	80.0	10	25.0	8	20.0
medium income	44	78.6	52	92.9	12	21.4	4	7.1
low income	28	82.4	32	94.1	6	17.6	2	5.9
Age (years):								
20 - 29	62	78.5	71	89.9	17	21.5	8	10.1
30 - 39	23	76.7	28	93.3	7	23.3	2	6.7
40 - 49	12	85.7	12	85.7	2	14.3	2	14.3
>50	5	71.4	5	71.4	2	28.6	2	28.6
Occupation:								
private sector	85	82.5	92	89.3	18	17.5	11	10.7
civil servant	17	63.0	24	88.9	10	37.0	3	11.1

Table 9.15. Fish species and medium chosen by respondents in buying canned fish

	Number *)	%
Fish species:		
Sardine	77	59.2
Mackerel	18	13.8
Tuna	33	25.4
Others (squid, shrimp, crab, milkfish and small tuna)	8	6.1
Medium:		
Tomato sauce	67	51.5
Vegetable oil	14	10.8
Brine	8	6.1
Vegetable oil and brine mixture	16	12.3
Others ("bumbu rujak", etc.)	21	16.1

Note: *) each respondent could choose more than one fish species and medium

9.5.3.5. Attitudes of respondent towards the product idea "canned fish with disguised fish oil"

Over 84% of the respondents were interested in the product idea of canned fish with disguised fish oil. Most of the respondents from each income bracket, age level and occupation type also showed interest in the proposed product idea, as shown in Table 9.16. Thus, the product idea was well accepted by all groups of respondents.

As shown in Table 9.17., 56.9% of respondents suggested the use of tomato sauce to disguise the fish oil as a medium for the proposed canned fish product. Some other respondents suggested disguising fish oil in vegetable oil (16.1%), brine (6.1%) and a mixture of vegetable oil and brine (12.3%).

Table 9.16. Respondent attitude to the idea of canned fish with disguised fish oil

	Product idea				Buying trend			
	Interested		not interested		Willing to buy		Not willing to buy	
	No.	%	No.	%	No.	%	No.	%
Income brackets:								
high income	32	80.0	8	20.0	28	70.0	12	30.0
medium income	47	83.9	9	16.1	50	89.3	6	10.7
low income	31	91.2	3	8.8	27	79.4	7	20.6
Age (years):								
20 - 29	69	87.3	10	12.7	63	79.7	16	20.3
30 - 39	25	83.3	5	16.7	25	83.3	5	16.7
40 - 49	12	85.7	2	14.3	13	92.9	1	7.1
>50	4	57.1	3	42.9	4	57.1	3	42.9
Occupation:								
private sector	57	83.8	11	16.2	82	79.6	21	20.4
civil servant	53	85.5	9	14.5	23	85.2	4	14.8

More than 80% of respondents are willing to buy the proposed canned fish product, if the product becomes available. This fact is supported by respondents from all income brackets, age levels and occupation types as shown in table 9.16. Respondents suggesting to use can types of 155 g, 185 g, 215 g and 415 g are 33.1%, 31.5%, 25.4% and 7.7% respectively. Approximately 37% of respondents suggested a purchase price of Rp.1000-1399. Other respondents are willing to buy the product if priced at Rp.400-999 (33.8%), Rp.1800-2599 (21.5%) and Rp.2600-3000 (4.6%) as shown in Table 9.17.

Table 9.17. Respondent preference to medium type, can size and price for proposed canned fish product

	Number *)	%
Medium types:		
Tomato sauce	74	56.9
Vegetable oil	21	16.1
Brine	8	6.1
Vegetable oil and brine mixture	16	12.3
Others ("bumbu rujak", etc.)	39	30.0
Can types:		
155 g	43	33.1
185 g	41	31.5
215 g	33	25.4
415 g	10	7.7
Product price:		
Rp. 400 - 999.-	44	33.8
Rp. 1000 - 1799.-	48	36.9
Rp. 1800 - 2599.-	28	21.5
Rp. 2600 - 3000.-	6	4.6

Note: *) each respondent could choose more than one medium type, can size and product price

9.6. DISCUSSION

9.6.1. Product type to be developed

Existing marketed canned fish shows that sardine is the most commonly used species for both locally produced and imported products. The market demand, exhibited by consumer survey results, reveals that most consumers chose canned sardine over the other species when buying canned fish. In addition, the cannery survey indicates that more canned sardine is produced for the local market than other canned fish products. The significantly lower price of canned sardine is probably the reason for the consumer preference. Producers could provide canned sardine at a lower price than other canned fish products, because the price of the raw material is significantly lower. As stated by one of the surveyed canneries, the prices of sardine, mackerel, skipjack and tuna are Rp.200-300/kg, Rp.500-800/kg, Rp.700-900/kg and Rp.1100-1300/kg respectively. Thus, the proposed canned fish product needed to use sardine as raw material, to enable the product to compete in the market.

Most local product types (86%) are canned in tomato sauce mediums, especially canned sardine. However Indonesia still imports canned sardine in tomato sauce - 4.000 tonnes in 1988 (Directorate General of Fishery, 1989). This was supported by the survey results where more than 30% of imported canned sardine are canned in a tomato sauce medium. The consumer survey also indicates that more than 50% of respondents purchased canned fish with tomato sauce medium. In addition, more than 55% of the consumers suggested the use of fish oil disguised in tomato sauce as a medium for the proposed product. As a response to market demand, most of the canneries produce canned fish in tomato sauce medium for the local market. The canneries also suggest the use of tomato sauce for the proposed product. The above results clearly indicate that the proposed canned fish product must use tomato sauce with disguised fish oil as a medium.

Most canned sardines in the market are canned in a 155g tall tube can. If the proposed product is developed for the local market, the product needs to be packed in a 155g tall tube can. This fact is supported by consumer survey results where more respondents are willing to buy the product if canned in 155g can.

The results obtained from the supermarket, cannery and consumer surveys indicated the same trend for the proposed product. The cannery survey is inclusive because managers were normally unwilling to answer questions about their activities as other factories could, possible, obtain this

confidential information. However this study proved that supermarket and consumer survey results are sufficient to generate the proposed product.

9.6.2. Prospects for proposed canned sardine with fish oil addition

The supermarket survey showed that the market for canned fish in tomato sauce, with disguised fish oil, is still open, since, to date, Indonesia imports canned sardine in tomato sauce to fulfil the market demand. Thus a competitive product already exists. However the proposed product is nutritionally better than the existing competitive product. The proposed product is richer in ω-3 fatty acids proved to have health benefits for the consumer. This fact will probably help the proposed product to be more competitive and successful.

In recent years the relationship between diet and health has received much publicity (Conning, 1990). This would help the proposed product to acquire consumer popularity through promotion and publicity, as all consumers are now far more aware of how diet affects health (Dennis, 1990). For example, the nutrition campaign being conducted by the Indonesian government through 10 PKK (Family Welfare Movement) programmes aims to guide Indonesians to improved family health and welfare. One of these concerns nutrition improvement. The nutritional benefits of the proposed product could be incorporated in the PKK programmes. However Conning (1990) states that while the food industry is satisfying consumer needs and wants, it remains true that responsibility for adequate nutrition is with the individual.

The consumer survey indicates that the respondents were willing to consume fish oil disguised in a canned fish product. Over 80% of the respondents were willing to buy canned fish with disguised fish oil. These results indicate that there are potential consumers already willing to purchase the proposed product. Thus, there is no reason for canneries not to produce. In fact, most of the surveyed canneries show significant interest for the proposed product and asked to be informed about the processing technology when developed. Most canneries have decided about production levels for this proposed product, which is another positive response.

9.7. CONCLUSIONS

The three surveys conducted, supermarket, cannery and consumer, indicate that the proposed canned fish product has to use sardine as raw material, tomato sauce as medium and 155 g tall tube can as container.

The survey also revealed that the proposed product had a good prospect in the market, the idea receiving a positive response from the canned fish producers. However a consumer test for the final product was still necessary to assess exact consumer acceptance.

Chapter 10

TOMATO SAUCE FORMULATION AND STERILIZATION CONDITION SELECTION FOR FISH CANNING

10.1. BACKGROUND

10.1.1. Tomato Sauce Formulation

Tomato sauce with fish oil disguised in it was chosen as the medium for the proposed canned fish product. As discussed in the previous chapter, the tomato sauce was suggested as the most acceptable medium by respondent to the a consumer survey. Tomato paste and water are the main ingredients in the tomato sauce medium, while sugar, salt and spices are added to create a desirable tomato sauce taste (Wiahayani, 1983; Novikov, 1984).

In the food industry, formulation plays an important role in the development of a new product, and must be selected carefully. In the development of a new tomato sauce with fish oil addition, with the objective of improving the nutritional value - particularly ω-3 fatty acids content - the formulation method must use a systematic determination with optimisation of ingredient levels. Trial and error methods, which waste time and money, must be avoided.

Factorial designs are usually unsuitable for the development of food products involving more than one ingredient. In mixture design experimentation, it is impossible to vary one ingredient or component, while holding all others constant. As soon as the proportion of one component is altered so is that of at least one other components altered, since the sum of all components is always 1.0. To cope with this situation a set of experimental plans, called mixture design, has been developed (Snee, 1971; Hare, 1974; Anderson, 1981a; Anderson and Earle, 1985). In a mixture experiment, the response to a blend or mixture of one or more ingredients depends only on the relative proportion of the ingredients present in the blend and not on the total amount of the blend (Cornell, 1979).

In mixture designs the sum of the variables must always be 1.0 or 100 percent. This constraint is not applicable in the study of independent variables. Fewer runs may be carried out if the experimenter is willing to accept less information about the mixture. The results of mixture design

can be subjected to vigorous analysis to obtain mathematical models relating the ingredient levels to some response variables as discussed by Hare (1974). Rigorous mathematical analysis is not always necessary, however, as one of the advantages of mixture designs is the relative ease of making subjective judgements for product improvement from a visual appraisal of the responses throughout the mixture space (Anderson, 1981a; Anderson and Earle, 1985).

Sensory evaluation involving trained panellists was used to determine the acceptability of the formula being developed. The usefulness of sensory testing in the product development is described by Erhardt (1978). Sensory tests provide the developer with corrective information and guidelines for product improvement. Tests can be used to determine which characteristics of the latest formulation do or do not meet the product model. Formulation can be also evaluated by sensory panels to determine whether optimization of the product quality has been achieved. Sensory evaluation can also be used to determine whether the addition of a certain ingredient affects the flavour of a product (Erhardt, 1978).

10.1.2. Sterilization

The term "sterile" refers to a condition in which no viable microorganisms are present, a viable organism being one that is able to reproduce under conditions optimum for its growth (Lund, 1975). The objective in the heat sterilization of foods is the destruction of heat resistant bacterial spores. Commercial sterilization requires that the spores of all pathogens and of all organisms likely to grow under the anticipated conditions of storage be destroyed during processing (Brody, 1971; Board, 1981; Adams, 1983). The process may be applied either within a sealed container, in the case of conventional canning, or prior to packing under aseptic conditions. Whatever the mode or method of hot sterilisation, the safety of a heat preserved product is not dependent upon the use of chemical additives or the control of temperature during storage and distribution (Hall and Pitcher, 1991). Profitability in sterilized products results from delivering, to the consumer, quality products safely and efficiently (Savage, 1984).

The application of sufficient heat to destroy food spoilage microorganisms and enzymes also results in some desirable and undesirable changes in the foods (Lund, 1973). The desirable effects of heat processing may be summarized as follows:

* favourable alteration of the characteristics of the product (browning reactions, textural changes, increased palatability, etc.);

* destruction of enzymes (peroxidase, ascorbic acid oxidase, thiaminase, etc.);
* improvement in availability of nutrients (gelatinization of starches and increased digestibility of proteins); and
* destruction of undesirable food components.

The undesirable effects of heat processing include changes in protein and amino acids, carbohydrates, lipids, vitamins and minerals. According to Leonard (1986), the breakdown of heat labile constituents in food is approximated as a first-order chemical reaction, mathematically similar to the destruction of bacteria.

The development in the processing of foods has been an attempt to optimize the thermal process for nutrient retention. For commercial sterilization, optimization of a thermal process is not so straightforward. The mode of heating within the product becomes an important factor. For those products which heat by convection, the high-temperature and short-time process results in optimum nutrient retention. Again, a comparison of z values indicates that the rate of destruction of nutrients is less temperature dependent than is the rate of destruction of microbial spores. Therefore, the high temperature and short time (HTST) process favours nutrient retention (Lund, 1975b).

As the effect of sterilization on fish oil stability, particularly on the ω-3 fatty acids has not been investigated, a further investigation is now undertaken to obtain the results of such a study in order to apply them to the processing of canned fish with disguised fish oil.

10.3. OBJECTIVES

The aims of the experiments were to obtain:

* selected combination levels of main ingredients in tomato sauce medium for the canned fish and optimal level of fish oil which could be disguised in tomato sauce to produce an acceptable tomato sauce; and
* sterilization condition for processing canned fish with fish oil addition with regard to fish oil stability, especially ω-3 fatty acid.

10.3. METHODOLOGY

10.3.1. Experiment 1: Tomato sauce formulation

10.3.1.1. Materials

The fish oil used in this experiment was crude fish oil. Tomato paste was obtained from J.Wattie Foods Ltd., Hastings. Salt, sugar, vinegar, shallots and garlic were purchased from supermarkets and retailers in Palmerston North.

10.3.1.2. Formulation

This experiment was planned to optimise the fish oil level disguised in a tomato sauce for canned fish, without reducing the acceptability of the product. The mixture-design was used to develop a new tomato sauce formula. The original formula used as the base in the development of the new tomato sauce formula was obtained from P.T.Bangka Pioneer Industries Ltd., Bangka (Wiahayani, 1983). The tomato sauce contains:

Ingredients	Percentage (%)
Tomato paste	18.6
Water	74.5
Salt	3.7
Sugar	3.1

In this experiment, salt and sugar contents were held constant, while tomato paste, fish oil and water quantities were subjected to change.

Requirements	low level (%)	high level (%)
Tomato paste (T)	15	50
Fish oil (O)	10	40
Water (W)	40	70

Optimisation of the new formula obtained from the above levels was also performed to determine the acceptability of tomato sauce by panellists. The formula was evaluated organoleptically by nine trained Indonesian panellists in terms of consistency, odour, colour, mouth feel, appearance and overall acceptability. The sensory sheet used for this purpose can be seen in Appendix 10.1. The samples were served both cold and warm. The experiment was performed with two replications.

10.3.1.3. Tomato sauce preparation

To prepare the tomato sauce, water was heated to boiling and then sugar and salt added, and boiled until dissolved. The tomato paste was added and subsequently homogenized using a hand mixer. Finally, fish oil was added and mixed thoroughly using a hand mixer.

10.3.2. Experiment 2: Simulation study on the selection of sterilization condition for canned fish with disguised fish oil

Both unrefined and resin refined fish oils were studied for their stability during the sterilization process. Fish oil (50ml) was contained in a can 6.6cm in diameter and 4.6cm in height. Vacuum condition in the can was created by application of vacuum pressure at 17.72-19.19inHg, during sealing.

Selected standard temperature and time combinations for salmon canning from the Food Processing Institute of the USA (Robertson, 1983) were applied to determine canning conditions suitable for processing canned fish with fish oil added. The temperature and time combinations were as follows:

a. 110°C and 139 minutes
b. 116.7°C and 79 minutes
c. 121.1°C and 64 minutes.

Sterilization was carried out using a pilot plant scale retort 90 cm long and 56 cm in diameter. The experiment was performed with two replications. Seven trained Indonesian panellists participated

in the sensory evaluation of the oil. The sensory sheet used is shown in Appendix 10.2..

10.4. RESULTS

10.4.1. Tomato sauce formulation

10.4.1.1. Tomato sauce formulation

Figure 10.1 shows the complete space available for the mixture design. The limits on the three ingredients given previously, restricted the area of experimentation to the shaded, feasible region. From that region, the vertices suitable for the experiments are as follows:

A = 15T + 15O + 70W
B = 15T + 40O + 45W
C = 20T + 40O + 40W
D = 50T + 10O + 40W
E = 20T + 10O + 70W
F = 34.4T + 30.2O + 35.4W

where, F shows the centre point of the region.

Based on these vertices and T + O + W = 186.4g, the tomato sauce formula developed to investigate the behaviour of those ingredients in the product were as follows:

27.96T + 27.96O + 130.48W
27.96T + 74.56O + 83.88W
37.28T + 74.56O + 74.56W
93.20T + 18.64O + 74.56W
37.28T + 18.64O + 130.48W
64.12T + 56.29O + 65.99W

} 7.4g salt + 6.2g sugar

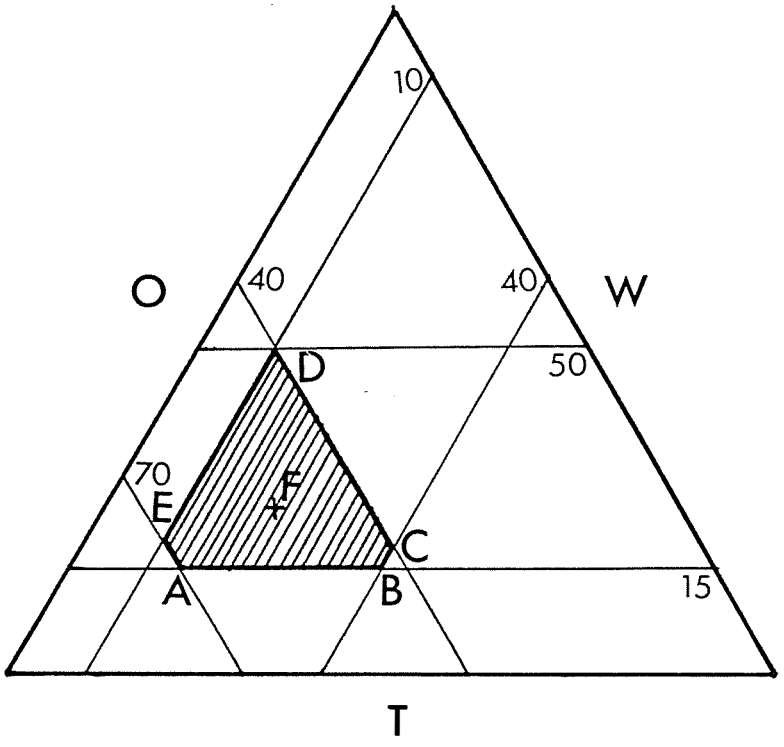


Figure 10.1. Mixture space showing areas of experiment

The products made using the above formulas were evaluated organoleptically and the total scores from all panellists for each parameter are shown in table 10.1.

Table 10.1. Total organoleptic score of the tomato sauce products of the first formulation

Sample	Code	Consistency	Odour	Colour	Mouth feel	Appearance	Overall acceptability	Coded Note
Cold	A	53	54	47	48	49	50	loT;loO;hiW
	B	51	51	48	49	46	49	loT;hiO;loW
	C	56	52	56	54	52	54	loT;hiO;loW
	D	36	48	50	45	43	42	hiT;loO;loW
	E	56	54	52	53	52	52	loT;loO;hiW
	F	49	50	52	47	50	49	mediant
Warm	A	50	48	47	47	45	48	loT;loO;hiW
	B	47	47	48	47	42	45	loT;hiO;loW
	C	53	48	56	53	51	53	loT;hiO;loW
	D	35	46	51	44	43	42	hiT;loO;loW
	E	54	51	54	53	51	51	loT;loO;hiW
	F	50	48	54	51	52	51	mediant

Note: lo = low
hi = high

10.9.1.2. Study of effects

Table 10.2 shows the effects of each of the three ingredients used to make tomato sauce on the sensory properties employed to evaluate the acceptability of the products. An example of the calculation to evaluate these effects is shown in Appendix 10.3.

Table 10.2. Effects of main ingredients on sensory properties of tomato sauce

Sample	Parameter	Tomato paste		Fish oil		Water	
		low amount	high amount	low amount	high amount	low amount	high amount
Cold	Consistency	54	32	47	54	46	55
	Odour	53	48	52	52	50	54
	Colour	51	50	50	52	51	50
	Mouth feel	51	45	49	52	49	51
	Appearance	50	43	48	49	47	51
	Overall acceptability	51	42	48	52	48	51
Warm	Consistency	51	35	46	50	45	52
	Odour	49	46	48	48	47	50
	Colour	51	51	51	52	52	51
	Mouth feel	50	44	48	50	48	50
	Appearance	47	43	46	46	45	48
	Overall acceptability	50	42	47	50	47	50

Samples served cold and warm did not show any significant effect on sensory properties, according to the panellists.

A low level of tomato paste gave a better consistency, odour, colour, mouth feel, appearance and overall acceptability than a high level.

In the observation using both cold and warm samples, a high level of fish oil in the tomato sauce was preferred in terms of consistency, colour and mouth feel. In cold sample, the high amount of fish oil gave a better appearance, but this effect was not detected in the warm sample. However the fish oil level had no effect on the odour of the sauce. Overall acceptability scores indicate that a high level of fish oil was preferred.

High levels of water in the tomato sauce resulted in a product with a better consistency, odour, mouth feel and appearance than the formula with low levels of water. A low level of water was preferred by panellists in terms the colour. Overall acceptability scores indicated that tomato sauce with a high level of water was more acceptable that the sauce with a low level of water.

10.4.1.3. Formula optimisation of tomato sauce

The above results suggest that the tomato paste level should be reduced and the fish oil level increased. The tomato paste, fish oil and water levels used for the optimisation of the tomato sauce formula are as follows:

Requirements	low level (%)	high level (%)
Tomato sauce (T)	10	20
Fish oil (O)	30	50
Water (W)	40	60

The new area in the mixture space is shown by the increase of fish oil level, and the reduced level of tomato paste. The new area of investigation to obtain the optimum level for each ingredient is shown in Figure 10.2.

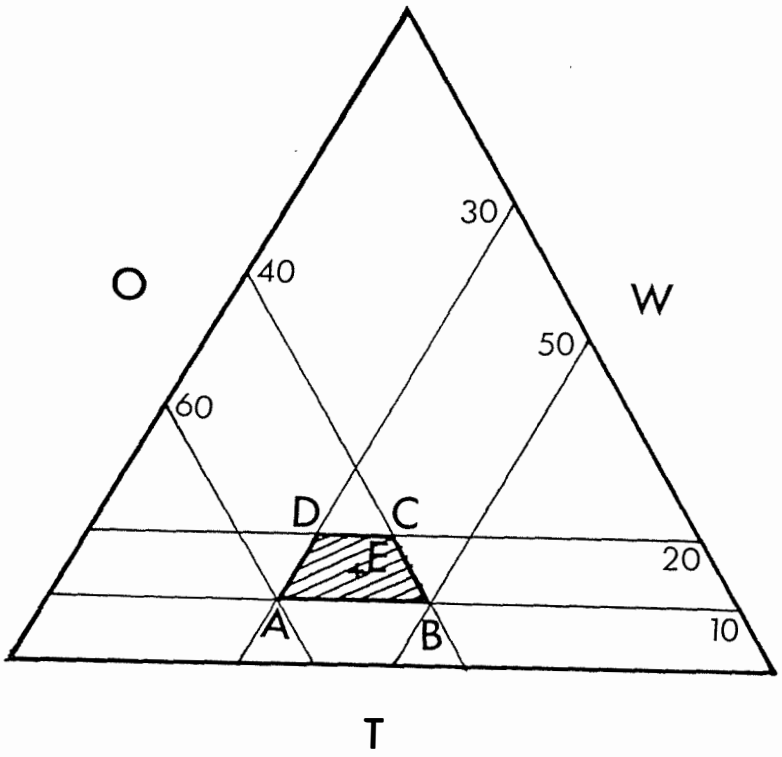


Figure 10.2. Mixture space showing new areas of experiment

The vertices for the new area of optimisation are A, B, C and D, while and E represents the centre point:

A = 10T + 30O + 60W

B = 10T + 50O + 40W

C = 20T + 40O + 40W

D = 20T + 30O + 50W

E = 15T + 37O + 48W (centre point)

Based on the above vertices, the recipes of tomato sauce to be used for the optimisation experiment are as follows:

18.64T + 55.92O + 111.84W
18.64T + 93.20O + 74.56W
37.28T + 74.56O + 74.56W
37.28T + 55.92O + 93.20W
27.96T + 68.97O + 89.47W
} + 7.4g salt + 6.2g sugar

Sensory evaluation results for the products made from the above recipes are shown in Table 10.3.

Table 10.3. Total organoleptic score of tomato sauce products of the optimisation experiment

Sample	Code	Consistency	Odour	Colour	Mouth feel	Appearance	Overall-acceptability
Cold	A	38	46	46	39	44	42
	B	41	47	47	44	44	43
	C	61	59	62	57	61	62
	D	63	58	62	59	61	62
	E	56	54	57	54	56	56
Warm	A	36	43	43	40	39	39
	B	36	41	46	42	43	40
	C	55	55	60	55	56	57
	D	59	56	59	57	60	60
	E	53	52	55	54	55	55

The sensory properties (consistency, odour, colour, mouth feel and appearance) of mixtures C and D were significantly better compared to three other mixtures. The overall acceptability showed the same trend, where products C and D were more acceptable to the panellists. In general, tomato

sauce processed from formula D was organoleptically better than tomato sauce produced from formula C using cold and warm samples. Thus, D was considered as the best formula for tomato sauce with fish oil disguised in it.

10.4.2. Stability of fish oil during sterilization

This experiment was intended to find the sterilization condition which could provide the optimal protection for ω-3 fatty acid from deterioration. To reveal the changes in fish oil due to the sterilization, the fish oil samples were analyzed for peroxide value, anisidine value, TBA value, free fatty acid value, refractive index, colour value, fatty acid profiles and sensory properties.

10.4.2.1. Effects of sterilization on peroxide value

Sterilization resulted in a significant decrease in peroxide value (PV) in refined and unrefined oils as shown in Figure 10.3. Vacuum treatment and various sterilization condition showed a pronounced effect on the changes in PV. A higher PV decrease was encountered in the oil contained in vacuum package than in the non-vacuum container. The PV reduction in unrefined oil occurred at higher value than in refined oil. Refined oil sterilized at 110°C for 139 minutes had a significantly higher PV than the oil sterilized at 121.6 for 64 minutes. The oil contained in vacuum package sterilized at 116.7°C for 79 minutes showed a markedly higher PV than the oil sterilized at 121.1° for 64 minutes.

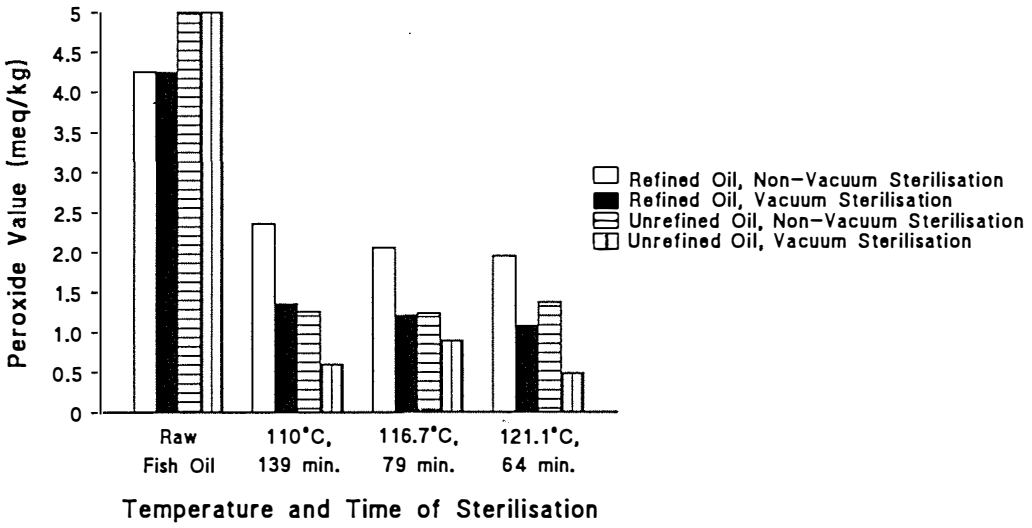


Figure 10.3. Peroxide value changes in fish oil during sterilization at various temperatures and times

10.4.2.2. Effects of sterilization on anisidine value

As shown in Figure 10.4, sterilization treatment of fish oil resulted in an increase of anisidine value in refined and unrefined oil from both vacuum and non-vacuum packages. The increase of anisidine value in the oil contained in the vacuum package was lower than in the oil contained in non-vacuum package. The unrefined oil had a higher anisidine value increase than the refined oil. Analysis of variance indicated that unrefined oil sterilized at 121.1°C for 64 minutes had a significantly higher anisidine value than the unrefined oil sterilized at 110°C for 139 minutes, and 116.7°C for 64 minutes.

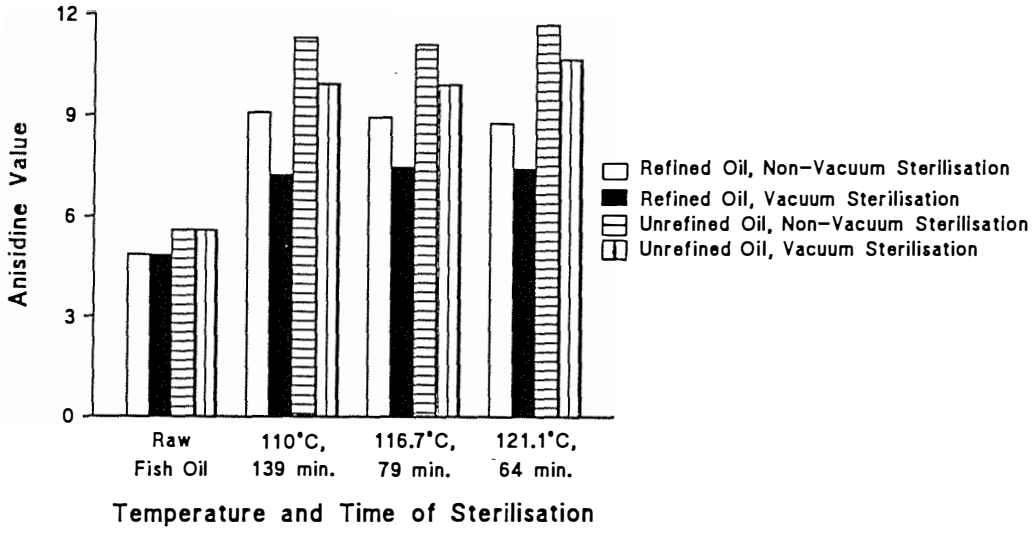


Figure 10.4. Anisidine value changes in fish oil during sterilization at various temperatures and times

10.4.2.3. Effects of sterilization on TBA value

A significant decrease in TBA value of refined and unrefined oils due to sterilization treatment was noted in the study as shown in Figure 10.5.

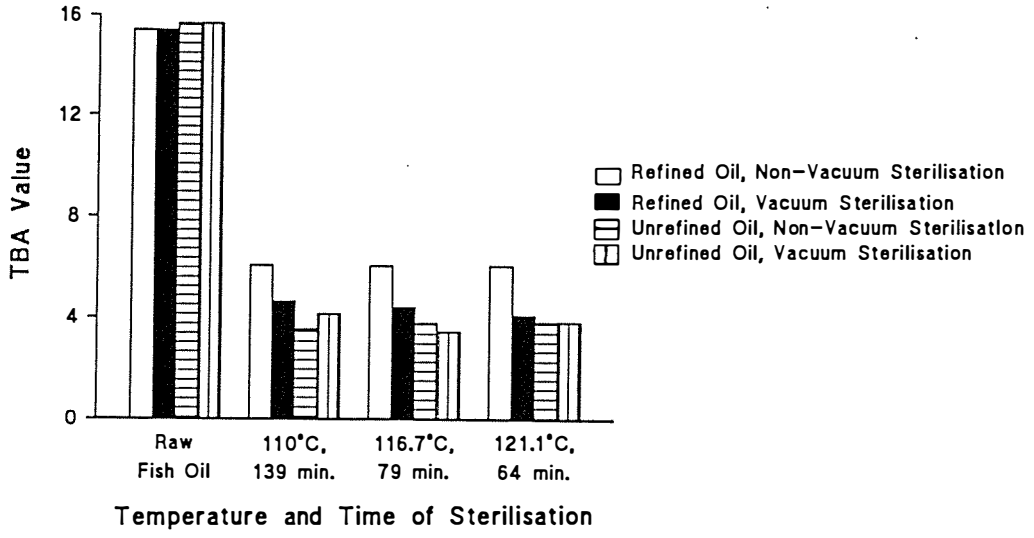


Figure 10.5. TBA value changes in fish oil during sterilization at various temperatures and times

The TBA value decrease in unrefined oil was registered at a higher value than in the refined oil. The reduction of TBA value in the oil contained in the vacuum package occurred at a higher value than in the oil packed in non-vacuum container. Various sterilization conditions did not induce any significant effect on the TBA values of the sterilized oils.

10.4.2.4. Effects of sterilization on totox value

Sterilization treatment of the fish oil resulted in the reduction of totox value for refined and unrefined fish oil as shown in Figure 10.6. However the reduction was insignificant in refined oil contained in non-vacuum package. In general, the vacuum package yielded sterilized fish oil having lower totox value than the non-vacuum package. Unrefined oil tended to show a higher totox value decrease than refined oil. Various sterilization conditions did not show a significant different in totox value for sterilized oils.

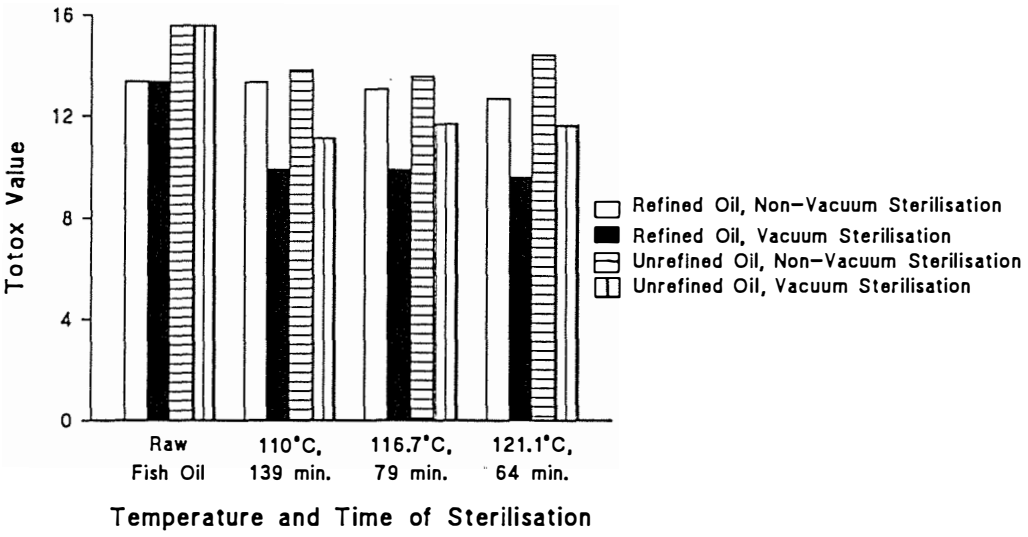


Figure 10.6. Totox value changes in fish oil during sterilization at various temperatures and times

10.4.2.5. Effects of sterilization on free fatty acid value

Analysis of variance indicated that sterilization did not result in any pronounced changes in free fatty acid (FFA) value of fish oil. However sterilized oils showed a tendency to have a lower FFA value than unsterilized oil as shown in Figure 10.7. Vacuum treatment did not have any significant effect on the FFA value of the fish oil during sterilization.

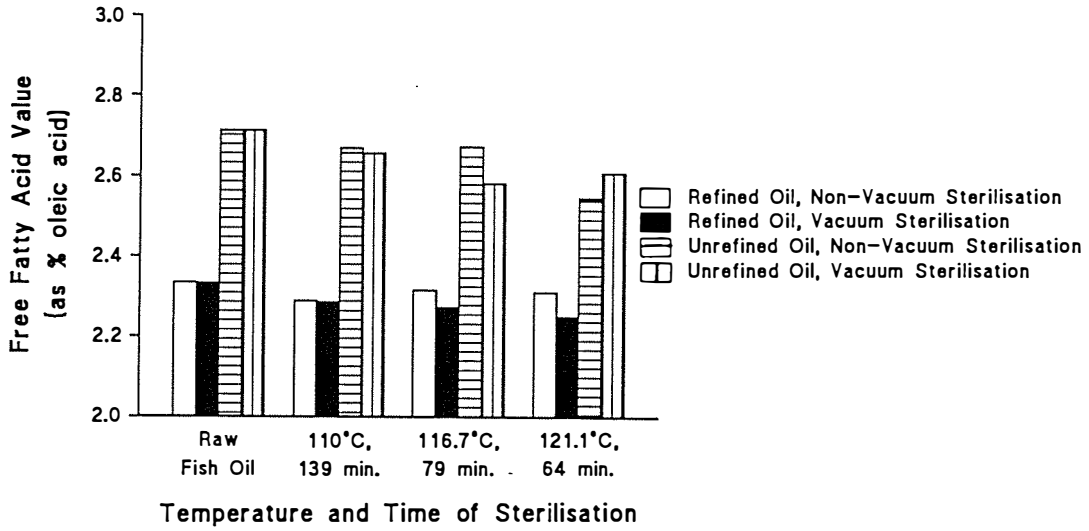


Figure 10.7. Free fatty acid value changes in fish oil during sterilization at various temperatures and times

10.4.2.6. Effects of sterilization on refractive index

Refractive index (RI) value of fish oil did not change significantly during sterilization. This was noted in refined and unrefined oils from both vacuum and non-vacuum packages.

10.4.2.7. Effects of sterilization on colour value

Photometric method was used to determine the colour value of both sterilized and unsterilized fish oils. The colour value of refined and unrefined fish oils increased due to the sterilization process as shown in Figure 10.8. This was found in both oils contained in vacuum and non-vacuum packages. The colour value increase in the oil packed in non-vacuum packages was higher than in the oil contained in vacuum package, and this was clearly exhibited in unrefined oil. T-test

indicates that unrefined oil sterilized at 116.7°C for 79 minutes had a lower colour value than unrefined oil sterilized at 110°C for 139 minutes and 121.1°C for 64 minutes. The non-vacuum packed oil sterilized at 116.7°C for 79 minutes had a significantly lower colour value than the non-vacuum packed oil sterilized at 110°C for 139 minutes.

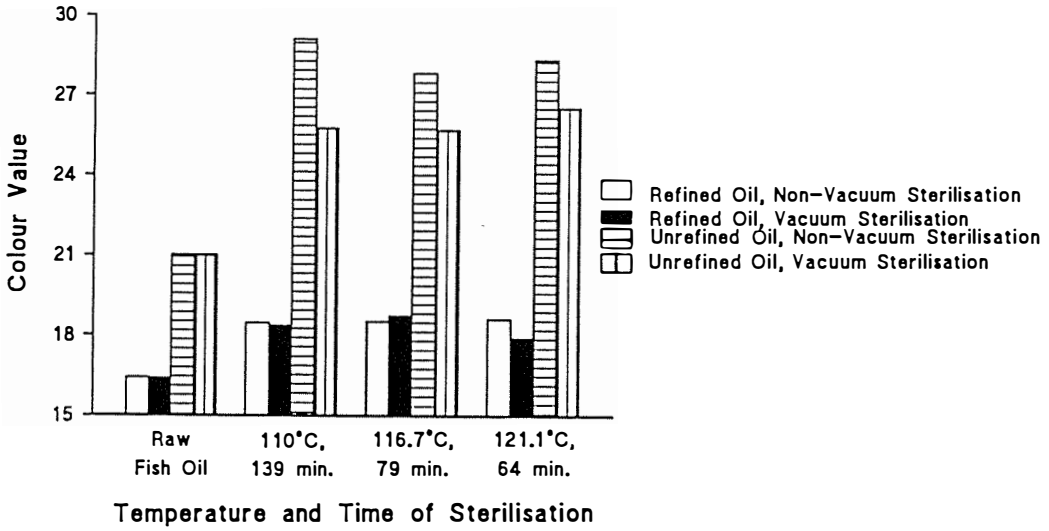


Figure 10.8. Colour value changes in fish oil during sterilization at various temperatures and times

10.4.2.8. Effects of sterilization on fatty acid profiles

Fatty acid profile changes in fish oil due to the sterilization process is shown in Table 10.4.

Table 10.4. Fatty acid profiles changes of fish oil during sterilization

Fish Oil	Fatty acids	Untreated Oil	110°C, 139 minutes		116.7°C 79 minutes		121.1°C 64 minutes	
			Vac.	Non-Vac.	Vac.	Non-Vac.	Vac.	Non. Vac.
Unrefined oil	SAFA	21.8	21.4	21.9	22.4	22.6	21.6	23.2
	MUFA	51.0	50.8	50.8	50.6	51.0	50.7	50.8
	PUFA	27.2	27.2	27.2	26.9	26.4	27.6	26.0
	ω-3FA	24.0	24.2	24.1	23.9	23.3	24.6	23.0
	EPA	11.2	10.9	10.7	11.0	10.2	11.1	10.2
	DHA	5.7	5.7	5.5	5.1	5.5	5.7	5.6
Refined oil	SAFA	22.0	22.0	21.7	21.5	22.5	21.3	20.7
	MUFA	50.9	50.6	51.2	51.0	52.0	50.4	52.2
	PUFA	27.1	27.3	26.7	27.4	25.5	28.3	26.9
	ω-3FA	24.1	24.3	23.7	24.4	22.4	25.3	23.8
	EPA	10.6	10.7	10.5	10.7	10.2	11.0	10.8
	DHA	5.4	5.7	5.7	5.7	5.6	6.0	5.9

Analysis of variance indicates that the relative values of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of fish oil were not affected by various sterilization treatments. However MUFA, PUFA, ω-3 fatty acids and EPA were significantly influenced by vacuum treatment during sterilization.

Vacuum package treatment yielded sterilized fish oil with a lower MUFA value, but a higher PUFA, ω-3 fatty acids and EPA values than non-vacuum package. In terms of condition, the sterilization at 121.1°C for 64 minutes yielded fish oil with higher PUFA and ω-3 fatty acid levels

than sterilization at 110°C for 139 minutes and 116.7°C for 79 minutes.

10.4.2.9. Effects of sterilization on sensory properties

The panellists indicated that sterilization caused the reduction of fishy odour and created an increase in rancid odour as shown in Figures 10.9 and 10.10. Vacuum treatment did not significantly affect the changes in fishy or rancid odour. Sterilization did not give a significant different in odour among sterilized oils.

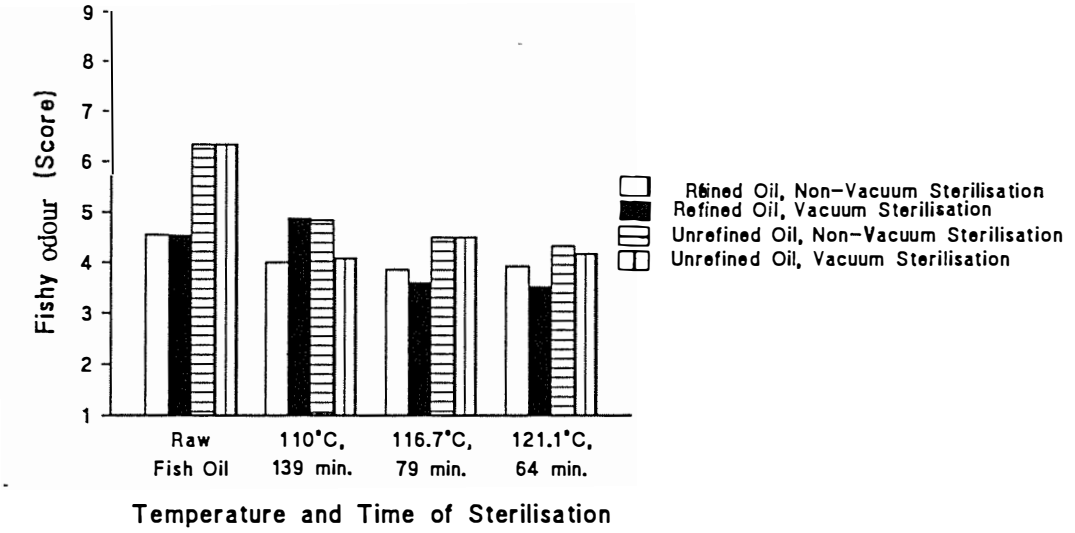


Figure 10.9. Fishy odour score changes in fish oil during sterilization at various temperatures and times

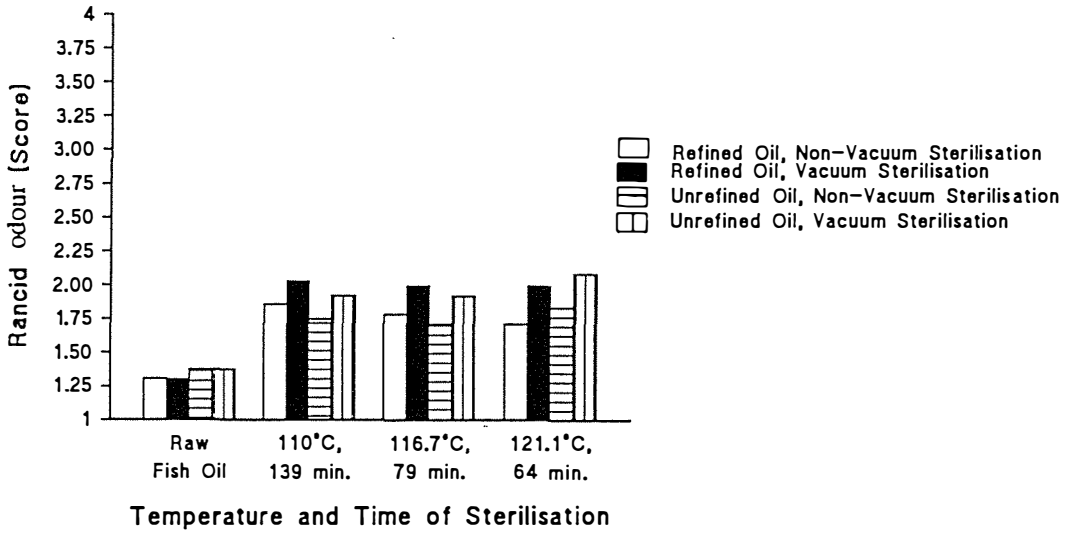


Figure 10.10. Rancid odour score changes in fish oil during sterilization at various temperatures and times

Sterilization changed the taste of the oil. As shown in Figures 10.11 and 10.12, the fishy taste decreased and the rancid taste increased. Vacuum treatment did not show any effect on taste. Sterilization did not induce a marked different in taste score.

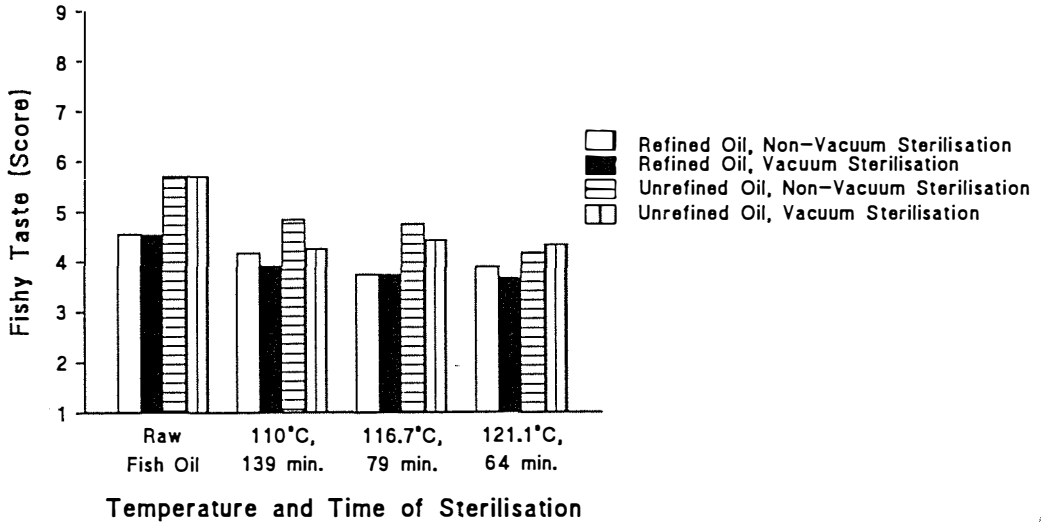


Figure 10.11. Fishy taste score changes in fish oil during sterilization at various temperatures and times

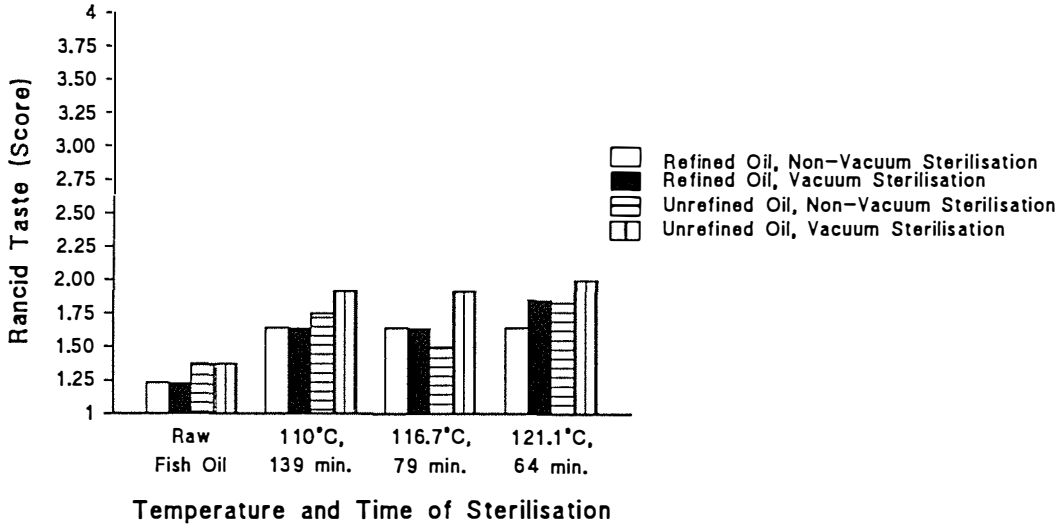


Figure 10.12. Rancid taste score changes in fish oil during sterilization at various temperatures and times

10.5. DISCUSSION

10.5.1. Tomato sauce formulation

The experiment showed that the mixture design was the most successful method to use in the formulation purpose. The design gave information about the formula, and the behaviour of the investigated ingredients. Thus the changes in ingredient levels could be made immediately without trial and error experiments. In this study, the effects of the ingredient quantities were investigated using sensory evaluation.

The experiment proved that fish oil present in tomato sauce improved the organoleptic properties of tomato sauce, such as consistency, colour, mouth feel and appearance. As revealed in the survey conducted by Fernandez (1987), the respondents pointed out that the unacceptable odour of fish oil was the main problem in fish oil consumption. However this was not found in this formulation study. Both high and low levels of fish oil, disguised in tomato sauce, showed the similar effect on odour properties.

In terms of overall acceptability, the formulation experiment indicated that fish oil added to tomato sauce and used as the medium in canned fish was acceptable to panellists. The experiment also revealed that a high level of fish oil was preferred, even higher than vegetable oil levels which are normally added to tomato sauce in fish canning at 3.4-10% (Novikov, 1984). The vegetable oil added to tomato sauce is to make the sauce shiny (Directorate General of Fishery, 1984). This was also evident in this study, where a higher level of fish oil gave a better appearance to the tomato sauce. Thus, the fish oil addition to the tomato sauce had two advantages: improved nutritional value, with more appealing appearance.

The above results suggest that the final tomato sauce formula could be manufactured using mixture D, considered by panellists as the best formula:

Tomato paste	= 18.6%
Fish oil	= 28.0%
Water	= 46.6%
Salt	= 3.7%
Sugar	= 3.1%

The quantity of tomato paste contributing in the final formula was the same as the level in the original recipe from P.T.Bangka Pioneer Industries Ltd., Bangka, 18.6%. The fish oil level disguised in the tomato sauce just replaced the water level.

10.5.2. Fish oil stability during sterilization

There are three categories of products of thermal oxidation: hydroperoxides; secondary oxidation products like hydroxy acids, keto acids, epoxy acids and carbonyl compounds formed by decomposition of the hydroxides; and cyclic and polymerized fatty acids (Lang, 1970). The products formed when fatty materials are heated in the absence of air can be roughly divided into three classes: volatile substances which can readily be distilled out; compounds which still contain about the original number of carbon atoms in the fatty acid chain; and dimers and polymers formed by attaching two or more fatty acid chain, together (Artman, 1969).

The above results reveal that the hydroperoxide content of fish oil, as shown by the peroxide value analysis, decreased during sterilization. Many theories have been developed to explain hydroperoxide behaviour in the heating treatment of fats. The formation and destruction of hydroperoxides are extremely rapid at high temperature (Nawar, 1985), since hydroperoxides are readily decomposed thermally (Hiatt and Irwin, 1968). When ethyl linolenate, for example, was heated in air at 250°C, hydroperoxides decomposition occurred so rapidly that a net peroxide value of zero was reached in less than 30 minutes (Lomanno and Nawar, 1982). Hydroperoxides also undergo a variety of scission and dismutation reactions to form a wide spectrum of carbonyl compounds, hydroxy compounds, short chain fatty acids, dimers and polymers (Dugan, 1968; Smouse, 1978). These processes may have induced the reduction of hydroperoxide value in the fish oil individually or collectively during sterilization.

Malonaldehyde content measured as TBA value also decreased during sterilization. Malonaldehyde is an intermediary in the decomposition of lipids (Finley, 1985). Sterilization may have accelerated the formation and decomposition rate of malonaldehyde, where, finally the fish oil showed a significantly lower TBA value than unsterilized oil. Sanders (1989) stated that malonaldehyde in fish tends to decrease with cooking.

Anisidine measuring α/β-aldehydes content increased during sterilization. This indicated that sterilization encouraged the formation of aldehyde. Nawar (1985) demonstrated that aldehyde

formation from hydroperoxides in thermal oxidative reactions. This occurrence could also be used to explain the reduction of hydroperoxides in the oil due to sterilization.

Free fatty acids (FFA) content of fish oil was relatively constant during sterilization. FFA resulted from the splitting of the attachment between the glycerol and fatty acids (Windsor and Barlow, 1981). Hydrolysis which is the reaction between water and triglycerides, could also produce FFA (Patterson, 1989). FFA can be oxidized by autoxidation and the oxidation is concerned primarily with the unsaturated fatty acid (Hamilton, 1989). Probably during sterilization, the rate of FFA formation and decomposition was insignificantly different, thus the process yielded fish oil with a relatively constant FFA value.

The sterilization process caused the fish oil colour to darken. A very complex process occurred affecting the colour of sterilized fish oil. Carotenoids, the most abundant natural pigments in fish oil, are unsaturated compounds and are therefore susceptible to oxidation giving rise to rancidity and bleaching. In addition, two types of isomerization can occur, namely, cis-trans isomerization and epoxy isomerization, which can cause the colour to lighten (Hall and Pither, 1991). Oxidized lipids and lipids soluble compounds may also decompose to form browning precursors (Sadler, 1987). Maillard browning reactions might also occur in the formation of the final colour of sterilized oil, where amino groups react with carbonyls from oxidized fat or aldehyde groups of reducing sugars (Burger and Walters, 1973). These indications suggest that browning reaction is a more important factor in the formation of sterilized fish oil colour than carotenoids decomposition.

Relative amounts of saturated and unsaturated fatty acids were insignificantly affected by sterilization. Sterilization at 121.1°C for 64 minutes tended to produce sterilized fish oil with higher relative quantity of ω-3 fatty acids than two other tested sterilization conditions. The higher relative quantity of ω-3 fatty acids could be obtained by vacuum treatment in the can. The advantages of vacuum treatment to reduce fish oil deterioration during sterilization were also shown by colour values, in which lighter oils were obtained. Less decomposition of hydroperoxides and malonaldehyde was observed in vacuum-packed-oils by exhibiting a lower anisidine value.

Sterilization reduced the fishy odour and taste strength of fish oil followed by an increase in rancid odour and taste. More aldehyde compounds may have imparted the odour and taste performance of sterilized fish oil. The odour and taste of fish oil disguised in tomato sauce seemed not to be a problem as discussed in the tomato sauce formulation experiment. Fish oil odour and taste were suppressed by the odour and taste of the tomato sauce, sugar and salt.

10.6. CONCLUSIONS

The tomato sauce formulation experiment proved that, organoleptically, fish oil addition to tomato sauce was acceptable by panellists and even improved the appearance and nutritional quality of the product. The final recipe obtained from the mixture design experiment is:

Ingredients	Level (%)
Tomato paste	18.6
Fish oil	28.0
Water	46.6
Salt	3.7
Sugar	3.1

However this tomato sauce formula could be further improved to enhance odour and taste by addition of spicies.

High temperature and short time (HTST) processing seems to be the best condition for sterilization of canned fish with fish oil disguised in it, particularly in order to protect ω-3 fatty acids from intensive deterioration. Vacuum treatment in the can was also suggested to give more protection to ω-3 fatty acids. Therefore, for canned fish with fish oil addition sterilized at 121.1°C with vacuum headspace in the can, is recommended.

Chapter 11

DETERMINATION OF IMPORTANT FACTORS IN FISH CANNING AND CANNING PROCESS OPTIMIZATION

11.1. BACKGROUND

11.1.1. Canned Fish

Canned fish is defined as fish packed in containers which have been hermetically sealed and sufficiently heated to destroy or inactivate all microorganisms liable to multiply at any temperature, while the product is in storage, canning spoilage or become harmful to the consumer (Codex Alimentarius Commission, 1976). The hermetically sealed container used also protects against damage and contamination with dirt (Connell, 1990). The most common sequence for canned fish processing are pre-treatment, pre-cooking, filling, sealing and retorting.

The initial treatment of fish after capture is of prime importance for all types of preservation including canning. The most important operation after capture is to chill the fish. This practice is valid for tropical fish and for fish from temperate or cold water (Perovic, 1977). Pre-treatment of fish covers the range of operations during which the product is prepared for canning including gutting, washing, nobbing, shucking, shelling, cutting, brining and dipping (Warne, 1988). The last washing can be combined with salting, to remove blood and slime and to improve flesh texture. The salting process also stabilizes the flavour of the canned product and brings out its characteristic taste (Van den Brook, 1965). Brining toughens the skin of the fish. When unbrined fish are canned, much of the skin adheres to the can. Brining also brightens the appearance of the fish (Burgess et al, 1967). Then the fish is packed manually on a conveyor which has supply conveyors for nobbed fish, empty cans and for fish already packed in cans (Perovic, 1977).

Pre-cooking is usually in steam, water, oil, hot air or smoke, or a combination of these. Fish are very often pre-cooked for the following reasons (Codex Alimentarius Commission, 1976; Warne, 1988):

* to free the flesh of fluid which would otherwise cook out during the heat process and remain in the container as an undesirable free liquid;
* to release body oil if the fish are excessively fat or if the oil has a very strong flavour;

* to improve texture or to condition the flesh for further processing;
* to obtain specific textural and flavour effects such as by frying in oil; and
* to loosen the shellfish meat from the shell and to make the meat firmer.

The amount of water released varies depending on the fat content: 17.5% for tuna and 19-34% for sardine (Van den Brook, 1965), and 20-30% for blue whiting (Karl, 1984). Pre-cooking can be combined with a dipping process, particularly for products which require additives to impart flavour or colour or in order to modify texture through the surface action of brines (Warne, 1985).

In filling containers, account must be taken of the fact that the contents will expand or contract in volume with changes in temperature (Codex Alimentarius Commission, 1976). Sauce or oil should be added to cans at a temperature in excess of 80-90°C (Bidenko et al, 1974; Novikov, 1984). Headspace is necessary for thermal expansion caused by heating the product. It is important that the temperature does not result in an excessive build up of pressure causing damage to the hermetic seal (Warne, 1985).

It is generally desirable to create a partial vacuum in containers of canned fish at the time they are sealed. This serves two purposes: it avoids excessive pressure from entrapped gas during heat processing, and it reduces the likelihood that internal gas pressure, which causes metal containers to swell if stored in warm places or exposed to low atmospheric pressures (Codex Alimentarius Commission, 1976). Vacuum may be obtained by using steam exhaustion, hot filling and steam vacuum closing. The best, and most consistent method of obtaining can vacuum is through the use of vacuum closing equipment (Landgraf, 1963). Sealing normally uses a double seaming operations. It is vitally important that the side seam and the double seams are completely hermetic (Clucas, 1982).

Careful control of the amount of heat treatment is extremely important. If the product is not heated sufficiently it may spoil, or it might become a potential health hazard. If heat treatment is too severe, the quality may be reduced by over-cooking. It is necessary to heat process canned fish at a temperature in the range 110-121°C. The higher temperatures are often preferred because of the saving in time and because it is found that texture, colour or flavour of some products may be preserved better by heating for a shorter time at a high temperature (Codex Alimentarius Commission, 1976).

11.1.2. Screening experimental design: Plackett and Burman

There is obviously a need for a practical method of screening the large number of variables in a process. If efficient preliminary screening could be accomplished, a relatively small number of important variables could be selected for further, more detailed study. One such method is the Plackett and Burman design, which is based on balanced incomplete blocks (Stowe and Mayer, 1966).

The Plackett and Burman design makes it possible to screen N-1 variables in N experiments. It is impossible to measure the effects of interactions, but this is not usually important in screening as the main objective is to isolate the important variables (Anderson, 1981a; Anderson and Earle, 1985). William (1963) compared various screening designs in a problem where 24 variables were studied. A Plackett-Burman analysis using 28 experiments was compared with a random-balance design using 28 experiments and with a fractional factorial using 32 experiments. The Plackett-Burman design is generally best suited for efficient screening and accuracy in selecting the important variables.

Plackett and Burman have provided the first row of the design matrix for investigating various numbers of factors. The remainder of the design matrix is generated by shifting this first row one space to the left N-2 times, where N equals the number of experiments. The last row of the matrix, a row of minus signs, is added to the bottom of the generated matrix (Stowe and Mayer, 1974; Anderson, 1981a; Anderson and Earle, 1985). For example, this study evaluated 8 factors, so 12 experiments were required. The first row is as follows (Plackett and Burman, 1946):

+ + - + + + - - - + -

where + signifies the high level of a variable and - the low level.
The full design matrix is shown in Table 11.1.

Table 11.1. Design matrix for screening important factors in fish canning

Treatments	Variables										
	ST	SVS	Brn	PC	GA	SA	VA	D	D	D	D
1	+	+	-	+	+	+	-	-	-	+	-
2	+	-	+	+	+	-	-	-	+	-	+
3	-	+	+	+	-	-	-	+	-	+	+
4	+	+	+	-	-	-	+	-	+	+	-
5	+	+	-	-	-	+	-	+	+	-	+
6	+	-	-	-	+	-	+	+	-	+	+
7	-	-	-	+	-	+	+	-	+	+	+
8	-	-	+	-	+	+	-	+	+	+	-
9	-	+	-	+	+	-	+	+	+	-	-
10	+	-	+	+	-	+	+	+	-	-	-
11	-	+	+	-	+	+	+	-	-	-	+
12	-	-	-	-	-	-	-	-	-	-	-

Note: ST = sterilization time; SVS = steam vacuum sealing; Brn = brining time; PC = pre-cooking of fish; GA = garlic addition; SA = shallot addition; VA = vinegar addition; D = dummy

Every Plackett and Burman design includes, for each variable, the same number of runs at the high level and the low level. To calculate the effect of any input variable, the average result at the low level of that variable is subtracted from the average result at the high level of the same variable. For example the effect of the sterilization time (ST) variable would be calculated as follows:

$$\text{Effects (ST)} = \frac{(X_1+X_2+X_4+X_5+X_6+X_{10})}{6} - \frac{(X_3+X_7+X_8+X_9+X_{11}+X_{12})}{6}$$

where X_n = value of response variable in run 1; n = number of run.

The calculated effects of the dummy variables are used to test the significance of the real effects. In cases where there is more than one dummy variable, their values can be pooled to estimate of the standard error:

$$\text{Standard error (effects)} = \sqrt{\frac{E_d^2}{n}}$$

where E_d = dummy effects; n = number of dummy effects.

Having obtained a measure of the standard error of the effects, the real effects can be subjected to a t-test to see if they are non-zero. t values are calculated for each of the effects by dividing the effects by the standard error calculated from the dummies:

$$t = \frac{\text{effect}}{\text{S.E.(effect)}}$$

These calculated t values are then compared to the table value where the degrees of freedom are equal to the number of dummy effects making up the error term. It is wise to use a significance level which reduces the risk of overlooking. For this reason 90%, rather than 95% or 98% significance is recommended (Anderson, 1981a; Anderson and Earle, 1985).

11.2. OBJECTIVES

This investigation was intended to identify the important factors in the processing of canned fish with fish oil disguised in it by the application of the Plackett and Burman design. This study was also aimed at investigating the optimal condition for factors selected from Plackett and Burman design to produce the most acceptable product by the panellists.

11.3. METHODOLOGY

11.3.1. Materials

Crude fish oil (mainly hoki) was used in this study. The oil was refined using macroporous strong acid cation resin. Pilchards (New Zealand sardine) for canning were obtained from Star Fish Supply Ltd., Napier.

11.3.2. Methods

11.3.2.1. Canned fish processing

The fish, received in frozen condition was thawed in running water. Head, tail and visceral parts were removed and then washed using clean water. In this study, some fish were treated by brining in 15% salt solution after washing. Four washed fish were packed in cans 8.2 cm in height and 5.1 cm in diameter. The fish was pre-cooked using steam at 98°C for 20 minutes, and the released water was discarded. Tomato sauce was added to each, leaving a headspace at the top of around 5-7.5mm. The cans were then sealed. During sealing, hot steam was passed over the surface of the can for approximately 2 seconds to provide vacuum headspace in the can. Finally, the canned fish were sterilized in the retort at 121.1°C.

11.3.2.2. Experiment 1: Determination of important factors in fish canning

Firstly, the Fo of the canned fish was determined using the method described in Chapter 3. The results revealed that the Fo of the product was 8.44 and the total heating and cooling time was 35 minutes. This total time was used as the low level for sterilization in the Plackett and Burman experimental design.

Table 11.2 shows the eight variables screened, and the upper and lower limits for these variables.

Table 11.2. Variables and limits for Plackett and Burman design of canned fish

Variables	Upper limit	Lower limit
A. Process variables:		
Sterilization time (min.)	60	35
Steam treatment during sealing (sec.)	2	0
Brining time in 15% salt solution (min)	10	0
Pre-cooking time (min.)	20	0
B. Seasoning variables:		
Garlic addition (%)	2	0
Shallot addition (%)	2	0
Vinegar addition (%)	1.2	0

The products were analysed chemically, physically and organoleptically. The chemical analyses performed were pH (fish and medium), moisture, protein, fat, salt contents, and peroxide value (fish and sauce). Hunter-L, -a and -b values of the fish meat colour were determined. Organoleptic evaluation was carried out for both the fish and tomato sauce medium by the 10 trained Indonesian panellists. The ideal ratio scoring method, which is basically a line-scale of descriptive testing, was employed during sensory evaluation and the sensory form used is shown in Appendix 11.1.

The salt level in the tomato sauce was reduced, as suggested by panel evaluation during the tomato sauce formulation, Chapter 10. The tomato sauce recipe used in this experiment contained 18.78% tomato paste, 46.95% water, 28.17% fish oil, 3.00% salt and 3.10% sugar. The percentages of garlic, shallot and vinegar added to the tomato sauce were based on the total quantity of tomato paste, water, fish oil, salt and sugar.

11.3.2.3. Experiment 2: Optimization of canned fish processing

As will be discussed later in this chapter, sterilization time required optimisation in a further investigation. The optimization experiment used sterilization times of 40, 50 and 60 minutes. The salt level in the tomato sauce was varied at 1.5 and 2.5%. The experiment was performed with two replications. The sensory evaluation method employed in the optimization experiment was a descriptore-scale of descriptive method, instead of the ideal ratio scoring method, since the panellists commented that the ideal ratio scoring method was difficult and confusing, and required intensive supervision during evaluation. The sensory evaluation form used for this purpose is shown in Appendix 11.2.

11.4. RESULTS

11.4.1. Determination of important factors in canned fish processing

11.4.1.1. Results of chemical, physical and sensory analyses

Table 11.3 shows the chemical analysis results for both fish flesh and tomato sauce. The moisture (MC), protein, fat and salt content of fish were in the range of 66.05-68.13, 23.33-24.19, 2.63-4.27 and 1.31-2.33% respectively. The pH of fish flesh and tomato sauce varied from 5.96 and 5.72 to 6.13 and 6.01 respectively. The peroxide values (PV) of fish flesh and tomato sauce were in the range of 23.14-433.67 and 10.63-32.65 meq/kg respectively.

Table 11.3. Results of chemical analysis of fish and tomato sauce

Treatments	Fish flesh						Tomato sauce	
	M.C. (%)	Protein (%)	Fat (%)	Salt (%)	P.V. (meq/ kg)	pH	P.V. (meq/ kg)	pH
1	66.17	23.52	3.69	1.51	171.43	5.96	10.75	5.72
2	66.05	23.54	3.90	1.98	272.24	6.01	15.17	5.90
3	66.16	23.08	4.27	2.07	563.79	6.04	16.08	5.86
4	66.71	23.51	4.05	2.06	258.86	6.00	15.34	5.89
5	67.68	24.19	4.03	1.31	23.14	6.05	16.04	6.01
6	67.86	23.33	3.67	1.40	163.73	5.97	14.51	5.90
7	66.66	23.75	4.17	1.56	498.75	6.04	16.82	5.81
8	68.03	23.42	3.73	1.94	818.66	6.12	32.65	6.01
9	67.96	23.73	2.89	1.46	246.97	6.04	10.85	5.94
10	66.99	23.33	4.07	2.06	240.39	5.99	10.63	5.82
11	68.01	23.36	2.63	2.33	371.13	6.13	21.52	6.00
12	68.13	23.27	3.37	1.34	433.67	6.11	27.63	5.99

Note: M.C. = moisture content; P.V. = peroxide value

Table 11.4 shows physical colour changes in fish flesh. The results showed that Hunter-L, -a and -b values of fish were in the range of 44.72-51.92, 1.19-3.56 and 12.69-14.81 respectively.

Table 11.4. Results of colour analysis of fish flesh

Treatments	L - value	a - value	b - value
1	47.72	3.53	14.81
2	46.72	3.17	14.70
3	49.69	1.73	14.07
4	46.13	3.42	14.82
5	44.72	3.56	13.74
6	44.92	2.88	13.34
7	51.92	1.69	14.58
8	49.52	2.00	14.87
9	49.34	1.19	12.62
10	46.84	3.22	14.59
11	49.39	1.38	13.29
12	50.64	1.76	14.44

The sensory evaluation results for fish and tomato sauce are shown in Tables 11.5 and 11.6 respectively. The scores of all sensory parameters showed variation, depending on the treatment.

Table 11.5. Results of sensory evaluation for fish

Treatment	Appear ance	Texture	Bone-Soft-ness	Salti-ness	Spici-ness	Sour-ness	Fishi-ness
1	1.35	1.27	0.78	0.81	0.70	1.14	1.31
2	1.43	1.01	0.87	1.35	0.97	1.19	1.42
3	2.62	1.26	0.49	1.48	0.94	1.39	1.64
4	1.96	1.28	0.65	1.49	0.96	1.29	1.57
5	3.08	1.00	0.68	1.34	0.94	1.08	1.42
6	2.08	0.88	0.90	1.33	0.84	1.27	1.28
7	1.78	1.17	0.52	1.05	0.83	1.03	1.66
8	2.87	1.03	0.42	1.30	1.06	0.97	1.63
9	2.58	1.27	0.42	1.11	0.98	1.03	1.23
10	2.28	1.14	0.81	1.14	0.91	0.93	1.22
11	1.86	0.99	0.40	1.20	0.98	1.13	1.40
12	1.45	1.21	0.31	1.08	0.81	0.85	1.77

Table 11.6. Results of sensory evaluation for tomato sauce and overall acceptability for canned fish product

Treatment	Tomato Sauce						Overall acceptability of the product
	Colour	Mouth feel	Salti-ness	Spici-ness	Sour-ness	Fish-ness	
1	0.96	1.35	1.01	0.90	1.14	1.42	0.54
2	0.90	1.43	1.13	1.11	1.10	1.42	0.58
3	0.80	1.43	1.15	1.01	1.14	1.34	0.45
4	1.01	1.41	1.45	0.95	0.99	1.28	0.60
5	0.80	1.17	1.22	0.86	0.94	1.48	0.58
6	0.89	1.40	1.13	0.93	1.21	1.34	0.54
7	0.71	1.64	0.99	1.04	1.11	1.15	0.54
8	0.73	1.20	1.44	1.16	1.17	1.33	0.47
9	0.83	1.42	0.99	1.11	1.03	1.19	0.54
10	0.70	1.05	1.16	1.06	1.02	1.29	0.70
11	0.97	1.33	1.21	1.02	1.24	1.14	0.60
12	0.83	1.11	0.91	0.93	1.10	1.17	0.53

11.4.1.2. Study on the effects

Tables 11.7 and 11.8 show the effects of each variable on chemical, physical and organoleptical parameters of fish and tomato sauce.

Sterilization time significantly decreased peroxide value, pH and Hunter-L value of fish flesh. This variable also significantly reduced the peroxide value and ideal ratio score of tomato sauce spiciness. Sterilization time increased Hunter-a value and the ideal ratio score of bone softness of fish, and increased the ideal ratio score of the fish taste of the tomato sauce. The overall acceptability of the product was improved by sterilization.

The application of steam vacuum sealing reduced the peroxide value of fish and tomato sauce. The ideal ratio score for tomato sauce spiciness was reduced by that variable as well. Steam vacuum sealing improved the ideal ratio score of tomato sauce colour.

Brining for 10 minutes in 15% salt solution reduced fish moisture content, but increased salt

content, peroxide value, the ideal ratio score for saltiness and spiciness. The brining treatment also increased the peroxide value, and ideal ratio scores for tomato sauce saltiness and spiciness.

Table 11.7. The main effects and significance levels of process variables on the characteristic of canned fish

Sample	Response variables	SP	SVS	Brn	PC
Fish	Moisture content	-1.21	-0.01	-1.71 a	-2.64 b
	Protein content	0.66	0.61	-1.27	-0.11
	Fat content	1.49	-0.86	0.51	0.95
	Salt content	-1.06	1.25	10.41 c	0.70
	Peroxide value	-3.81 c	-1.67 a	2.08 a	-0.16
	pH	-4.68 c	-0.19	1.12	-2.81 b
	Hunter value:				
	L	-5.61 c	-0.85	-0.23	1.65 a
	a	14.70 a	0.13	0.45	-0.69
	b	0.81	-1.20	1.07	0.33
	Sensory:				
	appearance	-0.32	0.52	0.23	-0.42
	texture	-0.70	1.27	-0.18	1.47
	bone softness	7.23	-1.39	0.10	1.80 a
	saltiness	0.38	0.28	1.96 a	-1.26
	spiciness	-0.75	0.21	1.92 a	-0.69
	sourness	0.80	1.31	0.80	0.19
	fishiness	-2.21 b	-0.82	0.42	-1.18
Tomato sauce	Peroxide value	-6.97 c	-4.34 c	2.39 b	-7.66 c
	pH	-1.24	-0.03	0.37	-2.50 b
	Sensory:				
	colour	1.19	1.86 a	0.27	-1.01
	mouth feel	-0.42	0.37	-0.32	0.92
	saltiness	0.87	0.57	2.73 b	-1.97 a
	spiciness	-2.03 a	-1.67 a	2.38 b	1.67 a
	sourness	-1.12	-0.66	0.37	-0.32
	fishiness	3.72 b	0.61	0.20	0.29
Canned fish	Overall acceptability	1.96 a	-0.24	0.62	0.14

Note: a = t-test significance level at 90%
b = t-test significance level at 95%
c = t-test significance level at 99%

SP = sterilization time
SVS = steam vacuum sealing
Brn = brining time
PC = pre-cooking of fish

Table 11.8. The main effects and significance levels of seasonings on the characteristic of canned fish

Sample	Response variables	GA	SA	VA
Fish	Moisture content	1.34	0.81	0.47
	Protein content	-0.19	0.91	-0.01
	Fat content	-2.19 b	0.11	-0.95
	Salt content	0.58	1.07	1.90 a
	Peroxide value	0.05	0.39	-1.06
	pH	-0.00	1.12	-1.12
	Hunter value:			
	L	-0.56	0.64	-0.11
	a	-1.80 a	1.80 a	-2.89 b
	b	-0.99	0.72	-1.29
	Sensory:			
	appearance	-0.33	0.36	-0.09
	texture	-1.23	-0.62	-0.10
	bone softness	1.12	-0.10	0.51
	saltiness	-0.76	-1.58 a	-0.06
	spiciness	0.37	-0.21	0.21
	sourness	0.26	-1.18	0.10
	fishiness	-2.01 a	-0.54	-1.66 a
Tomato sauce	Peroxide value	0.47	1.43	-4.63 c
	pH	0.30	-0.37	-0.43
	Sensory:			
	colour	1.31	-1.19	0.27
	mouth feel	0.42	-0.61	0.74
	saltiness	0.06	0.57	0.15
	spiciness	1.67 a	0.00	0.62
	sourness	1.70 a	0.14	0.03
	fishiness	0.53	0.29	-3.15 b
Canned fish	Overall acceptability	-0.62	0.91	1.77 a

Note: a = t-test significance level at 90%
b = t-test significance level at 95%
c = t-test significance level at 99%
GA = garlic addition
SA = shallot addition
VA = vinegar addition

Pre-cooking significantly reduced moisture content and pH of fish. Pre-cooking increased the Hunter-L value and the ideal ratio score for bone softness of fish. During pre-cooking the peroxide value, pH and ideal ratio score of tomato sauce saltiness decreased, while the ideal ratio score of tomato sauce spiciness increased.

Seasonings affected chemical, physical and organoleptical properties of fish and tomato sauce. Garlic reduced Hunter-a value of fish and the ideal ratio score of fishiness. Garlic increased the ideal ratio score of spiciness and sourness. Shallot increased Hunter-a value of the fish, but decreased the ideal ratio score of fish saltiness. The salt content of fish increased with vinegar addition. The vinegar addition reduced Hunter-a value of fish and ideal ratio score of fishiness. Vinegar addition resulted in the reduction of peroxide value and ideal ratio score for the tomato sauce fishiness. In addition, the added vinegar in canned fish improved the overall acceptability of the product.

11.4.2. Optimization of canning process

The above results indicate that all seasoning materials should be retained in the tomato sauce formula. Sterilization time needed to be optimized in terms of overall acceptability of the product. Pre-cooking and vacuum headspace in the can are essential, but brining treatment is unnecessary. The panellists complained that the tomato sauce was too salty for them, so the salt level in the sauce was varied during the optimization experiment. Thus optimization would concentrate on two variables: sterilization time and salt level in the tomato sauce. Optimization study for the sterilization time were 40, 50 and 60 minutes, while the salt levels in tomato sauce investigated were 1.5 and 2.5%.

The results of the optimization experiment are shown in Tables 11.9, 11.10 and 11.11.

Table 11.9. Chemical and physical changes in fish and tomato sauce during optimization experiment

Sample	Analysis		Salt in tomato sauce (%)	Sterilization time (minutes)		
				40	50	60
Fish	Moisture content (%)		2.5	67.41	67.52	67.00
			1.5	67.55	67.76	66.74
	Salt content (%)		2.5	1.80	1.90	1.86
			1.5	1.46	1.60	1.56
	pH		2.5	6.05	6.09	6.02
			1.5	6.07	5.98	5.95
	Hunter value	L	2.5	48.64	44.63	45.23
			1.5	46.52	44.38	44.52
		a	2.5	2.33	3.38	3.65
			1.5	2.41	3.35	3.54
		b	2.5	14.10	14.17	14.48
			1.5	13.62	13.87	14.55
Tomato sauce	pH		2.5	6.00	5.89	5.88
			1.5	5.98	5.90	5.90
	Peroxide value (meq/kg)		2.5	37.12	29.67	29.36
			1.5	38.14	24.19	25.26

Analysis of variance shows that moisture content of fish and pH of tomato sauce were not significantly affected by sterilization time and salt level in tomato sauce. Tomato sauce with 2.5% salt resulted in the fish having pH higher than tomato sauce with 1.5% salt. Sterilization time significantly influenced the Hunter-L,-a and -b values of fish. Fish sterilized for 50 and 60 minutes had a lower Hunter-L value than fish sterilized for 40 minutes. Fish sterilization time of 50 and 60 minutes resulted in a higher Hunter-a value than sterilization of 40 minutes. The fish sterilized for 40 and 50 minutes showed a lower Hunter-b value compared to the fish sterilized for 60 minutes. Fish canned in tomato sauce with 2.5% salt showed a higher salt content than the fish

canned in tomato sauce with 1.5%. Sterilization time also affected the peroxide value of tomato sauce, where tomato sauce with canned fish sterilized for 50 and 60 minutes had a lower peroxide value in comparison with the tomato sauce from the canned fish sterilized for 40 minutes.

Variables of sterilization time and salt level in tomato sauce did not affect, significantly, the sensory properties of the tomato sauce. Both variables did not show any pronounced effect on sourness, saltiness, spiciness and fishiness of the fish. However flesh texture and bone softness were significantly influenced by sterilization: the texture of fish sterilized for 40 minutes and 50 minutes were more acceptable than the texture of fish sterilized for 60 minutes. Sterilization time of 50 and 60 minutes resulted in fish having softer bone than fish sterilized for 40 minutes. The bone softness of fish sterilized for both 50 and 60 minutes was more acceptable than the bone softness for fish sterilized for 40 minutes. Statistically, the overall acceptability of the canned fish product was not affected by sterilization time, but the product sterilized for 50 minutes seemed to be more acceptable compared to the product sterilized for 40 and 60 minutes.

Table 11.10. Sensory changes in fish during optimization experiment

Attributes		Salt in tomato sauce (%)	Sterilization time (minutes)		
			40	50	60
Fish texture	tender - not tender	2.5	6.6	6.9	6.4
		1.5	6.9	6.8	6.3
	acceptability	2.5	7.0	7.2	6.7
		1.5	7.0	7.2	6.8
Bone softness	soft - not soft	2.5	5.4	6.1	6.5
		1.5	4.8	6.4	6.3
	acceptability	2.5	5.7	6.5	6.9
		1.5	5.5	6.8	6.9
Sourness	sour - not sour	2.5	5.4	5.1	5.7
		1.5	5.0	5.4	5.3
	acceptability	2.5	6.5	6.7	6.7
		1.5	6.4	6.8	6.2
Saltiness	salty - not salty	2.5	5.6	5.9	5.9
		1.5	5.2	6.0	5.3
	acceptability	2.5	6.5	6.7	6.5
		1.5	6.6	6.0	6.5
Spiciness	spicy - not spicy	2.5	6.4	6.3	6.1
		1.5	6.1	6.2	6.1
	acceptability	2.5	6.8	6.9	6.7
		1.5	6.4	6.9	6.8
Fishiness	fishy - not fishy	2.5	5.2	4.7	5.3
		1.5	5.3	5.3	5.2
	acceptability	2.5	6.5	7.0	6.5
		1.5	6.7	6.6	6.5

Table 11.11. Sensory changes in tomato sauce and overall acceptability of the product during optimization experiment

Attributes		Salt in tomato sauce (%)	Sterilization time (minutes)		
			40	50	60
Sauce colour	Bright red - not bright red	2.5	6.1	5.6	6.0
		1.5	5.9	6.3	5.7
	Acceptability	2.5	6.7	6.5	6.6
		1.5	6.7	6.8	6.5
Mouth feel	Oily - not oily	2.5	5.7	5.7	6.1
		1.5	6.2	5.9	6.0
	Acceptability	2.5	6.2	6.5	5.9
		1.5	6.3	6.5	6.4
Sourness	Sour - not sour	2.5	4.9	5.1	5.6
		1.5	4.9	5.5	4.8
	Acceptability	2.5	6.4	6.4	6.3
		1.5	6.2	6.5	6.5
Saltiness	Salty - not salty	2.5	5.9	6.0	5.8
		1.5	5.5	6.1	5.4
	Acceptability	2.5	6.5	6.4	6.4
		1.5	6.5	6.5	6.6
Spiciness	Spicy - not spicy	2.5	6.3	6.3	6.0
		1.5	6.4	6.5	6.4
	Acceptability	2.5	6.8	6.6	6.5
		1.5	6.7	6.8	6.9
Fishiness	Fishy - not fishy	2.5	4.9	5.4	5.4
		1.5	5.3	5.1	5.2
	Acceptability	2.5	6.4	6.5	6.1
		1.5	6.2	6.1	6.6
Overall acceptability of the canned fish product		2.5	6.5	6.8	6.5
		1.5	6.2	6.7	6.8

11.5. DISCUSSION

11.5.1. Changes in canned fish during processing

Brining treatment showed a decreased effect on moisture content and an increased effect of salt content of fish. The increment of salt content and the reduction of moisture content of fish are the common phenomenon in salting as reviewed by Stansby (1963), Burgess et al (1965), Voskresensky (1965) and Van Klaveren and Legendre (1965). The salt penetrates into the fish flesh because of the osmotic pressure differences in the fish and the salt surrounding the fish, and, at the same time, water is released from the fish. The increase of salt content was also affected by vinegar addition in the tomato sauce. Vinegar, which is basically acetic acid, may have accelerated penetration of salt from the tomato sauce into the fish flesh. According to Lee (1983), acid can induce denaturation of protein. Possibly, acid and sterilization treatments show a synergetic effect on denaturation of protein which finally allows the salt easy penetration into the fish flesh.

Peroxide value of fish and tomato sauce decreased due to the effect of sterilization time and vacuum headspace treatment in the can. The optimization experiment indicated that the peroxide value of tomato sauce from the canned fish sterilized for 40 minutes was higher than the peroxide value of tomato sauce from the canned fish sterilized for 50 and 60 minutes. As discussed in Chapter 10, hydroperoxides decompose during heat treatment. Thus the longer the sterilization time the greater decomposition effects on hydroperoxides, consequently, the lower the peroxide value in the product. Vacuum headspace in the can provided a much lower oxygen content compared to non-vacuum headspace. This condition could limit the production rate of hydroperoxides during sterilization.

Peroxide value of the fish was increased by brining treatment. Salt normally contains not only sodium chloride, but impurities such as copper, calcium salts, magnesium salts, sulphates and organic matters (Rawson, 1966; Tressler and Lemon, 1960; Burgess et al, 1965). Salt and copper have been known as pro-oxidants which act as catalyst agents in the oxidation process (Stuckey, 1972; Pokorny, 1987; Buck, 1991). These pro-oxidants may have enhanced the production of hydroperoxides in the fish treated with brine.

Pre-cooking of the fish decreased the peroxide value of the tomato sauce. As mentioned before, pre-cooking released water and oil with a high peroxide value. This study showed that the peroxide value of fish was significantly higher than the value in the tomato sauce. The pre-cooking

treatment could reduce the oil which might be released into the tomato sauce during sterilization. Thus the total peroxide value in tomato sauce was lower than the value in tomato sauce obtained from the canned fish without pre-cooking. The possibility of oil release during sterilization could also be used to explain the increase of peroxide value in the tomato sauce due to brining. The peroxide value of the tomato sauce also decreased due to vinegar addition. The acetic acid in vinegar might have the capability to act as a chelating agent removing pro-oxidants for the sauce. This could reduce the oxidation rate in the tomato sauce, and thus lead to the lower hydroperoxide content in tomato sauce with vinegar, in comparison with the tomato sauce without vinegar.

Both sterilization and pre-cooking showed a decreasing effect on the pH of fish, while the decreased pH value of the tomato sauce was significantly influenced by pre-cooking. The optimization experiment showed that fish in tomato sauce with 2.5% salt and sterilized for 50 minutes had a markedly higher pH than the fish with the same sauce sterilized for 60 minutes. The fish in tomato sauce with 1.5% salt and sterilized for 40 minutes showed a higher pH, compared to the fish of the same sauce sterilized for 50 and 60 minutes. In the canning experiment using cat fish, ocean perch and pollack, Paredes and Baker (1987) showed that these fish exhibited different pH changes as the results of sterilization effect where pH of both ocean perch and pollack increased, while the pH of cat fish decreased.

The Hunter-L value was decreased by sterilization, but increased by pre-cooking. The effect of sterilization was noted significant, while pre-cooking was insignificant. This indicated that the effect of sterilization time was stronger than the effect of pre-cooking. The higher Hunter-L value exhibited a lighter colour. The optimization experiment demonstrated that Hunter-L values of fish sterilized for 50 and 60 minutes were lower than the value in the fish sterilized for 40 minutes. This indicated that the longer sterilization time resulted in a darker fish colour. Sterilization time with shallot addition revealed an increasing effect on Hunter-a value at a significant level of 90% for each. However garlic and vinegar additions showed a decreasing effect on the Hunter-a value at significant levels of 90% and 95% respectively. The optimization experiment registered that the fish sterilization for 50 and 60 minutes had a higher Hunter-a value than the fish sterilized for 40 minutes. This indicated that the longer sterilization time tended to give a reddish colour to the fish. Hunter-b value, which was not significantly effected by any treatment in the Plackett and Burman experiment, was markedly influenced by sterilization time in the optimization experiment. Hunter-b values of fish sterilized for 40 minutes and 50 minutes were lower than the values of the fish sterilized for 60 minutes. That result indicated that the longer sterilization time produced fish with yellowish colour. In terms of Hunter values, Paredes and Baker (1987) showed a similar trend in the canning of ocean perch and pollack. Investigations by other researchers noted that when fish

is heated from one direction, three distinct changes of opacity can be discerned. An initial increase in translucence is followed by two successive increases in opacity. The second increase in opacity is due to precipitation of thermally denaturated sarcoplasmic proteins, which appears to begin about 45°C (Aitken and Connell, 1979). The increase in intensity of redness might be due to oxidation of iron pigments from ferrous to ferric derivates (English et al, 1988). A study in canned sardine revealed that pigments involved in the browning reaction were primarily lipophilic, and the presence of O_2 and fat were necessary for these reactions. It was found that volatile amines, such as trimethylamine, dimethylamine and ammonia react directly with oxidized lipids. The reactions were accelerated by high temperature (Wakao and Pazos, 1984). The Hunter-a and -b values could be affected by oxidation of the high myoglobin to metmyoglobin, a dark brown pigment, or by trimethylamine (TMAO) promoting the formation of the disulphide bond between denatured myoglobin and cysteine producing a green pigment (Francis and Clydesdale, 1975; English et al, 1988).

Panellists revealed that sterilization and pre-cooking significantly increased the bone softness of fish. Solubilization of organic matter of bone causes softening of fish bone due to heating process (Soesetiadi, 1977; Ishikawa et al, 1987). The inorganic matter includes calcium, phosphorous, magnesium, chlorine and fluorine (Soesetiadi, 1977). Pre-cooking may have resulted in preliminary organic matter solubilization, further solubilization occurred during sterilization. Acetic acid proved to accelerate the solubilization rate of organic matter from the fish bone (Ishikawa et al, 1989). However the panellist did not detect the effect of vinegar addition on fish bone softness in this study.

Brining treatment on fish significantly increased the saltiness of both the fish and tomato sauce. The higher saltiness of tomato sauce from the process with brining treatment compared to the sauce from the canned fish without brining treatment might be due to the less salt penetration from tomato sauce into the fish canned after brining, compared to canned fish without brining treatment. Brining treatment caused the fish to have a higher salt content, which finally reduced the penetration rate of salt from tomato sauce into the fish during sterilization. Shallot addition decreased the saltiness in fish. This might be because flavour compounds of shallot showed a stronger performance than flavour compounds of salt. Pre-cooking decreased the saltiness of the tomato sauce. Reduction of moisture content of the fish, due to pre-cooking, may have provided greater possibility for salt in tomato sauce to penetrate into the fish. This resulted in the tomato sauce with a less salty taste. However the increase of salt content caused by pre-cooking treatment was noted as insignificant.

Brining improved the spicy properties of both fish and tomato sauce, but sterilization time and vacuum headspace decreased the spiciness properties of tomato sauce. Sterilization may have induced decomposition of the spicy flavour compounds into smaller compounds, which could be volatile by heat during sterilisation, giving a less spicy taste to the tomato sauce. Pre-cooking treatment of fish could improve the spiciness of the tomato sauce. One of the purposes of pre-cooking is to develop desirable flavour properties (Codex Alimentarius Commission, 1976; Warne, 1988). By removal of undesirable flavour, such as fishy flavour during pre-cooking, the existence of a spicy flavour would be significant. Garlic significantly increased the spiciness of the tomato sauce. The principal chemical properties in garlic are diallyl disulphide, allicin, alliin and ajoene (Kritchevsky, 1991). Garlic also increased the sourness of the tomato sauce.

The fishiness of the fish was decreased as the result of sterilization, but the fishiness of the tomato sauce increased during this treatment. During sterilization, the fishy flavour compounds of the fish might be trapped in the tomato sauce showing an increase in fishiness. Garlic and vinegar additions decreased the fishiness of the fish, while the fishiness of the tomato sauce significantly decreased due to the effect of vinegar addition. This result indicated that garlic and vinegar have the capability to neutralize the fishy flavour in canned fish with disguised fish oil.

Sterilization time and vinegar addition in tomato sauce significantly increased the overall acceptability of the canned fish product. The above results show that sterilization had a significant effect on the bone softness of the fish. Thus, sterilization was necessary to the optimization experiment. Vinegar addition had a significant effect on the reduction of the fishiness of the fish. The vinegar addition in tomato sauce had to be retained in further experiments. Brining treatment was removed from the fish canning process, since this treatment increased peroxide value of the fish, and saltiness in the tomato sauce. By removing the brining treatment, the salt in the tomato sauce had more opportunity to penetrate into the fish during sterilization. This was expected to produce a more acceptable salty taste in the tomato sauce and fish. Pre-cooking of fish and vacuum headspace in the can as well as garlic and shallot addition in the tomato sauce are retained in the canning process, because they showed desirable effects on acceptability of the canned fish product.

11.5.2. Canning process optimization

Sterilization significantly affected the acceptability of fish flesh texture and bone softness. The texture of fish sterilized for 40 and 50 minutes was more acceptable than the texture of fish

sterilized for 60 minutes. Bone softness of fish sterilized for 50 and 60 minutes was more acceptable than the bone softness of fish sterilized for 40 minutes. By considering both texture and bone softness acceptability, the sterilization time of 50 minutes was regarded as the optimal sterilization time for canned fish with fish oil disguised in it. Generally, both flesh texture and bone softness are the most important factors in determining the acceptability of canned fish. The medium is also an important factor in the acceptance of canned fish by the consumer as indicated by the result of consumer survey. However the optimization experiment did not reveal any significant effect on sensory properties of the tomato sauce due to sterilization time and salt level.

Sterilization time and level of salt addition did not significantly influence the overall acceptability of the canned fish product. However the trend showed that the sterilization time of 50 minutes generally produced canned fish with a better overall acceptability than the other two sterilization times. Thus, all of the above explanations suggested to the use of 50 minute sterilization time for processing canned fish with fish oil disguised in it. Directorate General of Fishery (1984) recommended sterilization of canned sardine in tomato sauce at 117°C for 54 minutes. However the previous experiment, Chapter 10, proved that that temperature had less protection to ω-3 fatty acids in fish oil.

The salt levels of 1.5 and 2.5% in tomato sauce did not induce pronounced differences on the sensory properties of fish and tomato sauce. In terms of economic consideration, the salt level of 1.5% was recommended for the tomato sauce in processed canned fish with disguised fish oil.

11.6. CONCLUSIONS

The Plackett and Burman design proved to be very useful in identifying the important factors in the processing of canned fish with disguised fish oil. Pre-cooking, vacuum headspace, sterilization time, garlic, shallots and vinegar additions were considered as the important factors. However sterilization time and salt level in the tomato sauce needed optimization.

The optimization experiment recommended sterilizing the canned fish with disguised fish oil at 121.1°C for 50 minutes. The optimum salt level in the tomato sauce was found to be 1.5%.

Chapter 12

PROSPECT OF CANNED FISH PRODUCT WITH FISH OIL ADDITION IN INDONESIAN MARKET

12.1. BACKGROUND

The consumer survey indicated that the canned fish product being developed had good prospects in the Indonesian market, where approximately 80% of respondents intend buying the product. The supermarket survey revealed that this product was superior to the existing product, since the product was nutritionally better. During the development study, the sensory panel results suggested that the product was organoleptically acceptable. In the development process the product must now be exposed to a wider evaluation, using the Indonesian consumer, the target market, where more reliable information about the suitability of the product could be ascertained.

Measuring consumer response to products is considered as a critical part of the development process, so now major emphasis is given to this activity. Acceptance testing will indicate whether the product can be marketed, or improvement is needed (Dethmers, 1981).

The most practical approach to predicting consumer acceptance is through the use of sensory panels. Sensory panels measure human responses to sensory stimuli in food products. Consumers perceive product characteristics through the senses. Sensory cues, along with behavioral influences, provide the consumer with a basis for a judgemental value of acceptance or rejection (Ellis, 1970). The consumer may not have the level of skill for a specialized sensory taste; but, the consumer can provide information not obtainable, in an unbiased form, from the trained panellists; for example, preference, purchase intent, and so forth. All participants are important and have something to contribute (Stone, 1988).

A variety of tests may be employed, such as central location tests where the conditions are more controlled, or home placement tests where consumers can use the product under realistic conditions (Lyon, 1991). In this study, home placement tests were used as more questions could be asked and information could be obtained regarding the product and consumer attitudes toward product price, package label, etc; since the respondents are given enough time to answer the questionnaire (Gatchalian, 1981).

As the product was nutritionally better, containing a higher polyunsaturated fatty acid than existing products, particularly ω-3 fatty acids, a medical doctor survey was undertaken to reveal the medical perception of the developed product. The survey indirectly introduced the product to the medical doctors who were expected to contribute in the introduction of the health benefits of the developed product to the public, especially patients, who could benefit from its use.

12.2. OBJECTIVES

As the final stage in the process of product development, the objectives of this study were:

* to investigate the physical and chemical changes of the product during production trial;
* to show the acceptance of the product by Indonesian consumers in order to assess the prospect of the product in the market; and
* to show the response and comment from the Indonesian medical doctors about the product and the prospect of the product from the medical point of view.

12.3. METHODOLOGY

12.3.1. Materials

Fish, fish oil, tomato paste, salt, sugar, vinegar, shallots, garlic and cans were the same as used for the experiment in Chapter 11.

12.3.2. Methods

12.3.2.1. Production trial

The canned fish was processed, using the method described in Chapter 11, by employing sterilization at 121.1°C for 50 minutes. The product was processed at full capacity of pilot plant steamer for pre-cooking and retort (188 cans) in the Food Technology Department, Massey University. The tomato sauce formula used was 18.16% tomato paste, 45.10% water, 27.25% fish oil, 1.43% salt, 3.00% sugar, 1.90% shallot, 1.90% garlic and 0.95% vinegar. Weight, physical and chemical changes of fish, tomato sauce and the canned fish product during the production trial were determined.

12.3.2.2. Consumer test for product acceptability

Consumer testing of the developed canned fish was undertaken using the home placement test method in five cities in Java, Indonesia where approximately 60% of the Indonesian population lives, (Central Bureau of Statistic, 1990).

The samples for the consumer test were prepared in the Food Technology Department, Massey University, and flown to Indonesia for testing. Only one sample was given to each household or family. Three copies of the product testing questionnaire were given to each family, and one of the questionnaires was designed for the housewife or the person in charge of shopping and/or determining the daily family menu.

The samples were given to 158 families including 40 families in Jakarta (capital city of Indonesia), 30 families in Tangerang (West-Java), 37 families in Semarang (capital city of the Central-Java province), 26 families in Sragen (Central-Java) and 25 families in Lumajang (East-Java). The families were given freedom to organise their own testing by following directions on the questionnaire. They were asked to return the questionnaire to six survey coordinators, one coordinator in each city except Jakarta, where there were two coordinators. The questionnaire, as shown in Appendix 12.1, was designed as simply as possible by using a simple 5-scale hedonic method in "just right" form, so it could be completed by respondents who were able to read and write. The questionnaire prepared for the housewife or a person responsible for shopping and/or preparing the daily family menu included some supplementary questions.

Both types of questionnaires, in number, were returned as listed:

City	Family (number)	Housewife (number)	Total questionnaire (number)
Tangerang	30	30	81
Jakarta	37	36	104
Semarang	34	32	93
Sragen	26	23	60
Lumajang	24	22	64
Return rate	**95.6%**	**90.5%**	**84.4%**

12.3.2.3. Medical doctor survey

The survey was used to reveal the attitudes of medical doctors to fish oil and the comments about the product being developed. The questionnaire, as shown in Appendix 12.2, was distributed to doctors including pathologists, paediatricians, internists, obstetricians and general practitioners at the "Syaiful Anwar" general hospital, Malang, East-Java, the Medical Faculty of Brawijaya University, Malang, East Java, and public health service centres and general hospitals in the South Kalimantan Province. The questionnaire was also distributed to the general practitioners attending an obstetrics and gynaecology course in Malang, East-Java, in 1990. The responses to the survey numbered 163.

12.4. RESULTS

12.4.1. Changes during production trial

12.4.1.1. Weight changes

The initial raw fish weight was 35.51 kg. After preparation treatment involving heading, gutting and cutting, the net weight was 25.75 kg, a 27,49% weight loss.

The changes in fish weight in ten cans was monitored during the production trial. The changes are shown in Table 12.1.

Table 12.1. Fish and canned fish product weight changes during production trial

Can No.	Initial fish weight (g)	Pre-cooked fish weight (g)	Total fish and sauce weight (g)	Tomato sauce weight (g)
1	119.1	103.2	166.1	62.9
2	121.8	105.2	168.0	62.8
3	128.8	114.3	165.4	51.1
4	125.4	108.3	169.5	61.2
5	127.8	111.5	164.7	53.2
6	130.6	114.5	166.3	51.8
7	124.3	106.3	173.9	67.6
8	130.7	114.2	171.0	56.8
9	131.9	114.9	170.3	55.4
10	127.1	110.6	169.2	58.4
X ± SD	126.8 ± 4.1	110.3 ± 4.3	168.4 ± 2.9	58.1 ± 5.4

The average initial fish weight in each can was 126.76g. Pre-cooking by steaming reduced the fish weight to 110.31g. Fish weight loss due to steaming treatment was approximately 13%. The average weight of tomato sauce added to each can was 58.14g, approximately 34.5% of product net weight.

12.4.1.2. Colour changes

Colour changes in both fish flesh and tomato sauce medium were measured using HunterLab colour, and the results are shown in Table 12.2.

Table 12.2. Hunter-L, -a and -b values changes in both fish flesh and tomato sauce medium during production trial

Sample	L	a	b
Raw fish	34.15	4.21	8.86
Steamed fish	50.25	1.19	12.39
Sterilized fish	44.47	4.66	13.53
Tomato sauce medium before sterilization	26.44	12.10	8.32
Tomato sauce medium after sterilization	25.84	7.63	7.04
Blend of fish and medium of canned fish before sterilization	44.19	7.03	13.20
Blend of fish and medium of canned fish after sterilization	37.47	9.23	17.28

In terms of the fish colour changes, pre-cooking increased Hunter-L and -b values, but reduced Hunter-a values. Sterilization decreased Hunter-L value of steamed fish, but significantly increased Hunter-a value. The Hunter-b value increased slightly. Sterilization also decreased the Hunter-L value of the blend of fish, but medium increased both Hunter-a and -b values. Hunter-L, -a and -b values of tomato sauce medium decreased due to sterilization.

12.4.1.3. Chemical changes

As shown in Table 12.3 pre-cooking caused a significant change in the proximate composition of fish, especially in the significant decrease in moisture and ash content, and the increase of protein and carbohydrate contents. Sterilization did not markedly affect the proximate composition of pre-cooked fish. The oil addition to the tomato sauce medium significantly increased fat and carbohydrate contents, and reduced the protein content of the product.

Table 12.3. Proximate composition changes in the canned fish during production trial (%)

Parameters	Samples			
	Raw fish	Pre-cooked fish	Sterilized fish	Whole canned fish
Moisture content	71.8	69.4	69.4	68.2
Protein content	22.2	23.7	23.2	14.6
Fat content	2.4	2.9	3.0	10.1
Carbohydrate content (by difference)	0.5	1.5	1.9	4.4
Ash content	3.1	2.5	2.5	2.7

Stability of the oil in the tomato sauce was studied by extracting the oil and analyzing for peroxide, anisidine, TBA and totox values. The results are shown in Table 12.4. The increase of anisidine and TBA values were noted, while both peroxide and totox values registered a decreasing pattern.

Table 12.4. Results of stability study on the oil in tomato sauce medium due to sterilization treatment during production trial

Parameter	Tomato sauce	
	Before sterilization	After sterilization
Peroxide value (meq/kg)	49.5	19.4
Anisidine value	18.0	41.2
TBA value	19.1	29.7
Totox value	117.1	80.0

Table 12.5. Fatty acid profile changes in canned fish due to sterilization treatment during production trial

Samples	SAFA	MUFA	PUFA	ω-3 FA	EPA	DHA
Fish oil	28.4	48.0	23.5	20.4	6.6	8.3
Unsterilized tomato sauce	29.0	45.0	26.0	22.6	7.7	8.8
Sterilized tomato sauce	29.4	44.5	26.0	22.7	7.8	8.9
Raw fish	44.9	25.4	29.7	24.1	5.7	15.2
Sterilized fish	41.1	26.2	32.4	28.3	7.1	17.2
Unsterilized canned fish product	33.7	39.6	26.6	23.0	7.5	10.1
Sterilized canned fish product	33.2	40.7	26.1	22.8	7.6	9.9

Fatty acid profiles of fish, tomato sauce and the canned fish product during production trials are shown in Table 12.5. Fatty acid profiles of fish oil and tomato sauce with fish oil disguised in it showed differences in the relative amounts of monounsaturated fatty acid (MUFA), polyunsaturated fatty acids (PUFA) and ω-3 fatty acids. The relative amount of MUFA of tomato sauce was lower than the amount in fish oil, but the relative amount of PUFA and ω-3 fatty acid were higher than measured in the fish oil. The fatty acid profiles of tomato sauce were insignificantly changed during sterilization, but sterilization treatment increased significantly the relative amount of PUFA and ω-3 fatty acids, of fish, and decreased significantly the relative amount of SAFA. Fatty acid profiles of the whole canned fish product seemed relatively constant during sterilization.

12.4.2. Safety assessment of developed canned fish

As the canned fish produced from the production trial would also be used for consumer product testing, product safety had to be assured before releasing it to the Indonesian consumer. Sterility and incubation tests were used for this purpose.

The sterility test showed that no microorganisms grew on the nutrient agar plates during aerobic and anaerobic incubation at 30°C and 55°C. The incubation test performed by incubating five cans each at 30°C and 55°C for 14 days indicated that the can appearance was still in normal condition until the end of incubation period, and no undesirable changes, such as swelling were observed. Thus, sterilization and incubation test results revealed that the canned fish product was safe for consumption.

12.4.3. Product acceptability during consumer testing

12.4.3.1. Overall results for canned fish characteristics and acceptability

The frequencies of the "just right" score for each sensory characteristic of the developed canned fish product are shown in Table 12.6.

More than half of the respondents said that the texture of the fish flesh and bone softness was "just right", while a small number of respondents commented that the texture of fish flesh and bone was very strong. About 30% of respondents thought that the sauce colour was bright red, while 54% said that the sauce colour was "just right". Only 9% of respondents indicated that the sauce colour was dark red.

In terms of the taste of both fish flesh and sauce, over 50% of respondents said that the sourness, saltiness and spiciness of both fish flesh and tomato sauce medium were "just right", while approximately 20-35% of respondents commented that the fish flesh and tomato sauce lacked of sour, salt and spice taste. Only a minority of respondent thought that the product was very fishy. The respondents saying that the fish flesh was slightly fishy, "just right" and slightly non-fishy were 39%, 32% and 14% respectively. Respondents commenting that the tomato sauce medium was slightly fishy, just right and slightly non-fishy were 47%, 24% and 14% respectively. Most of the respondents said that the mouth feel of the tomato sauce medium was slightly oily (45%) and "just right" (31%).

With regard to overall acceptability of the canned fish product, a minority of respondents (18%) did not like the developed product. Approximately 38% of respondents liked the product, while approximately 45% neither liked nor disliked.

Table 12.6. Canned fish characteristics and acceptability in consumer testing

Parameter	Score									
	5		4		3		2		1	
	N	%*)	N	%	N	%	N	%	N	%
FISH										
Texture[a)]	50	13.4	56	13.6	267	66.4	25	6.3	0	0
Bone softness[a)]	99	24.6	73	18.1	208	51.7	22	5.6	0	0
Sourness[b)]	6	1.5	66	16.4	223	55.5	86	21.4	21	5.2
Saltiness[b)]	12	3.0	73	18.1	224	55.8	84	20.9	9	2.2
Spiciness[b)]	5	1.2	18	4.5	235	58.4	131	32.7	13	3.2
Fishiness[b)]	42	10.4	157	39.0	127	31.6	55	13.8	21	5.2
TOMATO SAUCE										
Colour[c)]	9	2.2	115	28.6	218	54.2	49	12.3	11	2.7
Mouthfeel[d)]	60	14.9	181	45.0	123	30.6	28	7.0	10	2.5
Sourness[b)]	8	2.0	54	13.4	214	53.2	110	27.4	16	4.0
Saltiness[b)]	15	3.7	66	16.4	202	50.3	109	27.1	10	2.5
Spiciness[b)]	3	0.7	25	6.2	216	53.8	144	35.8	14	3.5
Fishiness[b)]	30	7.5	189	47.0	98	24.4	58	14.4	27	6.7
CANNED FISH										
Overall acceptability[e)]	40	10.0	109	27.1	180	44.8	62	15.4	11	2.7

Note:

*) row percentage

a) score 5 very soft, 4 slightly soft, 3 just right, 2 slightly tough, 1 very tough

b) score 5 very strong, 4 slightly strong, 3 just right, 2 slightly lacking, 1 very lacking

c) score 5 very bright red, 4 slightly bright red, 3 just right, 2 slightly dark red, 1 very dark red

d) score 5 very oily, 4 slightly oily, 3 just right, 2 slightly non-oily, 1 very non-oily

e) score 5 like very much, 4 like slightly, 3 neither like nor dislike, 2 dislike slightly, 1 dislike very much

12.4.3.2. Acceptability of developed canned fish

The level of acceptability of the canned fish product in terms of demographic characteristics is shown in Table 12.7.

Respondents from Tangerang, Semarang and Lumajang showed a similar response to the acceptability of the developed product. Most respondents (47-57%) said that they neither liked nor disliked the product. Respondents who did not like the product were less than 15%, while 62% of the respondents from Sragen liked the product and 10% disliked the product. Jakarta showed a high number of respondents (48%) who did not like the product, while only 20% liked the product.

The survey results showed that the acceptability of the developed canned fish was independent of sex. The product was more acceptable to the consumer under 40 years of age than the consumer over 40 years.

In terms of respondent career, all career groups showed a lower percentage of respondent who did not like the product. The respondents who liked the product were mostly private sector workers, government officials and housewives, but less for pupil and college/university students. Only a minority of consumers from each family income bracket did not like the product. Therefore, consumers from all family income brackets showed as potential consumers.

When considering the total number of consumers, the results show that the consumer accepted the developed canned fish product. The mean score of overall acceptability is 3.26, or close to "like slightly" category.

Table 12.7. Acceptability of developed canned fish product in consumer test by demographic characteristics

Demographic characteristics	Score									
	5		4		3		2		1	
	N	%*)	N	%	N	%	N	%	N	%
Location:										
Tangerang	1	1.2	24	29.6	46	56.8	8	9.9	2	2.5
Jakarta	2	1.9	19	18.3	43	41.3	33	31.7	7	6.7
Semarang	9	9.7	27	29.0	44	47.3	11	11.8	2	2.2
Sragen	15	25.0	22	36.7	17	28.3	6	10.0	0	0
Lumajang	13	20.3	17	26.6	30	46.9	4	6.2	0	0
Sex:										
Male	11	7.1	49	31.8	71	46.1	22	14.3	1	0.6
Female	29	11.7	60	24.2	109	43.9	40	16.2	10	4.0
Age group (years):										
15 - 20	10	20.8	12	25.0	21	43.8	5	10.4	0	0
21 - 30	13	6.9	50	26.5	86	45.5	35	18.5	5	2.6
31 - 40	7	8.3	33	39.3	33	39.3	9	10.7	2	2.4
41 - 50	5	10.9	9	19.6	24	52.2	6	13.0	2	4.3
> 50	5	14.3	5	14.3	16	45.7	7	20.0	2	5.7
Career:										
Student (pupil - tertiary)	9	13.8	10	15.4	35	53.8	10	15.4	1	1.6
Private sector	13	6.8	59	31.1	87	45.8	27	14.2	4	2.1
Government official	6	13.7	14	31.8	17	38.6	7	15.9	0	0
Housewife/ family helper	12	11.7	26	25.2	41	39.8	18	17.5	6	5.8
Family income (xRp.1000.-):										
< 150	9	18.0	18	36.0	16	32.0	7	14.0	0	0
150 - 299	18	16.4	24	21.8	49	44.5	18	16.4	1	0.9
300 - 500	6	5.9	28	27.5	54	52.9	12	11.8	2	1.9
> 500	7	5.0	39	27.9	61	43.6	25	17.8	8	5.7

Note: *) row percentage

5 like very much, 4 like slightly, 3 neither like nor dislike, 2 dislike slightly, 1 dislike very much

12.4.3.3. Consumer buying trend of developed canned fish

The survey results of the buying trends in terms of demographic characteristics are shown in Table 12.8. More than 70% of respondents from Tangerang, Sragen and Lumajang intended to buy the developed product, and about 56% of respondents from Semarang indicated likewise. However more than 55% of respondents from Jakarta did not intend to buy the product.

Table 12.8. Buying trend of developed canned fish in consumer testing by demographic characteristic

Demographic Characteristics	Buying intention			
	Yes		No	
	N	%*)	N	%
Location:				
Tangerang	21	70.0	9	30.0
Jakarta	16	44.4	20	55.6
Semarang	18	56.3	14	43.7
Sragen	17	73.9	6	26.1
Lumajang	17	77.3	5	22.7
Age (years):				
15 - 30	35	55.6	28	44.4
31 - 40	29	72.5	11	27.5
41 - 50	14	63.6	8	36.4
> 50	11	61.1	7	38.9
Career:				
Private sector worker	39	62.9	23	37.1
Government official	9	56.3	7	43.7
Housewife/ family helper	41	63.1	34	36.9
Family income (xRp.1000.-):				
<150	8	44.4	10	55.6
150 - 299	25	62.5	15	37.5
300 - 500	26	72.2	10	27.8
>500	30	61.2	19	38.8

Note: *) row percentage

Respondents from all age groups showed a promising response to the developed product, where 55-73% of respondents from each age group intended to buy the developed product. Respondent careers did not significantly affect the buying trend, since more respondents from each career group wanted to buy the product than respondents not intending to buy the product.

Family income affected the buying trend. Mostly the respondents from middle and high income brackets planned to purchase the product. Approximately 56% of respondents from low income group decided not to buy the product.

Table 12.9 shows the buying trend according to respondent opinion about product acceptability and respondent experiences in buying canned fish. All respondents who liked the product very much wanted to buy the product. On the other hand, all respondents who did not like the product very much did not intend to purchase. The respondents commenting "like slightly" and "neither like nor dislike" were approximately 85% and 59% respectively, planned to buy the product. Approximately 15% of respondents who "did not like the product slightly" decided to buy the product.

For the respondents who have consumed present existing canned fish products, canned fish in tomato sauce and canned sardine products, more than 65% of them want to buy the product. More than 55% of respondents who have not consumed canned fish in tomato sauce and canned sardine intend to buy the developed canned fish product. However about 61% of respondents who have not bought any canned fish products decided not to buy this product.

Table 12.9. Buying trend of developed canned fish according to consumer testing acceptability and consumer experience in buying canned fish products

Information	Buying intention			
	Yes		No	
	N	%*)	N	%
Acceptability:				
Like very much	16	100	0	0
Like slightly	33	84.6	6	15.4
Neither like nor dislike	37	58.7	26	41.3
Dislike slightly	3	15.0	17	85.0
Dislike very much	0	0	5	100
Consume canned fish:				
Yes	80	66.7	40	33.3
No	9	39.1	14	60.9
Consume canned fish in tomato sauce:				
Yes	45	66.2	21	31.8
No	44	57.1	33	42.9
Consume canned sardine:				
Yes	34	69.4	15	30.6
No	55	58.5	39	41.5

Note: *) row percentage

Response to buying criterion, retail outlets, label information and selling price of the product are shown in Table 12.10. A majority of consumers agreed that the major reason for purchasing the developed product was convenience: the product is easy to store and serve. Only 28% and 17% of consumers considered buying the product because of its nutritional value and health benefit respectively.

Table 12.10. Buying criterion, retail outlet, label information and price of product suggested by consumer testing

Information	Consumer	
	Number	%*)
Buying criterion**)**		
Family preference	9	10.1
Convenience	65	73.0
Like to eat	8	9.0
Nutritional value	25	28.1
Reasonable price	15	16.9
Health benefits	15	16.9
Retail outlets**)**		
Supermarket	49	55.1
Retail shop	23	25.8
Food shop	21	23.6
Local market	3	3.4
Information on label**)**		
Brand	8	9.0
Ingredients	9	10.1
Composition	17	19.1
Name and address of factory	8	9.0
Product superiority	14	15.7
Net weight	11	12.3
All above	57	64.0
Price (Rp.)		
< 500	9	10.1
500 - 650	30	32.7
651 - 800	20	22.5
801 - 1000	16	18.0
> 1000	5	5.6

Note: *) Based on the respondents intending to buy the product
**) One respondent could give more than one answer

Most of the respondents (55%) suggested selling the product in supermarkets while others suggesting selling the product in retail shops and food shops 26% and 24% respectively. A majority of respondents said that all information mentioned on the label, as shown on Appendix 12.3, had to be retained. Some respondents suggested adding an expiry date, "no added

monosodium glutamate" and "100% halal". The statement "100% halal" means that the product can be eaten by Moslems as no forbidden ingredients, according to Islamic law, are used to process the product.

Half the respondents thought that the product should be sold at price between Rp. 500-800.

12.4.4. Opinions of Indonesian medical doctors to the product

12.4.4.1. Fish and fish oil

The results of the medical doctor survey, as shown in Table 12.11, indicates that many Indonesian doctors have not suggested their patients consume fish oil for health reasons. However more than 80% of the respondents from all demographic groups have advised patients to consume fish for their health. This indicated that the benefits from eating fish have been widely recognized by Indonesian medical doctors.

Table 12.11. Medical doctors advising the patients to consume fish and fish oil

Demographic characteristic	Fish oil				Fish			
	Yes		No		Yes		No	
	N	%*)	N	%	N	%	N	%
Speciality:								
Pathologist	0	0	1	100	1	100	0	0
Paediatrician	9	42.9	12	57.1	20	95.2	1	4.8
Internist	4	21.1	15	78.9	19	100	0	0
Obstetrician-gynaecologist	5	23.8	16	76.2	21	100	0	0
General practitioner	48	47.5	53	52.5	87	86.1	14	13.9
Experience (years):								
<5	40	50.6	39	49.4	68	86.1	11	13.9
5 - 10	10	41.7	14	58.3	23	95.8	1	4.2
11 - 15	8	25.8	23	74.2	29	93.5	2	6.5
16 - 20	2	18.2	9	81.8	10	90.9	1	9.1
> 20	6	33.3	12	66.7	18	100	0	0
Number of total patient/month:								
<100	10	62.5	6	37.5	14	87.5	2	12.5
100 - 300	24	39.3	37	60.7	54	88.5	7	11.5
301 - 500	17	34.0	33	66.0	46	92.0	4	8
501 - 700	8	38.1	13	61.9	19	90.5	2	9.5
> 700	7	46.7	8	53.3	15	100	0	0
Number of patient having heart problem/month:								
< 10	47	42.3	64	57.7	99	89.2	12	10.8
10 - 30	13	39.4	20	60.6	30	90.9	3	9.1
31 - 50	2	15.4	11	84.6	13	100	0	0
> 50	4	66.7	2	33.3	6	100	0	0

Note: *) row percentage

Table 12.12 shows the majority of the doctors surveyed recommended food products as a mean to deliver fish oil to consumers in order to increase the fish oil consumption. Only paediatricians and medical doctors having patients between 501 - 700 people/month tended to recommend encapsulated oil.

Table 12.12. The ways advised by Indonesian medical doctors to deliver fish oil to consumer

Demographic Characteristics	Capsule		Cooking oil		Tablespoon or direct consumption		Disguised in food products	
	N	%*)	N	%	N	%	N	%
Speciality:								
Pathologist	0	0	0	0	0	0	1	100
Paediatrician	14	66.6	0	0	1	4.8	6	28.6
Internist	4	44.4	1	11.1	0	0	4	44.4
Obstetrician-gynaecologist	1	4.8	3	14.2	1	4.8	16	76.2
General practitioner	25	24.8	8	7.9	0	0	68	67.3
Experience (years):								
< 5	22	27.8	7	8.9	0	0	50	63.3
5 - 10	5	20.8	1	4.2	0	0	18	75.0
11 - 15	7	22.6	3	9.7	2	6.4	19	61.3
16 - 20	3	27.3	0	0	0	0	8	72.7
> 20	7	38.9	1	5.6	0	0	10	55.5
Number of total patient/month:								
< 100	5	31.3	2	12.5	0	0	9	56.2
100 - 300	13	21.3	7	11.5	0	0	41	67.2
301 - 500	9	18.0	2	4.0	0	0	39	78.0
501 - 700	11	52.4	1	4.8	1	4.8	8	38.0
> 700	6	40.0	0	0	1	6.7	8	53.3
Number of patient having heart problem/month:								
< 10	32	28.8	10	9.0	0	0	69	62.2
10 - 30	9	27.3	1	3.0	2	6.1	21	63.6
31 - 50	1	7.7	1	7.7	0	0	11	84.6
> 50	2	33.3	0	0	0	0	4	66.7

Note: *) row percentage

12.4.4.2. Canned fish product

Comments of medical doctors on the developed canned fish product are shown in Table 12.13 and Appendix 12.4. About 70% of surveyed doctors said that the canned fish with fish oil disguised in it was a good idea to increase fish oil consumption for Indonesians. Appendix 12.4 indicates that paediatricians and medical doctors having 11-15 years experience did not give strong support to the product as only 38% and 40% of them respectively agreed with the idea. Approximately 63% of total surveyed doctors thought the product would have good prospects in the market in terms of medical suitability, while 34% said that maybe the product had good prospects in the market. In terms of demographic groups, each group of respondents had a less than 13% of respondents saying that the product did not have good prospects in the market.

Table 12.13. Comments of medical doctors on the product idea and the prospect of the product in the market

Questions	Respondents	
	N	%
1. Is the canned fish product with fish oil disguised in it the good way to improve fish oil consumption for Indonesian?		
YES	114	69.9
NO	34	20.9
MAYBE	15	9.2
2. Does this product have a good prospect in the market according to your medical point of view?		
YES	102	62.6
NO	5	3.1
MAYBE	36	34.3
3. Will you suggest your patients to consume this product, if the product is available on the market?		
YES	147	90.2
NO	14	8.6
MAYBE	2	1.2

The doctors showed strong support to the introduction of the product, as approximately 90% of them would suggest their patients consume this product. All demographic groups of respondents showed the same response by promising to advise their patients to purchase this product.

12.5. DISCUSSION

12.5.1. Chemical and physical changes in canned fish during production trial

In general, the changes in the product during the production trial were similar to those occurring in the experiment in Chapter 11. But, some new information was revealed.

The production trial proved that pre-cooking using steam induced weight loss in the fish, of approximately 13%. During cooking, water and water-soluble proteinaceous materials such as gelatin, nitrogen-containing extractives, and other substances are leached out of the fish (Lassen, 1965). The removal of water from the fish tissues is attained primarily by heat coagulation and an ensuing shrinkage of the flesh protein. This cooking is vital to remove tissue water, which, if not eliminated, will later appear in the can during sterilization, giving a watery, boiled-like fish into which the oil does not penetrate, with large drops of water in the oil phase (Cheftel, 1965).

Proximate analysis also indicated that the considerable changes in fish composition were found during pre-cooking as shown in the decrease in moisture and ash contents and the increase in protein, fat and carbohydrate contents. Sterilization did not induce any further changes in the proximate composition of the fish. This revealed that the stabilization in the fish structure has been formed by the application of pre-cooking. Proximate composition of the product showed that the fish oil addition into the medium significantly increased the fat content of the canned fish product. This means that the aim to deliver fish oil through the canned fish product in order to increase the fish oil consumption of Indonesians could be achieved.

As found in the experiment of Chapter 11, the peroxide value of the tomato sauce decreased after the sterilization process, while anisidine and TBA values measuring the secondary products of oxidation increased. This result indicates that sterilization caused the quick conversion of hydroperoxide to secondary products such as α/β-aldehyde and malonaldehyde measured as

anisidine and TBA values respectively. Hydroperoxide may also undergo a variety of scission and dismutation reactions to form a wide spectrum of carbonyl compounds, hydroxy compounds, short chain fatty acids, dimers and polymers (Dugan, 1968; Smouse, 1978). The total oxidation (totox) value of tomato sauce decreased due to sterilization treatment. This indicated that the destruction of hydroperoxide was more obvious than the conversion into the secondary products of oxidation.

Fat content of fish was significantly lower than the fat content of whole canned fish product due to the fish oil in the medium. Therefore, the changes in fatty acid profiles of fish were less important than the changes in tomato sauce or the whole product. The results of fatty acid profile analysis showed that the fatty acid profiles of tomato sauce were relatively constant during sterilization, while the relative quantity of fatty acid profile of fish changed due to sterilization process by exhibiting a higher relative amount of polyunsaturated fatty acids (PUFA) in the sterilized oil. The same indication was noted by Hale and Brown (1983) in the canning of spanish sardine, thread herring and chub mackerel. However the fatty acid profile of the whole canned fish product was relatively constant. This showed that the fatty acid profile of tomato sauce appeared to determine the final fatty acid profile of the product and needed more attention. Tomatoes are an ascorbic acid source (Hall, 1984) and ascorbic acid is known as a natural oxidation inhibitor (Pokorny, 1991). Ascorbic acid may have given protection to fatty acids, particularly unsaturated fatty acid from oxidation attacks during sterilization with remaining oxygen in the can.

Significant changes in canned fish colour occurred starting during pre-cooking, in which according to Hunter-L, -a and -b values the fish flesh colour shifted to white, grey and yellow direction respectively. Red colour, due to the presence of carotenoid, became discoloured as a result of the Hunter-a value sifting to grey. Sterilization induced the changes in Hunter-L, -a and -b values to the direction of black, red and yellow respectively. The detail discussion about the colour changes in the fish flesh has been given in Chapter 11. Sterilization caused the changes in Hunter-L, -a and -b values of the tomato sauce medium by exhibiting the reduction of white, yellow and red intensity respectively. The red colour in tomato sauce is formed by carotenoids, particularly lycopene (Belitz and Grosch, 1987). Two types of isomerisation can occur during heat treatment on carotenoids, namely cis-trans isomerisation and epoxide isomerisation, which can give rise to lightening of the colour (Hall and Pitcher, 1991).

12.5.2. Product safety and shelf life

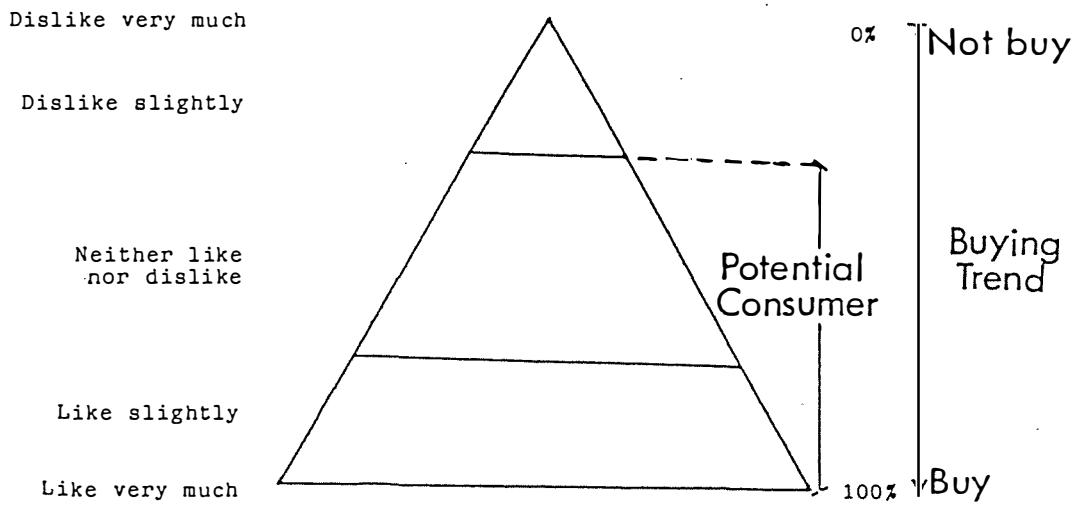
Sterility and incubation testing indicated that the canned fish for consumer testing in Indonesia was safe. Therefore, the possibility of spoilage due to the microorganisms surviving during the sterilization process was not considered, and the shelf life of the product depended on post-processing contamination.

Contamination of can contents after processing can occur through leaks in the can. Such leaks are often the result of faulty seaming and excessive corrosion (Van den Broek, 1965). The measured overlap of the double seam of the can in the production trial was 62.6% which was higher than the minimum standard of 45% (Warne, 1988). This overlap level was considered safe. Non-microbial spoilages which can occur and affect the shelf life of canned fish products are hydrogen swells, corrosion of cans, carbon dioxide swells, discoloration, filling problems and tainting as reviewed by Murrell (1978).

Shelf life prediction for a canned product is extremely difficult to estimate. Shelf life is determined by package composition, product and container compability and temperature of storage. For metal containers, internal corrosion and storage temperature are the most important considerations (Rees, 1991). According to the experiment conducted by Emshanova et al (1983) on the storage trial of canned mackerel, hake, turbot and cavalla in tomato sauce, the products were recommended for storage of up to two years. Bayley (1991) stated that canned fish which passes the incubation test may have a shelf life up to 20 months. Based on this information, the shelf life of canned fish with fish oil disguised in it is probably up to 20-24 months.

12.5.3. Prospect of developed canned fish in Indonesian market

The idea of incorporating fish oil into canned fish was approved by most surveyed medical doctors as a suitable way to deliver fish oil to the consumer. The consumer testing of the product showed that only a small percentage of consumers did not like the product. Most consumers commented "neither like nor dislike", "like slightly" and "like very much", thus indicating that the market of this product was promising. Crosstab analysis revealed that most consumers were willing to buy the product, especially the consumer who commented "neither like nor dislike", "like slightly" and "like very much". The projected buying trend of the product in terms of the product acceptability can be described using a triangular form as follows:



Potential consumers are expected from below the "dislike slightly" area. The percentage of consumers who are willing to buy the product increased from the top to the bottom of the triangle. This study showed that the buying trend of the prospective consumer commenting "dislike very much" and "like very much" to the product was 0% and 100% respectively.

Consumer experience in eating a similar product was important in influencing buying intention. The consumers, who have consumed similar products, gave more promising response to the product than the consumers who have not consumed similar products. The main reason for the consumer buying the product is convenience, as the product was easy to store and serve. Convenience products are found in more variety in large cities such as Jakarta and Semarang, providing more choice and product competitiveness. This may have affected the buying trend of the consumer to the developed product. The prospective consumers were found more in small cities (Tangerang, Sragen and Lumajang) than in large cities (Jakarta and Semarang). All information on the present label has to be retained. Since the majority of the Indonesian population is Moslem, it suggest that the words "100% guaranteed halal" be added, indication that no materials or ingredients used to produce the product were classified "not-halal". Some respondents commented that they did not intend to buy the product because canned fish was normally expensive. They preferred to consume fresh fish which is relatively cheap and easy to purchase. Actually, these respondent liked the developed product.

The use of sensory evaluation during the development process of this product in the laboratory seemed very useful. This is reflected in the results of consumer testing. The developed canned fish product processed using condition and formula selected through the sensory evaluation is accepted by most consumers and this revealed the usefulness of sensory evaluation to bridge laboratory trials and consumer desirability.

A majority of the surveyed gave strong support for the production of this product, as approximately 90% of the medical doctors were willing to advise their patients to consume the product for health reasons. The superiority of the product, according to the medical point-of-view, over the existing products in the market, was significant. The role of the medical doctor to introduce this product to prospective consumers (patient), is obvious and strengthens the claim that the product has health benefits.

12.6. CONCLUSIONS

Consumer product testing and the medical doctor survey show that the canned fish enriched with disguised fish oil has good prospects in the Indonesian market. Most of the consumers participating in the testing liked the product and most of them intend to buy. The role of the medical doctor is important in the introduction of health benefits of this product. Most prospective consumers suggested retention of the superiority claimed by this product, as stated on the label. The result of consumer testing gave information about the advantages of laboratory sensory evaluation to develop an acceptable product to the consumer.

Chapter 13

GENERAL DISCUSSION AND CONCLUSION

13.1. INTRODUCTION

Fish oil is a by-product of fish meal and canned fish production and has not received the proper attention during production and utilization by most Indonesian producers. At present, fish oil is mainly for non-food uses such as animal feed and leather tanning. No attempts have been made to use fish oil for human consumption. In terms of quality, most of the fish oil did not meet the requirements for human consumption, especially the undesirable odour which restricts use.

Quality improvement was necessary, particularly for reduction of the undesirable odour. Refining, which has been widely employed for vegetable oils, could be used to remove undesirable compounds in fish oil. This generated the idea for this study. The resin refining method developed by Fernandez (1986) was used in this study, and has been proved to give a high retention of polyunsaturated fatty acid, especially ω-3 fatty acid, since no heat is involved during the refining process.

However as also shown in the refining process of other oils using other refining methods, not only undesirable compounds were reduced, but also natural antioxidant. This would affect the stability of the fish oil during processing and storage.

13.2. FISH OIL REFINING

Only a minority of Indonesian fish oil producers provided the facility of fish oil refinement. All used the alkali refining method involving heat processing, which could affect the stability of the oil. This fact indicates that the prospect of the introduction of the resin refining process in Indonesia was promising. Most of the fish oil producers indicated their intention to adopt the technology.

Some aspects of the resin refining method have to be considered before its adoption. Quality of

the fish oil should be known before refining, especially in terms of odour. This study suggests that fish oil having a very strong undesirable odour must be passed through the refining process more than once, to reduce odour strength. The multiple refining did not only affect the odour of fish oil, but also the free fatty acid, colour and refractive index values. Multiple refining did not significantly affect fatty acid profiles. This indicated the superiority of the method.

In order to obtain the refined oil with optimum quality, the ratio of fish oil to resin volume had to be considered. The fish oil-resin volume ratio of 1:1 was recommended. A higher fish oil ratio will result in a lower refined fish oil quality. This showed that there is a limited capacity for the resin to retain the undesirable compounds of fish oil. When the resin has achieved its maximum capacity to retain undesirable compounds, the remaining compounds may pass through the column together with refined oil resulting in a lower oil quality. High column size tended to produce better oil quality, allowing more opportunity for the oil to contact with the resin. However the contact time did not affect the refined fish oil quality. This is shown in the experiment using vacuum pressure to accelerate the refining time, where the vacuum pressure did not influence the quality obtained.

Most of the Indonesian fish oil producers are located in an area near a beach. In constructing the refining unit, this has to be taken in account in the selection of materials used for the column. The construction materials must be anticorrosive and available locally, for easy purchase and maintenance. Automation would make the method more efficient. All of these requirements have to be considered to guarantee the success of the introduction of the resin refining method to Indonesian fish oil producers.

The resin refining process promoted toluene as the main flavour compound in the refined fish oil, making the odour and taste of the oil more acceptable. The odour was the most important factor in fish oil consumption, as shown in the survey conducted by Fernandez (1986). The Indonesian panellists, in this study, commented that the odour of the fish oil was easier to evaluate than the taste, but the panel ratings showed that the odour score was usually similar to the taste score.

13.3. FISH OIL STABILITY

Study in fish oil stability is very complex, since stability is affected by many factors, as shown in this study. Factors which may affect fish oil stability are fish oil type, refining treatment, storage

temperature, antioxidant addition, packaging type, and processing treatment.

Different fish oils had a different stability, as shown in Chapter 8. The level of natural antioxidant present in the oil may affect stability. The oil obtained from the fish meal processing was more stable than the oil collected from the fish canning operation, and the level of natural antioxidant, especially tocopherols in fish meal oil, was higher than in canning waste oil. Refined fish oil also has less stability when compared to the unrefined oil. The tocopherols content in refined oil was significantly lower than in the unrefined oil.

As storage conditions affected fish oil stability, storage condition manipulation seems to be a very effective way of improving stability. This study shows that the fish oil deterioration rate was the function of storage temperature. Chemical, physical and organoleptic deterioration could be significantly inhibited by storing the oil at low temperature. Oxidation is suspected as the main cause in fish oil deterioration, since the fish oil is rich with unsaturated fatty acid, especially polyunsaturated fatty acids. Oxidation occurring on the unsaturated fatty acid is shown in Figure 13.1. Oxidation could be inhibited by limiting oxygen levels in the container. In this study, the oxygen present in the package used to store fish oil was limited by using vacuum package. This method showed a significant effect in reducing oxidation rate.

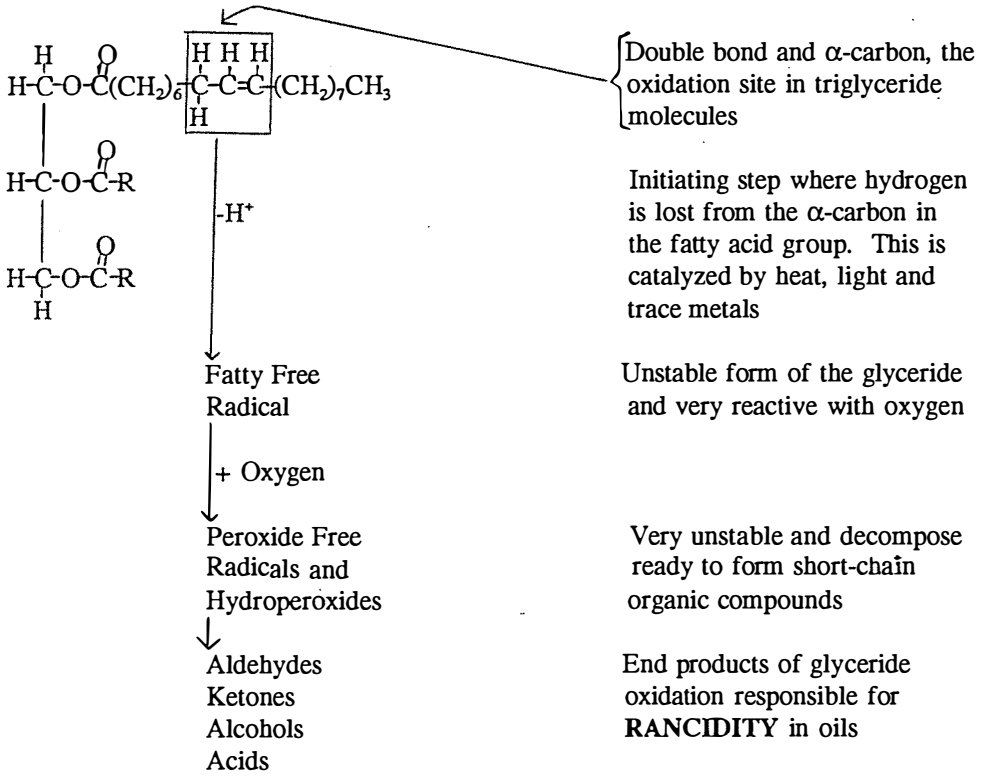


Figure 13.1. Oxidation of glyceride leading to rancidity of oil (Sherwin, 1990)

The sterilization process had an unusual effect on the fish oil and the changes occurring looked very complicated. High temperature may have accelerated all oxidation processes in the oil. Hydroperoxides might undergo a destruction process through scission and dismutation reaction resulting in carbonyl compounds, hydroxy compounds, short chain fatty acid, dimers and polymers including compounds known as secondary products of oxidation. The conversion of hydroperoxides into the secondary products of oxidation was shown by the increase of anisidine value measuring α/β-aldehyde formed during sterilization process. TBA value measuring malonaldehyde formation, which is also a secondary product of oxidation, decreased due to sterilization. This indicated that malonaldehyde was unstable when heated. The malonaldehyde formation mechanism is shown in Figure 13.2. However the sterilization process resulted in the increase in TBA value of the oil extracted from the tomato sauce medium. Tomato sauce may

have inhibited decomposition process of malonaldehyde.

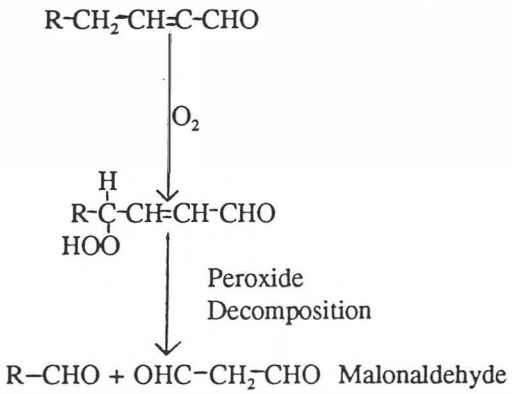


Figure 13.2. Mechanism of malonaldehyde formation (Erickson and Bowers, 1976)

Pigments found in fish oil are mostly caronetoids. Decomposition of carotenoids results in decolouration and this is possibly one factor involving in determining the colour of fish oil.

A darkening process may also occur in fish oil. This may be due to the reaction between protein with hydroperoxides and their degradation products producing browning process (Belitz and Grosch, 1987). The rate of both processes influences the colour of fish oil. If the former process is faster, the fish oil colour will be lighter. However if the later process is faster, the fish oil colour will be darker. Most of the oil studied for storage stability showed a lighter colour at the end of observation. In the study using canning waste oil, the oil colour showed a different pattern of colour changes, where the colour became darker at first, and then lighter during further storage. Sterilization resulted in a significantly different direction of colour change. The sterilized oil exhibited a darker colour than the unsterilized oil. This indicated that the browning reaction was more important than decomposition of carotenoids.

13.4. DEVELOPMENT OF CANNED FISH ENRICHED WITH FISH OIL

Canned fish was selected as a mean of delivering fish oil to the consumer, since the product showed the possibility of incorporating a high amount of fish oil without significantly affecting product acceptability. According to the survey results, this idea was well accepted by both potential consumers and medical doctors. Since the proposed product was market oriented, the medium used to disguise the fish oil had to be tested. A consumer survey endorsed the use of tomato sauce.

The laboratory sequences during development of this product can be seen in Figure 13.3, while the experimental designs used for each step are shown in table 13.1.

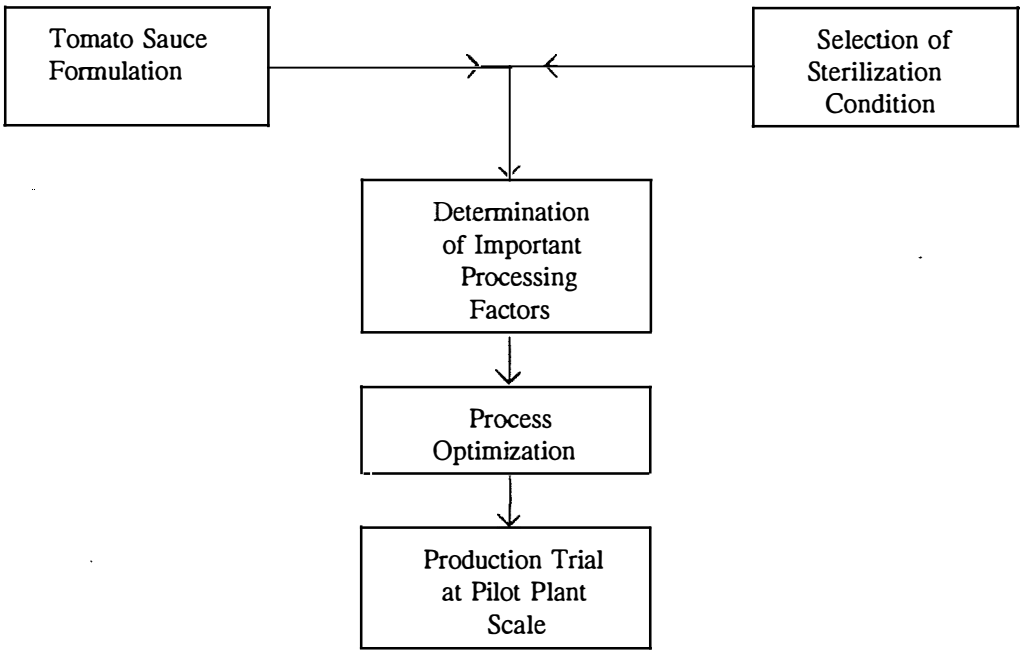


Figure 13.3. Experimental stages used to develop the canned fish with fish oil disguised in it

Table 13.1. Experimental design used for each experimental stage

Experimental Stage	Experimental Design
Tomato sauce formulation	Mixture design
Selection of sterilization condition	Factorial design
Determination of important factors in canning process	Plackett and Burman design
Process optimization	Factorial design

As the fish oil was to be incorporated into canned fish through a medium, the medium received the first attention with experiments on a tomato sauce formulation. A mixture-design was used to develop the formula. This design was very helpful giving directions of change in the levels of ingredients in the medium, and made the formulation experiment efficient. The panellists had no negative comments about the fish oil addition to the sauce during formulation sensory testing.

Since the fish oil addition was to improve nutritional value, its stability during sterilization, which employs a high temperature, had to be investigated to determine the sterilization condition which would give optimum prevention from deterioration. This study indicated that to reduce the risk of quality deterioration, the fish oil had to be sterilized at high temperatures for a short period of time with vacuum heat space in the can. Factorial experiment design was used in this study.

The next development step was the determination of important factors affecting product acceptability. The Plackett and Burman experimental design was used to select the important factors. This indicated that the Plackett and Burman design was a very effective tool for selecting the important factors over the factorial experimental design, since working with many factors, such as eight variables, was complex. According to the results, the variables considered important are pre-cooking, vacuum head space of can, garlic, shallot, and vinegar additions. These treatments must be retained in the processing. Sterilization time was optimized to obtain optimum acceptability of the product.

The selected variables obtained from the Plackett and Burman experiment underwent optimization

using the factorial experimental design. This design was used to select the optimum sterilization time and salt level, because only two variables were involved. T-test was used to reveal the significant difference among tested levels of each variable. The optimum levels were easily decided: optimum sterilization time and salt level in tomato sauce medium were 50 minutes and 1.5% respectively.

The final step in the development of the proposed canned fish was a production trial using a pilot plant scale at full capacity. The product was processed following the process flow as shown in Figure 13.4. The results showed that the changes in the product during processing were similar to those occurring in the optimization experiment. Since the product obtained from the production trial was to be distributed for Indonesian consumer testing, a safety assessment was undertaken. The results indicated that microbiologically the product was safe for consumption, as no microorganisms grew on nutrient agar plates during sterility testing. The incubation test revealed that no changes were found in the appearance of the can.

The above results show that the process flow developed from this study could be recommended for processing canned sardine enriched with fish oil disguised in tomato sauce. However if the technology is going to be applied to Indonesian sardine (Sardinella longiceps), which is commonly processed into canned fish in Indonesia, some changes in the process (e.g. sterilization time) will be required to obtain an acceptable product.

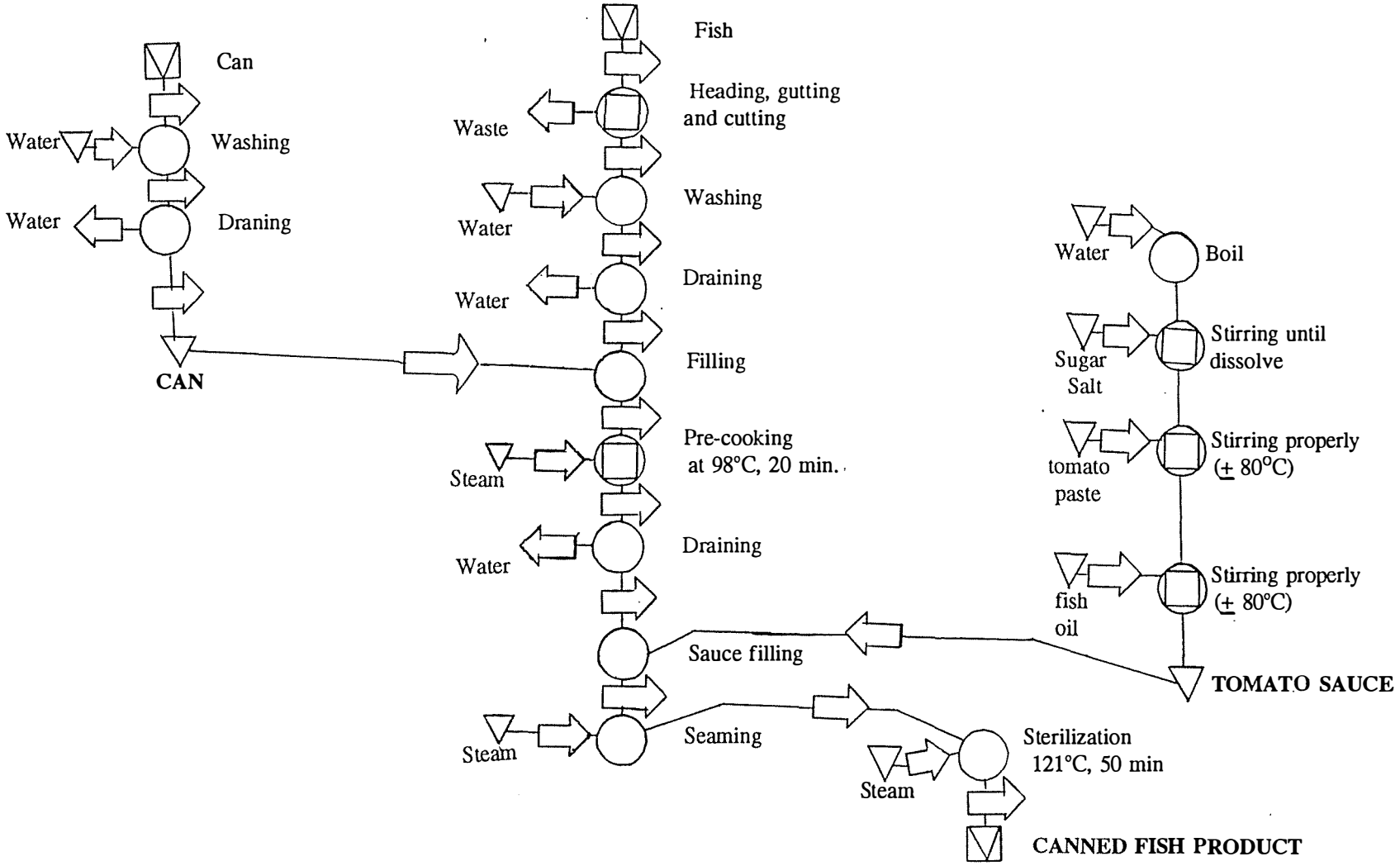


Figure 13.4. Process flow of canned fish with fish oil disguised in it

13.5. PROSPECT OF DEVELOPED CANNED FISH IN INDONESIAN MARKET

Incorporating fish oil disguised in tomato sauce in canned fish was aimed at developing a product which was nutritionally better than existing products. The fish oil addition aims to increase the ω-3 fatty acids level, as fish oil contains a high quantity of these acids. The results of the production trial showed that the fish oil enrichment increased the total fat content of canned sardine and consequently the ω-3 fatty acids level increased as well.

Consumer testing of the product in Indonesia revealed that only a minority of respondents said that they did not like the product. The majority, who can be classified as potential consumers, responded to the product with "neither like nor dislike", "like slightly" and "like very much". Consumer testing indicated that the majority were willing to buy the product. Product acceptability, by a consumer, significantly influences buying intention, where the consumer, from the group showing a higher acceptable level to the product, indicated a higher percentage of consumer intention to buy the product. If the product was released in the market, more effort is then required to convert consumers commenting "neither like nor dislike" and "like slightly" to "like very much", by influencing them using advertisements to promote the nutritional advantages of the product. Sloan (1987) has warned that food companies cannot afford to assume that consumers know the nutritional advantages of their product. Manufactures must clearly describe these nutritional values to consumers. Advertisements should convey specific nutritional information, which goes beyond the basics, to educate consumers. By giving consumers specific additional information, advertisements will engage consumer interest and make the product memorable. Thus, the health benefits of fish oil, particularly ω-3 fatty acids have to be mentioned clearly in promotional material, as well as on the label.

Another way to convey the health benefits of the product is through the help of medical doctors. The prospects for this approach are shown in the medical doctor survey. More than 90% of medical doctors surveyed were ready to advise their patients to consume the product for health reasons. This means that medical doctors provide another outlet access for the product. Most of the doctors thought that this product had good prospects in the Indonesian market in terms of medicinal value.

The consumer testing also shows that the experience of the consumer affects the buying trend. Before releasing the product to the market, information about the locations where high consumption of canned fish, especially canned sardine with tomato sauce medium occurs, has to be obtained. To assure success in marketing the product, the selling can be centralized to those locations first,

before other markets are developed. Most of the consumers said that the reason for buying the product was convenience. According to Kinsey (1992) rising incomes and the high value placed on time have driven consumers to buy convenience foods. This convenience reason, and the improving economic situation of Indonesians, would provide better opportunity for the developed product to be commercially successful.

13.6. ROLE OF SENSORY EVALUATION IN PROCESS AND PRODUCT DEVELOPMENT

Intensive sensory evaluation was used, as sensory characteristics can be the most critical elements in product success. If it does not taste "good" to consumers, the product probably will not be successful in the market place (Mook, 1984). The basic concern in all sensory testing is product acceptance (Ellis, 1970). This indicates the importance of sensory evaluation during product development.

Fernandez (1986) demonstrated the usefulness of sensory evaluation to select resin refining as the most appropriate method for refining fish oil. In this present study, sensory evaluation was used in optimization experiments for the refining process, storage, and sterilization. Descriptive analysis was undertaken using Indonesian trained panellists. The effect of the resin refining treatment can be shown quickly and clearly. Panellists showed that odour difference in fish oil was easier to detect than taste. The sensory evaluation for fish oil recommended only odour. This indicates that if the odour of fish oil is unacceptable, the panellists would hesitate and even rejected taste evaluation. Sensory evaluation can also be used to detect the changes in fish oil during storage and sterilization. In the storage study, the sensory evaluation results can be used to predict shelf life. A method of utilizing sensory evaluation to determine product shelf life has been reviewed by Dethmers (1979).

Sensory evaluation was used in all aspects of this study, as product development is a time-consuming, costly, and risky endeavour. It has been shown how laboratory sensory testing can reduce the lead time for introduction of the product to the market (Moskowitz and Rabino, 1983). Dethmers et al (1981) stressed that product developers need information on the sensory quality and relative acceptability of experimental prototype samples, as an input for marketability.

Various sensory testing methods were used during sensory evaluation. A nine-scale hedonic test

was used in the tomato sauce formulation. This test was very helpful in showing the acceptable level of ingredients. Descriptor-scales of descriptive testing were used in the experiment on the determination of the sterilization condition. The ideal ratio scoring method, which is basically a line-scale of descriptive testing, was used in the experiment on the determination of important factors of the fish canning process. However this method was not suitable for Indonesian panellists and they commented that the method was confusing and also required intensive supervision. Actually, the method has been used successfully in the Nham development for Thai market by Wiriyacharee (1990). In the canning process optimization experiment, two methods were used together: descriptor-scale of descriptive testing to evaluate product characteristics, and a nine-scale hedonic test to evaluate the acceptability of attributes and product. The methods seemed to be very convenience for the Indonesian panellists.

Trained sensory panellists, as employed in this study, can be useful during development of the consumer product testing questionnaire. They can identify and describe sensory characteristics applicable to the test product and generate meaningful terminology which can be utilized in the test questionnaire (Erhardt, 1978). Furthermore, the sensory method applied in the canning process optimization experiment considered suitable for Indonesians was chosen to generate a questionnaire for consumer testing of the product.

The "Just right" method, which is basically a descriptor-scale of descriptive testing was used in the consumer test. This method seemed very acceptable to the respondent. The home use testing method was used for this evaluation. Home use testing is the most natural testing situation, as the information obtained by home use testing reflects: the products assessment during an intensive situation, rather than a short taste-test situation; the product tasted without the standard controls; other influences, such as temperature and additional foods can be varied according to taste (Moskowitz and Chandler, 1979).

Sensory tests can provide data to confirm that changes in the product have been made in the direction indicated by consumer testing (Erhardt, 1978). This study revealed that the product most acceptable during laboratory sensory panel testing was also accepted by consumers participating in product testing.

13.7 THE ROLE OF THE CONSUMER IN PRODUCT DEVELOPMENT

In this study, the consumer was involved in two parts of the product development sequence: during the generation of the product type and product testing. Consumer participation is very important for market orientated product development.

To reveal whether the product idea generated by the product developer was interesting or not to the consumer, the consumer was asked for comments. Most of the prospective consumers were interested in the product idea. Furthermore, the involvement of the consumer during product design was aimed at obtaining the right product type to meet consumer wants. In terms of product type, the consumer was asked to contribute ideas about fish species to be canned, medium type, and can size. By using this information, the product development process could be started. During laboratory activities, the role of the consumer was replaced by a group of panellists who participated in the sensory evaluation of the product.

Further consumer participation was in the product testing involving more people than laboratory sensory evaluation and consumer survey. In this study, consumers participating in a consumer survey, laboratory sensory evaluation and consumer product testing was 130, 10 and 432 people respectively. According to Anderson (1981), consumer panels are an extremely important part of product development and marketing. They can provide invaluable information about consumer attitudes, which are a responses to all aspects of the marketing mix, and not simply to product formulation. In designing new products, consumer attitudes towards the total product image, created by the physical product, packaging, promotion, price and distribution, must be taken in account.

The survey on product marketability also involved the prospective consumer. Here, the consumer indicates information about buying trend, product price, buying motivation and market place. Using this information the product developer can arrange a marketing strategy.

13.8. RECOMMENDED FUTURE STUDIES

More studies relating the importance of fish oil to consumer health and well being, are still required. These includes:

* Investigation of the basic chemistry and physical process of resin action in the improvement of fish oil quality. So far, this information is unavailable.

* Construction of the resin refining unit, especially towards automation.

* The use of Indonesian sardine (Sardinella longiceps) for production of canned fish enriched with fish oil. Indonesian sardine contains up to 25% of fat which is significantly higher than New Zealand sardine.

* The use of fish oil in other Indonesian food types, such as fish sauce, sausage, mayonnaise and salad oil.

13.8. GENERAL CONCLUSION

Most Indonesian fish oils, especially those obtained from fish meal processing, definitely require the refining process to improve quality and acceptability. This study, using both Indonesian and New Zealand fish oils, proved that macroporous strong acid cation resin refinement could significantly improve fish oil quality and acceptability.

The refining process affected the fish oil stability due to the loss of quantities of natural antioxidant, particularly tocopherols. Fish oil stability could be improved by using low storage temperature, antioxidant addition and vacuum package. High temperature and short sterilization time could reduce the deterioration of fish oil during the process and this should be applied in the processing of canned fish and other products containing high amounts of fish oil.

The incorporation of fish oil into canned fish was organoleptically accepted by panellists during laboratory sensory evaluation and by the consumer during product testing. The prospect of the

product in the market is promising, as the majority of surveyed consumers were willing to buy the product. Medical doctors showed a positive response to the product, indicating their willingness to advise their patients to consume the canned fish for health reasons.

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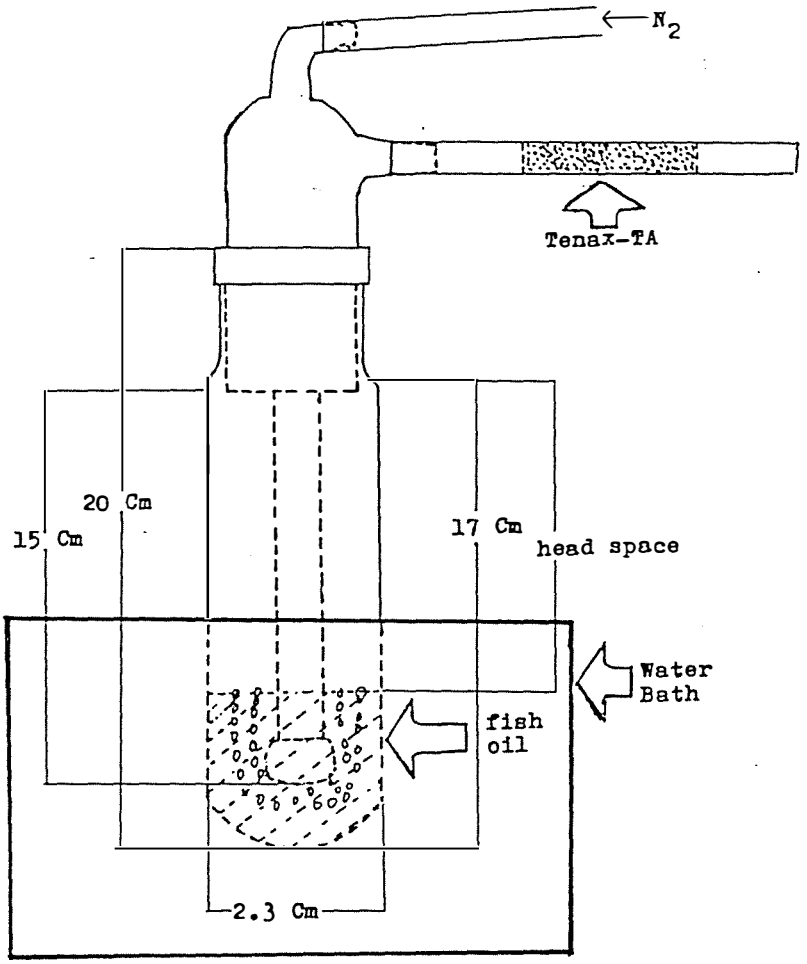
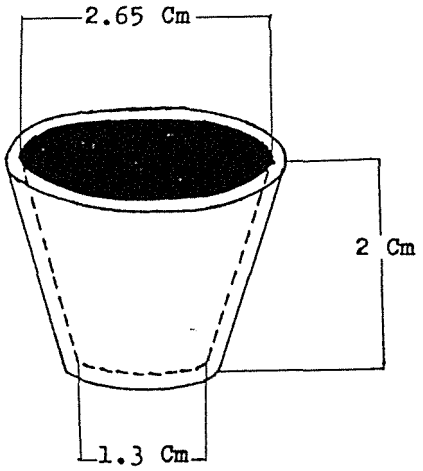
APPENDICES

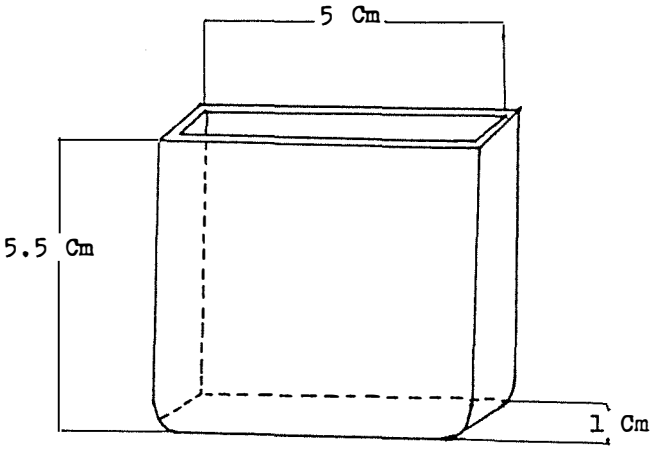
Appendix 3.1. Purging system for collection of volatile flavour compounds of fish oil



Appendix 3.2. Container used for colour analysis of fish flesh and tomato sauce



A. Container for fish flesh



B. Glass cuvette for tomato sauce

Appendix 4.1. Questionnaire used for fish meal factory survey

FISH MEAL FACTORY SURVEY

1. What is the position of fish meal production in your company?
   ( ) main product
   ( ) main product together with other main products
   ( ) by-product

2. Raw materials used for fish meal production in your factory (your answer can be more than one):
   ( ) one species: ( ) sardine
   ( ) mackerel
   ( ) scads
   ( ) others, ...
   ( ) mixture of fish species, mainly.....
   ( ) fish wastes: ( ) canning waste, mainly .....
   ( ) industry ....., mainly .....
   ( ) others, ...

3. In terms of the above raw materials, please specify the percentage of each raw material used in your factory.
   ....% one species: .... sardine
   .... mackerel
   .... scads
   .... others, ...
   ....% mixture of fish species
   ....% fish waste: .... canning waste
   .... industry .....
   ....% others, ...

4. What is the method used for fish meal production in your factory?
   ( ) wet rendering method
   ( ) dry rendering method
   ( ) other methods, please specify.....

5. Do you separate the oil from press liquor?
   ( ) Yes
   ( ) No
   If "YES", do you separate oil from all types of raw material?
   ( ) Yes
   ( ) No
   If "NO", what are raw materials which you do not separate their oils?
   Please specify,.....

6. Do you refine the oil to make it consumable for human?
   ( ) Yes
   ( ) No
   If "YES", what is the method being used to refine fish oil in your factory?
   ( ) alkali refine method
   ( ) molecular distillation

( ) thawing fractionation
( ) others,.....

==========

Additional Information:

Factory : .....
Location : .....
Fish oil packaging : .....
Fish oil selling price : .....
Fish oil buyers : ( ) food companies
( ) pharmaceutical company
( ) fish oil traders
( ) exporter
( ) others, ...

Raw material price: - sardine Rp.....
- mackerel Rp.....
- scad Rp.....
- mixture of fish Rp.....
- fish waste Rp.....

Appendix 4.2. Sensory evaluation sheet for Indonesian fish oil

SENSORY EVALUATION OF INDONESIAN FISH OIL

Name: .....

Date:.....

Instruction:

There are some samples in front of you and you are asked to evaluate their odour. Score the samples using the following scale:

Score	Descriptions
1	none fishy/undesirable odour
2	
3	slightly fishy/undesirable odour
4	
5	moderately fishy/undesirable odour
6	
7	strong fishy/undesirable odour
8	
9	extremely strong fishy/undesirable odour

Sample Code	Odour Score
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....
.....	.....

Appendix 4.3. Factories participating in the survey

Fish Meal Factories

1. C.V. Jaya Baru
2. P.T. Sinar Tratas
3. P.T. Avila Prima
4. Bpk. Salim
5. C.V. Bali Indah
6. P.T. Sumber Rejeki
7. P.T. Bali Maya Permai
8. P.T. Maya Muncar
9. C.V. Samudra Raya
10. P.T. Indo Bali
11. C.V. Harapan Lancar
12. P.T. Pengambengan Raya
13. P.T. Sinar Laut Jaya
14. P.T. Karya Manunggal Prima Sukses
15. N.V. Muncar
16. P.T. Sumber Yala Samudra
17. P.T. Sumino
18. P.T. Bali Raya
19. P.T. Blambangan Raya

Appendix 4.4. Fatty acid profiles of Indonesian Fish oil

Fatty Acid Profiles	Factory/Fish Oil Type/Quality									
	A/FM/-	B/FM/-	C/FM/-	D/FM/-	E/FM/-	F/FM/-	G/CW/-	H/FM/I	H/FM/II	H/FM/III
14:0	12.6	13.0	13.5	12.4	9.4	13.0	13.2	13.1	13.8	13.8
15:0	0.6	0.8	0.8	0.8	1.3	0.8	0.8	0.8	0.8	0.9
16:0	19.6	22.1	21.9	21.3	22.4	20.2	21.2	22.3	21.5	21.8
16:1	14.8	14.3	15.5	15.9	10.4	18.0	14.9	14.1	13.8	16.6
17:0	0.6	0.8	0.9	0.8	1.7	0.8	0.8	1.0	0.9	1.0
17:1	4.0	4.0	4.1	4.4	3.4	4.8	4.1	4.0	3.8	4.5
18:0	3.2	3.8	3.7	3.5	5.75	3.4	3.5	3.8	4.1	3.6
18:1	7.3	8.0	7.9	7.9	14.2	8.0	8.2	8.2	8.1	7.8
18:2	1.1	1.2	1.3	1.1	1.3	1.2	1.3	1.3	1.3	1.2
18:3	1.1	1.2	1.3	1.1	1.2	1.1	1.3	1.3	1.3	1.1
18:4	1.9	2.0	2.1	1.7	1.2	1.9	2.2	2.0	1.8	1.7
20:1	0.9	0.6	0.6	1.0	1.3	0.8	0.7	0.7	1.1	1.4
20:3	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
20:4	2.7	2.3	2.2	2.3	2.1	2.5	2.3	2.3	2.2	2.1
20:5	20.0	17.1	17.1	16.4	9.2	17.3	17.6	16.0	15.2	13.9
22:1	2.1	1.4	0.9	2.0	1.5	1.6	0.9	1.5	2.1	2.3
22:4	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1
22:5	1.2	1.1	0.9	1.3	0.8	0.9	1.0	1.1	1.4	1.1
22:6	5.8	6.1	5.0	5.7	12.5	3.5	5.8	6.3	6.4	5.0

Note: FM = Fish meal
CW = Canning waste
FWM = Fish waste meal

Continuation appendix 4.4

Fatty Acid Profiles	Factory/Fish Oil Type/Quality									
	H/CW/-	I/FM/-	I/CW/-	J/FM/-	K/FM/-	L/FM/ref	M/FM/A	M/FM/B	M/CW/-	N/FWM/-
14:0	11.5	13.3	12.8	11.6	12.5	12.4	8.8	10.9	11.8	12.8
15:0	0.9	0.7	0.8	0.8	0.9	0.9	0.9	1.0	0.8	0.8
16:0	21.3	20.7	21.3	20.7	21.1	21.4	21.6	21.0	22.9	21.3
16:1	15.3	15.6	14.8	16.4	16.3	14.6	11.7	14.3	15.8	14.8
17:0	1.0	0.7	0.9	0.8	0.9	1.0	1.3	1.1	0.9	0.9
17:1	4.4	4.3	4.1	4.5	3.2	4.0	3.7	4.5	4.4	4.1
18:0	3.9	3.4	3.7	3.8	4.6	4.0	5.0	4.3	3.5	3.7
18:1	8.7	7.2	8.2	8.1	11.5	8.4	12.0	9.6	8.1	8.2
18:2	1.4	1.2	1.3	1.3	1.2	1.4	1.3	1.4	1.3	1.3
18:3	1.4	1.2	1.3	1.2	1.1	1.4	1.2	1.3	1.2	1.3
18:4	2.3	2.0	2.1	2.1	1.7	2.0	1.6	2.0	1.9	2.1
20:1	0.8	0.7	0.8	0.8	1.4	0.8	1.0	0.7	1.5	0.8
20:3	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1
20:4	2.3	2.4	2.3	2.5	2.2	2.3	2.3	2.3	2.1	2.3
20:5	16.7	18.6	17.2	17.4	11.5	16.6	12.8	16.1	15.4	17.2
22:1	1.0	1.4	1.2	1.5	2.6	1.5	1.2	0.8	2.3	1.2
22:4	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1
22:5	1.0	1.0	1.1	1.1	1.7	1.1	1.0	1.0	0.9	1.1
22:6	5.9	5.3	5.8	5.2	5.3	6.1	12.2	7.4	4.9	5.8

Note: FM = Fish meal
CW = Canning waste
FWM = Fish waste meal

Continuation of appendix 4.4

Fatty Acid Profiles	Factory/Fish Oil Type/Quality			
	O/CW/I	O/FWM/II	P/FWM/-	Q/FWM/-
14:0	13.0	14.5	3.9	11.1
15:0	0.7	0.7	1.4	1.0
16:0	21.6	21.5	23.8	21.1
16:1	14.5	13.6	5.4	14.6
17:0	0.8	0.9	2.3	1.1
17:1	4.1	3.6	2.5	4.6
18:0	3.6	3.7	7.2	4.1
18:1	8.3	8.1	18.4	9.1
18:2	1.3	1.2	1.5	1.5
18:3	1.2	2.2	1.2	1.4
18:4	2.1	1.8	0.9	2.1
20:1	0.7	1.4	0.8	0.9
20:3	0.1	0.1	0.2	0.1
20:4	2.3	2.3	1.9	2.4
20:5	17.5	15.2	5.0	16.0
22:1	0.9	2.2	0.3	1.3
22:4	0.1	0.6	0.2	0.1
22:5	1.3	1.2	0.8	1.3
22:6	6.0	6.4	22.2	6.0

Note: FM = Fish meal
CW = Canning waste
FWM = Fish waste meal

Appendix 5.1. Results of chemical, physical and organoleptic analysis of fish oil as the effects of fish oil and resin ratio

Analysis : **Free fatty acid value - Crude oil (% oleic acid)**

Fish oil : Resin	Fraction -1	Fraction-2
Untreated fish oil	0.66	0.66
1 : 1	0.43	1.12
2 : 1	0.42	1.72
3 : 1	0.43	2.30
4 : 1	0.43	2.66

Analysis : **Free fatty acid value - Orange roughy oil (% oleic acid)**

Fish oil : Resin	Fraction -1	Fraction-2
Untreated fish oil	0.40	0.40
1 : 1	0.25	0.94
2 : 1	0.26	1.22
3 : 1	0.27	1.79
4 : 1	0.25	2.34

Analysis : **Refractive Index value - Crude oil (at 20°C)**

Fish oil : Resin	Fraction -1	Fraction-2
Untreated fish oil	1.4673	1.4673
1 : 1	1.4667	1.4710
2 : 1	1.4689	1.4715
3 : 1	1.4685	1.4720
4 : 1	1.4700	1.4728

Analysis : **Refractive Index value - Orange roughy oil (at 20°C)**

Fish oil : Resin	Fraction -1	Fraction-2
Untreated fish oil	1.4652	1.4652
1 : 1	1.4586	1.4641
2 : 1	1.4619	1.4632
3 : 1	1.4620	1.4631
4 : 1	1.4630	1.4620

Analysis : **Sensory analysis for odour - Crude oil**

Fish oil : Resin	Fraction -1	Fraction-2
Untreated fish oil	6.38	6.38
1 : 1	2.78	5.10
2 : 1	4.06	6.17
3 : 1	4.14	7.07
4 : 1	4.17	7.22

Analysis : **Sensory analysis for taste - Crude oil**

Fish oil : Resin	Fraction -1	Fraction-2
Untreated fish oil	5.88	5.88
1 : 1	2.88	4.19
2 : 1	3.22	6.28
3 : 1	3.64	5.98
4 : 1	3.79	6.50

Analysis : **Sensory analysis for odour - Orange roughy oil**

Fish oil : Resin	Fraction -1	Fraction-2
Untreated fish oil	4.06	4.06
1 : 1	3.64	5.79
2 : 1	3.44	6.38
3 : 1	3.71	6.57
4 : 1	3.86	6.86

Analysis : **Sensory analysis for taste - Orange roughy oil**

Fish oil : Resin	Fraction -1	Fraction-2
Untreated fish oil	4.15	4.15
1 : 1	3.19	5.71
2 : 1	3.44	5.50
3 : 1	3.43	6.63
4 : 1	3.57	6.93

Analysis : **Colour absorbance at 490 nm**

Fish oil : resin	Fraction -1 Crude oil	Fraction-2 Orange roughy oil
Untreated fish oil	0.90	1.96
1 : 1	0.81	1.71
2 : 1	0.82	1.74
3 : 1	0.82	1.75
4 : 1	0.83	1.76

Analysis : **Fatty acid profiles - Crude oil**

F.A.	Untreated Fish Oil	Fish oil : Resin							
		1 : 1		2 : 1		3 : 1		4 : 1	
		F-1	F-2	F-1	F-2	F-1	F-2	F-1	F-2
14:0	4.4	4.3	5.3	4.6	5.5	4.4	5.4	4.6	5.4
15:0	0.7	0.6	0.7	0.6	0.7	0.6	0.7	0.6	0.7
16:0	17.2	16.9	17.3	16.7	17.5	16.8	17.3	16.0	17.5
16:1	8.1	7.7	8.3	7.4	8.3	7.5	8.3	7.5	8.3
17:0	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.4	0.3
17:1	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
18:0	2.6	2.6	2.4	2.6	2.4	2.5	2.4	2.6	2.4
18:1	34.2	35.1	33.1	34.0	33.1	34.4	32.9	34.4	32.9
18:2	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
18:3	0.9	1.0	1.1	0.9	0.8	0.9	0.8	0.9	0.8
18:4	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
20:1	10.8	11.7	10.2	11.4	10.2	11.3	10.0	11.5	10.0
20:3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3
20:4	0.6	0.6	0.5	0.6	0.5	0.6	0.7	0.6	0.7
20:5	9.2	4.4	4.4	4.4	4.3	4.4	4.7	4.4	4.7
22:1	4.9	5.0	4.9	5.1	4.8	5.0	5.0	5.2	4.8
22:4	-	-	-	-	-	-	-	-	-
22:5	0.8	0.9	0.9	0.9	0.9	0.8	0.8	0.9	0.9
22:6	5.1	5.3	5.4	5.3	5.6	5.1	5.5	5.3	5.5

Note: F = Fraction

Analysis : **Fatty acid profiles - Orange roughy oil**

F.A.	Untreated Fish Oil	Fish oil : Resin							
		1 : 1		2 : 1		3 : 1		4 : 1	
		F-1	F-2	F-1	F-2	F-1	F-2	F-1	F-2
14:0	1.8	1.8	2.2	1.7	2.2	1.7	2.3	1.7	2.4
15:0	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.2
16:0	3.3	3.5	3.7	3.2	4.0	3.2	4.1	3.2	4.4
16:1	11.4	12.1	13.7	11.9	13.8	11.5	13.9	11.9	13.6
17:0	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.2
17:1	2.5	2.5	2.6	2.5	2.6	2.5	2.6	2.6	2.6
18:0	0.6	0.4	0.4	0.4	0.4	0.5	0.4	0.3	0.4
18:1	52.6	52.9	6.0	53.3	52.0	53.3	51.7	52.1	51.3
18:2	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.5
18:3	1.1	1.1	1.0	1.1	1.0	1.1	0.9	1.1	1.0
18:4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
20:1	14.8	14.7	13.2	14.8	13.6	15.0	12.8	14.6	12.8
20:3	0.3	0.3	0.2	0.2	0.2	0.3	0.2	0.3	0.2
20:4	0.4	0.3	0.4	0.3	0.3	0.4	0.5	0.4	0.5
20:5	1.4	1.4	1.4	1.3	1.4	1.4	1.5	1.5	1.7
22:1	5.7	5.6	5.6	5.8	5.4	5.8	5.4	5.9	5.3
22:4	-	-	-	-	-	-	-	-	-
22:5	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
22:6	1.1	1.0	1.1	1.0	1.2	1.0	1.3	1.0	1.5

Appendix 5.2. Results of chemical, physical and organoleptic analysis of fish oil as the effects of multiple refining using resin packed column

Analysis : **Free fatty acid value (% oleic acid) - Crude oil**

Refining/column	Fraction -1	Fraction-2
Untreated fish oil	0.63	0.63
I	0.48	1.04
II	0.41	0.71
III	0.35	0.62
IV	0.25	0.44

Analysis : **Free fatty acid value (% oleic acid) - Orange roughy oil**

Refining/column	Fraction -1	Fraction-2
Untreated fish oil	0.67	0.67
I	0.47	1.14
II	0.40	0.71
III	0.32	0.63
IV	0.26	0.50

Analysis : **Refractive Index value - Crude oil (at 20°C)**

Refining/column	Fraction -1	Fraction-2
Untreated fish oil	1.4740	1.4740
I	1.4665	1.4718
II	1.4625	1.4739
III	1.4601	1.4739
IV	1.4543	1.4737

Analysis : **Refractive Index value - Orange roughy oil (at 20°C)**

Refining/column	Fraction -1	Fraction-2
Untreated fish oil	1.4659	1.4659
I	1.4593	1.4630
II	1.4555	1.4649
III	1.4550	1.4651
IV	1.4506	1.4660

Analysis : **Sensory evaluation for Odour - Crude oil**

Refining/column	Fraction -1	Fraction-2
Untreated fish oil	5.70	5.70
I	3.19	6.75
II	2.90	6.20
III	2.70	6.00
IV	2.38	4.13

Analysis : **Sensory evaluation for Taste - Crude oil**

Refining/column	Fraction -1	Fraction-2
Untreated fish oil	6.00	6.00
I	3.00	6.94
II	2.90	5.78
III	2.90	5.84
IV	2.50	4.00

Analysis : **Sensory evaluation for Odour - Orange roughy oil**

Refining/column	Fraction -1	Fraction-2
Untreated fish oil	6.74	6.74
I	4.76	7.03
II	4.70	5.85
III	4.53	5.79
IV	4.14	5.64

Analysis : **Sensory evaluation for Taste - Orange roughy oil**

Refining/column	Fraction -1	Fraction-2
Untreated fish oil	5.72	5.72
I	4.60	6.92
II	4.60	5.73
III	4.11	5.51
IV	4.06	5.03

Analysis : **Colour absorbance at 490 nm**

Refining/column	Fraction -1 Crude Oil	Fraction-1 Orange roughy oil
Untreated fish oil	0.49	1.52
I	0.43	1.37
II	0.40	1.29
III	0.40	1.23
IV	0.36	1.13

Analysis: **Fatty acid profiles - Crude oil**

F.A.	Untreated Fish Oil	Refining - Column							
		I		II		III		IV	
		F-1	F-2	F-1	F-2	F-1	F-2	F-1	F-2
14:0	6.2	5.7	5.9	5.8	6.1	5.7	6.1	5.8	6.0
15:0	0.8	0.7	0.8	0.8	0.8	0.7	0.8	0.8	0.6
16:0	18.5	17.7	18.2	18.0	18.3	17.8	18.6	18.0	18.3
16:1	9.0	8.5	8.8	8.6	8.9	8.5	9.0	8.6	8.9
17:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
17:1	2.7	2.5	2.6	2.6	2.7	2.5	2.7	2.5	2.6
18:0	1.5	1.6	2.1	2.2	1.5	2.2	1.7	2.1	2.1
18:1	32.9	32.6	32.3	32.7	33.0	32.8	33.0	32.8	33.1
18:2	1.4	1.4	1.4	1.4	1.5	1.4	1.4	1.4	1.4
18:3	0.6	0.6	0.7	0.6	0.7	0.6	0.6	0.6	0.7
18:4	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.7
20:1	10.5	10.8	10.5	10.6	10.5	10.8	10.4	10.7	10.4
20:3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	03
20:4	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.5
20:5	3.9	4.2	3.9	3.9	3.8	3.9	3.8	3.9	3.8
22:1	4.2	4.7	4.2	4.3	4.2	4.4	4.0	4.4	4.2
22:4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
22:5	0.9	1.0	0.9	1.1	0.9	0.9	0.9	0.9	0.9
22:6	5.0	5.7	5.6	5.3	5.2	5.6	4.9	5.4	5.0

Note: F = Fraction

Analysis : **Fatty acid profiles - Orange roughy oil**

F.A.	Untreated Fish Oil	Refining - Column							
		I		II		III		IV	
		F-1	F-2	F-1	F-2	F-1	F-2	F-1	F-2
14:0	2.3	2.4	2.6	2.4	2.4	2.4	2.4	2.4	2.5
15:0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
16:0	4.8	4.6	5.0	4.7	4.8	4.8	4.7	4.7	4.8
16:1	13.0	12.5	13.2	13.0	13.0	12.8	12.7	12.9	13.1
17:0	-	-	-	-	-	-	-	-	-
17:1	2.3	2.6	2.6	2.7	2.6	2.6	2.6	2.6	2.6
18:0	1.0	1.5	1.5	0.6	0.9	1.3	1.0	1.4	0.6
18:1	52.7	52.3	52.0	52.6	52.0	52.6	51.8	52.2	51.8
18:2	1.5	1.5	1.4	1.4	1.4	1.4	1.5	1.4	1.4
18:3	0.5	0.5	0.5	0.4	0.5	0.4	0.5	0.4	0.5
18:4	0.4	0.4	0.4	0.4	0.4	0.4	0.6	0.4	0.2
20:1	13.4	13.7	13.0	13.7	13.8	12.9	13.7	13.0	13.3
20:3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
20:4	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3
20:5	1.1	1.2	1.1	1.2	1.2	1.3	1.6	1.3	1.8
22:1	4.9	5.1	4.5	4.8	4.8	5.0	4.9	5.2	5.4
22:4	-	-	-	-	-	-	-	-	-
22:5	0.2	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.2
22:6	1.2	1.1	1.2	1.2	1.2	1.2	1.2	1.1	1.1

Note: F = fraction

Appendix 5.3. Results of chemical, physical and organoleptic analysis of fish oil as the effects of vacuum pressure application during resin refining process

Analysis : **Free fatty acid value (% oleic acid)**

Treatment	Crude Oil		Orange roughy oil	
	Frac.-1	Frac.-2	Frac.-1	Frac.-2
Untreated fish oil	0.63	0.63	0.79	0.79
Without vacuum pressure	0.55	0.94	0.66	1.20
With vacuum pressure	0.48	0.76	0.68	0.94

Analysis : **Refractive Index (20°C)**

Treatment	Crude Oil		Orange roughy oil	
	Frac.-1	Frac.-2	Frac.-1	Frac.-2
Untreated fish oil	1.4731	1.4731	1.4651	1.4651
Without vacuum pressure	1.4710	1.4732	1.4631	1.4655
With vacuum pressure	1.4691	1.4735	1.4630	1.4660

Analysis : **Colour absorbance value 490 nm**

Treatment	Crude Oil		Orange roughy oil	
	Frac.-1	Frac.-2	Frac.-1	Frac.-2
Untreated fish oil	0.76	0.76	1.59	1.59
Without vacuum pressure	0.73	0.80	1.48	1.76
With vacuum pressure	0.72	0.80	1.51	1.72

Analysis : **Sensory evaluation for Odour**

Treatment	Crude Oil		Orange roughy oil	
	Frac.-1	Frac.-2	Frac.-1	Frac.-2
Untreated fish oil	6.06	6.06	6.31	6.31
Without vacuum pressure	4.25	5.56	3.63	6.00
With vacuum pressure	4.63	5.44	4.00	4.88

Analysis: **Sensory evaluation for Taste**

Treatment	Crude Oil		Orange roughy oil	
	Frac.-1	Frac.-2	Frac.-1	Frac.-2
Untreated fish oil	5.87	5.87	5.50	5.50
Without vacuum pressure	4.31	5.56	3.75	6.00
With vacuum pressure	3.94	5.00	3.75	5.19

Analysis: **Fatty acid profiles**

Fatty Acids	Untreated Oil		Crude Oil				Orange Roughy Oil			
	Crude Oil	Orange roughy oil	Without Vacuum Pressure		With Vacuum Pressure		Without Vacuum Pressure		With Vacuum Pressure	
			Frac.-1	Frac.-2	Frac.-1	Frac.-2	Frac.-1	Frac.-2	Frac.-1	Frac.-2
14:0	3.5	1.5	3.9	3.9	3.9	3.8	1.5	1.6	1.5	1.6
15:0	0.5	-	0.5	0.5	0.5	0.5	-	-	-	-
16:0	14.9	3.7	15.2	15.2	15.2	15.0	3.7	4.0	3.6	4.0
16:1	7.0	9.9	7.2	7.1	7.1	7.0	10.1	10.0	9.9	10.1
17:0	0.4	0.2	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3
17:1	2.2	2.1	2.0	2.0	2.1	2.1	2.1	2.3	2.1	2.1
18:0	-	0.8	-	-	-	-	0.8	0.9	0.8	0.8
18:1	36.0	51.4	35.9	36.1	35.7	36.5	51.3	50.4	51.6	51.4
18:2	1.8	1.5	1.7	1.6	1.7	1.7	1.4	1.4	1.4	1.4
18:3	1.1	1.3	1.1	0.9	1.1	1.0	1.2	1.1	1.1	1.1
18:4	1.0	0.6	0.9	0.8	0.9	0.9	0.4	0.5	0.4	0.4
20:1	12.0	15.6	11.7	11.8	12.0	11.9	15.8	15.6	15.9	15.5
20:3	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.3
20:4	0.6	0.3	0.6	0.5	0.6	0.6	0.4	0.4	0.4	0.3
20:5	4.6	2.1	4.4	4.4	4.4	4.6	1.9	2.1	1.8	1.8
22:1	5.4	6.5	5.3	5.5	5.1	5.2	6.3	6.3	6.5	6.4
22:4	-	-	-	-	-	-	-	-	-	-
22:5	0.9	0.5	1.1	1.0	1.1	1.0	0.4	0.4	0.4	0.4
22:6	7.7	1.2	7.7	7.7	7.7	7.3	1.9	2.1	1.9	2.1

Appendix 5.4. Results of chemical, physical and organoleptic analysis of fish oil as the effects of various height sizes of resin packed column

Analysis : **Free fatty acid value (% oleic acid)**

Diameter = 2.6 cm

Diameter : height	Fraction-1	Fraction-2
Untreated oil	2.62	2.62
1 : 5	2.20	2.71
1 : 10	2.21	3.21
1 : 15	2.16	3.33
1 : 20	2.07	3.73

Analysis : **Refractive Index value (20°C)**

Diameter = 2.6 cm

Diameter : height	Fraction-1	Fraction-2
Untreated oil	1.4706	1.4706
1 : 5	1.4650	1.4703
1 : 10	1.4645	1.4709
1 : 15	1.4650	1.4710
1 : 20	1.4650	1.4710

Analysis : **Colour absorbance value 490 nm**

Diameter = 2.6 cm

Diameter : height	Fraction-1	Fraction-2
Untreated oil	1.52	1.52
1 : 5	1.36	1.92
1 : 10	1.18	2.36
1 : 15	1.18	2.34
1 : 20	1.15	2.53

Analysis : **Sensory evaluation for Odour**

Diameter = 2.6 cm

Diameter : height	Fraction-1	Fraction-2
Untreated oil	6.72	6.76
1 : 5	4.11	5.04
1 : 10	3.64	5.32
1 : 15	3.82	5.25
1 : 20	3.36	5.39

Analysis : **Sensory evaluation for Taste**

Diameter = 2.6 cm

Diameter : height	Fraction-1	Fraction-2
Untreated oil	6.06	6.06
1 : 5	3.43	4.43
1 : 10	3.36	4.43
1 : 15	3.36	4.40
1 : 20	3.21	4.50

Analysis : **Fatty acid profiles**

Fatty Acids	Untreated Oil	Diameter : height							
		1 : 5		1 : 10		1 : 15		1 : 20	
		Frac.-1	Frac.-2	Frac.-1	Frac.-2	Frac.-1	Frac.-2	Frac.-1	Frac.-2
14:0	5.2	6.3	5.2	5.7	3.5	4.1	3.5	4.5	4.5
15:0	-	-	-	-	-	-	-	-	-
16:0	13.4	14.2	12.8	13.6	11.2	12.2	11.3	12.4	12.4
16:1	8.4	8.9	8.2	8.3	7.0	7.6	7.0	7.9	7.8
17:0	-	-	-	-	-	-	-	-	-
17:1	2.5	2.6	2.3	2.5	2.2	2.4	2.2	2.4	2.3
18:0	2.4	2.3	2.3	2.3	2.4	2.6	2.6	2.6	2.5
18:1	34.0	33.3	34.0	32.6	34.9	34.7	34.9	34.3	34.1
18:2	1.8	1.8	2.0	1.8	1.9	2.1	2.1	1.9	2.0
18:3	0.8	0.8	0.9	0.7	0.8	0.7	1.1	0.9	1.0
18:4	1.6	1.5	1.7	1.5	1.7	12.3	1.8	1.6	1.7
20:1	9.7	9.2	9.5	10.3	10.7	10.5	11.0	10.4	10.3
20:3	-	-	-	-	-	-	-	-	-
20:4	0.8	0.8	0.8	0.8	1.1	0.9	0.9	0.9	1.1
20:5	5.5	5.1	6.3	5.4	6.2	6.1	5.7	5.9	5.9
22:1	4.7	4.3	4.7	4.8	5.6	5.2	5.5	5.2	4.8
22:4	-	-	-	-	-	-	-	-	-
22:5	1.3	1.3	1.5	1.4	1.6	1.4	1.4	1.4	1.4
22:6	7.8	7.6	8.2	8.2	9.3	8.2	8.9	7.8	8.2

Appendix 5.4. Results of chemical, physical and organoleptic analysis of fish oil as the effects of various diameter sizes of resin packed column

Analysis : **Free fatty acid value (% oleic acid)**

Height = 39 cm

Diameter (Cm)	Fraction-1	Fraction-2
Untreated oil	1.82	1.82
1.65	1.53	2.01
2.60	1.44	2.13
3.20	1.53	2.23

Analysis : **Refractive Index Value (20°C)**

Height = 39 cm

Diameter (Cm)	Fraction-1	Fraction-2
Untreated oil	1.4700	1.4700
1.65	1.4670	1.4700
2.60	1.4655	1.4700
3.20	1.4660	1.4700

Analysis : **Colour absorbance value 490 nm**

Height = 39 cm

Diameter (Cm)	Fraction-1	Fraction-2
Untreated oil	0.99	0.99
1.65	0.91	1.07
2.60	0.90	1.13
3.20	0.88	1.18

Analysis : **Sensory analysis for Odour**

Height = 39 cm

Diameter (Cm)	Fraction-1	Fraction-2
Untreated oil	5.43	5.43
1.65	3.71	5.64
2.60	3.57	4.78
3.20	3.57	4.71

Analysis : **Sensory analysis for Taste**

Height = 39 cm

Diameter (Cm)	Fraction-1	Fraction-2
Untreated oil	5.36	5.36
1.65	3.71	6.28
2.60	3.50	5.50
3.20	3.78	6.00

Appendix 6.1. Score sheet used for sensory evaluation during fish oil storage experiment

SENSORY EVALUATION FOR FISH OIL STORAGE

Name : .....

Date : .....

Instruction: Please indicate the score that best reflects your attitude about the product.

Description	Scale
Bland	0
Suspicion of off-odour/taste	1
Noticeable but very slight off-odour/taste	2
More noticeable off-odour/taste	3
Distinct off-odour/taste	4
Disagreeable off-odour/taste, rancid	5
Markedly disagreeable off-odour/taste, very rancid	6

Sample Code	Odour	Taste
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....

Appendix 6.3. Linear relationship between the natural logarithm of rate constant and the reciprocal of absolute temperature for each parameter

Parameter	Fish Oil Sample	Storage Temp.(°C)	1/T(°K) (x 10^3)	lnk	linear equation
Peroxide Value	Refined oil	2	3.64	-0.06	ln k = 9.80 - 2690.7 1/T ; r^2 = 96.38%
		20	3.41	0.76	
		30	3.30	0.93	
		40	3.19	1.14	
	Unrefined oil	2	3.64	-0.17	ln k = 6.22 - 1737 1/T ; r^2 = 90.88%
		20	3.41	0.44	
		30	3.30	0.50	
		40	3.19	0.60	
Colour Absorbance Value (zero order reaction)	Refined oil	2	3.64	-4.60	ln k = 13.35 - 4918 1/T ; r^2 = 99.22%
		20	3.41	-3.30	
		30	3.30	-2.92	
		40	3.19	-2.37	
	Unrefined oil	2	3.64	-4.60	ln k = 7.94 - 3462 1/T ; r^2 = 98.14%
		20	3.41	-3.91	
		30	3.30	-3.58	
		40	3.19	-3.00	
Colour Absorbance Value (first order reaction)	Refined oil	2	3.64	-5.30	ln k = 14.83 - 5514 1/T ; r^2 = 99.01%
		20	3.41	-3.82	
		30	3.30	-3.44	
		40	3.19	-2.78	
	Unrefined oil	2	3.64	-5.81	ln k = 11.34 - 4705 1/T ; r^2 = 99.29%
		20	3.41	-4.60	
		30	3.30	-4.27	
		40	3.19	-3.65	
Odour score from: cold sample	Refined oil	2	3.64	-2.03	ln k = 4.62 - 1821 1/T ; r^2 = 98.73%
		20	3.41	-1.53	
		30	3.30	-1.38	
		40	3.19	-1.22	
	Unrefined oil	2	3.64	-2.35	ln k = 5.15 - 2077 1/T ; r^2 = 95.61%
		20	3.41	-1.99	
		30	3.30	-1.78	
		40	3.19	-1.38	
Odour score from: warm sample	Refined oil	2	3.64	-2.07	ln k = 4.62 - 1841 1/T ; r^2 = 99.77%
		20	3.41	-1.65	
		30	3.30	-1.47	
		40	3.19	-1.23	
	Unrefined oil	2	3.64	-2.54	ln k = 5.67 - 2373 1/T ; r^2 = 95.61%
		20	3.41	-2.17	
		30	3.30	-1.90	
		40	3.19	-1.48	
Taste score from: cold sample	Refined oil	2	3.64	-2.19	ln k = 4.839 - 1.932 1/T ; r^2 = 99.95%
		20	3.41	-1.76	
		30	3.30	-1.54	
		40	3.19	-1.32	
	Unrefined oil	2	3.64	-2.32	ln k = 4.12 - 1793 1/T ; r^2 = 90.38%
		20	3.41	-2.13	
		30	3.30	-1.83	
		40	3.19	-1.49	
Taste score from: warm sample	Refined oil	2	3.64	-2.24	ln k = 3.75 - 1666 1/T ; r^2 = 91.50%
		20	3.41	-2.06	
		30	3.30	-1.77	
		40	3.19	-1.48	
	Unrefined oil	2	3.64	-2.30	ln k = 6.12 - 2325 1/T ; r^2 = 97.77%
		20	3.41	-1.85	
		30	3.30	-1.62	
		40	3.19	-1.22	

Appendix 6.4. Linear relationship between the natural logarithm of shelf life (θ) versus the reciprocal of absolute temperature (°K)

Fish Oil Sample	Storage Temp (°C)	1/T (°K) (x 10^{-3})	ln θ	Linear Equation
Refined oil	2	3.64	3.401	ln θ = -3.36 + 1869.01 1/T ; r^2 = 99.91%
	20	3.41	2.996	
	30	3.30	2.773	
	40	3.19	2.565	
Unrefined oil	2	3.64	3.714	ln θ = - 3.93 + 2102.12 1/T ; r^2 = 99.88%
	20	3.41	3.258	
	30	3.30	2.996	
	40	3.19	2.773	

Appendix 6.5. Results of chemical, physical and organoleptic analysis of refined and unrefined fish oils during storage at various temperature

Analysis: **Peroxide value (meq/kg)**

Sample	Storage Temp. (°C)	Storage time (weeks)					
		0	2	5	10	15	20
Refined Oil	2	3.22	4.62	7.71	15.21	17.24	22.51
	20	3.22	10.40	14.68	21.52	24.97	35.42
	30	3.22	11.77	15.65	25.39	31.02	40.13
	40	3.22	12.64	19.07	29.04	33.91	
Unrefined Oil	2	4.28	5.94	8.80	13.52	15.59	20.53
	20	4.28	10.04	12.40	16.43	19.95	24.89
	30	4.28	10.36	12.80	18.47	20.36	24.66
	40	4.28	10.79	13.04	17.43	18.88	

Analysis: **Refractive Index Value (25°C)**

Sample	Storage Temp. (°C)	Storage Time (weeks)					
		0	2	5	10	15	20
Refined Oil	2	1.4670	1.4670	1.4670	1.4672	1.4672	1.4681
	20	1.4670	1.4671	1.4671	1.4679	1.4680	1.4690
	30	1.4670	1.4670	1.4673	1.4681	1.4682	1.4693
	40	1.4670	1.4675	1.4679	1.4688	1.4692	
Unrefined Oil	2	1.4720	1.4720	1.4720	1.4720	1.4721	1.4722
	20	1.4720	1.4721	1.4721	1.4721	1.4726	1.4729
	30	1.4720	1.4722	1.4722	1.4722	1.4729	1.4731
	40	1.4720	1.4722	1.4725	1.4728	1.4731	

Analysis: **Colour absorbance value 490 nm**

Sample	Storage Temp. (°C)	Storage time (weeks)					
		0	2	5	10	15	20
Refined Oil	2	1.86	1.84	1.80	1.76	1.71	1.68
	20	1.86	1.75	1.69	1.52	1.41	1.17
	30	1.86	1.72	1.60	1.36	1.10	0.83
	40	1.86	1.65	1.39	0.99	0.65	
Unrefined Oil	2	2.04	2.04	1.99	1.96	1.94	1.97
	20	2.04	1.96	1.95	1.90	1.83	1.73
	30	2.04	1.96	1.90	1.83	1.67	1.54
	40	2.04	1.92	1.80	1.63	1.31	

Analysis: **Odour score - Cold sample**

Sample	Storage Temp. (°C)	Storage time (weeks)					
		0	2	5	10	15	20
Refined Oil	2	0.93	1.00	2.14	2.40	2.87	2.92
	20	0.93	0.93	2.57	3.30	3.42	3.50
	30	0.93	1.36	2.54	4.07	4.42	4.42
	40	0.93	1.21	2.79	4.90	5.04	
Unrefined Oil	2	1.29	1.17	2.06	2.10	2.50	3.21
	20	1.29	1.17	2.27	2.90	3.47	3.62
	30	1.29	1.08	2.10	3.65	4.30	4.21
	40	1.29	1.33	2.50	4.90	5.20	

Analysis: **Odour score - Warm sample**

Sample	Storage Temp. (°C)	Storage time (weeks)					
		0	2	5	10	15	20
Refined Oil	2	1.04	1.00	2.14	2.10	2.70	2.33
	20	1.04	1.50	2.50	2.73	3.20	3.33
	30	1.04	1.64	2.29	3.37	4.15	4.08
	40	1.04	1.50	2.82	4.30	4.80	
Unrefined Oil	2	1.57	1.33	2.08	1.90	2.44	3.17
	20	1.57	1.33	2.24	2.70	3.12	3.54
	30	1.57	1.33	2.36	3.43	3.87	4.29
	40	1.57	1.25	2.81	4.35	4.94	

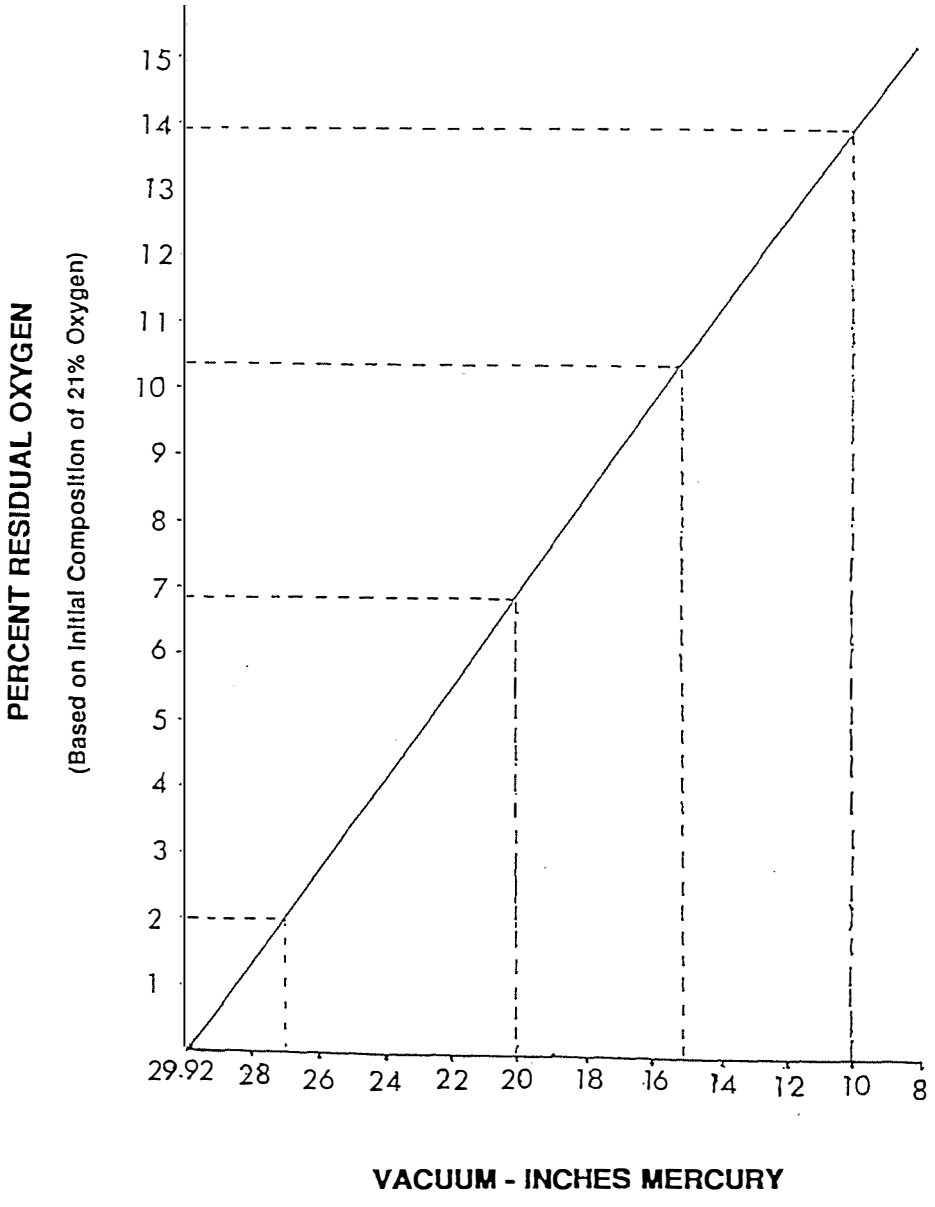
Analysis: **Taste score - Cold sample**

Sample	Storage Temp. (°C)	Storage time (weeks)					
		0	2	5	10	15	20
Refined Oil	2	0.93	0.86	1.86	2.20	2.54	3.00
	20	0.93	1.00	2.57	3.17	3.02	3.50
	30	0.93	1.29	2.36	3.40	3.90	4.17
	40	0.93	1.21	2.50	4.47	4.83	
Unrefined Oil	2	1.14	0.83	1.90	2.08	1.96	3.00
	20	1.14	1.08	2.13	2.55	2.79	3.50
	30	1.14	1.25	2.09	3.47	3.75	4.17
	40	1.14	1.25	2.44	4.55	4.79	

Analysis: **Taste score - Warm sample**

Sample	Storage Temp. (°C)	Storage time (weeks)					
		0	2	5	10	15	20
Refined Oil	2	0.86	0.79	1.71	2.10	2.40	2.75
	20	0.86	0.93	1.79	2.63	2.70	3.17
	30	0.86	1.11	1.89	3.10	3.30	3.50
	40	0.86	1.00	2.39	3.93	4.20	
Unrefined Oil	2	0.71	0.87	1.74	1.82	2.42	2.58
	20	0.71	0.96	1.90	2.40	2.71	3.17
	30	0.71	1.08	1.96	2.98	3.42	3.71
	40	0.71	1.33	2.19	3.88	4.58	

Appendix 7.1. Relationship between degree of vacuum and residual oxygen content (CIG Ltd, 1989)



Appendix 7.2. Permitted antioxidants to be used in Indonesian foods and drinks according to Health Ministry Regulation No.10178/A/SK/74

Foods	Antioxidants	Maximum allowable level (ppm)
Fat, oil and foods containing vitamin A (except foods contain vitamin A more than 100.000IU/gram)	a) propylgallate	100
	octylgallate	100
	dodecylgallate	100
	mixture three of them	100
	b) butylated hydroxyanisol (BHA)	200
	c) mixture a) and b)	100 a) 200 b)
	d) dibutyl hydroxitoluene (BHT)	200
	e) mixture of BHA and BHT	200
	f) nordihydroguaiaretic acid NDGA)	100
	g) α-tocopherol	100
Margarine	a) propylgallate	100
	octylgallate	100
	dodecylgallate	100
	mixture three of them	100
	b) BHA	200
	c) mixture a) and b)	100 a) 200 b)
	d) BHT	200
	e) mixture of BHA and BHT	200
	f) NDGA	100
	g) α-tocopherol	300
Butter for food production	a) propylgallate	80
	octylgallate	80
	dodecylgallate	80
	mixture three of them	80
	b) BHA	160
	c) BHT	160
	d) mixture BHA and b)	160
Food flavours	a) propylgallate	1000
	octylgallate	1000
	dodecylgallate	1000
	mixture three of them	1000
	b) BHA	1000
	c) mixture a) and b)	1000
	d) BHT	1000
	e) mixture BHA and BHT	1000
Materials containing vitamin A more than 100.000IU/gram	a) BHA	10
	b) BHT	10
	c) mixture BHA and BHT	10

Appendix 7.3. Results of chemical and physical analysis of fish oil as the effects of various antioxidant addition during storage at 63±2°C

Analysis: **Peroxide Value (meq/kg sample)**

Antioxidants added into refined fish oil	Storage time (days)						
	0	1	2	4	8	12	16
Control	12.47	17.65	18.52	23.10	29.64	63.57	104.84
0.02% BHA	11.76	18.19	19.74	25.02	28.85	36.98	51.16
0.02% TBHQ	9.76	14.49	14.47	14.81	22.42	31.14	45.51
0.1% Grindox	9.60	14.99	15.97	16.60	23.09	32.61	82.83
0.1% Tocopherol (heated prep.)	15.28	20.76	26.11	32.22	46.13	57.30	66.55
0.1% Tocopherol (direct addition)	20.20	21.62	23.78	32.39	46.61	52.18	66.23

Analysis: **TBA value (μmoles/g sample)**

Antioxidants added into refined fish oil	Storage time (days)						
	0	1	2	4	8	12	16
Control	13.56	21.14	21.47	29.84	46.01	120.83	176.86
0.02% BHA	16.46	18.75	18.06	29.24	46.07	64.10	86.96
0.02% TBHQ	13.11	15.57	16.06	23.18	34.89	48.85	82.69
0.1% Grindox	13.25	21.25	24.04	29.14	40.44	56.63	154.05
0.1% Tocopherol (heated prep.)	15.64	13.56	17.64	29.17	42.32	62.64	106.70
0.1% Tocopherol (direct addition)	13.67	14.03	21.07	29.43	43.18	64.11	106.75

Analysis: **Anisidine Value**

Antioxidants added into refined fish oil	Storage time (days)						
	0	1	2	4	8	12	16
Control	4.91	10.04	13.46	21.67	47.76	115.33	181.80
0.02% BHA	5.43	10.03	14.24	22.06	43.98	70.89	108.51
0.02% TBHQ	5.89	6.94	9.01	12.68	25.90	47.05	81.73
0.1% Grindox	4.56	7.73	11.40	19.53	36.14	61.96	141.90
0.1% Tocopherol (heated prep.)	4.38	8.67	13.98	22.39	49.19	82.85	127.25
0.1% Tocopherol (direct addition)	4.31	10.37	14.68	24.20	50.59	91.92	122.90

Analysis: **Totox Value (2 Peroxide Value + Anisidine Value)**

Antioxidants added into refined fish oil	Storage time (days)						
	0	1	2	4	8	12	16
Control	29.87	45.34	50.50	67.88	107.04	242.47	391.48
0.02% BHA	28.96	46.42	53.71	72.11	101.68	144.85	210.48
0.02% TBHQ	25.40	35.92	37.95	42.31	70.74	109.32	172.70
0.1% Grindox	24.34	37.70	43.34	52.73	82.33	127.18	307.56
0.1% Tocopherol (heated prep.)	34.95	50.19	66.20	86.83	141.46	197.46	260.36
0.1% Tocopherol (direct addition)	44.70	53.61	62.23	88.99	143.80	196.83	255.36

Analysis: **Refractive Index Value (25°C)**

Antioxidants added into refined fish oil	Storage time (days)						
	0	1	2	4	8	12	16
Control	1.4710	1.4729	1.4734	1.4745	1.4749	1.4751	1.4752
0.02% BHA	1.4720	1.4730	1.4740	1.4740	1.4744	1.4746	1.4745
0.02% TBHQ	1.4720	1.4730	1.4735	1.4740	1.4745	1.4745	1.4745
0.1% Grindox	1.4720	1.4729	1.4735	1.4740	1.4745	1.4746	1.4750
0.1% Tocopher. (heated prep)	1.4721	1.4730	1.4740	1.4742	1.4747	1.4750	1.4750
0.1% Tocopher. (direct addition)	1.4721	1.4732	1.4737	1.4746	1.4748	1.4749	1.4750

Analysis: **Colour absorbance value 490 nm**

Antioxidants added into refined fish oil	Storage time (days)						
	0	1	2	4	8	12	16
Control	2.20	2.15	2.05	1.78	1.15	0.35	0.10
0.02% BHA	2.25	2.22	2.02	1.82	1.30	0.80	0.31
0.02% TBHQ	2.25	2.19	2.13	2.05	1.81	1.39	0.63
0.1% Grindox	2.24	2.22	2.06	1.87	1.51	0.96	0.14
0.1% Tocopherol (heated prep.)	2.24	2.24	2.12	2.00	1.69	1.23	0.43
0.1% Tocopherol (direct addition)	2.24	2.16	2.08	1.97	1.63	0.98	0.49

Appendix 7.4. Results of chemical and physical analysis of fish oil as the effects of various levels of BHA addition during storage at 63±2°C

Analysis: **Peroxide Value (meq/kg)**

BHA dosage level added refined oil	Storage time (days)					
	0	2	5	9	13	17
Unrefined oil (control)	11.40	13.36	17.50	22.77	42.66	74.54
0.005%	10.57	15.61	24.03	31.33	50.51	80.10
0.010%	10.59	15.64	24.67	28.30	45.34	62.36
0.015%	10.27	16.94	24.09	26.26	40.37	54.61
0.020%	9.91	16.19	24.26	25.10	39.82	53.91

Analysis: **TBA Value (µmoles/g samples)**

BHA dosage level added refined oil	Storage time (days)					
	0	2	5	9	13	17
Unrefined oil (control)	17.44	13.73	27.21	49.97	90.23	162.61
0.005%	19.14	23.15	34.45	73.56	103.25	162.27
0.010%	18.07	23.78	33.49	57.08	86.78	130.05
0.015%	15.49	22.53	33.75	58.35	79.27	120.14
0.020%	15.07	20.94	31.54	52.34	76.95	107.95

Analysis: **Anisidine Value**

BHA dosage level added refined oil	Storage time (days)					
	0	2	5	9	13	17
Unrefined oil (control)	11.36	18.09	31.22	72.67	103.44	466.48
0.005%	9.54	17.50	35.29	82.28	114.27	457.64
0.010%	9.22	17.35	35.75	73.92	100.83	183.20
0.015%	8.55	18.32	34.47	73.47	96.27	159.32
0.020%	9.39	17.82	34.57	70.34	91.95	142.51

Analysis: **Totox Value**

BHA dosage level added refined oil	Storage time (days)					
	0	2	5	9	13	17
Unrefined oil (control)	34.15	44.82	66.22	118.21	188.77	615.57
0.005%	30.67	48.73	83.36	144.95	215.30	617.84
0.010%	30.39	48.62	85.08	130.53	191.50	307.92
0.015%	29.10	52.21	82.65	125.98	177.01	268.55
0.020%	29.20	50.21	83.09	120.55	171.59	250.33

Analysis: **Refractive Index Value (25°C)**

BHA dosage level added refined oil	Storage time (days)					
	0	2	5	9	13	17
Unrefined oil (control)	1.4750	1.4751	1.4750	1.4754	1.4755	1.4769
0.005%	1.4720	1.4742	1.4750	1.4753	1.4753	1.4760
0.010%	1.4720	1.4741	1.4750	1.4751	1.4752	1.4760
0.015%	1.4720	1.4742	1.4750	1.4750	1.4751	1.4760
0.020%	1.4720	1.4741	1.4750	1.4750	1.4750	1.4759

Analysis: **Colour absorbance value 490 nm**

BHA dosage level added refined oil	Storage time (days)					
	0	2	5	9	13	17
Unrefined oil (control)	0.95	0.88	0.74	0.47	0.33	0.31
0.005%	0.70	0.64	0.50	0.27	0.16	0.12
0.010%	0.71	0.64	0.51	0.30	0.20	0.12
0.015%	0.71	0.65	0.52	0.30	0.20	0.13
0.020%	0.72	0.65	0.53	0.32	0.22	0.13

Appendix 7.5. Results of chemical and physical analysis of fish oil during storage in vacuum package at 63±2°C and 30±2°C

Analysis: **Peroxide Value (meq/kg)**

Storage Temperature	Fish Oil	Packaging Condition	Storage time (**days**)						
			0	1	2	4	8	12	16
63 ± 2°C	Refined	non-vacuum	5.02	32.59	33.13	26.43	16.28	12.58	9.88
		vacuum	5.02	15.52	11.41	10.06	6.95	6.33	4.13
	Unrefined	non-vacuum	7.18	23.76	28.45	22.51	12.68	8.33	6.22
		vacuum	7.18	13.45	11.44	9.69	5.70	4.51	3.92
			Storage time (**weeks**)						
			0	1	2	4	8	12	16
30 ± 2°C	Refined	non-vacuum	6.17	32.58	66.24	48.75	40.77	38.98	38.62
		vacuum	6.17	20.91	23.82	18.61	18.44	18.31	17.93
	Unrefined	non-vacuum	6.79	44.80	60.22	43.23	31.98	29.96	27.80
		vacuum	6.79	23.04	22.66	16.93	13.86	15.31	13.84

Analysis: **TBA Value (μmoles/g)**

Storage Temperature	Fish Oil	Packaging Condition	Storage time (**days**)						
			0	1	2	4	8	12	16
63 ± 2°C	Refined	non-vacuum	9.83	62.34	59.79	42.82	29.11	26.52	21.76
		vacuum	9.83	29.16	19.90	13.70	10.74	10.88	9.59
	Unrefined	non-vacuum	10.29	37.90	39.82	23.39	14.32	13.73	12.27
		vacuum	10.29	17.56	13.21	7.73	5.55	5.66	5.55
			Storage time (**weeks**)						
			0	1	2	4	8	12	16
30 ± 2°C	Refined	non-vacuum	8.85	58.66	115.49	73.48	53.66	50.98	42.54
		vacuum	8.85	32.09	33.81	24.85	19.43	16.83	17.26
	Unrefined	non-vacuum	10.20	65.32	79.07	53.59	33.03	26.26	26.45
		vacuum	10.20	33.59	30.90	16.82	13.51	10.56	9.71

Analysis: **Anisidine Value**

Storage Temperature	Fish Oil	Packaging Condition	Storage time (**days**)						
			0	1	2	4	8	12	16
63 ± 2°C	Refined	non-vacuum	9.32	37.35	51.29	58.66	54.52	56.43	54.28
		vacuum	9.32	21.17	23.69	23.75	23.20	24.19	25.02
	Unrefined	non-vacuum	11.12	29.93	45.94	53.11	50.72	52.76	51.94
		vacuum	11.12	20.04	22.28	22.69	22.29	24.30	24.50
			Storage time (**weeks**)						
			0	1	2	4	8	12	16
30 ± 2°C	Refined	non-vacuum	9.13	18.96	46.37	43.22	42.32	43.72	42.23
		vacuum	9.13	15.74	18.87	20.03	19.62	19.37	19.96
	Unrefined	non-vacuum	10.96	21.21	34.83	37.95	36.92	37.34	40.97
		vacuum	10.96	15.37	18.38	18.81	18.83	19.58	19.63

Analysis: **Totox Value**

Storage Temperature	Fish Oil	Packaging Condition	Storage time (**days**)						
			0	1	2	4	8	12	16
63 ± 2°C	Refined	non-vacuum	19.35	102.54	117.54	111.53	87.09	81.61	74.04
		vacuum	19.35	52.20	46.51	43.72	37.09	36.84	33.28
	Unrefined	non-vacuum	25.47	77.44	102.84	98.13	76.09	69.41	64.38
		vacuum	25.47	46.93	45.16	42.07	33.69	33.32	32.34
			Storage time (**weeks**)						
			0	1	2	4	8	12	16
30 ± 2°C	Refined	non-vacuum	21.47	84.14	178.86	140.71	123.86	121.69	119.46
		vacuum	21.47	57.55	66.52	57.25	56.51	55.99	55.81
	Unrefined	non-vacuum	24.53	110.80	155.27	124.41	100.88	97.26	96.56
		vacuum	24.53	61.44	63.70	52.67	46.54	50.19	47.31

Analysis: **Refractive Index Value**

Storage Temperature	Fish Oil	Packaging Condition	Storage time (**days**)						
			0	1	2	4	8	12	16
63 ± 2°C	Refined	non-vacuum	1.4700	1.4711	1.4715	1.4717	1.4720	1.4720	1.4720
		vacuum	1.4700	1.4711	1.4711	1.4712	1.4712	1.4711	1.4711
	Unrefined	non-vacuum	1.4725	1.4730	1.4731	1.4735	1.4735	1.4735	1.4735
		vacuum	1.4725	1.4730	1.4730	1.4730	1.4730	1.4730	1.4731
			Storage time (**weeks**)						
			0	1	2	4	8	12	16
30 ± 2°C	Refined	non-vacuum	1.4690	1.4703	1.4730	1.4740	1.4730	1.4730	1.4726
		vacuum	1.4690	1.4702	1.4730	1.4740	1.4730	1.4731	1.4726
	Unrefined	non-vacuum	1.4729	1.4730	1.4750	1.4755	1.4750	1.4754	1.4750
		vacuum	1.4729	1.4730	1.4750	1.4751	1.4747	1.4754	1.4750

Analysis: **Colour absorbance value 490 nm**

Storage Temperature	Fish Oil	Packaging Condition	Storage time (**days**)						
			0	1	2	4	8	12	16
63 ± 2°C	Refined	non-vacuum	0.79	0.52	0.43	0.37	0.36	0.36	0.37
		vacuum	0.78	0.68	0.68	0.65	0.63	0.63	0.60
	Unrefined	non-vacuum	0.99	0.75	0.58	0.53	0.53	0.54	0.54
		vacuum	0.99	0.91	0.85	0.84	0.83	0.81	0.79
			Storage time (**weeks**)						
			0	1	2	4	8	12	16
30 ± 2°C	Refined	non-vacuum	0.77	0.61	0.29	0.30	0.28	0.28	0.34
		vacuum	0.77	0.68	0.58	0.68	0.58	0.62	0.62
	Unrefined	non-vacuum	1.04	0.81	0.50	0.51	0.55	0.58	0.64
		vacuum	1.04	0.95	0.78	0.81	0.83	0.84	0.88

Analysis: **Odour score for fish oil stored at 30 ± 2°C**

Fish oil	Packaging Condition	Storage time (**weeks**)					
		0	2	4	8	12	16
Refined	non-vacuum	0.83	2.93	3.67	3.62	4.50	4.37
	vacuum	0.83	2.43	2.25	2.00	3.00	3.50
Unrefined	non-vacuum	0.17	2.43	3.33	3.75	4.00	4.62
	vacuum	0.17	1.78	2.08	2.25	3.00	3.37

Appendix 8.1. Questionnaire used for fish meal factory survey

FISH MEAL FACTORY SURVEY

PLEASE CONSIDER THE FOLLOWING IDEA

I am going to introduce a new method for fish oil refining in order to produce fish oil for human consumption. In this method, the fish oil is passed through a resin packed column. The Refining unit is simple, easy to be designed and low in labour costs.

1. Do you think that the above idea is interesting?

( ) Yes

( ) No

2. Would you like to adopt the above refining method, if I can find the technology?

( ) Yes

( ) No

If YES, please continue to question 3.

If NO, you can stop answering this questionnaire, but please give your reasons,.....

3. How would you apply this refining method in your factory?

( ) replace the existing refining unit totally

( ) operate it together with the existing refining unit

4. What are the types of fish oils which will be refined using this method?

( ) all fish oil types produced

( ) certain fish oil type, please mentioned .....

==========

Additional Information:

Factory : .....
Location : .....

Appendix 8.2. Results of chemical, physical and sensory analysis of Indonesian fish oil as the effects of resin refining process

Analysis: **Free fatty acid value (% oleic acid)**

Sample	Fish Oil	
	Fish meal oil	Canning waste oil
Untreated oil	0.20	0.27
Fraction-1	0.17	0.19
Fraction-2	0.31	0.33

Analysis: **Refractive Index Value**

Sample	Fish Oil	
	Fish meal oil	Canning waste oil
Untreated oil	1.4791	1.4791
Fraction-1	1.4720	1.4750
Fraction-2	1.4791	1.4780

Analysis: **Colour absorbance value 490 nm**

Sample	Fish Oil	
	Fish meal oil	Canning waste oil
Untreated oil	1.51	0.33
Fraction-1	1.01	0.21
Fraction-2	2.31	0.78

Analysis: **Odour score**

Sample	Fish Oil	
	Fish meal oil	Canning waste oil
Untreated oil	7.7	3.9
Fraction-1	4.3	1.8
Fraction-2	7.3	4.9

Analysis : **Fatty acid profiles**

Fatty acids	Fish meal oil			Canning waste oil		
	Untreated Oil	Frac.-1	Frac.-2	Untreated Oil	Frac.-1	Frac.-2
14:0	13.3	13.8	12.6	12.7	12.6	12.0
15:0	0.6	0.7	0.7	0.8	0.8	0.9
16:0	20.0	20.3	24.9	21.2	21.0	21.4
16:1	15.3	15.8	16.1	14.9	15.0	14.8
17:0	0.6	0.6	0.8	0.8	0.9	1.0
17:1	4.2	4.4	4.2	4.0	4.2	4.0
18:0	3.1	3.0	2.8	3.5	3.4	4.0
18:1	7.2	7.0	7.1	8.0	8.3	8.3
18:2	1.1	1.1	1.1	1.2	1.3	1.3
18:3	1.0	1.0	1.1	1.2	1.2	1.2
18:4	1.8	2.0	1.9	2.1	2.2	2.2
20:1	0.9	0.9	0.8	0.8	0.9	0.9
20:3	0.1	0.1	0.1	0.1	0.1	0.1
20:4	2.6	2.6	2.4	2.4	2.4	2.3
20:5	19.0	17.8	15.5	17.2	16.9	16.5
22:1	1.8	1.5	1.2	1.2	1.2	1.2
22:4	0.1	0.1	0.1	0.1	0.1	0.1
22:5	1.0	1.2	1.0	1.2	1.2	1.2
22:6	5.7	5.5	4.8	6.1	6.3	6.0

Appendix 8.3. Results of chemical and physical analysis of Indonesian fish oil during storage at 63 ± 2°C

Analysis: **Peroxide Value (meq/kg)**

Fish Oil	Treatment	Storage Time (days)				
		0	2	4	7	11
Fish meal oil	Unrefined	21.45	19.80	21.68	18.35	33.65
	Refined	24.96	23.46	30.65	34.02	87.63
Canning waste oil	Unrefined	28.41	25.93	32.15	37.47	71.73
	Refined	31.61	34.53	51.94	92.24	118.69

Analysis: **TBA Value (µmoles/g)**

Fish Oil	Treatment	Storage Time (days)				
		0	2	4	7	11
Fish meal oil	Unrefined	27.62	25.13	27.15	38.62	73.87
	Refined	35.88	45.43	50.45	72.75	182.49
Canning waste oil	Unrefined	53.10	46.07	50.25	88.56	163.95
	Refined	58.33	64.74	84.60	189.35	274.22

Analysis: **Anisidine Value**

Fish Oil	Treatment	Storage Time (days)				
		0	2	4	7	11
Fish meal oil	Unrefined	16.08	21.68	29.63	45.11	71.98
	Refined	16.68	27.97	42.75	67.85	468.37
Canning waste oil	Unrefined	20.42	31.31	42.54	82.34	479.72
	Refined	18.93	32.11	54.45	160.20	471.74

Analysis: **Totox Value**

Fish Oil	Treatment	Storage Time (days)				
		0	2	4	7	11
Fish meal oil	Unrefined	58.98	61.29	73.00	81.81	139.28
	Refined	66.61	74.58	104.05	135.89	643.63
Canning waste oil	Unrefined	77.23	83.17	106.84	157.28	623.18
	Refined	82.14	101.18	158.32	344.68	709.12

Analysis: **Refractive Index Value**

Fish Oil	Treatment	Storage Time (days)				
		0	2	4	7	11
Fish meal oil	Unrefined	1.4770	1.4770	1.4770	1.4770	1.4770
	Refined	1.4731	1.4758	1.4763	1.4766	1.4773
Canning waste oil	Unrefined	1.4770	1.4770	1.4770	1.4770	1.4780
	Refined	1.4760	1.4760	1.4765	1.4773	1.4784

Analysis: **Colour absorbance value 490 nm**

Fish Oil	Treatment	Storage Time (days)				
		0	2	4	7	11
Fish meal oil	Unrefined	1.30	1.31	1.22	1.02	0.73
	Refined	1.14	0.99	0.83	0.60	0.21
Canning waste oil	Unrefined	0.34	0.55	0.69	0.60	0.46
	Refined	0.19	0.14	0.11	0.06	0.08

Appendix 9.1. Questionnaire used for supermarket survey

Supermarket: .....
City : .....

No.	Trade Mark	Fish Species	Ingredients/ medium	Weight (g)	Can Form	Can Size	Price (Rp.)	Product Origin/ Producer & Super-market

Appendix 9.2. Questionnaire used for cannery survey

CANNERY SURVEY

Direction: Please answer this questionnaire by giving thick mark (V)

1. What fish species are processed into canned fish in your company? Please give the quantity of canned fish processed from each fish species per year, if possible.

   ( ) ..... ton sardine
   ( ) ..... ton mackerel
   ( ) ..... ton tuna
   ( ) ..... ton skipjack
   ( ) ..... ton small tuna
   ( ) ..... others, please specify .....

2. What is the medium normally added canned fish in your company? Please give the amount of canned fish processed from each fish species per year, if possible.

   ( ) ..... ton tomato sauce
   ( ) ..... ton brine
   ( ) ..... ton vegetable oil
   ( ) ..... ton vegetable oil and brine mixture
   ( ) ..... ton others, please specify .....

3. Does your company export the canned fish product?

   ( ) YES ( ) NO

   If "YES", please specify the percentage of your product to be exported.....%

4. For local market, what fish species and medium type are processed the most? (Please give percentage for all products marketed locally)

Fish Species	**Medium Type**
( ) .....% Sardine	( ) ..... Tomato sauce
( ) .....% Mackerel	( ) ..... Vegetable oil
( ) .....% Tuna	( ) ..... Brine
( ) .....% Skipjack	( ) ..... Vegetable oil and brine
( ) .....% Small tuna	mixture
( ) .....% Others, please specify.....	( ) ..... Others, please specify
.....	

Please consider this product idea:

I am going to introduce canned fish enriched with disguised fish oil. This idea is aimed to optimize the fish oil utilisation and to produce canned fish which is nutritionally better than existing products in the market.

5. What is your comment about this idea, If the product is going to be marketed in Indonesia?

( ) interesting ( ) not interesting

6. What is the medium you prefer to use, if you produce this canned product?

( ) tomato sauce
( ) brine
( ) vegetable oil
( ) vegetable oil and brine mixture
( ) others, please specify .....

7. Would you like to be informed, if a processing technology for this product is developed?

( ) YES ( ) NO

If "NO", please give the reason .....

If "YES", please give the percentage of the product which is going to be produced, in terms of total production:

( ) 1 - 10%
( ) 11 - 20%
( ) 21 - 30%
( ) 31 - 40%
( ) Over 40%

Comments: .....
.....
.....

==========

Please complete this section. Your information is kept confidentially.

Name of factory : .....
Location : .....
Production capacity: .....ton/day
Annual production : .....ton/year
Fish price : sardine : Rp.....-...../kg
mackerel : Rp.....-...../kg
skipjack : Rp.....-...../kg
tuna : Rp.....-...../kg
small tuna : Rp.....-...../kg
..... : Rp.....-...../kg

Factories participating in the cannery survey:

1. P.T. Bali Raya, Benoa, Bali
2. P.T. Blambangan Raya, Muncar, East Java
3. P.T. Karya Manunggal Prima Sukses, Muncar, East Java
4. P.T. Sumino, Negara, Bali
5. C.V. Harapan Lancar, Muncar, East Java
6. C.V. Samudra Raya, Negara, Bali
7. P.T. Maya Muncar, Muncar, Bali
8. P.T. Bali Maya Permai, Negara, Bali
9. P.T. Sumber Yala Samudra, Muncar, Bali
10. P.T. Bali Raya, Negara, Bali
11. P.T. Sofcol, Bitung
12. P.T. CIP, Denpasar, Bali
13. N.V. Muncar, Muncar, Bali
14. P.T. Sinar Laut Jaya, Muncar, Bali
15. P.T. Pengambengan Raya, Negara, Bali
16. P.T. Indo Bali, Negara, Bali

Appendix 9.3. Questionnaire used for consumer survey

CONSUMER SURVEY

Dear Madam/Sir:

I am a PhD student in the Food Technology Department, Massey University, New Zealand. I am conducting a survey for a product development project on the use of fish oil in food for the Indonesian market.

The purpose of this questionnaire is to obtain information from the consumer for developing the product.

Please answer the questionnaire below and return it to the person distributing the questionnaire or to:

Hari Eko Irianto

c/- Ir.Giyatmi Irianto
Akademi Gizi Muhammadyah
Jln. Wonodri Dalam II/22
SEMARANG

I would like to thank you in advance for your contribution to this project.

Sincerely yours,

Hari Eko Irianto

I. Fish and Fish Product Consumption

1. Indicate your frequency in consuming fish or fish products in the following table

Products	frequency of consumption				
	once/ week	twice/ week	>twice/ week	twice/ month	once/ month
1. Fresh fish, including frozen and chilled fish					
2. Processed product: - dried salted fish - boiled salted fish - fermented fish/shrimp paste - pedah (moist fermented fish) - jambal (spongy fermented fish) - fish sauce - canned fish - smoked fish - softened bone fish - fish ball					

II. Fish Oil

Would you like to consume refined fish oil, if you knew that fish oil had health benefits?

( ) YES ( ) NO

How would you like to consume fish oil?

( ) capsule
( ) table spoon
( ) salad oil
( ) disguised into ordinary foods

III. Canned fish and canned fish containing fish oil

1. When buying canned fish, do you consider the kind of medium?

( ) YES ( ) NO

If "YES", what kind of medium most like?

( ) tomato sauce
( ) vegetable oil
( ) brine
( ) vegetable oil and brine mixture
( ) others, please specify.....

2. When buying canned fish, do you also consider the fish species?

( ) YES ( ) NO

If "YES", what kind fish species do you frequently buy?

( ) sardine
( ) mackerel
( ) tuna
( ) others, please specify .....

Please consider this idea:

I would like to introduce canned fish with disguised fish oil in order to optimise the fish oil utilisation and to produce canned fish nutritionally better than existing product in the market.

3. Do you find that product described above attractive?

( ) YES ( ) NO

4. As listed below, which medium of canned fish would you prefer the fish oil to be disguised?

( ) tomato sauce
( ) vegetable oil
( ) brine
( ) vegetable and brine mixture
( ) others, please specify .....

5. Would you like to buy this product?

( ) YES ( ) NO

6. In your opinion, what size would you like to buy?

( ) 155 g ( ) 185 g ( ) 215 g ( ) 415g

7. What price would you expect for this product?

( ) Rp. 400 - 999.-
( ) Rp. 1000 - 1799.-
( ) Rp. 1800 - 2599.-
( ) Rp. 2600 - 3000.-

==========

Please fill in this information. It will be used only for analysis and will be kept confidential. Your name and address will help me if I may need to conduct another survey for this product.

Name : .....
Address : .....
.....
.....
Age : .....years
Occupation : .....
Family income:

( ) >Rp. 500,000/month
( ) Rp. 150,000 - 500,000/month
( ) <Rp. 150,000/month

Appendix 9.4. Canned fish product being available in Indonesian market

No.	Trade Mark	Fish Species	Ingredients/medium	Weight (g)	Can form	Price (Rp.-)	Producer
1	Yamato	Sardine	tomato sauce, salt, sugar, MSG	400	oval	1030	Indonesia
2	Yamato	Sardine	tomato sauce	425	tube	960 - 1050	Indonesia
3	Yamato	Sardine	tomato sauce	155	tube	425 - 475	Indonesia
4	Bantan	Sardine	tomato sauce	400	oval	1010 - 1055	Indonesia
5	Bantan	Sardine	tomato sauce	425	tube	975 - 1075	Indonesia
6	Bantan	Sardine	tomato sauce	155	tube	415 - 455	Indonesia
7	Bantan	Mackerel	tomato sauce	400	oval	1210 - 1700	Indonesia
8	Del Monte	Sardine	water, tomato paste, salt, cellulose gum, natural flavouring	213	oval	1550 - 2020	Korea
9	Del Monte	Sardine	water, tomato paste, salt, cellulose gum, natural flavouring	425	oval	2555 - 3250	Korea
10	Gaga	Mackerel	tomato, garlic, chilli	155	tube	620 - 660	Indonesia
11	Gaga	Mackerel	tomato, garlic, chilli	425	tube	1500	Indonesia
12	Gaga	Sardine	tomato, garlic, chilli	155	tube	470 - 475	Indonesia
13	Botan	Mackerel	tomato sauce	425	oval	2675 - 3345	Indonesia
14	Botan	Mackerel	tomato sauce	425	tube	1700 - 1880	Indonesia
15	Botan	Mackerel	tomato sauce	155	tube	740 - 850	Indonesia
16	Botan	Sardine	tomato sauce	425	tube	1350 - 1795	Indonesia
17	Botan	Sardine	tomato sauce	155	tube	535 - 800	Indonesia
18	Botan	Sardine	tomato sauce	215	oval	1825 - 1945	Indonesia
19	Botan	Sardine	tomato sauce	425	oval	3100 - 3350	Indonesia
20	CIP	Sardine	tomato sauce	400	oval	1400 - 1675	Indonesia
21	CIP	Sardine	tomato sauce	155	tube	615	Indonesia
22	Three Star	Sardine	tomato sauce	425	tube	975 - 1055	Indonesia
23	Three Star	Sardine	tomato sauce	155	tube	400 - 415	Indonesia
24	King's Fisher	Sardine	tomato sauce, salt	155	tube	410 - 440	Indonesia
25	King's Fisher	Sardine	tomato sauce, salt	425	tube	1000 - 1065	Indonesia
26	King's Fisher	Tuna	oil, onion, green peas, spices	185	tube	1030 - 1055	Indonesia

Continuation of appendix 9.4.

No.	Trade Mark	Fish Species	Ingredients/medium	Weight (g)	Can form	Price (Rp.-)	Producer
27	Bottom	Mackerel	tomato sauce	155	tube	550 - 650	Indonesia
28	Bottom	Mackerel	tomato sauce	425	tube	1350 - 1920	Indonesia
29	Calmex	Sardine	tomato sauce, salt	425	oval	2240 - 2290	Mexico
30	Pronas	Tuna chunk	vegetable oil, salt	185	st.tube	1200 - 1995	Indonesia
31	Pronas	Tuna	water, salt	200	st.tube	1215 - 1455	Indonesia
32	Pronas	Sardine	tomato sauce	425	tube	1150 - 1250	Indonesia
33	Kiku	Sardine	tomato sauce	155	tube	395 - 425	Indonesia
34	Swallow	Sardine	tomato sauce	155	tube	410 - 480	Indonesia
35	Kokin	Sardine	tomato sauce, palm oil, onion, sugar, salt	155	tube	400 - 425	Indonesia
36	Deho	Chunk light Tuna	vegetable oil, salt	185	st.tube	975	Indonesia
37	Billtan	Mackerel	water, tomato paste, salt, cellulose gum, natural flavouring	425	oval	1085	Indonesia
38	Billtan	Mackerel	tomato sauce	425	tube	1075 - 1450	Indonesia
39	Billtan	Mackerel	tomato sauce	155	tube	450	Indonesia
40	Maya	Sardine	tomato sauce	425	tube	1175	Indonesia
41	Maya	Sardine	tomato sauce	155	tube	450	Indonesia
42	Maya	Sardine	tomato sauce with cilli	155	tube	525	Indonesia
43	Katan	Tuna	fried chilli, spice, onion, garlic, vegetable oil, coconut milk, glutamat salt (sambel goreng)	185	st.tube	1850	Indonesia
44	Fried Dace	Dace	black bean, vegetable oil, soy sauce, salt, spice	227	oval	1925 - 2580	PR China
45	Ayam	Sardine	tomato sauce, salt	425	oval	4110 - 4275	Korea
46	Ayam	Sardine	tomato sauce, salt, olein	215	oval	1550 - 2050	Korea
47	Ayam	Sardine	tomato sauce, salt, olein	425	tube	2950 - 3720	Korea
48	Ayam	Sardine	tomato sauce, salt olein	230	tube	1825	Korea
49	Ayam	Sardine	tomato sauce, salt, olein	155	tube	1600	Korea

Continuation of appendix 9.4.

No.	Trade Mark	Fish Species	Ingredients/medium	Weight (g)	Can form	Price (Rp.-)	Producer
50	Heinz	Greenseas sandwich tuna	vegetable oil, salt, water	170	st. tube	4675 - 7500	Australia
51	Heinz	tuna	vegetable oil, salt, water	180	st. tube	4715	Australia
52	Heinz	greenseas light meat chunk style tuna	salt, water	180	st. tube	9750	Australia
53	Star Kist	solid light tuna	spring water, vegetable broth, salt	184	st. tube	5000	USA
54	S & W	chunk light fancy tuna	vegetable oil, salt, hydrolysed protein	184	st. tube	3100 - 3265	USA
55	S & W	solid light fancy tuna	soya oil/cotton seed oil, salt	184	st. tube	3375 - 3545	USA
56	S & W	fancy red sockeye blue black salmon	salt	220	st. tube	4135	USA
57	S & W	fancy red sockeye tuna	salt	220	st. tube	7250 - 13500	USA
58	John West	chunk style tuna	vegetable oil, salt	185	st. tube	5105 - 5280	Australia
59	John West	tuna	water, salt	185	st. tube	4840 - 5280	Australia
60	John West	sandwich tuna	vegetable oil, salt	185	st. tube	4840 -5280	Australia
61	John West	pink salmon	salt	105	st. tube	4800	Australia
62	John West	pink salmon	salt	210	st. tube	6350 - 8400	Australia
63	John West	pink salmon	salt	440	tube	7700 - 11600	Australia
64	John West	tuna	onion, vegetable oil, tomato puree, vinegar, sugar, salt, spices	185	st. tube	4840 - 5280	Australia
65	John West	Red salmon	salt, water	105	st. tube	4800	Australia
66	John West	herring fillets	tomato puree, water, soya oil, vinegar, modified starch, spices	200	oval	5700	Australia
67	John West	skipper filter	water, salt	200	oval	5700	Australia

Continuation of appendix 9.4.

No.	Trade Mark	Fish Species	Ingredients/medium	Weight (g)	Can form	Price (Rp.-)	Producer
68	John West	skippers smoked brisling	vegetable oil, salt, liquid smoke	106	oval	4200 - 4375	Australia
69	Hormel	skinless & boneless chunk pink salmon	water, salt	141	st. tube	6960 - 8020	USA
70	Bumble Bee	sockeye red salmon	salt	210	st. tube	8190	Canada
71	Bumble Bee	sockeye red salmon	salt	440	tube	15895	Canada
72	Duchef	tuna chunk	vegetable (soya bean) oil	170	st. tube	1500 - 1700	Australia
73	Ligo	sardines	tomato sauce	425	tube	1925 - 2300	Chile
74	Smiling Fish	fried sardine	sugar, chilli soy sauce, vegetable oil	155	tube	2160	Thailand
75	Minerva	sardine	salt, soy oil	125	rectangular	2970	Portugal
76	Minerva	sardine	tomato, vegetable oil, salt	125	rectangular	2970	Portugal
77	Plumrose	mackerel fillets	tomato concentrate, salt, water	125	oval	4200 - 4395	Denmark
78	Plumrose	mackerel fillets	vegetable oil, salt	125	oval	4200 - 4395	Denmark
79	White Rose	chunk light tuna	water seasoned with vegetable broth, salt, pyrophosphate	184	st. tube	1275 - 1495	Indonesia
80	Gold Cup	sardines	tomato sauce	400	oval	1150	Indonesia
81	Gold Cup	sardines	tomato sauce	425	tube	1150	Indonesia
82	Ayam	sardines	fish oil, salt	106	rectangular	3000	Norway
83	Sea Gift	smoked sardine	sardine oil, salt	106	rectangular	2970	Norway

Appendix 9.5. Dimensions of can found in the market

Can Size (g)	Height (Cm)	Diameter
Tall tube can		
155	8.8	5.3
425	11.3	7.5
440	11.3	7.5
Short tube can		
141	4.1	8.5
170 a	4.5	8.5
170 b	4.2	8.5
180	4.5	8.5
184	4.2	8.5
185 a	4.6	8.5
185 b	4.0	8.3
200	4.5	8.5
210	4.8	8.5
220	5.0	8.4/7.7
Oval can		
213	3.1	8.5/12.4
227	4.7	7.2/14.5
400	3.9	10.8/15.7
420	3.3	10.9/15.7
Rectangular can	Length	Wide/height
106/125	10.6	8.7/1.8

Appendix 9.6. Chi-square, degree of freedom and Cramer's V of Crosstab analysis results for consumer survey

Variable	Chi Square	DF	Cramer's V
Age*Fish oil consumption method	131.628	8	0.709
Age*Fish species selection	131.703	8	0.709
Age*Medium selection	134.072	8	0.715
Age*Product idea	135.595	8	0.719
Age*Buying trend	135.044	8	0.718
Income*Fish oil consumption method	133.206	6	0.713
Income*Fish species selection	131.593	6	0.709
Income*Medium selection	136.198	6	0.721
Income*Product idea	132.813	6	0.712
Income*Buying trend	136.685	6	0.722
Occupation*Fish oil consumption method	131.386	4	0.708
Occupation*Fish species selection	135.881	4	0.720
Occupation*Medium selection	131.004	4	0.707
Occupation*Product idea	131.259	4	0.708
Occupation*Buying trend	131.431	4	0.712

Note: DF = degree of freedom

Cramer's V classification (Craft, 1990):

< 0.10	= weak association
0.11 - 0.25	= weak to moderate association
0.26 - 0.40	= moderate association
0.41 - 0.50	= moderate to strong association
> 0.50	= strong association

Appendix 10.1. Sensory form used for evaluating tomato sauce acceptability

SENSORY EVALUATION FOR TOMATO SAUCE USED IN CANNED FISH

Name : .....
Date : .....

Instruction: Please indicate the score that best reflects your attitude about the tomato sauce

Description	**Score**
Extremely very acceptable	9
Very acceptable	8
Acceptable	7
Slightly acceptable	6
Not sure	5
Slightly unacceptable	4
Unacceptable	3
Very acceptable	2
Extremely very unacceptable	1

Attributes	**Sample code**				
	.....	.....	.....	.....	.....
Consistency	.....	.....	.....	.....	.....
Odour	.....	.....	.....	.....	.....
Colour	.....	.....	.....	.....	.....
Mouth feel	.....	.....	.....	.....	.....
Appearance	.....	.....	.....	.....	.....
Overall acceptability	.....	.....	.....	.....	.....

Comments: .....
.....

Have you ever eaten canned fish in tomato sauce medium? yes/no

Appendix 10.2. Sensory form used for evaluating sterilized fish oil

SENSORY EVALUATION OF STERILIZED FISH OIL

Name :.....
Date :.....

Instructions: please evaluate samples in front of you in terms of taste and odour. Score the samples using the following scale:

Fishy taste and odour

1 no fishy taste/odour
2
3 slightly fishy taste/odour
4
5 moderately fishy taste/odour
6
7 strong fishy taste/odour
8
9 extremely strong fishy taste/odour

Rancid taste and odour

1 Typical and fresh fish oil
2 Bland-indicates incipient rancidity
3 Rancidity just noticeable
4 Rancidity clearly noticeable

Sample Code	Taste		Odour	
	Fishy	Rancid	Fishy	Rancid
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....
.....	.....	.....	.....	.....

Appendix 10.3. The example of the calculation of ingredient effects

STUDY ON FISH OIL EFFECTS

1. Effect on sauce consistency

Cold observation

high amount of fish oil

$$\frac{51+56}{2} = 54$$

low amount of fish oil

$$\frac{53+32+56}{3} = 47$$

Warm observation

high amount of fish oil

$$\frac{47+53}{2} = 50$$

low amount of fish oil

$$\frac{50+32+56}{3} = 46$$

Comments: high amount of fish oil was preferred in terms of sauce consistency from both cold and warm samples

2. Effect of sauce odour

Cold observation

high amount of fish oil

$$\frac{51+52}{2} = 52$$

low amount of fish oil

$$\frac{54+48+54}{3} = 52$$

Warm observation

high amount of fish oil

$$\frac{47+48}{2} = 48$$

low amount of fish oil

$$\frac{48+46+51}{3} = 48$$

Comment: The amount of fish oil did not affect the odour of tomato sauce from both cold and warm observation

Appendix 10.4. Results of chemical, physical, sensory analysis of fish oil as the effect of sterilization treatment

Analysis: **Peroxide Value (meq/kg)**

Fish oil	Sterilization condition	Initial value	Temperature and Period of Sterilization		
			110.0°C 139 min.	116.7°C 79 min.	121.1°C 64 min.
Unrefined	Vacuum	4.99	0.60	0.89	0.49
	Non-vacuum	4.99	1.26	1.23	1.37
Refined	Vacuum	4.25	1.36	1.21	1.09
	Non-vacuum	4.25	2.35	2.06	1.95

Analysis: **TBA value (μmoles/g)**

Fish oil	Sterilization condition	Initial value	Temperature and Period of Sterilization		
			110.0°C 139 min.	116.7°C 79 min.	121.1°C 64 min.
Unrefined	Vacuum	15.66	4.14	3.42	3.81
	Non-vacuum	15.66	3.54	3.76	3.82
Refined	Vacuum	15.40	4.65	4.42	4.12
	Non-vacuum	15.40	6.07	6.03	6.04

Analysis: **Anisidine value**

Fish oil	Sterilization condition	Initial value	Temperature and Period of Sterilization		
			110.0°C 139 min.	116.7°C 79 min.	121.1°C 64 min.
Unrefined	Vacuum	5.59	9.91	9.88	10.62
	Non-vacuum	5.59	11.28	11.06	11.64
Refined	Vacuum	4.85	7.24	7.48	7.42
	Non-vacuum	4.85	9.08	8.93	8.74

Analysis: **Totox value**

Fish oil	Sterilization condition	Initial value	Temperature and Period of Sterilization		
			110.0°C 139 min.	116.7°C 79 min.	121.1°C 64 min.
Unrefined	Vacuum	15.58	11.11	11.67	11.60
	Non-vacuum	15.58	13.80	13.53	14.38
Refined	Vacuum	13.36	9.95	9.91	9.60
	Non-vacuum	13.36	13.34	13.04	12.64

Analysis: **Free Fatty Acid Value (% oleic acid)**

Fish oil	Sterilization condition	Initial value	Temperature and Period of Sterilization		
			110.0°C 139 min.	116.7°C 79 min.	121.1°C 64 min.
Unrefined	Vacuum	2.71	2.66	2.58	2.61
	Non-vacuum	2.71	2.67	2.67	2.54
Refined	Vacuum	2.33	2.29	2.27	2.25
	Non-vacuum	2.33	2.29	2.31	2.31

Analysis: **Refractive Index value**

Fish oil	Sterilization condition	Initial value	Temperature and Period of Sterilization		
			110.0°C 139 min.	116.7°C 79 min.	121.1°C 64 min.
Unrefined	Vacuum	1.4691	1.4691	1.4692	1.4692
	Non-vacuum	1.4691	1.4692	1.4693	1.4692
Refined	Vacuum	1.4660	1.4661	1.4662	1.4661
	Non-vacuum	1.4660	1.4662	1.4662	1.4662

Analysis: **Colour value (Photometric method)**

Fish oil	Sterilization condition	Initial value	Temperature and Period of Sterilization		
			110.0°C 139 min.	116.7°C 79 min.	121.1°C 64 min.
Unrefined	Vacuum	21.01	25.77	25.68	26.51
	Non-vacuum	21.01	29.11	27.84	28.33
Refined	Vacuum	16.41	18.37	18.75	17.92
	Non-vacuum	16.41	18.45	18.51	18.61

Analysis: **Fishy Odour**

Fish oil	Sterilization condition	Initial value	Temperature and Period of Sterilization		
			110.0°C 139 min.	116.7°C 79 min.	121.1°C 64 min.
Unrefined	Vacuum	6.33	4.08	4.50	4.17
	Non-vacuum	6.33	4.83	4.50	4.33
Refined	Vacuum	4.55	4.89	3.61	3.54
	Non-vacuum	4.55	4.00	3.86	3.93

Analysis: **Rancid Odour**

Fish oil	Sterilization condition	Initial value	Temperature and Period of Sterilization		
			110.0°C 139 min.	116.7°C 79 min.	121.1°C 64 min.
Unrefined	Vacuum	1.37	1.92	1.92	2.08
	Non-vacuum	1.37	1.75	1.71	1.83
Refined	Vacuum	1.30	2.04	2.00	2.00
	Non-vacuum	1.30	1.86	1.79	1.71

Analysis: **Fishy Taste**

Fish oil	Sterilization condition	Initial value	Temperature and Period of Sterilization		
			110.0°C 139 min.	116.7°C 79 min.	121.1°C 64 min.
Unrefined	Vacuum	5.70	4.25	4.42	4.33
	Non-vacuum	5.70	4.83	4.75	4.17
Refined	Vacuum	4.55	3.93	3.75	3.68
	Non-vacuum	4.55	4.18	3.75	3.89

Analysis: **Rancid Taste**

Fish oil	Sterilization condition	Initial value	Temperature and Period of Sterilization		
			110.0°C 139 min.	116.7°C 79 min.	121.1°C 64 min.
Unrefined	Vacuum	1.37	1.92	1.92	2.00
	Non-vacuum	1.37	1.75	1.50	1.83
Refined	Vacuum	1.23	1.64	1.64	1.86
	Non-vacuum	1.23	1.64	1.64	1.64

Analysis: **Fatty acid profiles (% fatty acids)**

Fatty acids	Unsterilized oil		Sterilization Condition											
			110°C for 139 minutes				116.7°C for 79 minutes				121.1°C for 64 minutes			
	UR*)	R**)	Vacuum		Non-vacuum		Vacuum		Non-vacuum		Vacuum		Non-vacuum	
			UR	R	UR	R	UR	R	UR	R	UR	R	UR	R
14:0	5.8	5.8	5.1	5.8	6.0	5.9	6.1	5.9	6.1	6.2	5.8	5.7	6.2	4.5
15:0	0.7	0.4	0.6	0.6	0.7	0.7	0.7	0.5	0.4	0.7	0.4	0.6	0.7	0.3
16:0	12.8	12.9	12.9	12.8	12.9	12.9	13.1	12.8	13.2	13.3	12.7	12.5	13.4	13.4
16:1	8.0	8.1	8.2	8.1	8.3	8.2	8.4	8.3	8.5	8.5	8.1	8.0	8.4	8.6
17:0	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
17:1	2.3	2.2	2.4	2.0	2.4	2.4	2.4	2.3	2.4	2.7	2.4	2.3	2.4	2.5
18:0	2.5	2.6	2.5	2.5	2.0	2.0	2.2	2.0	2.5	2.0	2.5	2.2	2.6	2.3
18:1	28.6	29.1	28.9	29.1	29.0	29.2	28.9	29.1	29.1	29.5	28.8	28.6	29.0	29.7
18:2	1.7	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
18:3	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.9	0.7	0.8	0.8	0.8
18:4	2.1	2.1	3.0	2.9	2.9	2.6	3.0	3.0	2.9	1.9	2.4	3.0	2.9	2.1
20:1	7.8	7.8	7.8	7.8	7.7	7.8	7.5	7.8	7.6	7.7	7.9	7.8	7.6	7.8
20:3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3
20:4	0.7	0.7	0.8	0.7	0.8	0.7	0.7	0.7	0.7	0.7	0.8	0.8	0.7	0.7
20:5	11.0	10.6	10.9	10.7	10.7	10.5	11.0	10.7	10.2	10.2	11.1	11.0	10.2	10.8
22:1	3.7	3.7	3.5	3.5	3.5	7.6	3.4	3.6	3.4	3.5	3.6	3.7	3.4	3.6
22:4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
22:5	4.8	4.4	3.9	4.4	4.4	4.3	4.1	4.4	4.1	3.9	4.8	4.7	3.6	4.4
22:6	6.0	5.4	5.7	5.7	5.5	5.7	5.1	5.8	5.5	5.6	5.7	6.0	5.6	5.9

Note: *) UR = unrefined oil
**) R = refined oil

Appendix 11.1. Sensory sheet for Plackett and Burman experiment of canned fish

PLACKETT AND BURMAN EXPERIMENT OF CANNED FISH

Name : .....
Date : .....

Please evaluate each sample for the following attributes by placing a vertical line on the scale at the point which you think best describes the product and put the sample code at the top of this line. Please also make a line where your **IDEAL** canned fish would be and label this line with **"I"**

Example:

342 123 421
I
. . . . .

FISH

1. Appearance

. . . . .
not broken very broken

2. Flesh texture

. . . . .
tender tough

3. Bone softness

. . . . .
not soft very soft

4. Sourness

. . . . .
not sour very sour

5. Saltiness

. . . . .
not salty very salty

6. Overall spiciness

. . . . .
not spicy very spicy

7. Fishiness

not fishy ——•——•——•——•—— very fishy

TOMATO SAUCE

1. Colour

bright red ——•——•——•——•—— not bright red/dark

2. Mouth feel of sauce

not oily ——•——•——•——•—— very oily

3. Sourness

not sour ——•——•——•——•—— very sour

4. Saltiness

not salty ——•——•——•——•—— very salty

5. Overall spiciness

not spicy ——•——•——•——•—— very spicy

6. Fishiness

not fishy ——•——•——•——•—— very fishy

OVERALL ACCEPTABILITY

not acceptable ——•——•——•——•—— very acceptable

Thank you for your time

Appendix 11.2. Sensory sheet for experiment on canning process optimization

FISH CANNING PROCESS OPTIMIZATION

Name: .....
Date: .....

Please indicate the score that best reflects your attitude about each sample in terms of product characteristics and acceptability

Score	Product Characteristics	Score	Product Acceptability
9	Extremely very	9	Extremely very acceptable
8	Very	8	Very acceptable
7	.....	7	Acceptable
6	Slightly	6	Slightly acceptable
5	Not sure	5	Not sure
4	Slightly not	4	Slightly unacceptable
3	Not	3	Unacceptable
2	Very not	2	Very unacceptable
1	Extremely very not	1	Extremely very unacceptable

Note: ..... means followed by description placed under evaluated characteristics

Attributes	**Product characteristic/Product acceptability** SAMPLE CODES				
	.....	.....	.....	.....	.....
FISH					
1. Flesh texture (tender-not tender or tough	.....	.....	.....	.....	.....
2. Softness of bone (soft-not soft)	.....	.....	.....	.....	.....
3. Sourness (sour-not sour)	.....	.....	.....	.....	.....

4. Saltiness ....
(salty-not salty)

5. Overall spiciness ....
(spicy-not spicy)

6. Fishiness ....
(fishy-not fishy)

TOMATO SAUCE

1. Sauce colour ....
(bright red-not bright red or dark red)

2. Mouth feel of sauce ....
(oily-not oily)

3. Sourness ....
(sour-not sour)

4. Saltiness ....
(salty-not salty)

5. Overall spiciness ....
(spicy-not spicy)

6. Fishiness ....
(fishy-not fishy)

OVERALL ACCEPTABILITY

....

COMMENTS:.....

.....

Thank you for your time

Appendix 12.1. Questionnaire form used for consumer product testing

CONSUMER TEST

Dear Sir/Madam

Attached to this questionnaire is a sample of canned sardine. The product has been developed in the Food Technology Department, Massey University, New Zealand for the Indonesian market. I would like you to taste the sample and answer the questions below.
Thank you for your time and cooperation.

Hari Eko Irianto
PhD Student
Food Technology Department
Massey University
Palmerston North
New Zealand

INSTRUCTION: 1. Taste this product the way you would normally eat canned fish, but do not add anything, e.g. spices, water, salt, etc.
2. Please answer the questions by ticking the appropriate blank

I. How did you eat the canned fish?

(a) treatment:
.... the samples was heated (warmed)
.... no treatment at all

(b) with other foods
.... with rice and/or other foods, e.g. vegetables....
.... with rice only
.... the sample only
.... other, please specify .....

II. Question about the FISH

1. How is the flesh texture when you bite it?
.... very tender
.... slightly tender
.... just right
.... slightly tough
.... very tough

2. How is the bone softness when you bite it?
   .... very soft
   .... slightly soft
   .... just right
   .... slightly hard
   .... very hard

3. How is the sourness of the fish?
   .... very sour
   .... slightly sour
   .... just right
   .... slightly lacking in sourness
   .... very lacking in sourness

4. How is the saltiness of the fish?
   .... very salty
   .... slightly salty
   .... just right
   .... slightly lacking in saltiness
   .... very lacking in saltiness

5. How is the overall spiciness of the fish?
   .... very spicy
   .... slightly spicy
   .... just right
   .... slightly lacking in spiciness
   .... very lacking in spiciness

6. How is fishiness of fish?
   .... very fishy
   .... slightly fishy
   .... just right
   .... slightly non-fishy
   .... very non-fishy

III. Questions for the TOMATO SAUCE

1. What is the colour of tomato sauce?
   .... very bright red
   .... slightly bright red
   .... just right
   .... slightly dark red
   .... very dark red

2. How does the mouth feel when eating the tomato sauce?
   .... very oily
   .... slightly oily
   .... just right
   .... slightly non-oily
   .... very non-oily

3. How is the sourness of the tomato sauce?
   .... very sour
   .... slightly sour
   .... just right
   .... slightly lacking in sourness
   .... very lacking in sourness

4. How is the saltiness of the tomato sauce?
   .... very salty
   .... slightly salty
   .... just right
   .... slightly lacking in saltiness
   .... very lacking in saltiness

5. How is the overall spiciness of the tomato sauce?
   .... very spicy
   .... slightly spicy
   .... just right
   .... slightly lacking in spiciness
   .... very lacking in spiciness

6. How is the fishiness of tomato sauce?
   .... very fishy
   .... slightly fishy
   .... just right
   .... slightly non-fishy
   .... very non-fishy

IV. Overall acceptability
   .... like it very much
   .... like it slightly
   .... neither like nor dislike
   .... dislike it slightly
   .... dislike it very much

V. Personal Data

1. Sex : .... male .... female

2. Age (years): Under 20
   20 - 30
   31 - 40
   41 - 50
   Over 50

3. Carrier : - school pupil
   - college or university student
   - government officer
   - working at company
   - private work
   - others, specify.....

4. Family income: Under Rp.150,000
Rp.150,000 - Rp.299,999
Rp.300,000 - Rp.500,000
Over Rp.500,000

5. City:.....

QUESTIONNAIRE FOR HOUSEWIFE OR PERSON BEING RESPONSIBLE FOR SHOPPING

INSTRUCTION: 1. Taste this product the way you would normally eat canned fish, but do not add with anything, e.g. spices, water, salt, etc
2. Please answer the questions by ticking the appropriate blank

I. How did you eat the canned fish?

(a) treatments:

.... the sample was heated (warmed)

.... no treatment at all

(b) with other foods:

.... with rice and/or other foods, e.g. vegetables....

.... with rice only

.... the sample only

.... others, please specify.....

II. Question about the FISH

1. How is the flesh texture when you bite it?

.... very tender

.... slightly tender

.... just right

.... slightly tough

.... very tough

2. How is the bone softness when you bite it?

.... very soft

.... slightly soft

.... just right

.... slightly hard

.... very hard

3. How is the sourness of fish?

.... very sour

.... slightly sour

.... just right

.... slightly lacking in sourness

.... very lacking in sourness

4. How is the saltiness of fish?

.... very salty

.... slightly salty

.... just right

.... slightly lacking in saltiness

.... very lacking in saltiness

5. How is overall spiciness of fish?

.... very spicy

.... slightly spicy

.... just right

.... slightly lacking in spiciness

.... very lacking in spiciness

6. How is fishiness of fish?
   .... very fishy
   .... slightly fishy
   .... just right
   .... slightly no fishy
   .... very no fishy

III. Questions for the TOMATO SAUCE

1. What is the colour of tomato sauce?
   .... very bright red
   .... slightly bright red
   .... just right
   .... slightly dark red
   .... very dark red

2. How is the mouth feel when eating the tomato sauce?
   .... very oily
   .... slightly oily
   .... just right
   .... slightly not oily
   .... very not oily

3. How is the sourness of the tomato sauce?
   .... very sour
   .... slightly sour
   .... just right
   .... slightly lacking in sourness
   .... very lacking in sourness

4. How is the saltiness of the tomato sauce?
   .... very salty
   .... slightly salty
   .... just right
   .... slightly lacking in saltiness
   .... very lacking in saltiness

5. How is the overall spiciness of the tomato sauce?
   .... very spicy
   .... slightly spicy
   .... just right
   .... slightly lacking in spiciness
   .... very lacking in spiciness

6. How is the fishiness of tomato sauce?
   .... very fishy
   .... slightly fishy
   .... just right
   .... slightly not fishy
   .... very not fishy

IV. Overall acceptability

.... like it very much
.... like it slightly
.... neither like nor dislike
.... dislike it slightly
.... dislike it very much

V. Buying trend Survey

1. Would you buy this product?
   .... yes
   .... no, please give a reason.....
   If "no", you do not need to continue to further questions.

2. How often would you buy it?
   .... everyday
   .... twice a week
   .... once a week
   .... once every two weeks
   .... once a month
   .... occasionally

3. What do you think would be a reasonable price for this product?
   Rp.....

4. When you inspect the label, what information do you prefer to retain?
   .... healthy information
   .... nutritional information
   .... ingredients
   .... net weight
   .... trade mark
   .... name and address of processor
   .... other information needed to be added, specify .....

5. Where would prefer to buy this product?
   .... drugstore
   .... supermarket
   .... food shop
   .... retailer
   .... others, please specify.....

6. What is your reason for buying this product?
   .... healthy reason
   .... nutritional reason
   .... family preference
   .... convenience, easy to serve
   .... reasonable price
   .... you like to eat it
   .... others, please specify .....

VI. If you have any comments about this product, please write them here!

.....

.....

.....

V. Personal Data

1. Sex : .... male .... female

2. Age (years): Under 20
20 - 30
31 - 40
41 - 50
Over 50

3. Carrier : - school pupil
- college or university student
- government officer
- working at company
- private work
- others, specify.....

4. Family income: Under Rp.150,000
Rp.150,000 - Rp.299,999
Rp.300,000 - Rp.500,000
Over Rp.500,000

5. City:.....

Appendix 12.2. Questionnaire form for medical doctor survey

QUESTIONNAIRE FOR DOCTOR SURVEY

Dear Sir/madam,

I am a post-graduate student in the Food Technology Department, Massey University, New Zealand, conducting research into the development of a new fish product for the Indonesian market.

The aim of this questionnaire is to obtain the opinion of the medical fraternity for my research into canned fish with fish oil added.

Hopefully, you can help me by completing this questionnaire. I appreciate your help and cooperation.
Thank you.

Hari Eko Irianto
Post Graduate Student
c/-Food Technology Department
Massey University
NEW ZEALAND

(1) A lot of experiments on fish oil have been conducted in order to show the benefit to our health, especially the reduction of coronary disease risk.

(a) Have you ever suggested your patients consume fish oil for their health?

( ) yes
( ) no

(b) Have you ever suggested your patients eat more fish for their health?

( ) yes
( ) no

(2) If you think that the fish oil is good for our health, in which form you find that your patients should consume fish oil.

( ) capsule ( ) table spoon
( ) salad oil ( ) disguised in ordinary foods
( ) others, specify .....

Please consider this idea:
I would like to introduce canned fish which contains fish oil disguised in the medium. The rational behind this idea is to optimise the fish oil utilization and to produce canned fish nutritionally better than the existing in the market.

(3) Do you think that the product described above is a good way to increase fish oil intake of consumer?

( ) yes
( ) no
( ) maybe

(4) Do you think that this product has a good prospect in the market from the medical point of view?

( ) yes
( ) no
( ) maybe

(5) If this product is available in the market, would you advice your patients to buy for health reason?

( ) yes
( ) no
( ) maybe

==========

Please fill in information below:

1. How long have you been a doctor ? ..... years
2. How many patients do you have in average each month? .....
   people
3. Where are you from originally? .....
4. Speciality .....

Appendix 12.3. Information on the label of the developed canned fish product distributed during consumer testing

"OMEGITA" - Ikan sardine dalam saus tomat

* diperkaya dengan minyak ikan
  (asam lemak omega-3)
* tanpa bahan pengawet

Bahan:
ikan pilchard, air, minyak ikan, pasta tomat, gula, bawang merah, bawang putih, garam dan cuka

Komposisi:
Air 68.2%, protein 14.6%, lemak 10.1%, karbohidrat 4.3%, abu 2.8%

Departemen Teknologi Pangan
Massey University, SELANDIA BARU

English translation of the label:

"OMEGITA" Sardine in tomato sauce

* enriched with fish oil (omega-3)
* no preservatives

Ingredients:
pilchard, tomato paste, water, fish oil,sugar, shallot, garlic and vinegar

Nutritive values:
Moisture 68.2%, protein 14.6%, fat 10.1%, carbohydrate 4.3%, ash 2.8%

Food Technology Department
Massey University, NEW ZEALAND

Appendix 12.4. Medical doctor's comments on developed canned fish

Demographic characteristic	Can canned fish be used for fish oil consumption improvement?			Does canned fish have a good prospect in Indonesia?			Will you suggest your patient to consume this product?		
	YES	NO	MAYBE	YES	NO	MAYBE	YES	NO	MAYBE
Speciality:	(N/%)	(N/%)	(N/%)	(N/%)	(N/%)	(N/%)	(N/%)	(N/%)	(N/%)
Pathologist	0/0	1/100	0/0	1/100	0/0	0/0	0/0	1/100	0/0
Paediatrist	8/38	1/5	12/57	20/95	0/0	1/5	20/95	0/0	1/5
Internist	14/74	3/16	2/10	19/100	0/0	0/0	18/95	0/0	1/5
Obstetrician-gynaecologist	19/90	2/10	0/0	17/81	1/5	3/14	21/100	0/0	0/0
Gen.prac.	73/72	27/27	1/1	46/46	3/3	52/51	88/87	13/13	0/0
Experience (years):									
<5	58/73	18/23	3/4	41/52	3/4	35/44	70/89	9/11	0/0
5 - 10	20/83	4/17	0/0	13/54	0/0	11/46	22/92	2/8	0/0
11 - 15	15/48	8/26	8/26	22/71	2/6	7/23	27/87	3/10	1/3
16 - 20	8/73	2/18	1/9	10/91	0/0	1/9	11/100	0/0	0/0
>20	13/72	2/11	3/17	16/89	0/0	2/11	17/94	0/0	1/6
Number of total patient/month:									
<100	9/56	6/38	1/6	8/50	2/13	6/37	13/81	3/19	0/0
100 - 300	38/62	20/33	3/5	30/49	3/5	28/46	50/82	10/16	1/2
301 - 500	41/82	6/12	3/6	34/68	0/0	16/32	49/98	1/2	0/0
501 - 700	15/71	0/0	6/29	18/86	0/0	3/14	21/100	0/0	0/0
>700	11/73	2/13	2/13	12/80	0/0	3/20	14/93	0/0	1/7
Number of patient having heart problem/month									
<10	75/68	27/24	9/8	62/56	5/4	44/40	97/87	13/12	1/1
10 - 30	24/73	5/15	4/12	22/67	0/0	11/33	32/97	1/3	0/0
31 - 50	11/85	1/8	1/8	13/100	0/0	0/0	12/92	0/0	1/8
>50	4/67	1/17	1/17	5/83	0/0	1/17	6/100	0/0	0/0

Appendix 12.5. Fatty acid profile changes in fish oil and canned fish product during production trial

Fatty acids	Fish oil	Tomato sauce		Fish		Canned fish product	
		Unsterilized	Sterilized	Unsterilized	Sterilized	Unsterilized	Sterilized
14:0	4.4	4.2	4.3	6.7	6.3	5.6	6.1
15:0	0.8	0.8	0.8	1.8	1.3	0.9	0.9
16:0	17.0	17.3	17.6	27.4	26.2	20.1	19.6
16:1	5.5	5.2	5.4	3.7	5.0	4.9	5.6
17:0	0.7	0.7	0.8	1.9	1.2	0.9	0.9
17:1	1.4	1.3	1.3	1.4	1.5	1.5	1.3
18:0	5.5	5.9	5.9	7.1	6.1	6.1	5.6
18:1	33.9	32.7	32.2	15.0	15.5	28.6	28.9
18:2	1.8	2.0	1.9	3.0	2.0	2.0	2.0
18:3	1.7	1.9	1.8	1.7	1.7	1.8	1.7
18:4	2.4	2.6	2.6	1.2	1.5	2.3	2.3
20:1	5.1	4.1	4.0	3.3	2.9	3.5	3.6
20:3	0.2	0.2	0.2	0.3	0.3	0.2	0.2
20:4	0.8	0.8	0.8	1.6	1.2	0.9	0.8
20:5	6.6	7.7	7.8	5.7	7.1	7.5	7.6
22:1	2.1	1.7	1.6	2.1	1.3	1.1	1.3
22:4	0.2	0.1	0.2	0.2	0.2	0.1	0.1
22:5	1.6	1.8	1.8	0.7	1.2	1.6	1.4
22:6	8.3	8.8	8.9	15.2	17.2	10.1	9.9

Appendix 12.6. Chi square, degree of freedom and Cramer's V of crosstab analysis results from Consumer product testing

Variables	Chi-Square	DF	Cramer's V
Age*Fish texture	419.528	20	0.510
Age*Bone softness	421.842	20	0.512
Age*Fish sourness	452.120	25	0.470
Age*Fish saltiness	413.287	25	0.453
Age*Fish spiciness	433.078	25	0.464
Age*Fish fishiness	424.518	25	0.459
Age*Sauce saltiness	416.768	25	0.455
Age*Sauce sourness	420.914	25	0.457
Age*Sauce fishiness	444.056	25	0.469
Age*Sauce spiciness	418.504	25	0.456
Age*Sauce mouth feel	440.45	25	0.468
Age*Sauce colour	432.182	25	0.463
Age*Overall product acceptability	427.18	25	0.460
Carrier*Fish texture	428.809	32	0.516
Carrier*Bone softness	426.178	32	0.514
Carrier*Fish sourness	478.524	40	0.487
Carrier*Fish saltiness	428.680	40	0.461
Carrier*Fish spiciness	442.877	40	0.469
Carrier*Fish fishiness	441.814	40	0.468
Carrier*Sauce saltiness	442.583	40	0.469
Carrier*Sauce sourness	450.692	40	0.473
Carrier*Sauce fishiness	432.255	40	0.463
Carrier*Sauce spiciness	435.783	40	0.465
Carrier*Sauce mouth feel	442.048	40	0.468
Carrier*Sauce colour	434.724	40	0.464
Carrier*Overall product acceptability	446.308	40	0.471
City*Fish texture	445.671	20	0.526
City*Bone softness	426.886	20	0.515
City*Fish sourness	450.078	25	0.473
City*Fish saltiness	440.099	25	0.467
City*Fish spiciness	449.642	25	0.472
City*Fish fishiness	479.457	25	0.488
City* Sauce saltiness	443.173	25	0.469
City*Sauce sourness	435.087	25	0.465
City*Sauce fishiness	491.112	25	0.494
City*Sauce mouth feel	450.611	25	0.473
City*Sauce colour	443.263	25	0.469
City*Overall product acceptability	483.294	25	0.490
Income*Fish texture	433.829	16	0.519
Income*Bone softness	436.183	16	0.520
Income*Fish sourness	432.871	20	0.518
Income*Fish saltiness	435.713	20	0.520
Income*Fish spiciness	430.547	20	0.517
Income*Fish fishiness	434.469	20	0.519

Continuation of appendix 12.6

Variables	Chi-Square	DF	Cramer's V
Income*Sauce saltiness	421.648	20	0.511
Income*Sauce sourness	429.599	20	0.516
Income*Sauce fishiness	415.842	20	0.508
Income*Sauce spiciness	428.009	20	0.515
Income*Sauce mouth feel	420.234	20	0.511
Income*Sauce colour	418.506	20	0.510
Income*Overall product acceptability	431.055	20	0.517
Sex*Fish texture	405.297	8	0.709
Sex*Bone softness	417.379	8	0.720
Sex*Fish sourness	405.240	10	0.709
Sex*Fish saltiness	404.864	10	0.709
Sex* Fish Spiciness	412.524	10	0.715
Sex*Fish fishiness	410.556	10	0.714
Sex*Sauce saltiness	407.731	10	0.711
Sex*Sauce sourness	405.117	10	0.709
Sex*Sauce fishiness	406.215	10	0.710
Sex*Sauce spiciness	410.026	10	0.713
Sex*Sauce mouth feel	407.081	10	0.711
SeX*Sauce colour	406.246	10	0.710
Sex*Overall product acceptability	411.316	10	0.714
Age*Buying trend	148.382	10	0.718
Age*Buying frequency	158.558	30	0.469
Carrier*Buying trend	150.610	14	0.723
Carrier*Buying frequency	171.705	42	0.446
City*Buying trend	153.624	10	0.730
City*Buying frequency	184.264	30	0.506
Income*Buying trend	148.002	8	0.717
Income*Buying frequency	163.210	24	0.532
Sex*Buying trend	145.290	4	0.710
Sex*Buying frequency	148.157	12	0.717
Canned fish customer*Buying trend	150.27	4	0.722
Canned fish customer*Buying frequency	152.43	12	0.728
Fish species selection*Buying trend	145.633	4	0.711
Fish species selection*Buying frequency	152.023	12	0.727
Medium selection*Buying trend	145.856	4	0.712
Medium selection*Buying frequency	148.334	12	0.718
Overall prod.accept*Buying trend	189.896	10	0.812
Overall prod.accept*Buying frequency	200.731	30	0.528

Note: DF = degree of freedom
Cramer's V classification (Craft, 1990):

< 0.10	= weak association
0.11 - 0.25	= weak to moderate association
0.26 - 0.40	= moderate association
0.41 - 0.50	= moderate to strong association
> 0.50	= strong association

Appendix 12.7. Chi-square, degree of freedom and Cramer's V of crosstab analysis results from medical doctor survey

Variables	Chi-square	DF	Cramer's V
Speciality*Fish oil consumption suggestion	172.258	10	0.725
Speciality*Fish consumption suggestion	171.319	10	0.723
Speciality*Fish oil consumption method	195.114	20	0.545
Speciality*Product idea	240.312	15	0.699
Speciality*Product prospect	234.070	15	0.690
Speciality*Product consumption suggestion	188.780	15	0.619
Num.of HP*Fish oil consumption suggestion	169.314	8	0.718
Num.of HP*Fish consumption suggestion	166.284	8	0.712
Num.of HP*Fish oil consumption method	176.591	16	0.519
Num.of HP*Product idea	167.689	12	0.584
Num.of HP*Product prospect	176.556	12	0.599
Num.of HP*Product consumption suggestion	173.476	12	0.594
Num.of GP*Fish oil consumption suggestion	168.439	10	0.717
Num.of GP*Fish consumption suggestion	166.208	10	0.712
Num.of GP*Fish oil consumption method	188.007	20	0.535
Num.of GP*Product idea	189.886	15	0.612
Num.of GP*Product prospect	182.844	15	0.610
Num.of GP*Product consumption suggestion	181.925	15	0.608
Work exp.*Fish oil consumption suggestion	172.869	10	0.726
Work exp.*Fish consumption suggestion	168.972	10	0.718
Work exp.*Fish oil consumption method	176.626	20	0.519
Work exp.*Product idea	183.614	15	0.611
Work exp.*Product prospect	181.813	15	0.608
Work exp.*Product consumption suggestion	172.682	15	0.592

Note: HP = Patients having heart problem
GP = General patients
DF = degree of freedom
Cramer's V classification (Craft, 1990):

< 0.10	= weak association
0.11 - 0.25	= weak to moderate association
0.26 - 0.40	= moderate association
0.41 - 0.50	= moderate to strong association
> 0.50	= strong association