EFFECT OF SUPERHEATED STEAM (SHS) PRE-TREATMENT ON QUALITY OF FISH OIL FROM CATFISH (*Clarias batrachus*)

by

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TABLE OF CONTENTS

CHAPTER 1 – INTRODUCTION

CHAPTER 2 – LITERATURE REVIEW

CHAPTER 3 – MATERIALS AND METHODS

CHAPTER 4 - RESULTS AND DISCUSSION

CHAPTER 5 – OVERALL CONCLUSIONS AND RECOMMENDATIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Symbols/Abbreviation	**Caption**
PUFA	Polyunsaturated fatty acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
MYR	Malaysian Ringgit
SHS	Superheated steam
NH3	Hydrogen nitride (Ammonia)
NH_4^+	Ammonium
SFE	Supercritical fluid extraction
O_2	Oxygen
CO_2	Carbon dioxide
FAO	Food and Agriculture Organization
BIOHAZ	Biological Hazard
EFSA	European Food Safety Authority
FFA	Free fatty acid
CFAM	Cyclic fatty acid monomers
POP	Persistent organic pollutants
PCB	Polychlorinated biphenyls
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSC	Differential scanning calorimeter
FA	Fatty acid
FAME	Fatty acid methyl esters
GC	Gas chromatography
MUFA	Monounsaturated fatty acid
SFA	Saturated fatty acid
AV	Anisidine value
NaOH	Sodium hydroxide
CH_3COOH	Acetic acid

$CHCl_3$	Chloroform
KI	Potassium iodide
dH_2O	Distilled water
$Na_2S_2O_3$	Sodium thiosulfate
mEq/kg	Miliequivalents per kilogram
IUPAC	International Union of Pure and Applied Chemistry
RSC	Radical scavenging capacity
BF_3	Borontrifluoride
ANOVA	Analysis of variance
KOH	Potassium hydroxide
GOED	Global Organization for EPA and DHA
TOTOX	Total oxidation

KESAN STIM PANAS LAMPAU (SPL) ATAS IKAN KELI (*Clarias batrachus*) TERHADAP KUALITI MINYAK IKAN YANG DIPERLOEHI

ABSTRAK

Stim panas lampau (SPL), iaitu stim kering yang mempunyai suhu yang lebih tinggi daripada takat didih dan beroperasi dalam kondisi tiada/kurang oksigen digunakan untuk merawat ikan keli, *Clarius batrachus*, sebelum minyak diekstrak melalui mesin "hydraulic press". Ikan dibersihkan dan dipotong kepada saiz yang lebih kecil sebelum dirawat pada tiga suhu berbeza iaitu, 150°C, 200°C, 250°C, untuk 10, 15, 20 minit dalam setiap suhu. Selepas dirawat, ikan dimasukkan kedalam acuan dan minyak diperah dengan "hydraulic press" yang hasil perahan kemudiannya diemparkan dan minyak yang terpisah dikumpulkan. Kualiti minyak ikan dikaji dari segi hasil, warna, pengoksidaan, analisis terma dan komposisi asid lemaknya. Minyak ikan keli menunjukkan hasil yang lebih tinggi apabila dipanaskan pada suhu SPL yang lebih tinggi, yang mana hasil tertinggi didapati daripada rawatan 250°C, 20 minit (8.91 gram/gram ikan) berbeza signifikan pada $p > 0.05$. Walau bagaimanapun, hasil ini lebih rendah daripada pengekstrakan kimia dan fakta ini telah terbukti dalam literatur yang lepas. Analisis warna menunjukkan kencendurangan kepada warna kuning yang lebih dalam apabila suhu dan masa rawatan dipanjangkan. Dari segi nilai asid lemak bebas, minyak ikan keli mentah dari ikan mentah yang diekstrak menggunakan pengekstrakkan kimia mempunyai nilai yang tertinggi (2.56% asid oleik) dan nilai ini lebih tinggi dari standard untuk minyak ikan. Walau bagaimanapun, kebanyakan nilai peroksida tidak ketara. Tahap oksidasi jelas dalam nilai p-anisidine yang berbeza secara signifikan, yang mana minyak ikan yang terhasil pada 250°C, 20 minit pra-rawatan SPL, menunjukkan peningkatan mendadak kepada 12.05 yang mana nilainya berbeza secara signifikan. Nilai ini masih dalam had standard minyak ikan. Nilai TOTOX juga menunjukkan nilai yang paling tinggi secara signifikan dalam minyak ikan yang terhasil dari 250°C, 20 minit rawatan SPL (15.79) tetapi masih dalam had standard minyak ikan (maksimum 26). Begitu juga nilai iodine menunjukkan penurunan dalam minyak ikan yang terhasil dari 250°C rawatan SPL, yang mana nilai-nilai ini adalah berbeza secara signifikan apabila dibandingkan dengan nilai-nilai dari suhu rawatan 150°C dan 200°C. Minyak ikan keli terhasil dari ikan yang tidak drawat mempunyai keupayaan mengaut radikal yang tertinggi yang mempunyai perbezaan yang signifikan. Asid lemak utama yang hadir dalam minyak ikan keli termasuk C16 (asid palmitik), C18:1 (asid oleik), dan C18:2 (asid linoleik) yang mana minyak ikan yang terhasil dari 250°C, 20 minit rawatan SPL menunjukkan penurunan nisbah asid lemak tak tepu kepada asid lemak tepu. Takat lebur minyak ikan keli mentah daripada sampel ikan dirawat dengan SPL adalah dalam julat -2.36°C ke 10.56°C kecuali minyak ikan mentah; -6.24°C ke 3.07°C. Penggunaan SPL menunjukkan indeks pengoksidaan yang lebih rendah dalam banyak aspek berbanding minyak ikan keli diekstrak menggunakan pengekstrakan kimia asalkan suhu SPL tidak melebihi 200°C.

EFFECT OF SUPERHEATED STEAM (SHS) PRE-TREATMENT ON QUALITY OF FISH OIL FROM CATFISH (*Clarius batrachus)*

ABSTRACT

Superheated steam (SHS), a dry steam with temperature higher than boiling point that operates without the presence of oxygen is used to pre-treat catfish, *Clarius batrachus,* before being subsequently pressed via hydraulic press to obtain fish oil. Fish were first cleaned and reduced in size before being treated with SHS at 150°C, 200°C, 250°C, for 10, 15, 20 min for each temperature. Fish are then transferred into a mould, pressed using a hydraulic press and the resulting aliquot is centrifuged and oil is collected. Quality of the resulting fish oil is studied in terms of yield, colour, oxidation, thermal analysis and fatty acid composition. Yield of the catfish oil showed higher values when heated with higher temperatures of SHS, the highest being the 250°C, 20 minutes pre-treatment (8.91 gram/gram fish, significantly different at $p> 0.05$) which was incidentally lower than solvent extraction and this fact has been proven in past literature. Colour analysis showed tendency towards a darker yellow colour as treatment time and temperature increased. In terms free fatty acids (FFA) values, solvent extracted raw catfish oil had the highest and most significant value (2.56% oleic acid) and this reflected in high acid value beyond that of acceptable edible oil standard. However, peroxide values were mostly insignificantly different. The extent of oxidative damage was obvious in the p-anisidine values whereby at 250°C, 20 minutes pre-treatment, there was a sharp rise to 12.05 which, although significantly different, was still within edible oil standard. TOTOX value also showed the highest significantly different value at 250°C, 20 minutes SHS pre-treatment but was still within edible oil standard. Similarly iodine value also showed significantly different value decrease at 250°C SHS pre-treatment temperature when compared to values of 150°C and 200°C temperature treated fish oils. Raw catfish oil had highest significant radical scavenging capacity. Major fatty acids present in catfish oil include C16 (palmitic acid), C18:1 (oleic acid), and C18:2 (linoleic acid) whereby, the 250°C, 20 minutes SHS pre-treatment showed difference in terms of a lower unsaturated fatty acids (UFA) to saturated fatty acids ratio. Melting points of crude catfish oil from fish samples pre-treated with SHS ranged from -2.36°C to 10.56°C except for crude fish oil from raw samples that ranged from -6.24°C to 3.07°C. SHS pre-treatment showed lower oxidation indexes in many aspects when compared to solvent extracted catfish oil given that the temperature does not exceed 200°C.

CHAPTER 1

INTRODUCTION

1.1 Background

Global market for high quality fish oils has notably increased both for consumption and as a constituent in industries such as pharmaceutical (Abdulkadir et al., 2010; Aberoumand, 2012). Fish oils are also coveted because it is the primary natural source of omega-3 polyunsaturated fatty acids (Rubio-Rodríguez et al., 2012; García-Moreno et al., 2013). Fish oils are effective against a plethora of disease that range from asthma to cardiovascular diseases mainly due to the presence of these omega-3 polyunsaturated fatty acids (PUFA) (Ramakrishnan et al., 2013). Long chain PUFAs that particularly contribute to the health benefits of fish oil consumption are namely eicosapentaenoic (EPA) and docosahexaenoic (DHA) (Calder, 2004; de Castro et al., 2007). Other importance of fish oils include usage as raw material for the processing of biofuels (Jayasinghe et al., 2013). Meeting demands of fish oils have brought about the need to locate new sources and propelled research towards elucidating characteristics of these sources and methods to procure them (Einig and Ackman, 1997; Aberoumand, 2010).

The catfish, known as Keli in Malaysia is a widely accepted freshwater fish popularly consumed due to its abundance, being good source of protein, and cheaper prices compared to other types of freshwater fish (Sivakumar et al., 2000; Kiew & Mat Don, 2013). Since catfish in Malaysia has been predominantly intended for daily consumption, an attempt to increase commercial value is a new frontier therefore not limiting it as just food and widening its use in other industries (Kiew & Mat Don, 2013). Besides that, catfish aquaculture was valued at approximately 46, 522 tonnes (166.95 million MYR) in Malaysia in 2012 (Department of Fisheries, Malaysia, 2012). Catfish has also been reported to contain considerable fat content of about 6.91% (Islam et al., 2013). It is considered a moderately fatty fish similar to likes of salmon (Dean, 1990), which is a source of commercial edible fish oil (Crouzier et al., 2011). Thus, utilising such inexpensive natural resources for conversion into commercial products (in this case fish oil) would enable economic provisions to both the fisheries industry and local fishermen in Malaysia (Kiew & Mat Don, 2013). Many species of fish have contributed to the abundance of a variety of fish oils (Kris-Etherton et al., 2002).

Fish oil is commonly prepared in the industries by cooking and pressing usually involving some form of heating followed by mechanical extraction and finally centrifugation to separate fat components from other constituents (Dauksas et al., 2005; Ramakrishnan et al., 2013). However, since the method involves use of heat, its application would inevitably damage PUFA resulting in products of secondary lipid oxidation eventually giving way to rancidity and formation of compounds that cause off-flavour in food (Aubourg & Medina, 1997; Kong et al., 2008).

Lipid oxidation, an inescapable occurrence in fish oils, involves a rather complicated mechanism but since oxidation gives way to several mechanisms and several different products of oxidation, the resulting products of oxidation and oxidation pathways intertwine making the entire phenomenon difficult to decipher (Indrasena & Barrow, 2011). Contributing factors such include light, heat, enzymes, and microbes, all of which gives rise to the development of undesirable odours and nutrient loss (Shahidi & Zhong, 2005). Fish oils are especially susceptible to the complex mechanism of lipid oxidation due to the presence of long chain PUFA (Kaitaranta, 1992), therefore there is a demand for processing technologies that can reduce oxidative damage without compromising content. Lipid oxidation also pose significant economic importance when it comes to fish oils in particular and thwarting oxidation paramount in ensuring prolonged shelf life and slowing down or disrupt the deterioration of important nutrient components in (Wasowicz et al., 2004).

Superheated steam (SHS) is a dry steam that is derived when heat is applied to wet steam subsequently raising steam temperature higher than the resulting boiling point at a given pressure (Head et. al., 2010). SHS operates without the presence of oxygen which can essentially reduce susceptibility to ignition and oxidation, allowing retention of important organic compounds that add value to the end-product (Mujumdar & Law, 2010). Besides that, low energy usage and little or no discharge from the process adds to the fact that treatment with SHS results in better end-product quality (Bórquez, 2003). These criteria make it an attractive alternative to heat fish to enable oil expression with minimal oxidative damage and improved oil quality.

As it can be ascertained no work has been done on the effects of SHS heat pre-treatment on the quality of *Clarias batrachus* oil recovered using mechanical extraction. This thesis attempts to study the effects of SHS treatment followed by mechanical pressing on the quality of catfish (*Clarias batrachus*) oil.

1.2 Problem statement

Fish oils are highly susceptible to oxidation and thus require proper heat treatment and extraction. Conventional heating treatments tend to cause oxidation due to heating in a non oxygen-free environment. SHS has been so far used predominantly as a drying technology and it is possible that the conditions of SHS may improve fish oil quality.

1.3 Importance of study

SHS has only been associated with drying and these results would provide insight into the possibility of cooking raw materials prior to fish oil extraction without compromising on crude fish oil quality. Fish oils resulting from this study could be higher in quality than commercially produced fish oils as it would be heat-treated without the presence of oxygen and thus reducing lipid oxidation.

1.4 Objectives

The main objectives of this study are:

1. To determine the effects of superheated steam treatment at 150, 200 and 250 °C for 10, 15 and 20 minutes on yield and colour of catfish oil extracted via hydraulic pressing.
2. To determine the effects of superheated steam treatment at 150, 200 and 250 °C for 10, 15 and 20 minutes on lipid oxidation of catfish oil.

3. To determine the effects of superheated steam treatment at 150, 200 and 250 °C for 10, 15 and 20 minutes on fatty acids content of catfish oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Fish Oil

Over many years, global fish oil production numbers have persisted between 1 million and 1.25 million tons; an indication of the sustainability of the fishing industry and post production of fisheries (Pike and Jackson, 2010). Deepening concerns toward nature and the ecosystem coupled with the urge to live a healthier lifestyle has become consumer-favorite notions, pushing developers to adopt chemical free, eco-friendly processing of products (Febrianto and Yang, 2011). However, where fish oils are concerned, post processing of crude oil is deemed necessary because unpurified fish oil contain various impurities (free fatty acids, primary oxidation products, minerals, pigments, moisture, phospholipids etc) which decrease shelf life and negatively impact oil quality (Huang and Sathivel, 2010).

Fish oils have been revered for the abundance of nutritional value and the health benefits that entail them namely polyunsaturated fatty acids (PUFA). In the past decade alone numerous research and clinical trials have been performed to validate its potency in terms of therapeutic effects against an array of health conditions i.e. reduction of risk of cardiovascular diseases (He, 2009), promotion of immune function (Gray et al., 2012), promotion of the expression of genes regulating anti-inflammatory response (Bouwens et al., 2009), therapeutic against rheumatoid arthritis (Berbert et al., 2005) and many others. Generally fatty acids can be synthesized by human body, but omega-3 and omega-6 fatty acids - the essential fatty acids - are not synthesizable and therefore are consumed via diet (Rubio-

Rodríguez et al., 2010). Therein lays its importance and necessity to human health. The essential fatty acids, PUFAs specifically, are the omega fatty acids abundant in fish and among them, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are frequently cited as vastly beneficial (Cottin et al., 2011; Sorgi et. al., 2007; Zhang et. al., 2007).

2.2 Sources of Fish Oil

As knowledge of the masses accumulate with regards to the benefits of PUFAs and also taking into account its limited dietary sources, PUFA concentrates have become significantly important in terms of demand, leading to its presence in value-enhanced end products especially in pharmaceutics, food additives, and health supplements (Sahena et al., 2010).

Fish sources for fish oil, namely fish that are high in EPA and DHA, include red mullet (*Mullus surmuletus*), menhaden (*Brevoortia tyrannus*), and anchovies (*Engraulis encrasicolus*) among others (Rubio-Rodríguez et al., 2010). Table 2.1 details important PUFAs, EPA and DHA content in several fish sources. By products of fish fillet production is a coveted source of raw material for fish oil production, for example, from 10,000 kg cod fillets produced, more than 1000 kg marine lipids can be sourced from its by-products alone. Of this 1000 kg lipid 30% contain n-3 fatty acids. Lipid is extractable from liver, viscera, trimmings, muscle and gonads (Falch et al., 2006). Fishing industry in Alaska also holds substantial salmon by-products i.e. heads, skin, and viscera, all of which can be utilised for oil production (Huang and Sathivel, 2010).

Table 2.1: EPA and DHA content in some popular fish modified from Kris-Etherton and others' work.

Fish	EPA+DHA Content, g/3-oz Serving Fish (Edible Portion)
Tuna	0.24-1.28
Sardines	0.98-1.70
Salmon, Atlantic, farmed	1.09-1.83
Salmon, Atlantic, wild	0.9-1.56
Salmon, Chum	0.68
Salmon, Sockeye	0.68
Salmon, Pink	1.09
Salmon, Chinook	1.48
Mackerel	0.34-1.57
Herring, Pacific	1.81
Herring, Atlantic	1.71
Trout, rainbow, farmed	0.98
Trout, rainbow, wild	0.84
Halibut	0.4-1.0
Cod, Pacific	0.13
Cod, Atlantic	0.24
Haddock	0.2
Catfish, farmed	0.15
Catfish, wild	0.2
Flounder/sole	0.42

Values are rough estimates because oil content can vary depending on species, season, diet, packaging and cooking methods (Kris-Etherton et al., 2002).

Although most of these species are of marine origin, freshwater fish have also been studies to view fatty acid contents whereby fish like tilapia (*Oreochromis niloticus*), elephant fish (*Hyperopisus bebe*) and the Nile perch (*Lates niloticus*) have been compared to marine fish sources. In this particular study, the author concluded that due to higher content of linoleic acid (18:2n-6) and arachidonic acid (20:4n-6), freshwater fish oils are much higher in quality compared to marine fish oils (Ugoala et al., 2008). Similarly, Wahidu and others studied tilapia *(Oreochromis niloticus)*, and two types of catfish (*Pangasius pangasius* and *Clarias batrachus*). Fat was extracted from fish muscles and by-products (unspecified) and recorded high fat content (Zzaman et al., 2014).

2.3 Catfish

The catfish *Clarias batrachus* is a significant freshwater fish for culture in Malaysia due to its suitability for culture and also characteristics like the ability to survive in conditions of low oxygen and with an efficient growth rate (Cheah et al., 1990). Such conditions of catfish habitat can even extend to murky and shallow waters where other kinds of fish that can be cultured may not thrive as well (Jayakumar & Paul, 2006).

2.3.1 Description of catfish

The catfish is a freshwater fish belonging to the family Clariidae, under sub-order Siluroidei (Armbruster, 2011). Locally known as "ikan keli", it is an important aquaculture fish variety in Malaysia, second to only tilapia in percentage cultured besides being a staple for daily consumption due to being more cost-effective (Kiew & Mat Don, 2013). The catfish is usually more or less cylindrical with a gray or silver body, a large mouth, and no scales. Probably the most noticeable characteristic of the fish that has garnered the name 'catfish' would be presence of barbels near the mouth (Fink and Fink 1981; Armbruster, 2011). The catfish is omnivorous being able to feed on a broad array of prey and is also able to live in conditions of low oxygen and murky waters (Siraj et al., 2009).

Although every country on Earth has catfish which people most associate with common descriptions, there are 37 families of catfishes known now with approximately 3,407 species whereby just about 10.8% of all fishes and 5.5% of all vertebrates happen to be catfishes (Armbruster, 2011). According to the Annual Fisheries Statistics by the Department of Fisheries, Malaysia, freshwater catfish aquaculture was valued at 166.95 million MYR in 2012 (at approximately 46, 522 tonnes), which increased 9% in value from the previous year (2011; 153.06 million MYR). Catfish (keli kayu/ keli bunga) is classified as freshwater catfish represented by two species, *Clarias batrachus* and *C. marcrocephalus* (Department of Fisheries, Malaysia, 2012).

Figure 2.1: Catfish, *Clarias batrachus*

2.3.2 Study of catfish

Growing interest in "catfish science" or study of the order Siluriformes, has seen addition to scientific literature in the form of published articles, particularly from the years 2005 to 2010 in areas of biology, ecology, techniques, fisheries management, conservation, and aquaculture (Kwak et. al., 2011). Various studies have been done on catfish in the past which included it in the form of fish oil in terms of refining (Sathivel & Prinyawiwatkul, 2004); in terms of oil production from crude fish oil (Sathivel et. al., 2003c); and effects of thermal degradation on fatty acids (Sathivel et. al., 2003b). *Clarias batrachus* has been studied in terms of growth and feed ratio whereby authors studied effects of feed carbohydrate-to-lipid ratio on its growth and found out that extreme ratios caused poor feed conversion (Erfanullah, 1998).

Other catfish species was also used in studying palm oil by-product used as feed in terms of growth, vitamin E content, and oxidative stability in fish muscle (Ng et. al., 2004). The African catfish (*Clarias gariepinus*) is highly tolerant to ammonia toxicity made possible by its defence strategy of active NH_4^+ excretion (Ip et. al., 2004) and is this has been further investigated to reveal that catfish can withstand 0.34 mg NH3-N/L water without significant changes (Schram et. al., 2010). It has also been studied for extraction of collagen (Kiew & Mat Don, 2013) and has been reported to be a good source collagen (Sivakumar et. al., 2000).

More recent advances have gone into the study of *Clarias batrachus* catfish oil content which was recorded at 9.28% in muscle and 17.16% from its by-products (Zzaman et al., 2014) putting it in the high fat content fish category that has more than 8% fat according to Ackman and, where oil from its by-products is concerned, is also considered high fat fish according to Dean (Ackman, 1989; Dean, 1990).

Catfish oil has also been used with response surface method for optimisation of oil recovery whereby catfish oil was extracted via an optimal SHS treatment of 180°C for 18 minutes (with oil recovery of approximately 5.27% per wet weight). Catfish oil from this optimisation was evaluated for lipid oxidation using peroxide value, p-anisidine value, free fatty acids and iodine value results of which met standards for edible oil (Latip et. al., 2014).

2.4 Fish Oil Processing

The expression of oils and fats from various sources; be it animal based or plant based raw materials, oil extraction have been done over millennia ago with the purpose of obtaining high yield/extractability without compromising on quality (O'Brien, 2004). There have been, in the past various studies on the methods of extraction that can be used to extract these edible oils (Shah et al., 2005; Rai et al., 2010; Okada & Morrissey, 2007).

2.4.1 Extraction methods

These processing conditions include several types and are made of up different processing levels. Among these processing types include solvent extraction as exhibited by Aryee and Simpson in extracting fish oil from salmon skin with different solvent systems. Fish oil from these different solvent systems was then analyzed for efficiency in terms of oil yield and quality. Solvents used include hexane–isopropanol and chloroform-methanol. Extraction methods used include Soxhlet extraction and Randall extraction (Soxtec). Results indicated that salmon skin was rich in oil (23.32–61.53% dry weight basis) and fared well in quality post extraction (considerably low in free fatty acid content, 0.60–1.19%). Study also reported that the Soxtec method with hexane as a solvent gave the highest yield, 62% on a dry weight basis (Aryee and Simpson, 2009).

Herring fish (*Clupea harengus*) was also studied by Adeniyi, (2006) for its feasibility for oil recovery. The extraction was carried out via Soxhlet apparatus with frozen herring fish although the study did not mention its usage of the said method in an industrial scale. Author also suggested the possibility of using resulting herring oil which was refined via degumming, neutralizing, drying, and decolorizing, in the pharmaceutical, soap, and food industries (Adeniyi, 2006). However, production of edible fish oil for human consumption does not utilise solvent extraction due to disadvantages of using solvents plus the added restrictions within the food industry since usage of solvent poses the risk of leftover solvent in the product which, if and when consumed, can cause adverse health effects (Rubio-Rodríguez et al., 2010; Sarker et al., 2012).

The mechanical counterpart to solvent extraction include the use of pressure and force to expel lipid content from the raw material; homogenisation and pressing. Pre-treatments like cooking and drying further facilitate the expulsion of lipids from raw material while centrifugation and straining separate the oil from solids and unwanted particles (FAO, Fisheries Department, Fisheries Industries Division, Italy, 1986). The production of fish oil deals with the separation of fatty substances (lipids) from other constituents of the fish. Production of fish oils usually starts from the treatment of the raw material and ends at the purification of crude fish oil. Hydraulic pressing is commonly used methods in the fish oil industry; fish oil is obtained from moderately cooked fish that has high fat content and then expression by pressing the fish in several working batches (Sahena et al., 2009). Where mechanical pressing is concerned, albeit a less efficient process, it has advantages over chemical extraction in terms of high investment, energy requirement and use of dangerous chemicals (Sharma et al., 2002).

2.4.2 Mechanical oil recovery

Mechanical means of oil recovery is basically the use of force to break down cells (cell walls in the case of plant cells), aimed at releasing oils from within the cells (Mercer & Armenta, 2011). Although the basic principle is the same, there are different methods to achieve it which include, hydraulic pressing, screw press and rolling press (Deli et al., 2011; Bamgboye & Adejuno, 2007). Mechanical oil recovery has advantages aplenty since it is principally safe, the process is fairly simple with better conserved inherent properties, and, the end-products are chemical free, although where efficiency is concerned solvent extraction has an upper hand (Uquiche et al., 2008).

2.4.2.1 Hydraulic pressing

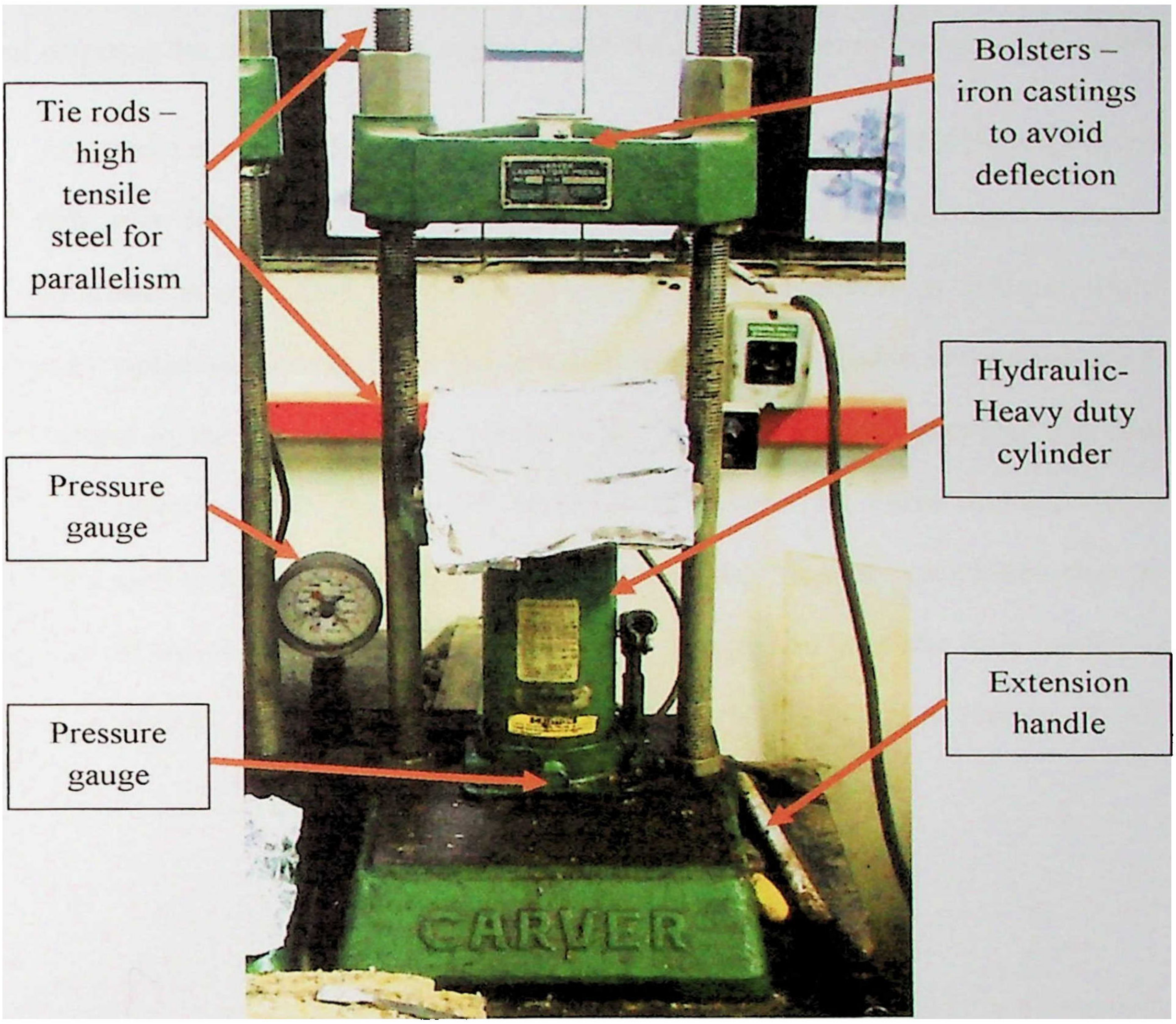

Figure 2.2: A bench-top manual Hydraulic press (labels retrieved from catalogue).

The hydraulic press was initially patented by Joseph Bromah (1795) from England and was founded from the mechanism of the hydraulic ram (Sorin-Stefan et al., 2013; Dunning, 1953). It is a process that involves a batch by batch operation (Santoso et al., 2014), whereby a mass of treated fish (usually partially cooked fish) is pressed for the separation of fatty substance (Adeniyi & Bawa, 2006). Hydraulic press involves compression via hydraulic force, ideal for lesser amounts of raw materials which has better pressing due to both sheer force and compression pressure. The hydraulic press is also more economical because energy consumption is lower and disposes the need of a water supply to aid the process (Pérez-Gálvez et al., 2009).

Hydraulic press has been used in recent times in past literature for the expression of fish oils like tuna fish oil from precooked and non-precooked samples (Chantachum et al., 2000). It was also employed for the recovery of sardine aliquot whereby optimisation was done to maximise yield and minimise suspended solids left behind in the aliquot (Pérez-Gálvez et al., 2009). Hydraulic pressing was also used to examine differences in effectiveness of several oil extraction/expression whereby catfish and herring was used as raw material. Authors concluded that the mechanical means of obtaining catfish oil and herring oil had the best quality in terms of peroxide value and also yield that was notably high (NhẬt & Hoàng, 2010).

2.4.2.2 Screw expeller

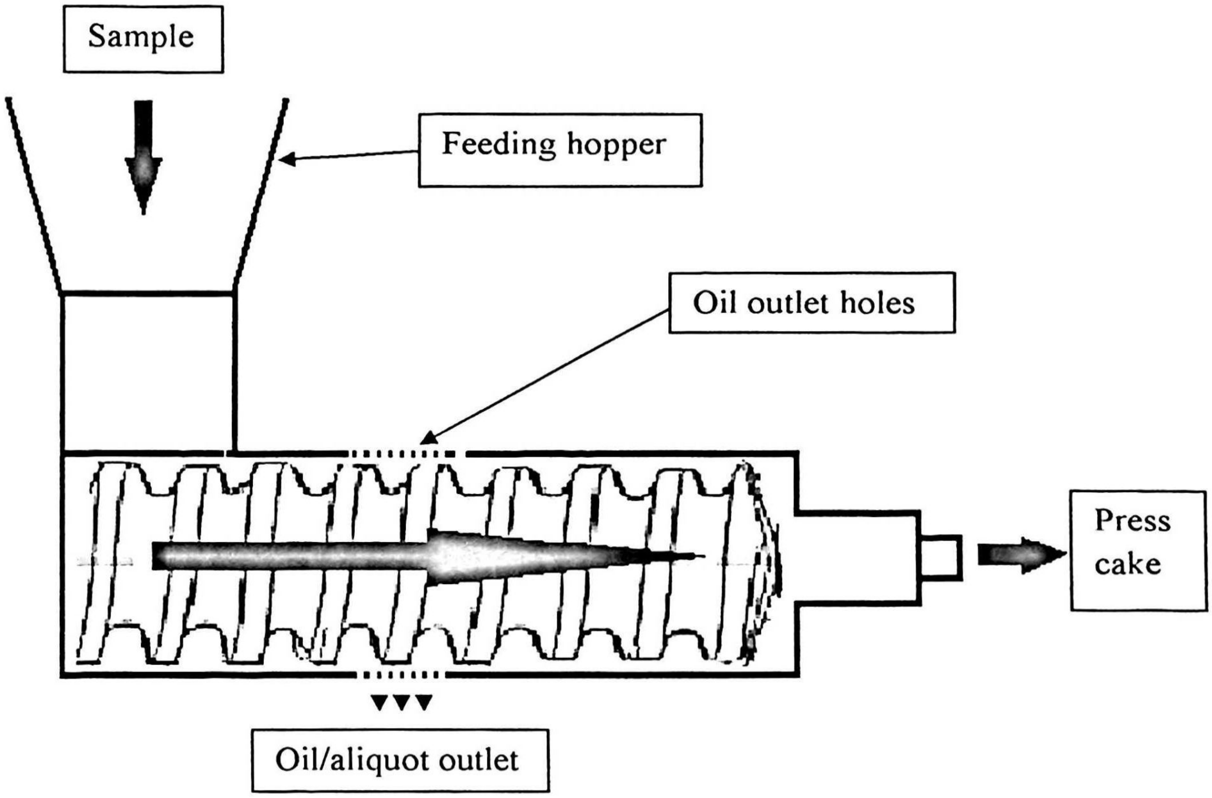

Figure 2.3: Simplified diagram of a screw press; arrows indicate route of samples.

Unlike the hydraulic press the screw expeller is a continuous system whereby samples can be fed uninterrupted to the device (Santoso et al., 2014). It is commonly employed for the use of expelling vegetable oil, more precisely, oilseeds (Pietsch & Eggers, 2011; Omobuwajo et al., 1997). V.D. Anderson was responsible for the invention of the first screw press from the United States in 1900. Since the device had undeniable advantages which included its continuous working process and its superior working capability made it eventually used for the expression of oil from oilseeds. The screw expeller also worked without high shocks and vibrations, with adjustable pressures screw presses making it quickly the favourable option in place of the hydraulic press (Biris et al., 2009; Sorin-Stefan et al., 2013). Force that works continuously to press the samples reaches a point whereby compression leads to breakage of sample particles releasing cell content and in this case oil/lipid (Sorin-Stefan et al., 2013).

2.4.3 Laboratory preparations

These include preparations that isn't commonly commercially carried out for the production of fish oil due to only being used in laboratory levels for research purposes or experimental procedures that have not yet been up-scaled. Work of Sathivel and others especially involved laboratory research, prior to oil production, in order to collect results of analysis of methods leading up to oil production (Sathivel et al., 2003c).

2.4.3.1 Homogenization

In laboratory preparations, the fish samples are sometimes homogenized and strained before centrifugation as it was done with production of catfish oil. The raw material was frozen and then homogenized and filtered through cheesecloth to remove solid particles. Resulting liquid is then centrifuged and the resulting oil is collected (Sathivel et al., 2003c). In another study analysing fish oil properties, raw fish material was cooked in boiling water for 30 minutes and left to settle down before the oil portion was collected and kept for further analysis (Aberoumand, 2010).

2.4.3.2 Enzymatic extraction

Enzymatic extraction of fish oil has also been studied in the past using Nile perch (*Lates niloticus*) and salmon with the aid of the enzyme protease without adjusting pH levels or adding water. Only the heads of both the fish were used as it constituted by-products from the fish fillets which are mostly sold away or wasted. The study recorded notable results albeit yield of fish oil was slightly lower when compared to solvent extraction. It was also known to be cost effective due to the utilisation of low cost food grade protease enzyme and improved yields without using harsh processing conditions (i.e. lower temperatures, shorter periods of time in prescribed condition, no usage of solvent) making it a much appreciated alternative method to procure fish oil (Mbatia et al., 2010).

2.4.3.3 Supercritical fluid extraction (SFE)

The other method for oil extraction or recovery is the SFE which has been studied by researchers for over two decades (Sahena et al., 2009). Supercritical CO_2 extraction was employed to extract oil from hake fish (*Merluccius capensis* – *Merluccius paradoxus*) off-cuts; a by-product of hake filleting. SFE results recorded more than 96% oil recovery in three hours. The oil was reported to be high in DHA and EPA albeit high free fatty acids content which was explained to be high even when solvent extraction was used in the same study. The team also observed the need to prevent against pro-oxidation as the oil seemed to be highly susceptible to lipid peroxidation, recording elevated initial levels of peroxide value (4.11 mequivalent O_2/kg oil) (Rubio-Rodríguez et al., 2008). Comparisons have been made with other extraction methods by the same team of researchers on SFE, cold extraction (centrifugation), wet reduction and enzyme extraction. SFE was found to be effective in producing fish oil from four different seafood by-products (hake; *Merluccius capensis–Merluccius paradoxus*, orange roughy; *Hoplostethus atlanticus*; salmon, *Salmo salar*; jumbo squid; *Dosidicus gigas*). The method was explained to be useful in preventing lipid oxidation of fish oils high in omega-3 PUFAs and also significantly reduced amounts of pollutants. Given that raw materials used are fresh, SFE proved to be efficient in extraction high quality fish oil even from inputs that are low in fat (in the case of freeze-dried samples) (Rubio-Rodríguez et al., 2012).

There has been many more research done in the past years with regards to supercritical fluid extraction for fish oils due to its unobtrusive nature and absence of the usage of solvents whereby researchers cited high recovery of important nutrient components using supercritical CO_2 extraction (Davarnejad et al., 2008; Aguiar et al., 2012; Chang et al., 2008). In association to producing fish oils SFE seems desirable albeit higher costs than conventional methods.

2.4.4 Industrial level oil processing

According to the Food and Agriculture Organization of the United Nations (FAO), industrial levels of fish oil processing starts with the heating/cooking of the samples with the goal being to liberate oil from the fish; a way of treating the raw material for succeeding stages. Generally, raw material is heated to 95-100 °C for about 15 to 20 min in a steam cooker. The resulting cooked fish is then drained of excess liquid via a strainer conveyer and then pressed with the purpose squeezing out as much liquid as possible from the solid material via a screw press.

Centrifugation is another means of recovering oil from the fish press aliquot that works to separate the layers present in the resulting fish liquid. Resulting aliquot of pressing is then separated into sludge, water and oil in a decanter or desludger and then centrifuged to remove the stickwater (water layer of liquid from the press). The oil is then further polished via hot water and centrifuged before being stored away as crude fish oil (FAO, Fisheries Department, Fisheries Industries Division, Italy, 1986). It is noteworthy that crude fish oil is not used as a final product due to the presence of impurities that would eventually lead to shorter shelf life and formation of off-flavours (Bimbo, 1998).

2.4.5 High quality oil processing

High quality fish oil processing conditions are set in a way that does not compromise the content of PUFAs (EPA and DHA) or the bioactive components like vitamins readily available in the fish oil [EFSA Panel on Biological Hazards (BIOHAZ), 2010]. Producing edible fish oil requires refining because crude fish oils contain certain amounts of non-triglyceride constituents. These components sometimes aid in retarding lipid oxidation but most often cause undesirable odours, and cause it to smoke or foam when further processed (Bimbo, 1998). Compounds that are naturally occurring like tocopherols, vitamins and pro-vitamins, and some flavour compounds are desirable whereas others are considered undesired or impurities. These impurities include hydrolytic and oxidative components like moisture, insoluble matter, free fatty acids, trace metals, and oxidation products (Young, 1986). Another complication in conventional fish oil processing is that heat application is desirable for higher yields but undesirable for quality reasons; higher temperatures increase yields but facilitate oxidation (Aidos et al., 2003).

Various patents have also been published that deal with the expression of fish oils and the various methods of doing so (Dijkstra, 2012);

i) conventional methods via steam treatment and treatment with organic acid prior to separating oil from the raw materials;
ii) addition of antioxidants to fish oil for the stabilisation of oxidation levels and separation of solids and stickwater; and
iii) treatment of crude fish oil with acids for removal of contaminants; etc.

Emergence of new technologies taking into account both cost and sustainability, there have been constant efforts to produce high quality fish oils (De Greyt, 2012). As mentioned before, various efforts have been put in to produce high quality fish oil that breaks away from conventional fish oil processing, namely enzymatic treatments and supercritical fluid extraction (Mbatia et al., 2010; Sahena et al., 2009). "Extra low oxidized oils' from very high quality (i.e. very fresh; just after filleting; processed immediately after catching) fish like salmon or herring involving lower treatment temperature (90-95°C) just to allow expression of oil. Fish meal is obtained from the same process used for production of marine protein hydrolysates. The Norwegian virgin cod liver oil is produced in the same manner that concentrates long chain PUFAs (PUFA; omega-3 EPA and DHA), retains beneficial bioactive components that are present naturally in the fish oil (Vitamins A and D), while removing impurities [EFSA Panel on Biological Hazards (BIOHAZ), 2010].

2.4.6 Oil refining

As mentioned before, due to the presence of undesirable components in crude fish oil, refining it is deemed necessary. Refining steps include degumming, neutralizing, bleaching, and deodorizing. Each step serves a purpose (Sathivel et al., 2003c; Young, 1986);

i) Degumming – removal of insoluble and soluble impurities;

ii) Neutralization – removal of free fatty acid (FFA) done with caustic soda

iii) Bleaching – removal of soap, trace metals, sulfurous compounds; and

iv) Deodorization – removal of residual FFA, aldehydes, and ketones unacceptable oil odor and flavor.

Although widely accepted as standard practice for the production of oils in general, refining is slowly being seen upon as hazardous especially in vegetable oils that are used for domestic practices. Usage of chemicals and processes of very high temperatures (of over 400 degrees) for processing and deodorizing (rendering the oil colourless and nearly tasteless, to promote acceptability) (Cook, 2005), is slowly but surely making consumers shy away from highly processed vegetable oils. However, research regarding the effects of conventional refining steps to the detriment of fish oil quality is an area that is yet to be properly explored (Fournier et al., 2006; Berdeaux et al., 2007).

Deodorization in particular involves usage of high temperature and can be detrimental to stability of oils (Čmolík & Pokorný, 2000). While it is aimed at eliminating compounds that cause undesirable flavour, taste and smell of fish oil, deodorization can cause a concurrent loss of valuable components identifiable from the degradation products in the fish oil. These degradation products include polar compounds (mostly oligomers), cyclic fatty acid monomers (CFAM) and geometrical isomers (trans-fatty acid isomers) (Fournier et al., 2006).

Refining methods have also been "refined" in order to prevent degradation of long chain PUFAs. In deodorization, efforts have been made to improve the process in terms of lowering the processing temperature. Chung and Lee studied the absorption of trimethylamine, an odour causing agent in fish oil associated with foul fish smell, via zeolite catalysts (microporous crystalline solids with well-defined structures) using low temperatures in deodorization (Chung & Lee, 2009). Also the use of adsorbents like magnesol XL and passive filtration to refine oil (a form of physical refining) has been optimised for use in improving quality of sardine oil (Suseno et al., 2011).

The use of membrane technology as an alternative for conventional refining methods was done by using a hydrophobic nonporous denser membrane. Membrane technology presents a simple, low-energy consuming process at ambient temperatures to refine oil without loss of nutrient components. The study was conducted using *Engraulis japonicus* and *Maurolicus japonicus* fish oils and results showed that peroxide and anisidine values were significantly lowered without the loss of fatty acids in the oil (Miyagi et al., 2009).

Activated carbons (adsorbent) have been used for refining fish oils to remove contaminants like persistent organic pollutants (POP) and polychlorinated biphenyls (PCB). Activated carbon refinement of fish oils offers a milder processing condition when compared to conventional deodorization yet effective in removing the aforementioned contaminants. Work has been done to study its effect on its efficacy and effects of nutritional content of fish oil (Maes et al., 2005) and also optimisation