CHAPTER II

LITERATURE REVIEW

1. RICE

Rice is a species (*Oryza sativa L.*) of grass, native to tropical, subtropical southern , southeastern Asia and Africa, which together provide more than one fifth of the calories consumed by humans. Rice is an annual plant, growing to 1–1.8 m tall, occasionally more, with long slender leaves 50–100 cm long and 2–2.5 cm broad. The small wind-pollinated flowers are produced in a branched arching to pendulous inflorescence 30–50 cm long. The seed is a grain (caryopsis) 5–12 mm long and 2–3 mm thick.

1.1 Rice Component

A rice kernel consists of 20% hull, 72% starchy endosperm, 6% bran, and 2% germ (Juliano and Bechtel, 1985; Lu and Luh, 1991). Rice bran is the soft germ and several soft layers, pericarp, seed coat, nucellus and aleurone layer, surrounding the hard starchy endosperm as shown in Figure 1. Rice bran contains 16-32 % oil, generally the same as soybeans, 12-16% proteins, 34-52% available carbohydrates, 7-11% crude fiber, and 7-10% ash. The typical crude RBO is composed of 68-71% triacylglycerols, 2-3% diacylglycerols, 5-6% monoacylglycerols, 2-3% free fatty acids, 5-7% glycolipids, 3-4% phospholipids, 2-3% waxes, and 4.2% unsaponifiable fraction (McCaskill and Zhang, 1999; Sayre and Saunders, 1990). Free fatty acids, monoacylglycerols and diacylglycerols are associated with enzymatic hydrolysis. Sterols, existing as free or esterified, are the major portion in the unsaponifiable fraction and classified as 4-desmethylsterols, 4-monomethylsterols and 4, 4'dimethylsterols, also known as triterpene alcohols. RBO contains more non-triacylglycerol components than most vegetable oils but these are lost during refining processes.

1.2 Rice Bran

Rice bran is a rich source of nutrients for humans. Many years, mostly of rice bran is sold as animal feed. Numerous potential food uses for full-fat and defatted rice bran have been well proven in laboratory tests but are not yet in commercial practice (Saunders, 1986). One of the challenges facing the rice industry is to utilize the food value of rice bran more effectively (Marshall, 1994).

The greatest restriction to the use of rice bran as a food ingredient, or even as a source of edible oil, is its naturally occurring enzymatic activity. This cause rapid hydrolytic of the bran after milling, produce bitter taste. In addition, oxidative rancidity results in strong, unpleasant "rancid" odor.

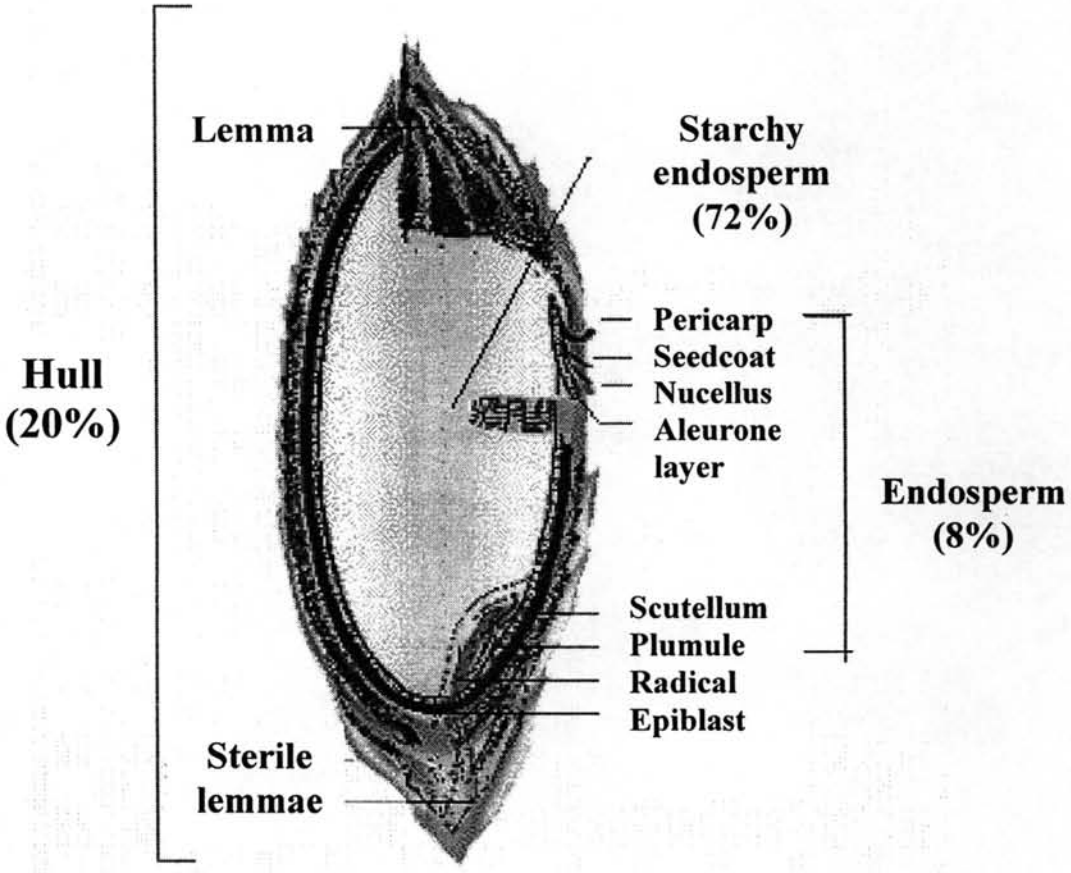


Figure 1 Rice kernel structure (Orthoefer, 1996)

1.3 Rice Bran Oil (RBO)

.RBO is extracted from the stabilized bran after milling. The oil may be removed from the bran using mechanically pressing or solvent extraction. Mechanically pressing results in only 50% oil recovery, therefore solvent extraction, generally with hexane, is preferred. Once extracted, RBO is stable and may be stored for subsequent processing. Crude RBO can be refined into a high quality salad oil and contains several other components that may be isolated as commercial products.

The crude oil is usually dark greenish-brown, depending upon the extraction method (Kochhar, 2002). Moreover, RBO has characteristic odor. Color bodies should be absent, and off-odors are difficult to mask in cosmetic preparation.

1.4 RBO Component

RBO consists of two components. The first one is saponifiable components including saturated and unsaturated fatty acids or essential fatty acids. Another component is unsaponifiable components as beneficial antioxidants. The lipid composition of crude RBO is presented in Table 1. Unsaponifiable component is present in Table 2.

Table 1 Composition of Crude RBO (Sayre, 1988)

Composition of Crude RBO	% w/w
Saponifiable Lipids	**90-96**
Neutral Lipids	88-89
Triglyceride	83-86
Diglycerides	3-4
Monoglycerides	6-7
Free fatty acid	2-4
Waxes	3-4
Glycolipids	6-7
Phospholipids	4-5
Unsaponifiable Lipids	**4.2**
Phytosterols, Sterol esters, Hydrocarbons	
Triterpene Alcohols, Tocopherols	

Table 2 Composition of Unsaponifiable Lipids in Crude RBO (Sayre, 1988)

Unsaponifiable Lipids in Crude RBO (% of Crude Oil)	
Total Unsaponifiable Lipids	4.2
Sterols	1.8
β-Sitosterol	0.88
Campesterol	0.51
Stigmasterol	0.27
4-Methyl Sterols	0.4
Citrostadienol	0.17
Gramisterol	0.16
Obtusifoliol	0.03
Triterpene Alcohols	1.2
24-Methylene-cycloartanol	0.49
Cycloatenol	0.48
Cycloatanol	0.11
Less Polar Compounds	0.86
Hydrocarbons : Squalene	0.75
Tocotrienols	0.04
Tocopherols	0.07

1.4.1 Saponifiable Components

The lipid component of RBO consists of neutral lipids, glycolipids and phospholipids. Neutral lipids consist mostly of triacylglycerols, monoacylglycerols and few diacylglycerols, sterols and free fatty acids. The major phospholipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidic acid (Hamavathy and Phabhakar, 1987). Palmitic, oleic and linoleic fatty acid constitute 93-95% of the fatty acid portion of glycerol esters (Kochhar, 2002). Linoleic acid belongs to the polyunsaturated fatty acid (PUFA) and it is an essential

fatty acid because it has to be supplied by the diet (Polo, 1988). It can be utilized in the skin for its presumed benefit on scaling phenomena with no chemical modification (Rieger, 1994). RBO has linoleic acid about 29-45% of fatty acid portion (Kochhar, 2002). Fatty acid esters are responsible for the excellent slip and lubrication properties of RBO. Thus, RBO is used in skincare for its good moisturizing and emollient properties on dry skin.

1.4.2 Unsaponifiable Component

RBO contains 3-5% unsaponifiable lipids (Sayre and Sounders, 1990) which is higher than other vegetable oil sources (Roger et al., 1993). Unsaponifiable components of RBO contain many bioactives such as carotenoids, vitamin B, vitamin E, and γ-oryzanol, but two unsaponifiable components of RBO have been investigated for possible health benefits. These are γ-oryzanol and vitamin E (Roger et al., 1993, Lloyed et al., 2000)

1.5 Reaction of Fats and Fatty Acids

1.5.1 Saponification

Saponification involves the reaction of a fat (triglyceride) with an alkali to yield glycerol and a salt (also called soap). This reaction is fundamental in soap making.

$$\underset{\text{Triglyceride}}{C_3H_5(OOCR)_3} + \underset{\text{Alkali}}{3NaOH} \longrightarrow \underset{\text{Glycerol}}{C_3H_5(OH)_3} + \underset{\text{Fatty Acid Salt (Soap)}}{3NaOOCR} \quad \text{..... (1)}$$

Under controlled condition, reacting free fatty acids with an alkali results in the formation of a salt and set free a water molecule.

$$RCOOH + NaOH \longrightarrow RCOONa + H_2O \quad \text{..... (2)}$$

When fatty acids are used, soap formation may be effected with sodium carbonate. This reaction produces a soap, water and carbon dioxide.

$$2RCOOH + Na_2CO_3 \longrightarrow 2RCOONa + H_2O + CO_2 \quad \text{..... (3)}$$

After neutralization, the batch is generally boiled with an excess of caustic soda to saponify the small amount of neutral.

1.5.2 Esterification

Esterification of fatty acid generally occurs with alcohols such as glycerol. The reaction is reversible and proceeds to completion only when water is removed. Acid catalysts promote this reaction.

$$\underset{\text{Glycerol}}{C_3H_5(OH)_3} + \underset{\text{Fatty acid}}{3HOOCR} \longrightarrow \underset{\text{Triglyceride}}{C_3H_5(OOCR)_3} + \underset{\text{Water}}{3H_2O} \quad \text{..... (4)}$$

1.5.3 Hydrolysis

The reverse reaction is known as hydrolysis and is similarly catalyzed. Tailor made triglycerides of any desired composition may be made by hydrolyzing the fats to fatty acids, purifying the fatty acid by fractional distillation, and recombining the fatty acids in desired proportions with glycerol in the process of esterification. The products find many uses ranging from food additives to non-edible industrial emulsifiers.

$$\underset{\text{Triglyceride}}{C_3H_5(OOCR)_3} + \underset{\text{Water}}{3H_2O} \longrightarrow \underset{\text{Glycerol}}{C_3H_5(OH)_3} + \underset{\text{Fatty Acid}}{3HOOCR} \quad \text{..... (5)}$$

1.5.4 Lipolytic Hydrolysis

During the dehulling of rice, the layers of the kernel are disrupted, allowing the oil to make contact with lipases. The lipases catalyze the rapid hydrolysis of triglycerides to free fatty acids. Lipases produced by bacteria and mold on the surface of the kernel also come into contact with the oil, further promoting the hydrolysis reaction. Therefore, the rate of free fatty acid formation is dependent on the degree of disruption of the kernel, the quantity of lipase present, the moisture content and temperature. In rice, approximately 30 % of the oil can be converted to free fatty acids in the period of one week, under high humidity, and temperature conditions (Enochian et al., 1980). High levels of free fatty acids in oil lead to soapy taste.

1.5.5 Oxidation

Rancid off-flavours and off-odors in oil are the result of oxidative deterioration. The reaction can be either enzyme catalyzed or non-enzymatic. Both reactions result in the production of hydroperoxides that react to yield secondary oxidation products such as aldehydes and ketones. These products produce the off-flavors and off-odors associated with racidity.

1.5.5.1 Enzymatic Oxidation

Enzymatic oxidation is catalyzed by lipoxygenase, an enzyme found in germ. It is responsible for the oxidation of saturated free fatty acids. As lipoxygenase acts only on saturated free fatty acids. The extent of oxidation by this mechanism depends on the amount of free fatty acid made available by lipolytic hydrolysis.

1.5.5.2 Non-enzymatic Oxidation

Non-enzymatic oxidation may be catalyzed by metal ions, light, high-energy radiation, and heat. The reaction can occur by free radical oxidation (auto-oxidation) or photo-oxidation routes. In free radical oxidation, lipid molecules produce free radicals by their interaction with oxygen in the presence of a catalyst. Hydroperoxides are the initial reaction products. In photo-oxidation, singlet oxygen is formed by the reaction of an excited photosensitive molecule with oxygen. The singlet oxygen goes on to react with fatty acids to produce the hydroperoxides. Non-enzymatic oxidation, unlike other mechanisms, is inhibited by antioxidants present in the rice bran.

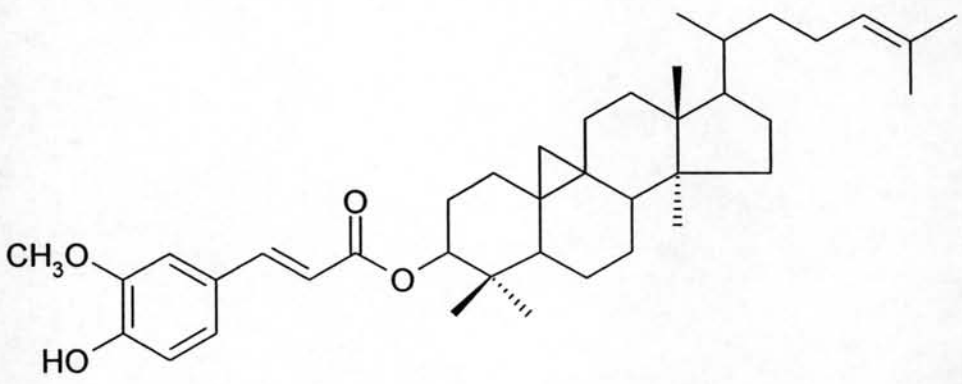
2.Antioxidant Activity of Components Found in RBO

The antioxidant compounds in RBO have health benefits as well as antioxidant characteristics for improving the stability of foods. Several studies have reported the effects of RBO on metabolic activities including reduced plasma cholesterol in laboratory animals and humans (Yoshino et al., 1989, Qureshi et al., 1991, Hegsted et al., 1990, Kahlon et al., 1992, Hegsted and Windhauser, 1993). Oryzanol has been studied for its ability to reduce cholesterol absorption (Rong et al., 1997). Komiyama et al., 1992 and Nesaretnam et al., 1998 reported anticancer activity associated with tocotrienols. Thus, rice bran is viewed as a potential source of these high-value antioxidants for use as additives in foods, pharmaceuticals, and cosmetics.

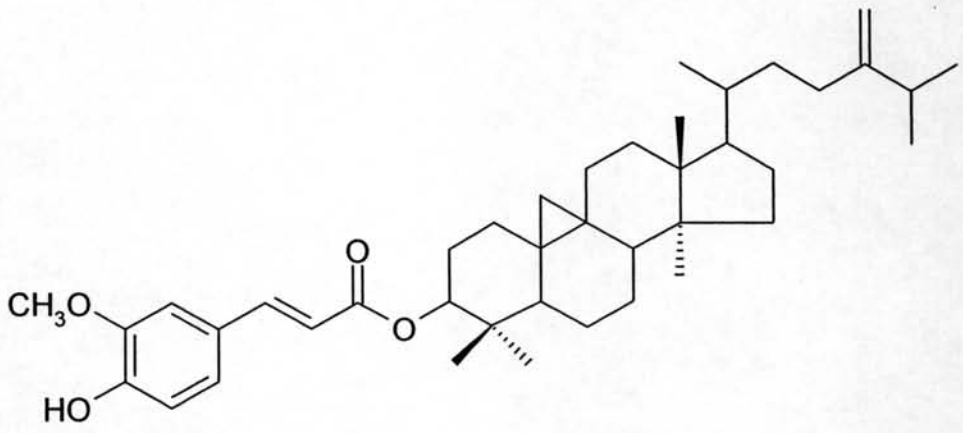
2.1 Gamma-oryzanol (γ-oryzanol)

The most characteristic component of RBO is γ-oryzanol; oryzanol was first separated from RBO by Kaneko and Tsuchiya in 1954 and thought to be a single compound. Subsequently, it was shown to be a mixture of ferulic acid esters of triterpene alcohols and plant sterols (Shimizu et al., 1957, Ohta and Shimizu, 1957, Ohta and Shimizu, 1958). The oryzanol is composed of four esters of ferulic acid as

shown in Figure 2. These ferulic acid esters have been identified as cycloartenyl ferulate (15-30%), 24-methylene cycloartanyl ferulate (10-40 %), campestryl ferulate (25-50 %) and β-sitosteryl ferulate (15- 25 %) (Iijima and Sano,1986, van Amerongen et al., 2002).

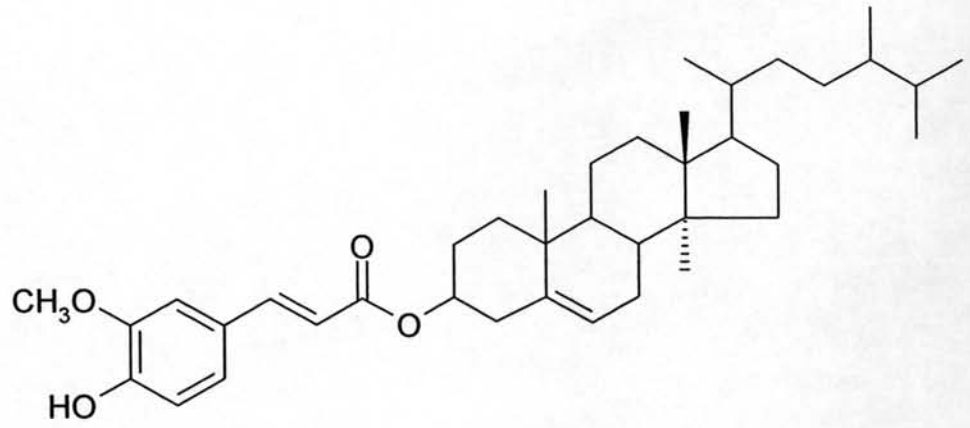


Cycloartanyl ferulate (15-30%)

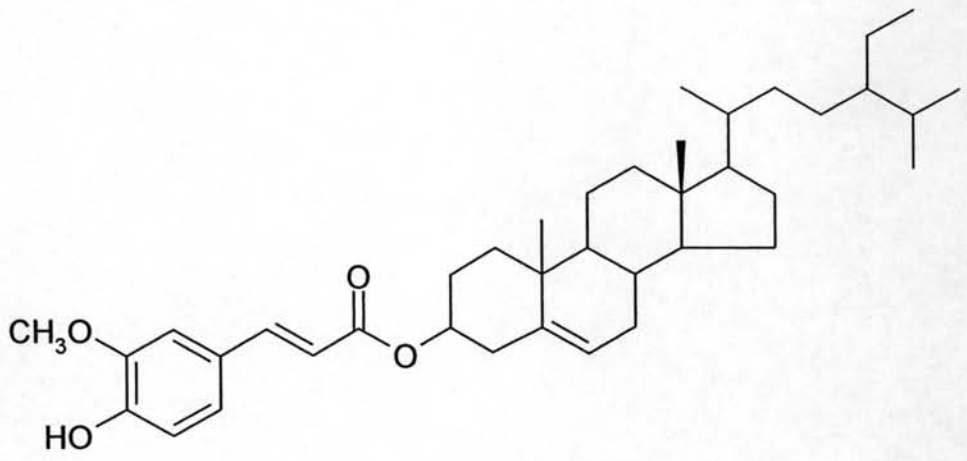


24-methylene cycloartanyl ferulate (10-40 %)

Figure 2.1 Major Components of γ-Oryzanol (Diack and Saska, 1994)



Campesteryl ferulate (25-50 %)



β-sitosteryl ferulate (15- 25 %)

Figure 2.2 Major Components of γ-Oryzanol (Diack and Saska, 1994)

γ-Oryzanol is a white or slightly yellow crystal or crystalline powder, stable at room temperature (Tarnagawa et al., 1992). It has a melting point of 137.5 °C - 138.5 °C and shows ultraviolet absorption maxima at 231, 290 and 315 nm in heptane (Tsuchiya and Kaneko, 1954). Oryzanol is soluble in solvents such as diethyl ether, methylene chloride, acetone and alcohol and is only somewhat soluble in non-polar solvents like petroleum ether and hexane (Seetharamaiah and Prabhakar, 1986).

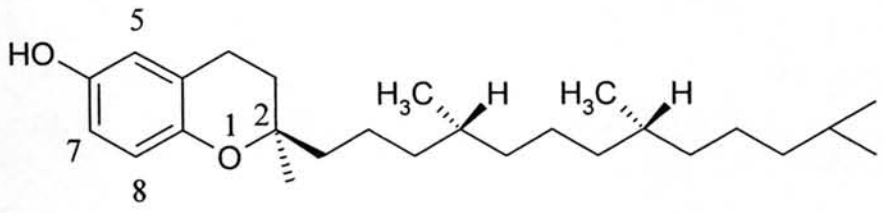
γ-Oryzanol has been considered to be the major antioxidant in RBO because the quantity of γ-Oryzanol in RBO is up to 10 times higher than vitamin E (Xu et al., 2001). Moreover, γ-Oryzanol has been shown to be very safe (Deckere and Korver, 1996). Side effects have not been reported in animal studies using doses of up to 1,000 mg per day of γ-Oryzanol or up to 1,500 mg per day of ferulic acid. Poor absorption appears to be the reason for the lack of side effects associated with higher doses (Hirose et al., 1999).

2.2 Tocopherols and Tocotrienols

Moreover, RBO is also a rich source of tocotrienols, the content and biological activities of tocotrienol are higher than those of tocopherols (Sugano and Tsuji, 1997; Qureshi et al., 2001). Tocotrienols differ from tocopherols in having three double bonds in the isoprene side chain. The structural differences among tocotrienols are relative to the number and location of methyl groups on the chroman rings (Figure 3).

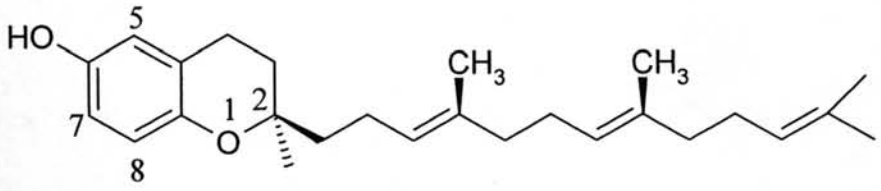
The study by Qureshi et al. (1996) showed the structural differences among tocotrienols may influence their biological activities. δ-Tocotrienol is the most potent cholesterol inhibitor among the known tocotrienols, followed by γ-tocotrienol and α-tocotrienol.

(a)



Tocopherol

(b)



Tocotrienol

Position of methyl Group	Tocopherols	Tocotrienols
5,7,8 –Trimethyl	α-T	α-T3
5,8 –Dimethyl	β-T	β-T3
7,8-Dimethyl	γ-T	γ-T3
8-Monomethyl	δ-T	δ-T3

Figure 3 Molecular structures of tocopherols and tocotrienols (Lee et al., 2004)

The four common isomers of tocopherols and tocotrienols that occur in nature are α, β, γ, and δ (Rogers, 1993). Tocopherols are powerful antioxidants with a potent

vitamin E activity and have higher activity against cardiotoxicity. Furthermore, RBO is relatively rich in tocotrienols which have been investigated for possible health benefits (Cheruvanky, 2000). Like tocopherols, tocotrienols possess antioxidant activity. In addition, other physiological actions attributed to the tocotrienols are decreasing serum cholesterol, decreasing hepatic cholesterol synthesis and having anti-tumor activity (Roger, 1993)

2.3 Cosmetic Uses of RBO

The components in RBO, γ-oryzanol and ferulic acid, have actions in preventing skin aging and smoothening wrinkles without any adverse effects on living bodies when used as components for general pharmaceutical and cosmetic preparations such as ointments, creams and lotions (Tatsu et al., 1993). Functions of RBO and γ-oryzanol in skin formulations are skin conditioning agent, emollient, moisturizer and sun protection factor (SPF) booster (Wenniger and McEwen, 1992)

3 . Production Methods of RBO

3.1 Traditional Method

3.1.1 Ghani Oil Extraction

Ghanis are powered by animals or motors (power-ghanis) (Figure 4) although sometimes human power (Figure 5) is used. The mortar is firmly fixed in the ground and as the pestle rotates oil is released by friction and pressure and runs out of a small aperture at the base of the mortar.

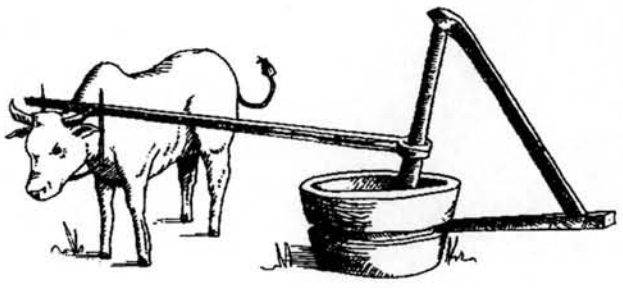


Figure 4 Traditional animal powered ghani (Minor crop, 1992)

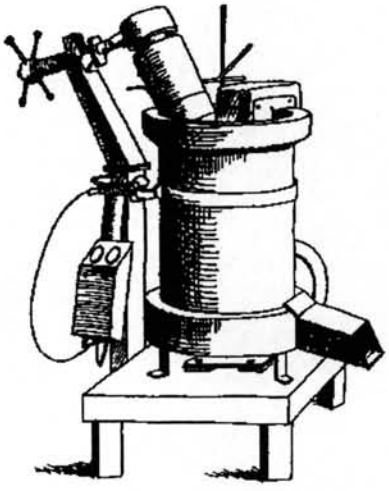


Figure 5 Power Ghani (Minor crop, 1992)

3.1.2 Manual –Pressed

Two basic types of manual presses are plate presses and ram presses. In the first type (Figure 6), plate or piston is forced into a perforated cylinder containing the oil bearing material by means of a worm. In some cases hydraulic jacks have been used, care is needed to make sure there is no leakage of hydraulic fluid that might contaminate the edible oil. In a ram press (Figure 7) a piston forces the oil seed forward in a perforated cage fitted with an adjustable choke at the outlet, which controls the pressure. Ram presses provide a greater shearing action than simple screw presses and have been found to be considerably more efficient for some raw

materials. It is important that when selecting a particular type of press its suitability for the raw material to be processed is confirmed.

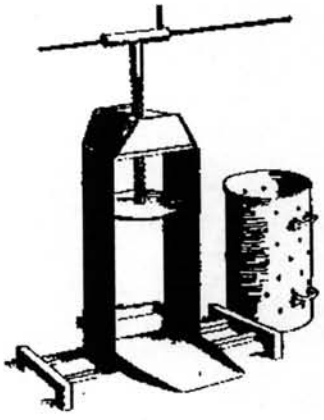


Figure 6 Plate press (Minor crop, 1992)

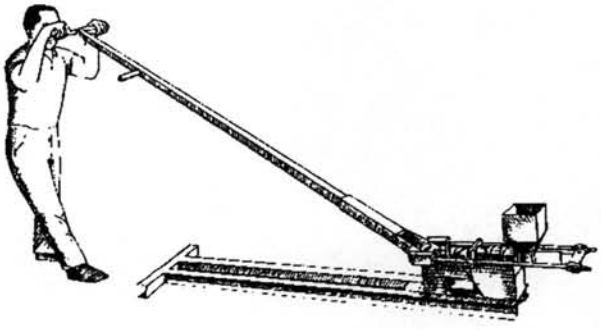


Figure 7 Ram Press (Minor crop, 1992)

3.1.3 Power- Pressed

Powered devices and the oil expeller is the most common to use to obtain greater through puts and extraction efficiencies. The raw material, which may have been previously heated to aid in the release of oil, is fed continuously to the expeller where it is fed by the funnel into a horizontal cylinder (Figure 8). A controllable pressure is built up in the cylinder by means of an adjustable choke at the cylinder exit. The internal pressure ruptures oil cells in the material and oil flows out

through perforations in the cylinder cage. Some care has to be taken when selecting an expeller for a particular commodity.

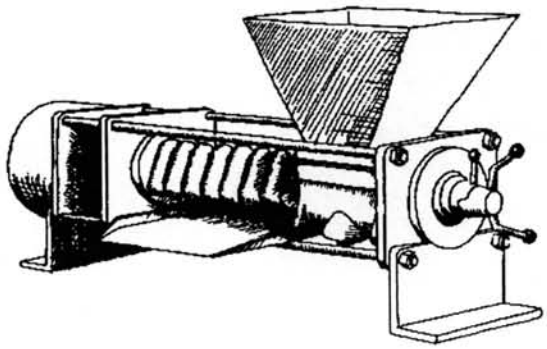


Figure 8 Expeller Pressed (Minor crop, 1992)

3.1.3.1 Cold- Pressed

Cold pressed is term used to describe oils that have been mechanically pressed slowly so that temperatures do not rise above 60°C. The steps of cold-pressed are shown in Figure 9.We can call cold- pressed in other name such as unrefined, unprocessed, and expeller-pressed, etc., and sold in dark containers. Cold pressed oils are more intense in taste and color than refined oils

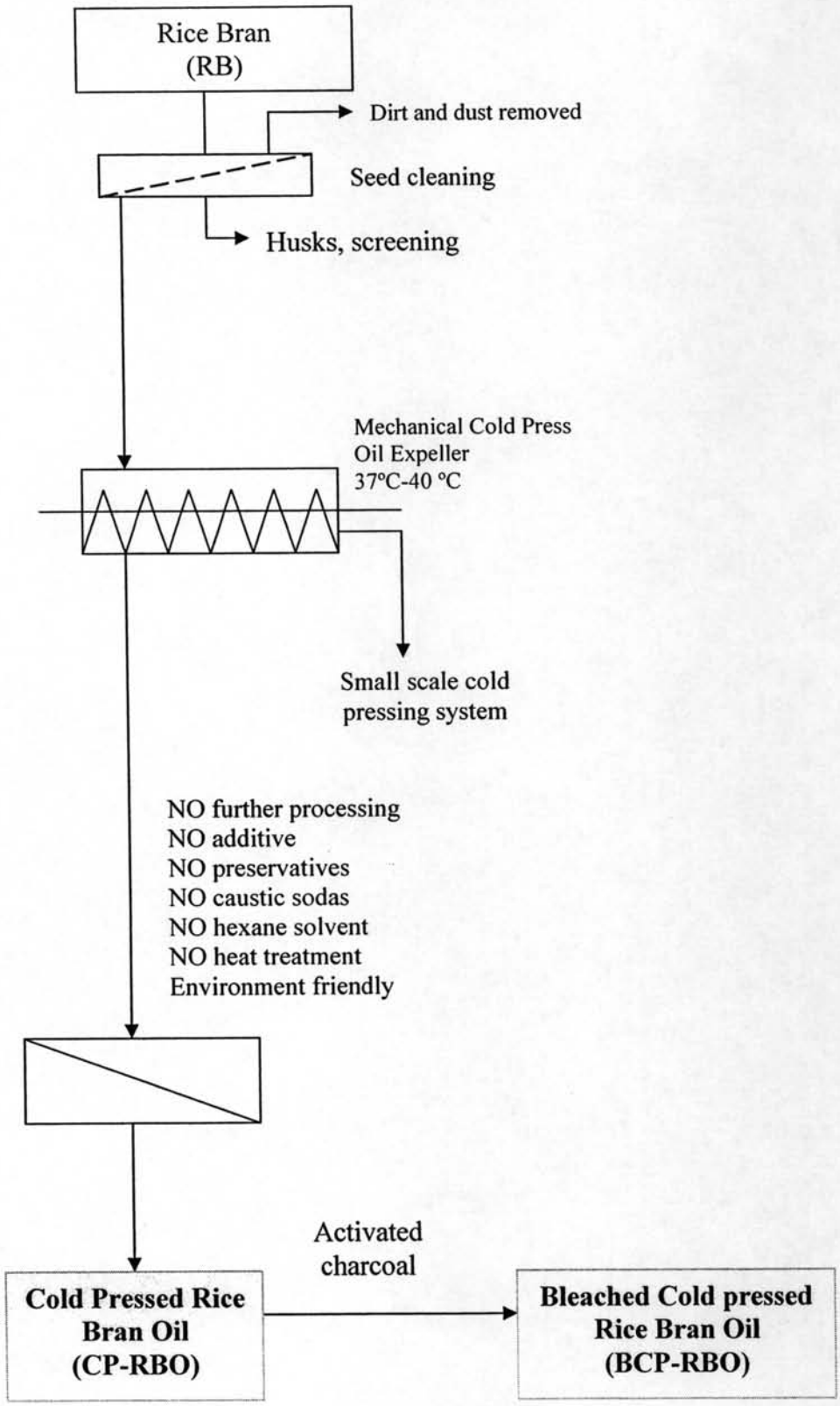


Figure 9 Cold pressed RBO steps

3.2 Solvents and Solvent Extraction

A solvent is usually a liquid that dissolves a solid, liquid, or gaseous substance (the solute), resulting in the formation of a solution. Solvents are used to extract soluble compounds from a mixture. As a solvent dissolves a compound, it will create various weak chemical interactions with the solute in order to solubilize it. The most common of these interactions, in increasing strength, are the Van der Waals interactions consisting of induced dipole interactions, the dipole-dipole interactions, and the hydrogen-bond interactions (Miller et al., 1981). Solvents and solutes can be broadly classified as polar (hydrophilic) or non-polar (lipophilic). Because of this polarity of a solvent that determines what type of compounds which is able to dissolve and with what other solvents or liquid compounds is miscible. Relatively the more polar solvents the more polar compounds dissolve as same as the more non-polar solvents non-polar compounds dissolve.

For determination of solvents used in this experiment, previous research was reviewed and shown that hexane efficiently extracts all of the major lipid components from cereal grains; however, hexane is believed to cause health implications when used in food-grade lipid extractions. The process of edible oil productions by solvent extraction is shown in Figure 10.

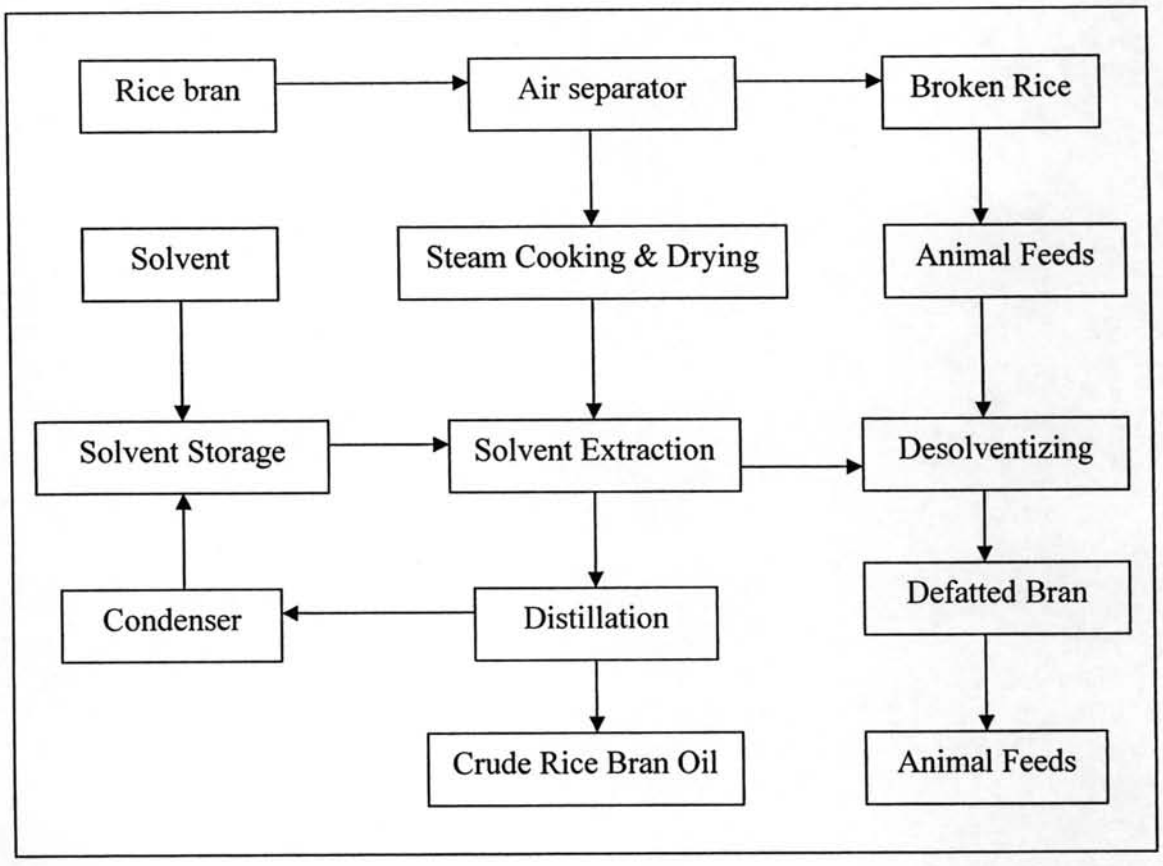


Figure 10 Solvent Extraction in Edible oil productions

3.3 Refining Process

Refining processes of RBO generally require only the triglyceride portion of the crude oil. Some components, such as unsaponifiables, pigments, and partial esters, are more difficult to remove but do not detract the oil from food utilization. Processing crude oil involves dewaxing, degumming, neutralization, bleaching, winterization, and deodorization, which are described below (Figure 11). The process of solvent extraction and refining of RBO are shown in Figure 12.

3.3.1 Dewaxing

Dewaxing of rice bran oil is done while still in miscella form by cooling, crystallization of waxes, and centrifugation or filtration for wax removal.

3.3.2 Deguming

Gums, which are polar lipids having surface-active properties. Gums are removed by degumming agents such as phosphoric or citric acid. The hydrolyzed gums are separated from the oil by centrifugation.

3.3.3 Neutralization

Neutralization, also known as refining, involves the removal of free fatty acids (FFA) by caustic soda. The acids are converted to sodium soaps, which are hydrated and also removed by centrifugation.

3.3.4 Bleaching

Activated bleaching clays are added to the oil to remove pigments, oxidized lipids and polar components from the oil. The clay is then removed from the oil by filtration.

3.3.5 Winterization

Winterization, generally performed before deodorization, removes the high melting point triglycerides from the fraction of the oil that remains lipid at refrigeration temperature. The triglycerides are crystallized out of the oil by chilling and subsequently separated via filtration.

3.3.6 Deodorization

Deodorization is a vacuum stream distillation process that removes odor, flavors, and free fatty acids. Volatile compounds found in the deodorizer distillate include aldehydes, ketones, peroxide, and a portion of the tocopherols and sterols present in the oil.

Processing of RBO causes significant variation in the levels of unsaponifiables present in commercial RBOs. Up to, 90% of the oryzanol and tocotrienol content of crude oil can be lost. From these reasons, it is evident that cold-pressed method must be use to preserve the active components in RBO.

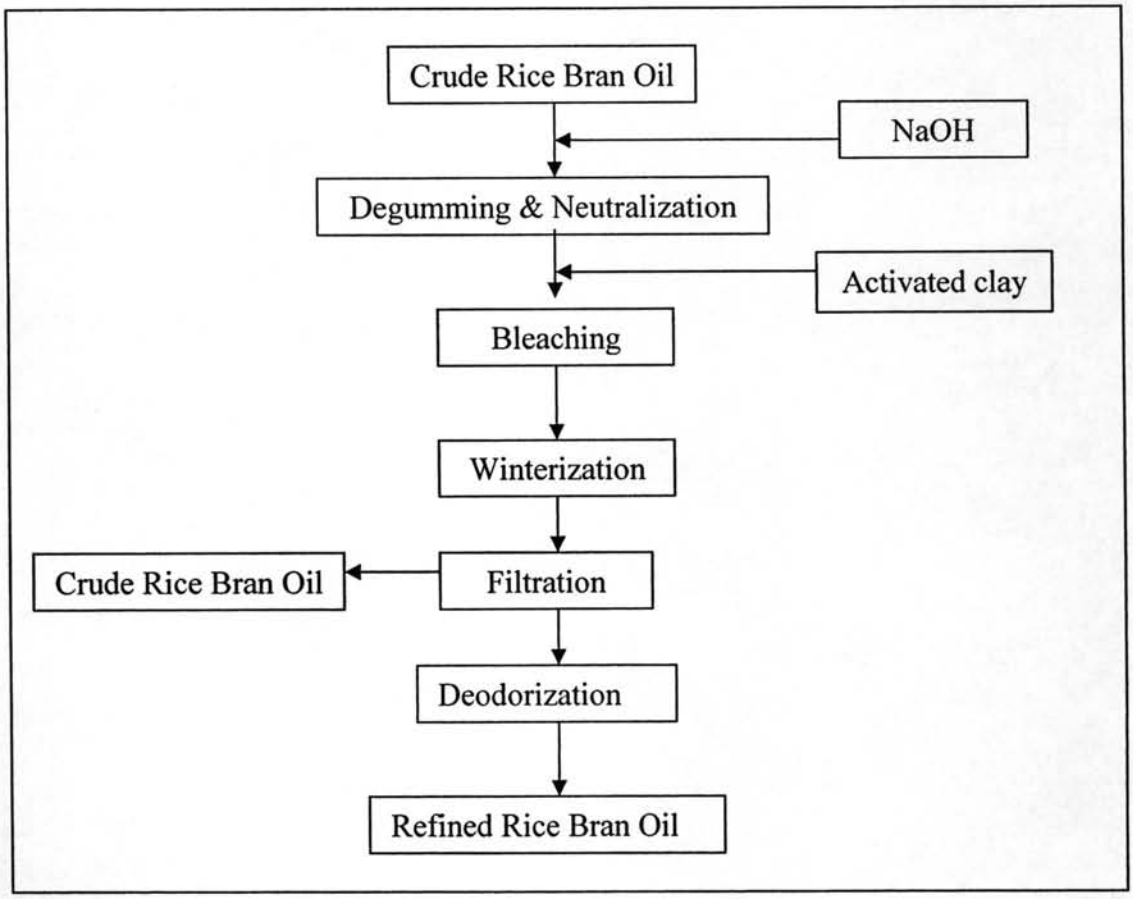


Figure 11 Rice bran oil refining process

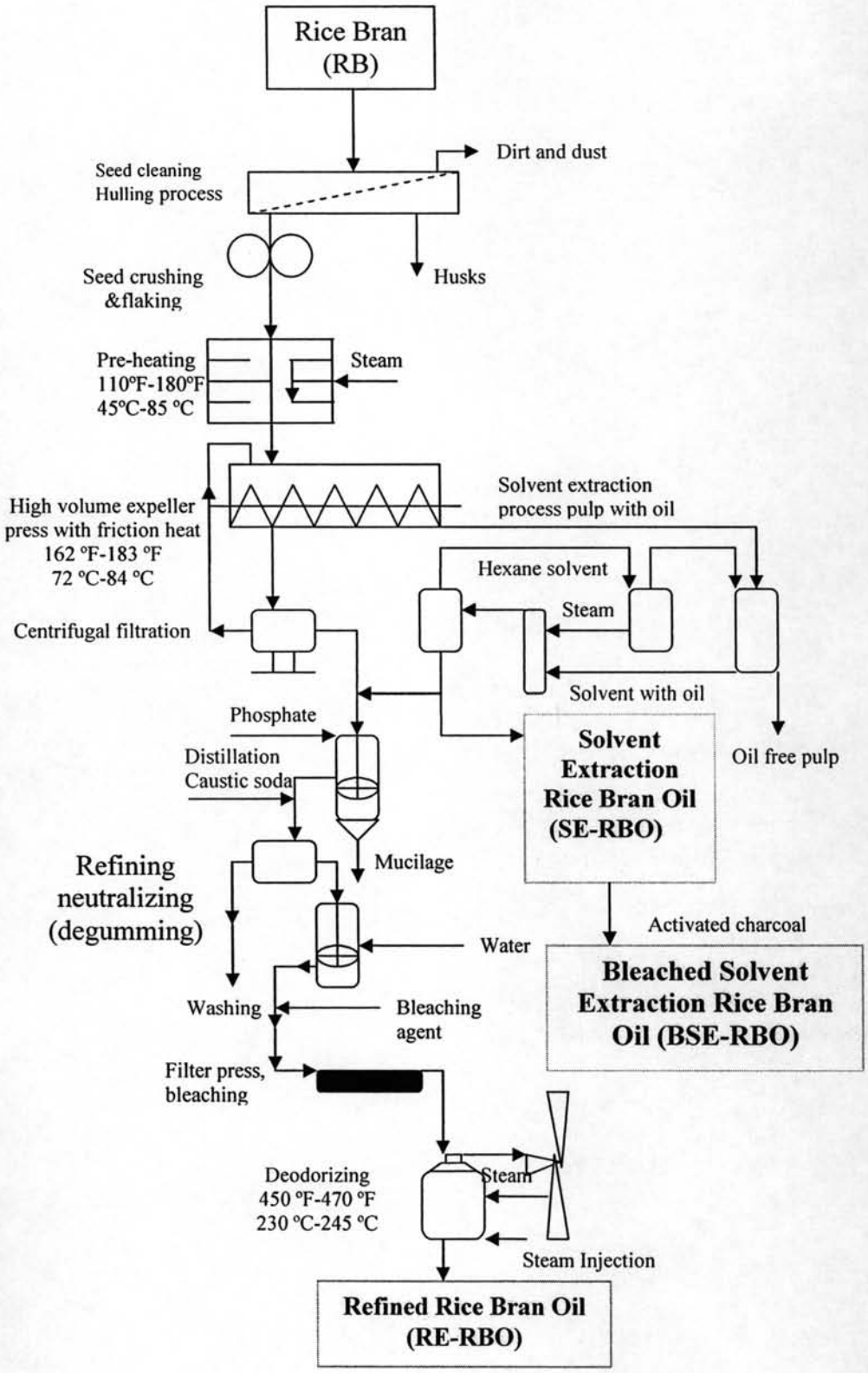


Figure 12 Solvent Extraction and Refining process of RBO.

4 . Analysis Method

4.1 Lipid Oxidation Tests

Tests for lipid oxidation are either predictive tests or indicator tests. Predictive tests use accelerated conditions to measure the stability of a fat or finished product. These tests may be used to determine ingredient quality, measure effectiveness of preservatives, or estimate product shelf life. Indicator tests are intended to quantify product or ingredient rancidity. Some of the more commonly used tests are described briefly in the following paragraphs.

4.1.1 Active Oxygen Method (AOM)

This method predicts the stability of a fat by bubbling air through a solution of the fat using specific conditions of flow rate, temperature, and concentration. At intervals, peroxides and hydroperoxides produced by this treatment are determined by titration with iodine. The AOM value is defined as the number of hours required for the peroxide concentration to reach 100 meq/kg of fat. The more stable the fat, the longer it will take to reach that level. For products other than fats and oils, the fats must first be gently extracted with solvents. The method is very time-consuming since a stable fat may require 48 hours or more before reaching the required peroxide concentration. While still used today, the AOM method is being supplanted by faster automated techniques.

4.1.2 Oxidative Stability Index (OSI)

The method is similar in principle to the AOM method, but it is faster and more automated. Air is passed through a sample held at constant temperature. After the air passes through the sample, it is bubbled through a reservoir of deionized water. Volatile acids produced by lipid oxidation are dissolved in the water increasing its conductivity. Conductivity of the water is monitored continuously

and the OSI value is defined as the hours required for the rate of conductivity change to reach a predetermined value (Figure 13). Multiple samples can be tested simultaneously and software controls instrument parameters and data collection (Figure 14). The method has been collaboratively studied and accepted by Association of Official Chemists (AOCS).

4.1.2.1 Rancimat Method

The Rancimat method is an accelerated oxidation test that is run at elevated temperatures and the sample exposed to air. This results in auto-oxidation in a few hours, instead of weeks or months.

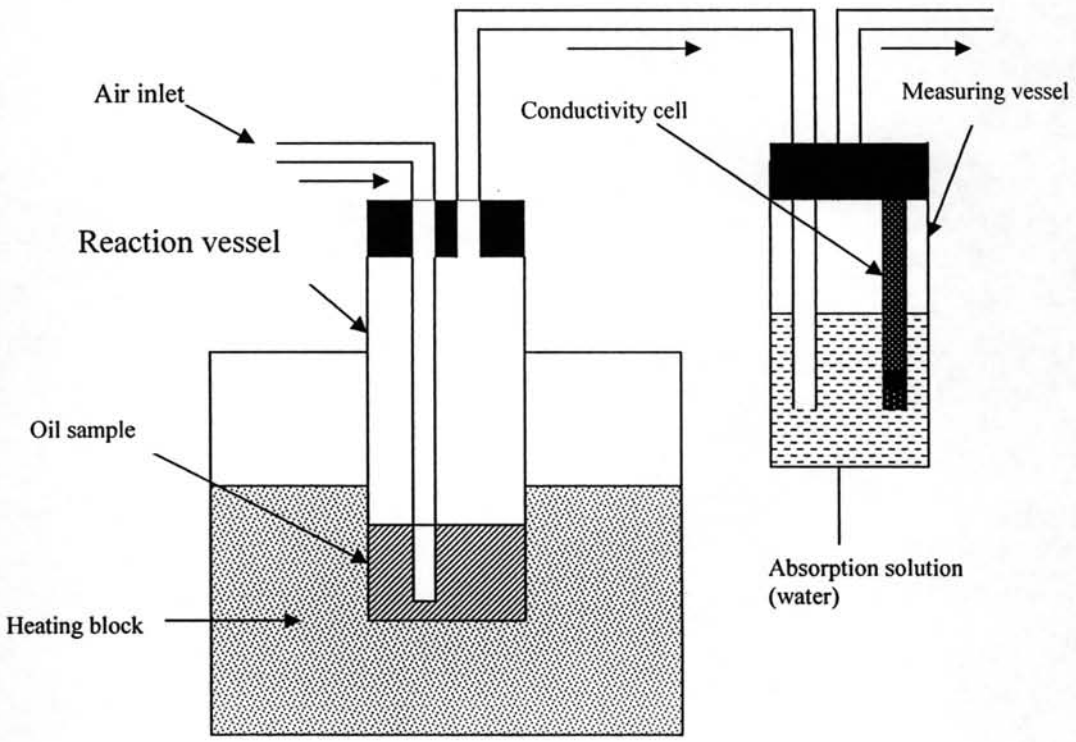


Figure 13 The process of OSI

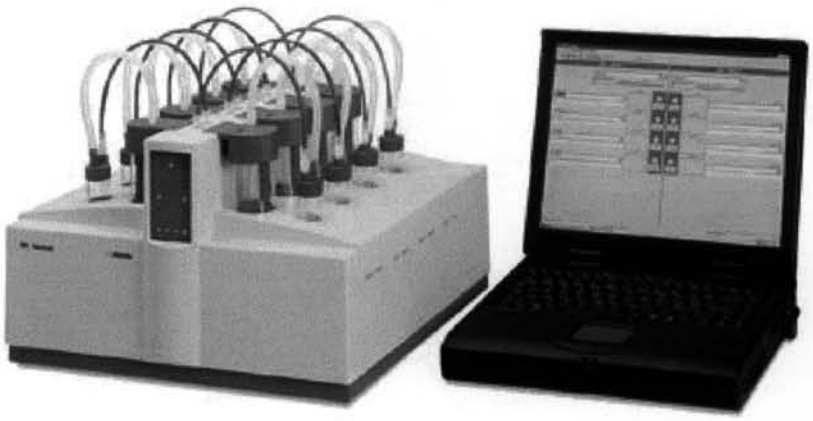


Figure 14 The OSI instrument 743 Rancimat®

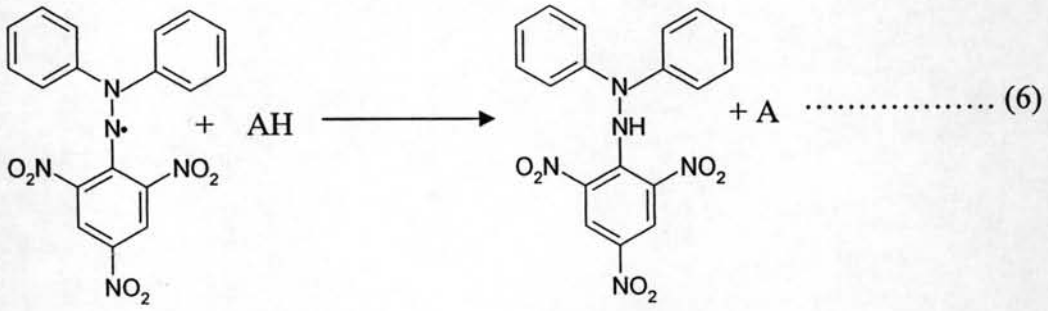
4.2 Analytical Method for Testing Antioxidant Properties (free radical scavenging) of RBO

There are large numbers of oxidant capacity assays that use chemical chromogenic/fluorescent redox reactions to test antioxidant properties of different chemical components. Other methods are based on the properties of the biological systems; for example, the integrity of the cellular membrane is correlated with LDH (lactate dehydrogenase) leakage (Yu, 1999).

Among the oxidative species used *in vitro* for testing the scavenging capacity of different antioxidants, hydrogen peroxide, peroxynitrite, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) or ABTS, and 1,1- diphenyl-2-picrylhydrazyl (DPPH) are most commonly used (Miller, 2001; Hoelzl et al., 2005; Miller et al., 1993). DPPH method was reported in the literature as a suitable means to evaluate the antioxidant activity of different vegetable oils and it was selected as the method of choice in the present study to measure antioxidant activity of the RBO (Espin et al., 2000; Parry et al., 2005; Oufnac et al., 2007)

4.2.1 Hydogen-donating Activity (DPPH radical scavenging activity)

The diphenylpicrylhydrazyl (DPPH) method is a simple colorimetric assay (Prakash, 2001) of antioxidant activity based on the decrease in absorbance at 517 nm of the DPPH radical (deep purple) after the addition of an antioxidant compound or a hydrogen donor in an ethanolic solution (Chaudhuri, 2002). The addition of an antioxidant (AH) results in the changing of this color to yellow. The structure of DPPH and its reduction product by an antioxidant are shown in Figure 15.



DPPH• (Purple)

DPPH-H (Pale yellow)

DPPH = 1, 1-diphenyl-2-picrylhydrazyl

AH = antioxidant

Figure 15 Structure of DPPH and reaction with an antioxidant (Prakash, 2001)

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to from the reduce DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured. The DPPH method has also been used to quantify antioxidants complex biological systems in recent years. This method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample (Prakash, 2001).

4.3 Analysis Method for Determination of γ-Oryzanol in RBO

4.3.1. HPLC Theory

High performance liquid chromatography (HPLC) is based on the principles of classical chromatography but achieves a higher resolution of solutes. This is accomplished by reducing the particle size of the stationary phase. The smaller particle size allows the solute to diffuse rapidly between the mobile and stationary phases. This increases the rate of equilibrium between the two phases and results in more uniform migration paths. The use of finer particles creates a much higher resistance to solvent flow. It is therefore necessary to use high pressure, approximately 7 - 40 MPa, to force the solvent through the column. Normal-phase chromatography refers to the use of a polar adsorbent, such as silica, and a less polar solvent for the mobile phase. Also commonly used, reverse-phase chromatography employs a non-polar adsorbent and a more polar mobile phase. The adsorbent is generally a bonded phase, such as C18, attached to the surface of the silanol groups. Reverse-phase chromatography provides excellent separations. It eliminates tailing problems associated with adsorption of polar compounds by polar packing and is less sensitive to polar impurities, such as water, in the eluent (Hams, 1991).

Rogers et al., (1993) reported the identification and quantitation of individual γ-oryzanol component and simultaneous assessment of tocopherols and tocotrienols in RBO (Figure 16). A method was developed for their separation by reverse-phase HPLC. For γ-oryzanol, detector was set at a wavelength of 325 nm. Vitamin E was analyzed by using the same procedures as γ-oryzanol, but the detector wavelength was set at 290 nm.

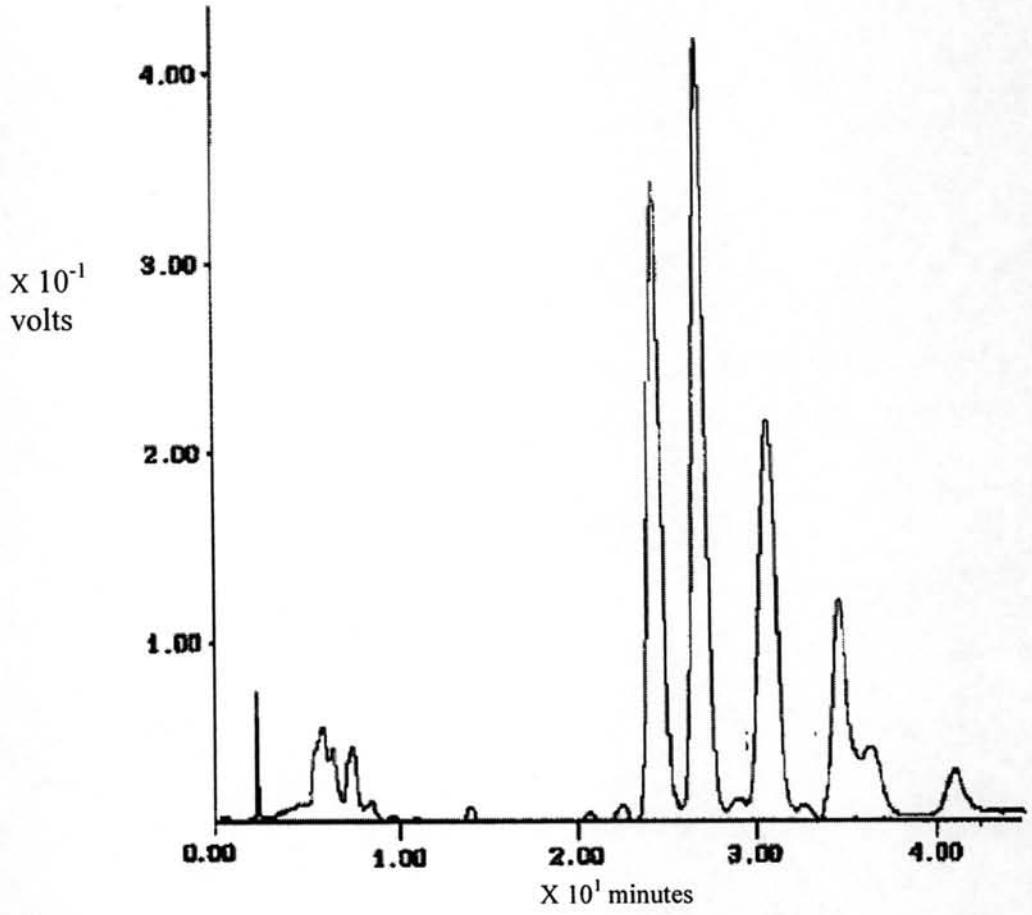


Figure16 HPLC Chromatogram of Crude RBO

(Rogers et al, 1993)

4.4 Analysis Method for Determining Stability of γ-Oryzanol in RBO from Various Production Methods.

Stability testing usually begins during the early stages of product development. The main purpose of stability testing is to establish a product shelf-life. According to the long duration of room-temperature shelf lives, stability tests are often performed under stressed conditions (e.g. elevated temperatures) to accelerate the degradation process (Ertel and Carstensen, 1990). Garrett has introduced the principles of chemical kinetic to evaluate drug stability at higher temperature (Garrett, 1962). The room-temperature stability or any lower temperature stability could be extrapolated from accelerated data by using Arrhenius relation. The Arrhenius equation is expressed mathematically as:

$$k = Ae^{-Ea/RT} \quad \text{..... (7)}$$

Where k is the reaction rate constant of any order, R denotes the gas constant (1.987 calories degree^{-1} mole^{-1}), A is the frequency factor, Ea is the activation energy (Kcal/mol) and T is the absolute temperature.

5. Emulsions Preparation

5.1. Emulsions

Almost of cosmetic products, the emulsion is the form that is probably the most used. For reasons of skin feeling, consumer appeal, and ease of application, emulsions are preferred to waterless oil and lipids along with gels. The main components of emulsions are lipid (lipophilic compounds) and water (and/or hydrophilic compounds). These two immiscible phases are allowed to remain in metastable mixed state by an amphiphilic component, an emulsifier. Emulsion can either be of the oil-in-water (o/w) and water-in-oil (w/o) types. If emulsions are liquid, they are generally called lotions. Creams are emulsions occurring in semisolid

form. Under gravitation, creams do not flow out through the orifice of reversed containers because of the behavior consistency in comparison with lotions.

5.1.1. Oil-in-Water Emulsions

The high acceptance of o/w emulsion (Figure 17) is based on the following reasons: feel light and not greasy when applied, showed good spreadability and penetration and an active hydration effect by the external water phase. Also, it gave a cooling effect because of the evaporation of the external aqueous phase.

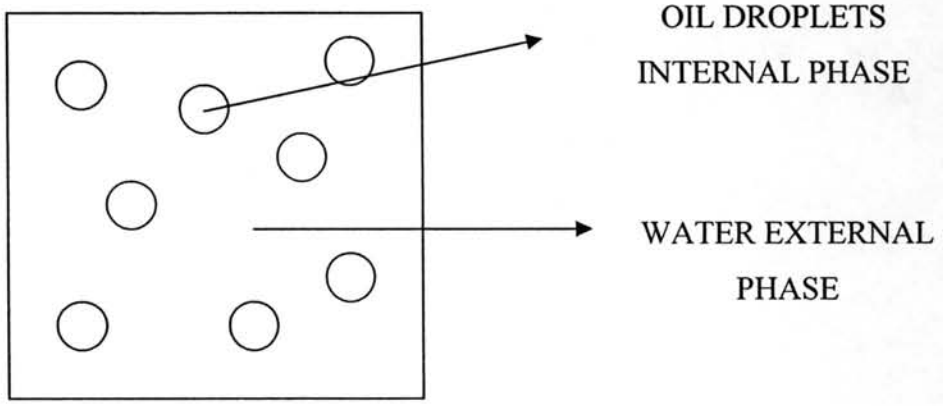


Figure 17 oil-in-water emulsions

5.1.2. Water-in-Oil Emulsions

Water-in-oil (w/o) emulsions may still be regarded as heavy, greasy, and sticky although during recent years great progress has been achieved in the preparation of pleasant w/o emulsions. Therefore, the w/o emulsion type is not only the basis for water-resistant sun protection, baby cream, or night creams, but also for protective day creams. The advantages of w/o emulsions are close resemblance to the natural protective lipid layer in the stratum corneum, efficient skin protection attributable to formation of a continuous layer of lipids on skin after application, sustained moisturization because on skin a continuous semiocclusive barrier is formed that reduces evaporation of skin water and that in addition actively releases the incorporated water from the internal phase, generally several times more

efficient than o/w emulsions, improved penetration into the lipophilic stratum corneum coupled with improved carrier function of lipophilic active substances, and even of hydrophilic substances incorporated in the internal aqueous phase

5.2. Components of Oil-in-Water Emulsions (Kennerth, 2005)

These components are commonly used in the oil-in-water formulation as shown in Table 3.

Table 3 Components of an oil-in-water emulsion

Components of an oil-in-water emulsion

Material	%	Function
Water	60-95	Diluent
Humectant	2-5	Improves stability, affects skin feel, solubilizer for preservatives
Emollient (water soluble)	1-2	Improves skin feel, reduces tackiness
Thickener	0.2-1.0	Improves stability, modifies skin feel, suspending agent
Preservative	0.1-1.0	Preservative
Emulsifier	2.0-5.0	Stabilizes emulsion
Emollient (oil soluble)	5-15	Improves skin feel
Silicone	2-5	Reduces skin whitening (soaping) improves skin feel
Wax	1-3	Affect skin feel
Color	As needed	Consumer appeal
Fragrance	0.15-0.5	Consumer appeal
Alcohol	0.0-20	Reduces tackiness, provides cooling effect

5.3 Oil-in-Water Emulsions Materials (Preston, 2003)

These materials are commonly used in the formulation of oil-in-water emulsions using RBO as active ingredient.

5.3.1 RBO (HLB = 7): Emollient oil with antioxidant and free radical scavenging property. Natural source of vitamin.

5.3.2 Stearic acid (HLB = 15): One of the most common natural fatty acid occurring in vegetable fat used as an emollient.

5.3.3 Cetyl alcohol (HLB =15.5): Noncomedogenic emollient emulsifier and thickener

5.3.4 Bee wax (HLB = 12): Natural wax produced by bees. Used as naturally non-comedogenic oil-absorbing ingredient.

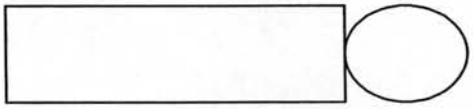
5.3.5 Glycerin: A humectant with water-attracting/binding properties that allows it to draw and absorb water from the air and help the skin retain moisture.

5.3.6 Propylene glycol: Appears in many cosmetic as a solvent, conditioning, and humectant

5.3.7 Paraben concentrate: Paraben concentrate consists of 20 % w/v methyl paraben and 2 % w/v propyl paraben in propylene glycol using as preservative.

5.4 Emulsions and the HLB System

In 1949, William C. (Bill) Griffin developed the Hydrophile-Lipophile Balance System (HLB) when he was a chemist at the Atlas Powder Company, which eventually became ICI Surfactants and is part of Uniqema today. All emulsifier have two parts; like a bar magnet. A bar magnet has a north pole and a south pole. Nonionic emulsifiers also have two poles or parts. An emulsifier molecule has one part that loves water and one part loves oil. The water loving part is called hydrophilic. The other part of the emulsifier molecule is lipophilic (Figure 18).



Lipophilic **Hydrophilic**

Figure 18 Hydrophilic part and a lipophilic part

Therefore, an emulsifier has a hydrophilic part and a lipophilic part. The balance of these two portions of the emulsifier gives us the Hydrophile-Lipophile Balance. The HLB of emulsifiers can be calculated or determined through trial and error.

5.4.1 HLB Determination

The HLB of oil phase was calculated as shown in Table 4.

Table 4 HLB Calculation of RE-RBO in oil phase

Oil phase ingredient	%	HLB no.	Fraction	HLB x fraction
RBO	5	7	5.0 / 8	4.38
Stearic acid	0.5	15	0.5 / 8	0.94
Cetyl alcohol	2	15.5	2.0 / 8	3.88
Bee wax	0.5	12	0.5 / 8	0.75
Total	8			9.94

HLB of emulsifying agent

Required HLB = (fraction GMS SEx $HLB_{GMS\ SE}$) + (fraction Tween 60 x $HLB_{Tween60}$)

Required HLB = (X x 5.8) + ((1-X) x 14.9)..... (8)

9.94= (X x 5.8) +((1-X) x 14.9)

X= 0.54

Emulsifying agent 5 %

GMS SE = 2.7 g ,

Tween 60 = 2.3 g

6. Stability Consideration

6.1 Physical Stability

Creaming, sedimentation, flocculation, coalescence, Ostwald ripening and phase inversion are the most important processes associated with emulsion instability. They are illustrated in Figure 19.

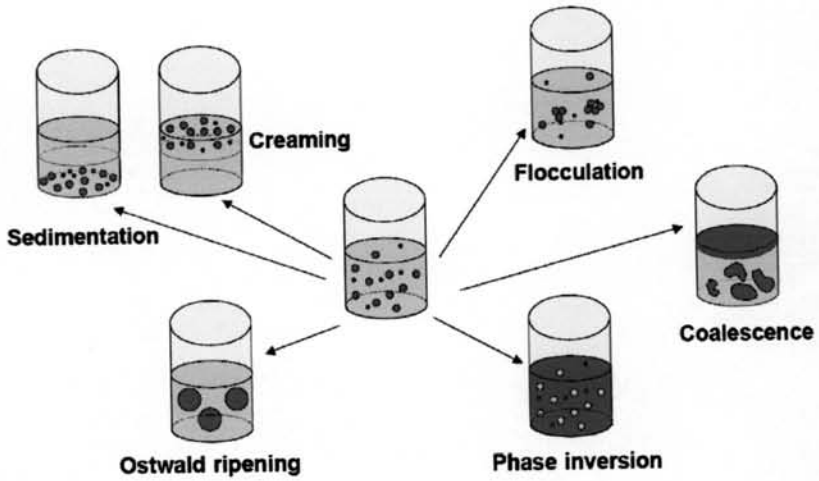


Figure 19 Main breakdown processes of emulsions (Tadros, 2004)

6.1.1 Flocculation (aggregation)

This process is characterized by a weak, reversible association between droplets of the emulsions internal phase. Each individual droplet maintains its own identity; thus there is no change in the basic droplet size. Flocculation represents a less serious sign of instability, which can be reversed by shaking the system.

6.1.2 Creaming

When particles of an emulsion aggregate, there is a tendency for upward sedimentation. This causes a partial separation of the emulsion into two emulsions, one of which is richer in the internal phase and the other richer in the external phase. As in the case of flocculation, this stability problem can be reversed by agitation.

6.1.3 Coalescence

An aggregation between two particles can, if the two particles combine, lead to the formation of one larger particle. This process, known as coalescence, represents a more serious stability problem. A related phenomenon is that of Ostwald ripening, in which the particles all tend to become the same size. Both of these processes are irreversible and can eventually lead to complete separation of the internal and external phases of emulsion. For stability considerations, this change is typically undesirable, since it will change the physical properties of product.

6.1.4 Ostwald Ripening

Ostwald ripening results from the difference in solubility between small and large droplets and occurs mainly in nanoemulsions. It is characterised by the increase of droplets size with time. The reduction of the interfacial energy by incorporating surfactants into the dispersed phase which strongly adsorb at the interface and have low solubility in the continuous phase may reduce Ostwald ripening significantly. Another method is to add a second disperse phase component, concentrated in smaller droplets and being insoluble or less soluble than the first one in the continuous phase.

6.1.5 Phase Inversion

Phase inversion is a process whereby the internal and external phases of the emulsion suddenly invert (O/W changing to W/O). This phenomenon can be induced either by changing the volume fraction of one of the phases or by changing the affinity of the surfactants towards the two phases (transition inversion). In the first case, the increase of one fraction above a critical value induces a phase inversion; the latter accounts for certain nonionic surfactants, which in an O/W system can alter the phase affinity with temperature. The critical temperature at which a phase inversion may take place is referred to as the phase inversion temperature (PIT) and is an important parameter to be taken into account when preparing nanoemulsions.