CHAPTER 3

Surface Active Properties of Palm Oil

3.1 Introduction

Emulsifiers can be divided broadly into natural and synthetic. The synthetics are chemically synthesized based on established synthetic pathways, incorporating a hydrophobic chain linked to some hydrophilic functional groups. At present, the number of synthetic surface active materials available commercially reaches into thousands with more than 700 being of relatively pure materials (1). In comparison, natural emulsifiers are fewer in numbers. Proteins, lecithin and monoglycerides (partially natural as they are usually derived from natural sources) are the most common natural emulsifiers used in food. Other natural emulsifiers include the sphingolipids, glycolipids and bile salts.

Monoglycerides and their derivatives account for about 75% of the world production of food emulsifiers (2). The most often used are the relatively pure distilled monoglycerides derived from natural oils and fats, although some derivatives of monoglycerides are known to exhibit surface active properties too.

Monoglycerides belong to the class of nonionic emulsifiers. Their surface activity is a consequence of the asymmetric structure of the molecule which give rise to the amphiphilic chemical nature. The hydrocarbon chain of the fatty acid forms the hydrophobic moiety of the monoglyceride. It ranges from C12 to C22 carbons depending on the source of triglycerides they are derived. The hydrophilic moiety consists of the two polar hydroxyl groups formed by the glycerol fragment together with the ester bond of the fatty acid ester (Fig.1).

The common type of phospholipids used commercially is usually a crude mixtures obtained from the degumming of soybean or rapeseed. It is commonly known as "lecithin" and contains various phospholipids, besides phosphatidylcholine. Various types of modified phospholipids with different degree of purity are used commercially both in the food and non-food industries (3). Phospholipids belong to the class of ionic emulsifiers. The hydrophilic moiety consists of the polar phosphate derivatives while the lipophilic moiety consists of the alkyl chains of the fatty acids (Fig. 1).

In view of the amphiphilic nature of these emulsifiers irrespective of the phase in which the emulsifiers are dissolved, they will tend to become located at the interface if present in air/water or oil/water interface. The hydrophilic headgroup will tend to locate itself in the aqueous phase while the lipophilic hydrocarbon chain orientates towards the oil phase. This situation is thermodynamically more favourable than complete dissolution in either phase. This phenomenon of strong adsorption of the emulsifiers at the interface in the form of an orientated monolayer is termed surface activity and results in the lowering of the interfacial tension at the oil/water interface. This process plays a critical role in determining the behaviour of emulsion droplets where there is ample oil water interface formed by the dispersed oil or water droplets. Thus the behaviour of the emulsifiers at the interface determines the behaviour of the oil/water system. When the interfacial tension of the oil/water interface is lowered, deformation of the interface is easily obtained resulting in the formation of small oil droplets (4). Subsequent stablisation of the oil droplets depends on the interaction of the various surface forces (5), steric effect due to complex formation as in mixed emulsifiers (6) or large biopolymers of proteins (7). Further emulsifier adsorption resulting in formation of bilayers (8) or liquid crystals (9) can contribute to enhanced emulsion stability.

Many other macroscopic phenomenan eg. flotation, detergency, lubrication and wetting also depend on the interfacial interaction or adsorption of emulsifiers at the solid/liquid interface in which interfacial tension is one of the critical parameters affecting them.

Palm oil has a wide range of potential applications but there seems to be a dire lack of information on its surface activity. The acquisition of interfacial tension data of the palm oil/water system in the presence of some natural emulsifiers can certainly lead to a better understanding of the various related processes such as palm oil milling, refining and emulsification of palm oil. The adsorption behaviour of various surface active materials at the palm oil/water interface need to be understood for better utilisation of palm oil.

3.2 MATERIALS

Chemically refined palm oil (NBDPO) was used as the standard palm oil in all the interfacial tension and surface tension measurement. NBDPO contained 95.97 wt % triglycerides, 3.99 wt % diglycerides and 0.04 wt % monoglycerides. The fatty acid composition and quality of the oil used are shown in Tables 1,2 and 3.

In this study various additives were introduced into the NBDPO and the interfacial tension determined. They are as described. Monoglycerides of 98-99% purity were purchased from Sigma Chemical Co. and used as received. Monoglycerides used were 1-monolauryl (C12:0), 1-monomyristic (C14:0), 1-monopalmitoyl (C16:0), 1-

monopalmitoleoyl (C16:1, [cis]-9), 1-mono-stearoyl (C18:0), 1-monooleoyl (C18:1, [cis]-9), 1-monolinoleoyl (C18:2), [cis]-9,12) and 1-monolinolenoyl (C18:3, [cis]-9,12,15) and referred to as monoglycerides C12:0, C14:0, C16:0, C16:1, C18:0, C18:1, C18:2 and C18:3 respectively in this study.

Palmitic and stearic acids were 99 wt % pure. Diolein was (C18:1 [cis]-9) mixed isomers of approx. 85 wt % 1,3 and 15 wt % 1,2 isomers. Dipalmitin (C16:0) was mixed isomers of approx. 50 wt % 1,2 and 50 wt % 1,3 isomers of 99% purity. Cholesterol (5[6]-Cholesterol-3ß-ol) was of 95 wt % purity. They were all purchased from Sigma Chemical Co. and used as received.

Commercial monoglyceride, PVP, consisting of 90% monopalmitin and 10% monoolein of 90% purity was supplied by Grindsted Products A/S, Denmark.

Three types of lecithin from Sigma Chemical Co. were used. They were i) type IV-S: soybean lecithin (containing approx. 40% phosphatidylcholine), ii) Lyso-Phosphatidylcholine and iii) Type XI-E. (99%): L-α-Phosphatidylcholine from fresh egg yolk.

Three fractions of the $CHCl_3$/MeOH extract from the oil droplets of palm oil mill sludge were studied (see chapter 2). They were a) the chloroform fraction b) acetone fraction and c) methanol fraction.

Monoglycerides from the chloroform fraction was obtained by thin layer chromatography. The monoglycerides band was scraped and washed twice with 15 ml chloroform. The solvent was then rota evaporated.

3.3 METHODS

The Kruss Digital Tensiometer Model K10T was used for the measurement of surface tension and interfacial tension. All water and oil samples were melted and placed in a 60°C oven before transferring to the measuring system. The instrument was checked daily by measuring the surface tension at 60°C of twice distilled water from an all glass water still before measurement. Double distilled water was used in all the measurements.

All the additives were added to the refined palm oil (NBDPO) except the alcohol insoluble residue which was dissolved in water.

3.3.1 Surface Tension Measurement

The tensiometer with the attached ring in air was tared. The sample was then brought up until the ring was submerged. The system was allowed to stabilise for half an hour and measurement began by slowly lowering the sample stage. The ring pulled a collar of liquid into the air. The maximum force necessary to break the film in air was taken as the surface tension of the liquid.

The ring was cleaned with methanol, acetone and finally flamed after every measurement before the next.

3.3.2 Interfacial Tension Measurement

The tensiometer was tared in the lighter phase (oil) by submerging the ring completely in it. Zero adjustment in the oil was necessary to compensate for buoyancy and surface tension which acted on the shaft of the ring. The ring was cleaned as described above.

After that a new sample container was half filled with the heavy phase (water, 12.5g) and the cleaned ring submerged into it. The lighter phase (oil, 12.5g) was added carefully to the top of the water. The system was allowed to stabilise for half an hour before the ring was pulled through the oil/water interface. The maximum force necessary to break the film in the oil was taken as the interfacial tension of the system. The mean interfacial tension of the refined palm oil (NBDPO) against water was 20.6 mN m^{-1} at 60°C based on measurements of 10 samples of the same batch of oil. The measured values ranged from 19.2 to 21.6 mN m^{-1} with an S.D. of ± 0.72 mN m^{-1}.

3.3.3. Extraction of Alcohol Insoluble Residue (AIR) of Sludge from Palm Oil Mill

Sludge from the centrifuge of the palm oil mill was fractionated by centrifugation as decribed in section 2.2.1, Chapter 2. The oil droplets were separated and the supernatant collected. The total solid content of the supernatant was determined gravimetrically by drying a known weight of the supernatant at 105°C. The residual dried weight was calculated as the total solid content.

In the extraction of AIR, 500 ml of supernatant was transferred to a 2 litre beaker and placed in a water bath at 40°C. Very slow stirring was carried out by a blade rotor mixer. 1,500 ml. of methanol was introduced slowly into the beaker. Flocs appeared gradually and stirring was stopped. The flocs was removed by centrifugation at 10,000 rpm and the flocs sedimented as a layer of compact precipitate. The precipitate was filtered under vacuum and washed twice with 120 ml of 60% methanol followed by twice of 150 ml 95% methanol. The AIR was then vacuum dried at 30°C, ground and sieved through a 0.1 mm sieve. The yield of the extract is calculated on a dry basis.

$$\text{Yield on dry basis (\%)} = \frac{\text{Wt. of dried AIR obtained} \times 100}{\text{Wt. of total solid in known volume of supernatant used}}$$

The protein, and oil and grease content were determined according to PORIM Test Methods (10,11).

3.4 Calculation of Surface Excess, Γ and Area per Molecule, σ

The surface excess, Γ and surface area per molecule σ of emulsifier were computed according to the Gibb's adsorption equation. For very dilute solution of a non-ionic surfactant; the concentration C is taken as the activity.

$$\Gamma = \frac{-1}{RT} \frac{d\gamma}{d \ln C} \text{ Mol m}^{-2}$$

where Γ = surface excess of solute (Mol m^{-2})
R = Gas constant
T = Absolute temperature (K)
γ = Interfacial tension (mNm^{-1})
C = Concentration of emulsifier (Mol dm^{-3})

Computation of the Best Fit Curve for the interfacial tension vs ln (concentration) graph was carried out using the Cricket Graph IBM version 1.3.2 software (Table 4). The coefficient of determination, r^2 in every case was greater than 0.99 when the second degree polynomial was fitted. The negative gradient indicated there was positive adsorption of emulsifier at the interface. The first derivative, $d\gamma/d \ln C$ was deduced from the best fit equation obtained. The surface area per molecule, σ, was calculated according to

$$\sigma = \frac{1}{\Gamma\ N_{AV}}\ m^2/\text{molecule}$$

where N_{AV} = Avogadro's Constant

3.5 RESULTS AND DISCUSSION

3.5.1 Interfacial Tension of Palm Oil/Water System

Interfacial tension measurements of the triglyceride water interface was first carried out by Lord Raleigh using castor oil in 1890 (12). Interfacial tensions of various vegetable oils had been reported in the literature (13-16) and big variations were found. Fisher et. al. (13) attributed this variation of interfacial tension values not to the different structures of the triglycerides but to the methods of measurement. Using the pendant-drop technique, a range of 22.80 to 24.77 mNm^{-1} was found for five types of commercial triglycerides studied but no detail chemical analysis of the oils was provided. Under alkaline pH of the aqueous phase, hydrolysis of the oil forming mono- and diglycerides was shown to occur which could lower the interfacial tension (14). Various purified vegetable oils and commercial vegetable oils (palm oil excluded) showed different interfacial tensions due to the presence of surface active impurities (15). Goanker (16) studied different commercial oils and detected anomalous behaviour where the interfacial tension (in the presence of sodium chloride) increased with increasing monoglyceride concentration. He deduced that the difference in interfacial tensions among oils was due to the presence of mixtures of monoglycerides and formation of reverse micelles in the oil.

The effect of various food grade emulsifiers on the interfacial tension of the soyabean oil/water system with and without milk protein added to the water was compared in an attempt to understand the real, complex food system (17). Anionic emulsifiers (sodium stearoyl-2-lactylate and diacetyl tartaric acid ester) and strongly polar non-ionic emulsifiers (Polysorbate 60) were found to be very active in reducing the interfacial tension of the soyabean oil/water system when the water phase contains proteins. Similarly interfacial tension studies of some natural surfactants have been carried out on corn oils and safflower oil/water system (18). However there seems to be a general lack of data on and understanding of the surface and interfacial behaviour of palm oil. This is probably because that palm oil is only recently recognised as a commercially available quality vegetable oil comparable to other more traditional vegetable oils like soyabean and corn oil.

The surface tension of various commercial palm oil products had been measured previously at specific temperature but no significant variations were found

(19). The present study showed that at 60°C the surface tensions of palm oil at various stages of refining were essentially similar within a narrow range of 30.6 - 31.7 mNm^{-1} (Table 5). On the other hand the interfacial tension of the crude palm oil against water was found to range from 6.0 to 9.6 mNm^{-1} indicating a much higher interfacial activity compared to the other partially refined palm oils. The surface activities of palm oil and its products are clearly manifested in their low interfacial tension values.

Crude palm oil is processed to remove the undesirable impurities and thus rendering them bland, colorless and chemically more stable. There are basically two types of refining processes practised by the Malaysian industry. They are chemical and physical refining processes as illustrated in the flow chart (Fig. 2). Chemically refined oils have higher interfacial tension ranging from 25.0 to 26.4 mNm^{-1} compared to physically refined oil which ranges from 10.6 to 12.9 mNm^{-1} (Table 5).

Fig. 3 shows the decrease in interfacial tension when various partially processed and crude palm oils were added to chemically refined palm oil. In physical refining after the degumming and bleaching stage, many surface active components originally present in the crude palm oil still remained. When degummed and neutralised palm oil from the chemical refining process was added individually to the chemically refined oil, there was only a slight decrease in interfacial tension. This indicates that chemical neutralisation and subsequent washing of the palm oil with water removed most of the surface active substances compared to physically degummed and bleached palm oil. The rate of deterioration in the quality of physically refined oil was much faster compared to that for chemically refined oil (20). The presence of surface active materials could be one of the major contributory factors.

Possibly the chemical neutralisation process removed the surface active materials more effectively. This is illustrated in the wide variation of the interfacial tension of the various partially processed oils measured against water (Table 5). The surface active materials may include the monoglycerides, glycolipids and phospholipids. Hydrolysis products such as fatty acids, aldehydes, ketones, hydroperoxides and peroxides were some of the impurities removed in the refining process. Carotene breakdown products are also subsequently removed in the refining process. At each stage of degumming, neutralisation and bleaching of the oil subsequently more surface active substances are removed as shown by the increase in interfacial tension of the oil against water. In chemical refining the degumming and neutralisation seemed more effective than bleaching in the removal of surface active substances from the oil as the changes in interfacial tension increases from a mean of 8.00 mN/m to 20.8 mN/m (difference of 11.2 mN/m) after degumming and neutralisation while there was an increment from 20.8 mN/m to 25.0 Mn/m (difference of 4.2 mN/m) after bleaching. Based on the data collated from the commercial samples analysed a higher standard deviation was noted for the degummed and neutralised oil to that of the neutralised, degummed and bleached oils.The efficiency of removal of the surface active substances is dependent not only on the type of process used but also on the type (surface activity) and dosage of bleaching earth used, the duration and temperature of the respective treatment (21).

Finally deodorisation at high temperature has a strong influence on the amount of volatile impurities removed but not so much the removal of the associated surface active compounds, since the interfacial tension was not much changed in both the physically and chemically refined oils, before and after the deodorisation process.

Since the interfacial tension of the crude palm oil and chemically refined palm oil (NBDPO) are so different, an attempt was made to study this parameter by adding to NBDPO some common constituents that are known to be present in crude palm oil. These include fatty acids, carotene, cholesterol, phospholipids and partial glycerides of mono and diglycerides.

The effect of various additives on the interfacial tension of the NBDPO/water system is shown in Fig. 4. Palmitic and stearic acid changed the interfacial tension only marginally even when added at as high as 5 % w/w was added separately to the refined oil. In fact the interfacial tension was increased slightly with the addition of palmitic acid. A very slight reduction in interfacial tension was observed when cholesterol was added to the oil. Similar findings were reported by Ogino (18) on oleic acid and cholesterol which were shown to have insignificant effect on the corn oil/water interfacial. Carotenes up to as high as 400ppm added to NBDPO did not have any effect on the interfacial tension of the NBDPO/water system. Neither did diglycerides of dipalmitin or diolein at as high as 3 % w/w changed the interfacial tension of the NBDPO/water interface. The NBDPO used contained 3.99% diglycerides, thus it appears the additional diglycerides added did not result in any significant surface activity changes at the oil-water interface.

When commercial monoglycerides PVP, a mixture of 90% monopalmitin and 10% monoolein was added to NBDPO, the decrease in interfacial tension at the NBDPO/water interface was gradual below 0.2% w/w (Fig 4). Above 0.2%

w/w a rapid decrease in interfacial tension with PVP concentration was noted. The interfacial tension was reduced from 19 mN/m to about 6.5 mN/m at 1.25% w/w PVP. Various extent of lowering of interfacial tension of purified corn oil/water interface by mixtures of monoglycerides had been reported (15). The reduction of interfacial tension depends on the concentration of the monoglycerides mixture added. Interfacial tension close to that of unpurified commercial soyabean oil (approx. 24 mN/m) was found for Florisil column purified soyabean oil at 0.055% w/w of mixtures of monopalmitate;monoolein; monolinoleate (3:5:12). At 0.075% w/w the interfacial tension was reduced to as low as a 5mN/m. Reduction of interfacial tension of NBDPO palm oil, like other vegetable oils, was also sensitive to the concentration of mixtures of monoglyceride added to the oil.

When soya lecithin was added to the NBDPO, there was a rapid decrease in interfacial tension at the oil/water interface (Fig 4). At 0.25% w/w the interfacial tension was reduced to about 5 mN/m. At the same concentration the decrease in interfacial tension by lecithin was much greater compared to the monoglycerides PVP (16mN/m). Lecithin has been found to be surface active in corn oil and safflower oil/water system too. At a concentration of 0.1% w/w lecithin in the oil, the interfacial tension was reduced to as low as about 2 mN/m (18).

3.5.2 Surface Activity of Aqueous Supernatant of Sludge

In the milling of oil palm fruits, besides the aqueous juice from the palm fruits, steam and hot water are added to the pressed fibers and crude oil respectively. The steam aids extraction while water helps clarification. The water also dissolves some water soluble components from the fibrous mesocarp and crude oil. It was in this intimate mixture of oil and water under agitation and pumping that emulsions are formed, rendering the separation of the oil from water a time consuming and tedious process in the clarification station. The stable small oil droplets formed are not easily recoverable in the present milling technology. The surface active materials from the palm fruits contribute significantly to the formation and stabilisation of the emulsified oil droplets.

Sludge has a total solid content of about 5.3-6.9% w/w, of which about 50% w/w are soluble and 50% w/w insoluble (22). High speed centrifugation at 10,000 G separates the insoluble solids, and gave a clear brownish supernatant.

The surface tension of the clarified supernatant was 30 mN m^{-1} compared to 66 mN m^{-1} for water at 60°C (Fig. 5). The surface tension of the clarified supernatant decreased slightly from 32 to 29 m N m^{-1} on increasing the temperature from 40°C to 80°C but the interfacial tension of NBDPO/supernatant increased from 19 to 23 mN m^{-1}. The surface tension of the supernatant increased when it was diluted with water (Fig. 6). The interfacial tension of crude palm oil and refined oil (NBDPO) against supernatant increased also when the supernatant was diluted.

In the crude palm oil/supernatant system which could be approximated to the real palm oil milling process, the interfacial tension was much lower than that for refined oil. It could be reduced to less than 3 mN m^{-1} which was conducive to emulsification under intense agitation during milling. Surface active materials from both the aqueous and oil phase were possibly acting synergistically in reducing the interfacial tension of the crude palm oil/water system favouring emulsification during the milling process.

The supernatant separated from the sludge had a solid content of about 3.9-5.1% w/w with a mean of 4.49% w/w. The pH ranged from 3.90 to 5.03 with a mean of 4.62. An attempt was made to extract some of the alcohol insoluble compounds (AIR) from the supernatant via flocculation under acidic conditions. This method was commonly used in the extraction of pectin (23,24) but other soluble compounds may also be simultaneously extracted.

The AIR extract was surface active when redissolved in water (Fig. 7). It lowered the interfacial tension of the NBDPO/water interface to 13.6 mNm^{-1} at 1% w/w conc. The AIR has a high molecular weight of 40,000 dalton (Table 6) and a protein content of 29.6% w/w. Much inorganic compounds measured as ash content (1.93% w/w) were simultaneously precipitated. Soluble carbohydrates, pectin, sugars and phenolics have been identified previously in the soluble fraction of the combined liquid discharge of palm oil mill sludge (25). Some of these are possibly present in the AIR. Further detailed identification and separation of each component which is surface active or possesses synergistic efect with surface active compounds in the supernatant would be useful in elucidating the surface activity of the sludge from the mill. The water soluble proteins could possibly have a profound effect on the surface activity at the crude palm oil/water interface in the presence of other surface active materials present in crude palm oil.

3.5.3 Surface Active Substances Associated with the Oil Droplets from the Aqueous Sludge

Oil droplets were separated from the separator sludge of the palm oil mill and extracted with chloroform and methanol as described in Chapter 2. The $CHCl_3$/MeOH extract of the oil droplets from the separator sludge was surface active. When added to NBDPO, it caused a sharp decrease in the interfacial tension of the refined palm oil (NBDPO)/water interface (Fig. 8). At 0.8% w/w in oil it reduced the interfacial tension to 1.4 mN m^{-1}. Its interfacial behaviour is comparable to that of soya lecithin that contains 40% phosphatidylcholine. There was no significant changes in surface tension of NBDPO when the $CHCl_3$/MeOH extract was added to the oil.

The extract was separated into three fractions by column chromatograph using different solvents: namely chloroform, methanol and acetone. The dried fractions were added individually to NBDPO and the greatest reduction of interfacial tension was noted for the acetone faction which was found to contain glycolipids (Chapter 2). The methanol fraction known to contain phospholipids showed only slight surface activity at the concentration used. The simple lipids which contained mainly triglycerides with 8% diglyceride and 0.5% monoglycerides were surface active only at very high concentration. The monoglycerides separated from the chloroform fraction showed slight surface activity at the concentrations added.

The unfractionated $CHCl_3$/MeOH extract in total showed comparable interfacial reduction at the oil/water interface as the commercial soya lecithin. At the same concentration the unseparated $CHCl_3$/MeOH extract from the oil droplets was most surface active compared to the other individually separated fractions. The results show that the $CHCl_3$/MeOH extract which contains a mixture of compounds acted synergistically in interfacial tension reduction at the oil/water interface.

Separation and collection of the various components of the extract by column and thin layer chromatography was technically tedious. Relatively large amount was needed for the interfacial tension measurement. Thus only low concentrations were measured but an indication of its surface activity was adequately illustrated from the data obtained.

3.5.4 Surface Activity of Low Concentration of Pure Monoglycerides and Phospholipids at the Refined Palm Oil (NBDPO)/Water Interface

Lipids form monomolecular surface film at the air/water interface. The molecular orientation of the monoglycerides at the air/water interface depends on the different phases of the lipids at various temperatures and pressure.

Various terms are used to describe the monolayer phase. In relation to structural formation, the gaseous phase (G) is one in which the molecules are distributed independent of each other at the interface (26). In the liquid expanded state (LE) the molecules are in a lamellar liquid crystalline phase, where the hydrocarbon chains are compressible (this being due to significant chain mobility thus its area may differ). In the liquid condensed phase (LC), the molecules are in a more rigid tilted crystalline form. Using the force-area isotherm, at the transition from LE to LC states at 25°C, cross sectional areas of 28.5 $Å^2$and 22.5 $Å^2$ have been determined for molecules of 1-monomyristin monolayers at the air/water interface. They have been designated as Form 1 and Form 2 corresponding to the LE and LC states respectively (27).

Most of the measurements of the lipid monolayer and understanding of its orientation and area covered are usually made at the air/water interface. This include the fatty acids (28), phosphatides (29) and monoglycerides (30). Different triglycerides have different equilibrium spreading pressure (ESP) and will spread at different rates at the air/water interface. The orientation of the triglycerides is such that the three fatty alkyl chains will be directed away from the water to allow interaction between the polar part of the molecule and water. Competitive spreading of the triglycerides with other polar/non polar surface active materials is such that the lipid with the higher spreading pressure will displace the lipid with the lower spreading pressure (26). Mixed monolayers of different types of phospholipids (30) and mixtures of monoglycerides (31) have also been investigated. Their behaviour depends on the compatibility and solubility of the individuals molecules in the mixture. Study on the effect of some synthetic surfactants at the hydrocarbon oil/water interface has been reported. The packing of the surfactant molecules at the oil/water interface may be influenced by the type or chain length of the mineral oil used (32).

Differences between different oils (triglycerides or hydrocarbon oils)/water and air/water interfaces thus would arise. This is illustrated by the interfacial tension of triglyceride/water interface ranging from 20-25 mN m^{-1} while that for hydrocarbon oil/water is about half this value (33). Thus adsorption can be very selective. While both the fatty acids and the monoglycerides adsorb strongly at the hydrocarbon/water interface, fatty acids are completely soluble in triglycerides. These differences arise because triglycerides are "polar" oils having distinct hydrophobic head and hydrophilic tail regions. Uniqueness in different vegetable oils is further reinforced by the inherent minor components associated with them. Palm oil is known to have a particularly high content of diglycerides and carotenes, besides a distinct composition of triglycerides.

At the palm oil (various types of palm oil and their products)/water interface, when emulsifiers are introduced into the oil phase, mixed monolayers of emulsifiers may be formed while competitive adsorption of the natural surfactants and the major glycerides of di and triglycerides may possibly occur too. In the study of mixed monolayers of tripalmitin and cholesterol at the air/water interface, mixed monolayers were formed but at higher pressure tripalmitin was squeezed out. Critical ratios of monoglycerides and diglycerides could even displace proteins from corn oil/water droplets interface (34). The degree of unsaturation of the monoglycerides may also affect the surface activity (35). Mono-unsaturated monoglycerides was found to displace proteins from fat globules in ice cream mix better than the saturated monoglycerides. In an attempt to understand the surface forces involved, Pezron et. al (36) coated mica surfaces with monolayers of monopalmitin (in fluid/gel state) and various degree of repulsion/ attraction were found when immersed in water, depending on the temperature and the separation distance of the adsorbed layers. In contrast, monoolein was found to be unstable and readily transformed into undefined structures.

The interfacial tension of some vegetable oils with respect to the concentration and type of monoglycerides was found to be affected by the chain length of the monoglycerides added to the oil (37). In this study the interfacial tension of refined palm oil (NBDPO)/water interface was observed to decrease rapidly with increasing concentration of various monoglycerides (Fig. 9). At very low concentration, the effect of the chain length of the fatty acyl group was not clear. At higher concentration, the monoglyceride with the shortest chain monolauryl C12:0, was the most surface active whereas that for the longer acyl chain of monostearin C18:0 was the least. Monoglycerides of carbon chain lengths C14:0 and C16:0 were about similar and intermediate in surface activity between those for C12:0 and C18:0.

The interfacial tension of the NBDPO/water interface is also affected by the degree of unsaturation of the fatty acyl chain of the monoglyceries added to the oil phase. This is illustrated in the comparison of monoglycerides C18:0 with C18:1, C18:2 and C18:3 and C16:0 with C16:1 (Fig. 10 and 11). Generally unsaturation of the acyl chain increased the interfacial activity. At low concentration the effect was less marked. Higher unsaturation such as C18:2 and C18:3 showed much lower interfacial tension at the same concentration of the monoglycerides.

Using the Gibb's Equation of adsorption, the calculated surface area for a molecule of the monoglyceride decreased with an increase in its concentration (Fig.12) indicating compression of the monolayer at the interface. There is an increase in surface area/molecule with an increase in the acyl chain length of the monoglycerides. Only C12:0 has a much smaller molecular area (25Å^2) compared to other monoglycerides C14:0 - C18:0. At 60°C, the short range forces among the hydrocarbon chains are diminished (the liquid condensed phase where the hydrocarbon chains are still interactive may not be pronounced at 60°C). Thus the much larger surface area per molecule estimated at approximately 25-40Å^2 seems comparable to the surface area of the molecule (22.5 - 28.5 Å^2) in the solid condensed to liquid condensed state of the air/water monolayer of 1-monomyristin determined at 25°C (27).

At 60°C and low pressure,the monoglycerides possibly assume a less vertical orientation and thus may sweep out an area much bigger than the surface area of the molecule in a liquid condensed phase. Hiemenz (38) proposed as a model for highly expanded state an extreme situation such that the hydrocarbon chain may possibly lie "flat" on the interface, occupying an area of πl^2 where l is the length of the hydrocarbon "tail". The intermediate areas determined for the monoglycerides at various concentrations indicate that they may sweep out an area much larger than the surface area of the molecule in a liquid condensed phase but may not completely orientate flat at the interface. Palm oil may have a different affinity for the alkyl chain accounting for the slight variation in surface area per molecule of the different monoglycerides. The difference arising from the alkyl chain length variation may not be fully manifested in our system of triglyceride mixture. Palm oil is also slightly polar due to the presence of oxidative breakdown products of the oil itself. This possibly affects the orientation of the polar moiety of the monoglyceride which consists of the glycerol fragment of two hydroxyl groups together with the carbonyl ester bond.

The surface area for the monounsaturated, C18:1 was very much larger compared to the saturated C18:0 (Fig 13). This is possibly due to the co-planar ethylenic bond of C18:1 which is very rigid and flat. It is also favourably orientated to the more polar water phase as it is enriched in electrons. The attached hydrocarbon chain tends to lie flat on the interface when allowed to do so sterically (39). However, the incorporation of two or more double bonds at the 12th and 15th carbon positions of the acyl chain did not seem to affect the surface area as much as the monounsaturated (Fig 13). Similar observation was noted on the significant increase in surface area per molecule of the monounsaturated C16:1 compared to the saturated C16:0 (Fig. 14).

The surface activity of some phospholipids at the palm oil (NBDPO)/water interface was also studied (Fig. 15). Phospholipids are generally more surface active than the monoglycerides. Lyso-phosphatidylcholine consists of only one fatty acid chain (Fig. 16) in the C1 position while L-α-phosphatidylcholine contains two fatty acid chains. The polar head group of choline are similar in both compounds. In lyso-phosphatidylcholine, the hydroxyl group attached to the C-2 affects its polarity differently as in contrast to that of the ester group of the L-α-phosphatidylcholine. Difference in polarity of the emulsifier would affect its surface activity at the NBDPO/water interface. The soya lecithin used contains only 40% phosphatidylcholine but its surface activity falls in between that of lysophosphatidylcholine and L-α-phosphatidylcholine.

The plot of the calculated surface area per molecule versus concentration of L-α-phosphatidylcholine and Lyso-phosphatidylcholine (Fig. 17) indicated a possible limiting area of approximately 70 $Å^2$/molecule. A rapid decrease in surface area with concentration of the phospholipids was observed for lysphophatidylcholine before its limiting value was reached. In the case of L-α-phosphatidylcholine, the surface area did not vary much at lower concentration. This is probably due to steric effect of the molecule. The molecule of L-α-phophatidylcholine with two fatty acid chains is bulkier than lyso-phosphatidylcholine and its film would appear also less compressible.

Changes in surface area of various synthetic phospholipids at various pressure in a monolayer at the air-water interface was studied by Deenan et. al. (29). The closest stable packing of distearoyl homologues of various phospholipids ranged from 36 to 40Å^2 on a phosphate buffer substrates at pH 7.4, i.e. phospholipids containing different head groups showed only small difference in the force-area curves. Shortening of the chain length and particularly unsaturation of the fatty acid constituents greatly expanded the film of the L-α-lecithin studied to approximately 60Å^2. A smaller area of 40Å^2/molecule of phosphatidylcholine from egg yolk was reported by Handa (40) in his experiment which was carried out at the triolein/water interface at 25°C.

In this study the limiting surface area of approximately 70Å^2 determined for both the lyso and L-α-phophatidylcholine was slightly high compared to those determined by Deenan (29) and Handa (40). As the system studied was a palm oil/water interface, differences are expected in terms of relative favourable orientation of the phospholipid molecule at the interface where possible interactions between the palm oil and phospholipid take place, in particular the apolar moiety of one or two alkyl chains of the lyso and L-α-phosphatidylcholine respectively. Hydration could also be involved. Temperature variation also significantly affects the packing configuration and surface area covered by some surface active substances at the interface (41).

Lecithin is known to consist of a variable mixture of phospholipids together with its various minor components and fillers used (42,42).Due to non availability of more technical information on the specification regarding the exact composition of the lecithin (specified as containing 40% phosphatidylcholine) used in this study, the calculated surface area/molecule of 170 Å^2 seemed relatively high compared to the other two types of phospholipids studied where the range of values determined was comparable to that observed by Deenan (29). It is also noted that the surface area of the lecithin was independent of its concentration over a wide range similar to that of the L-α-phosphatidylcholine. It is speculated that the other 60% of the lecithin mixture could possibly be other types of phopholipids which would then increase the concentration of the surface active molecules and reduced the calculated surface area/molecule significantly.

3.6 CONCLUSION

Under current practice, the refining of palm oil is not intensively monitored except for colour and free fatty acid content. These parameters are more for specification and quality control purpose than for process monitoring. In this study, the interfacial property of palm oil refined to various extent of purity was investigated using interfacial tension measurement. It was found that interfacial tension could potentially be used to monitor the efficiency of each stage of refining as an indicator of the "impurities" removed. A simple interfacial measurement is recommended for incorporation as a test to determine how efficiently the oil has been refined. There is a need by the industry for specific tests to be adopted to differentiate between physically and chemically refined oil (44). As the interfacial tensions of the chemically and physically refined oils are very different, this simple physical measurement is recommended. Simultaneously this parameter could also be employed by end users to determine the suitability of any particular oil in product formulation where interfacial tension is one of the factors affecting manufacturing process such as emulsification.

Natural surface active compounds were extracted from the oil droplets and from the supernatant of the palm oil mill centrifuge sludge respectively. The most surface active constituent from the oil droplets was the acetone fraction of the $CHCL_3$/MeOH extract although the $CHCL_3$/MeOH extract in total is the most surface active and has surface activity comparable to that of the soya lecithin studied. Natural surface active constituents of palm oil possess synergistic effect which may manifest in the utilisation of palm oil.

The surface active constituents of the aqueous supernatant from the palm oil mill sludge could be precipitated using methanol. However, further characterisation is needed to determine the specific compounds that contribute to its surface activity at the NBDPO/water interface, although early indication is that they contain phosphorus (phospholipids), are pectinic and proteinaceous in nature.

At the NBDPO/water interface, the interfacial tension data indicated that the order of surface activity is as follows: L-α-phosphatidyl-choline > soya lecithin > lysophosphatidylcholine > mono-glycerides. In monoglycerides the chain length and degree of unsaturation of the alkyl chain affect the surface activity as follows: C12:0>C14:0≅C16:0>C18:0 while C16:1>C16:0 and C18:2>C18:3>C18:1>C18:0.

Fatty acids,carotenes,cholesterol and diglycerides when added to refined palm oil did not exhibit significant surface activity at the NBDPO/water interface.

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Table 1. Fatty Acid Composition of Chemically Refined Palm Oil (NBDPO)

Fatty Acid	wt. %
C12:0	0.2
C14:0	1.0
C16:0	43.9
C18:0	4.0
C18:1	40.6
C18:2	9.6
C18:3	0.3

Table 2. Quality of Chemically Refined Palm Oil (NBDPO)

Peroxide Value	2.408 meq/kg
$UV^{1\%}_{233}$	1.5509
$UV^{1\%}_{269}$	0.3637
Carotene	1 ppm
Anisidine Value	1.3121

Table 3. Lipid Composition of Partially Processed Palm Oil

Glycerides Composition wt. (%)	Crude Palm Oil	Degummed and Neutralised Oil[b]	Degummed and Bleached Oil[a]	Refined Palm Oil[a]	Refined Palm Oil[b] (NBDPO)
Free fatty acids	3.25	0.14	2.92	0.04	0.02
Monoglycerides	0.27	0.17	0.17	0.04	0.04
Diglycerides	6.71	5.85	5.41	6.61	3.99
Triglycerides	89.75	93.84	91.50	93.31	95.90
Phosphorus[c] (ppm)	14.6	8.8	2.6	7.0	2.2

a = Physical Refined oil
b = Chemically refined oil
c = To convert to Phospholipids multiply by factor 25

Table 4. Derived Best Fit Equation based on the Interfacial Tension vs ln Concentration of Additives added to Palm Oil (NBDPO)

Additive	^{a}Best Fit Equation	Coefficient of Determination, ^{b}r2
Monoglycerides		
C12:0	$y = -23.020-14.501x-1.2488x^2$	1.000
C14:0	$y = -15.873-11.37726x-0.92175x^2$	0.990
C16:0	$y = -14.001-9.99985x-0.71361x^2$	0.996
C16:1	$y = 7.1878-2.9352x-0.20814x^2$	0.990
C18:0	$y = -17.194-12.021x-0.98122x^2$	0.992
C18:1	$y = 2.0372-4.5294x-0.2676x^2$	0.985
C18:2	$y = -10.609-4.6455x$	0.985
C18:3	$y = -22.032-11.757x-0.83101x^2$	0.998
PVP	$y = -21.005-14.552x-1.3047x^2$	0.996
Lyso-PC*	$y = -31.219-10.452x-0.53838x^2$	0.970
L-α-PC*	$y = -68.723-9.5272x-0.17262x^2$	0.955
Lecithin	$y = -10.307-2.6704x$	0.961

* PC = Phosphatidylcholine

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^{a}y is the interfacial tension, x is the ln concentration of emulsifier in oil.

$^{b}r^2$ is defined as $1-\frac{\Sigma(y-y')^2}{\Sigma(y-\bar{y})^2}$

Table 5. Interfacial Tension of Palm Oil/Water at Various Stages of Refining Measured at 60°C

Oil*	Interfacial Tension against Water (mNm^{-1})			Surface Tension (mNm^{-1})		
	Range	Mean	S.D	Range	Mean	S.D
[a]Crude Palm Oil	6.0-9.6	8.0	1.2	30.6-30.9	30.8	0.1
[b]Degummed and bleached palm oil	10.1-15.6	12.7	1.7	30.8-31.0	30.9	0.1
[b]Refined, bleached and deodorised palm oil (RBDPO)	10.6-12.9	11.8	1.1	30.8-31.1	30.0	0.1
[c]Degummed and Neutralised Palm Oil	18.9-24.2	20.8	1.8	31.0-31.7	31.2	0.1
[c]Degummed, neutralised and bleached	24.7-25.4	25.0	0.3	30.7-31.0	30.9	0.1
[c]Neutralised, bleached and deodorised palm oil (NBDPO)	25.0-26.4	25.6	0.5	30.8-31.1	31.0	0.1

a - Based on 18 samples
b - Based on 11 samples (physical refining from 4 refineries)
c - Based on 7 samples (chemical refining from 2 refineries)

*Point of sampling. Refer to Fig. 2.

Table 6. Properties of AIR precipatated using 1:1 (v/v) (Methanol: Supernatant) at pH 3, at 25°C

Ash content	1.93%
Acid insoluble ash	0.001%
Protein*	29.6%
Molecular Weight	40,000
Oil and Grease	16.5%

*Calculated from nitrogen content

Fig. 1. Possible Orientation of Emulsifiers at the Oil-Water Interface.

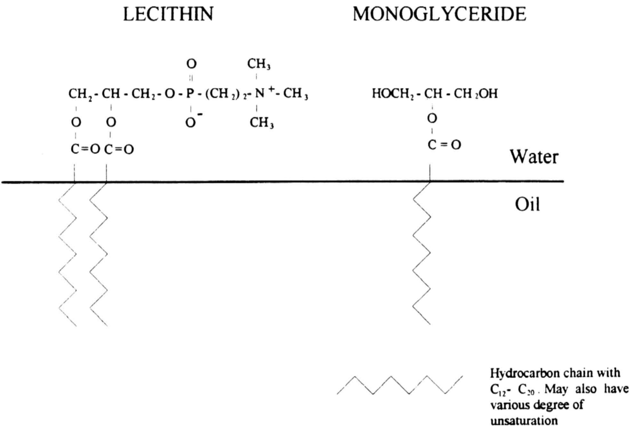

Fig. 2: Refining Processes for Crude Palm Oil

Chemical refining

Crude Oil

↓ 0.05-0.10% orthophosphoric acid added

Degummed oil

↓ Caustic soda added

Degummed and neutralised oil

↓ Water washed and vacuum dried

*Neutralised oil (NPO)

↓ Added bleaching earth and filtered

*Degummed, Neutralised and bleached oil (NBDPO)

↓ Steam deodorisation

*Neutralised bleached deodorised oil (NBDPO)

Physical refining

Crude oil

↓ Added orthophosphoric acid

Degummed oil

↓ Bleaching earth

Degummed and bleached oil

↓ Steam Deodorisation

*Refined bleached and deodorised palm oil (RBDPO)

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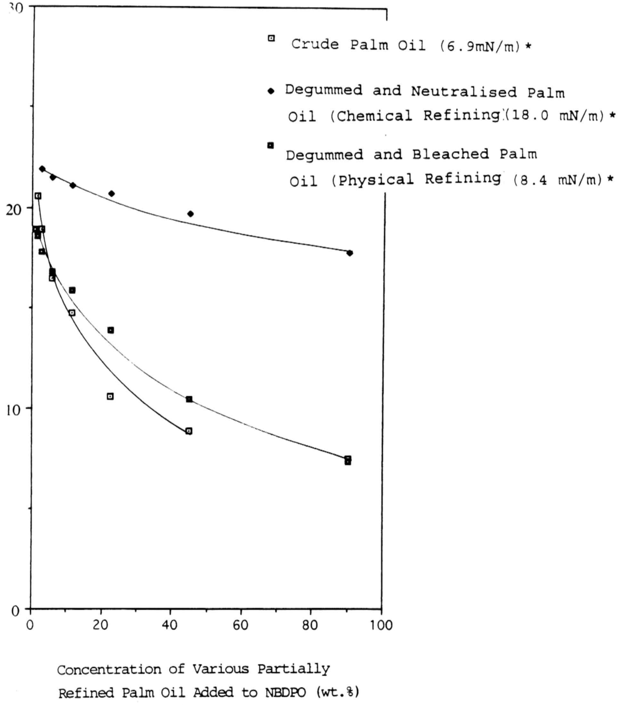
*Commercially available

Adapted from "Selected Readings on Palm Oil and Its Uses "Ministry of Primary Industries, Malaysia, 1993, 150-174.

3: The Effect of Various Types of Partially Refined Oil on the Interfacial Tension of Chemically Refined Palm Oil (NBDPO)/Water Interface



*Interfacial Tension of Oil used

Fig. 4. The Effect of Various Additives in Palm Oil on the Interfacial Tension of Chemically Refined Palm Oil (NBDPO) Water Interface

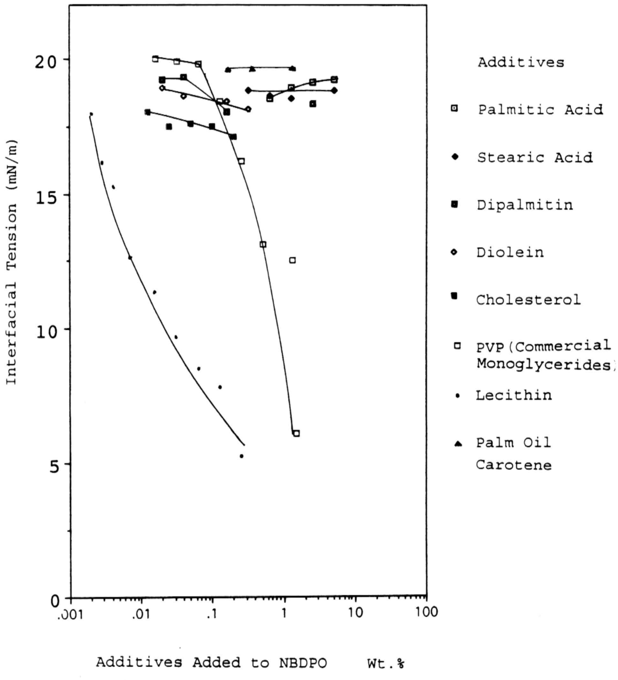


Fig. 5. Interfacial Tension and Surface Tension of Supernatant from the Sludge at Various Temperatures

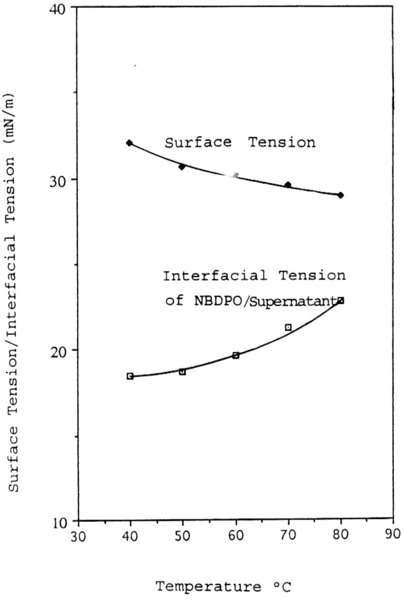


Fig. 6. Effect of Dilution with H_2O at 60°C on the Surface Tension of Supernatant and Interfacial Tension of Palm Oil (NBDPO) and Crude Palm Oil (CPO)/Supernatant.

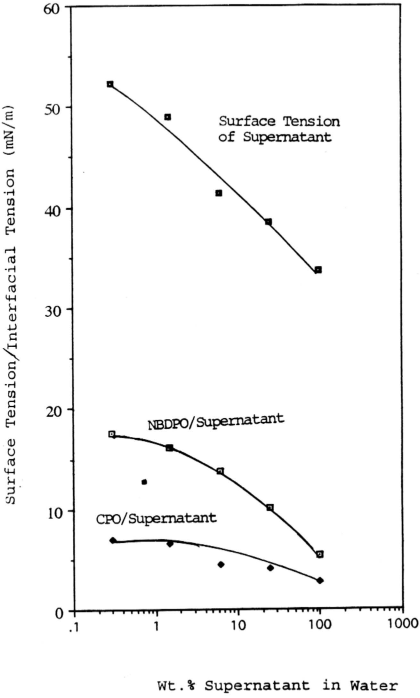
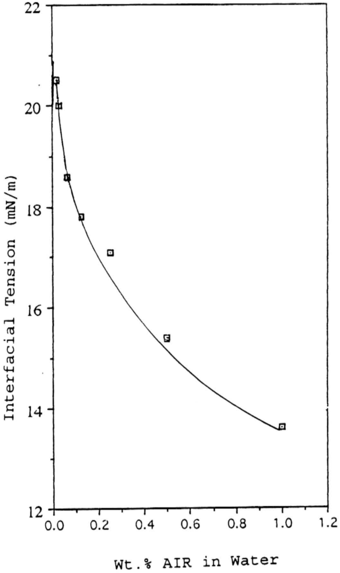


Fig. 7. Interfacial Tension of the Alcohol Insoluble Residue (AIR)* in Water against Palm Oil (NBDPO) as a function of the Concentration of AIR in Water.



*AIR was precipitated at 40°C with sludge supernatant: ethanol at (1:5 v/v) pH 4

Fig. 8. Effect of Additives on the Interfacial Tension of Palm Oil (NBDPO)/Water System.

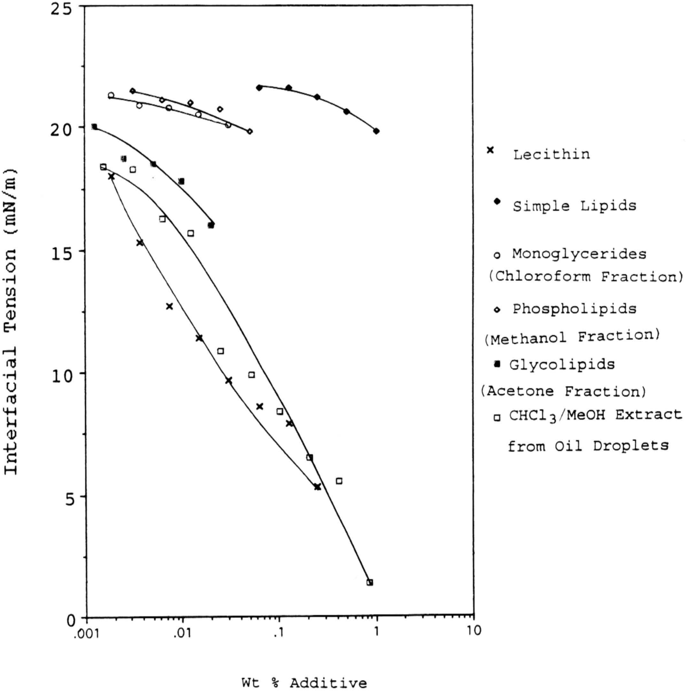


Fig. 9. Influence of Chain Length of Monoglycerides on the Interfacial Tension of Palm Oil (NBDPO)/ Water System at 60°C.

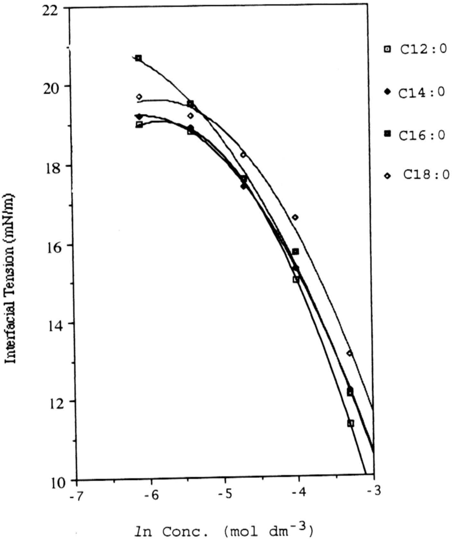


Fig. 10. Influence of Unsaturation of the Monoglycerides (18 carbon atoms) on the Interfacial Tension of Palm Oil (NBDPO)/Water System at 60°C.

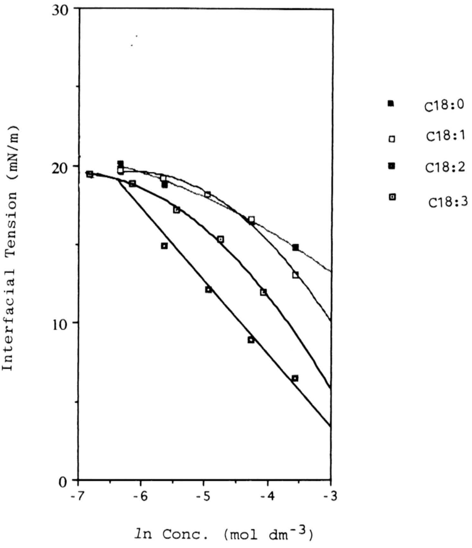


Fig. 11. Influence of Unsaturation of the Monoglycerides (16 carbon atom) on the Interfacial Tension of Palm Oil (NBDPO)/Water System at 60°C.

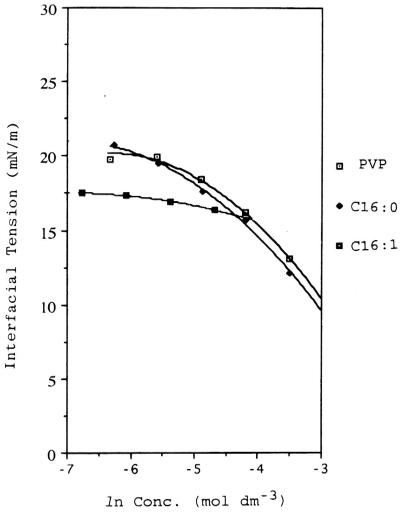


Fig. 12. Influence of Chain Length of Monoglycerides on the Molecular Area of the Monoglycerides at the Palm Oil (NBDPO)/Water Interface.

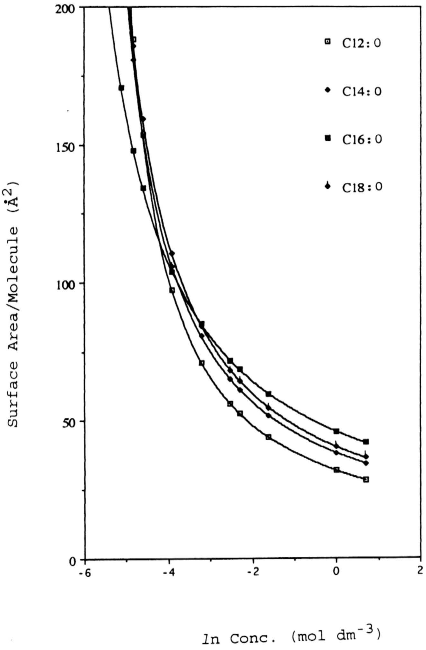


Fig. 13. Influence of Unsaturation of Monoglyceride (18 carbon atom) on the Molecular Area of the Monoglycerides at the Palm oil (NBDPO)/Water Interface.

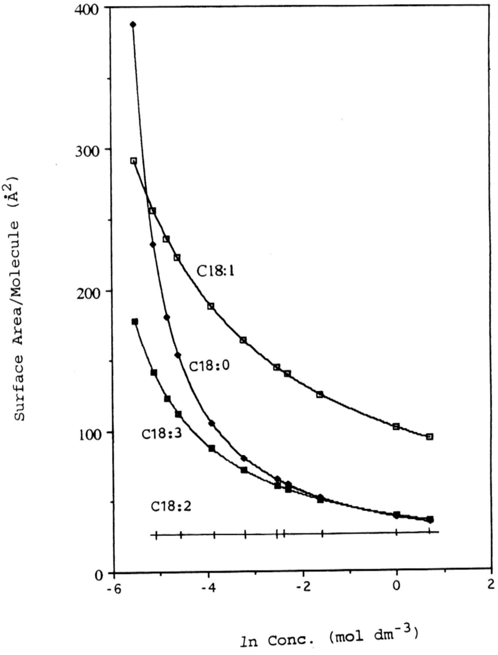


Fig. 14. Influence of a Double Bond on the Molecular Area of Monopalmitin at the Palm Oil (NBDPO)/ Water Interface.

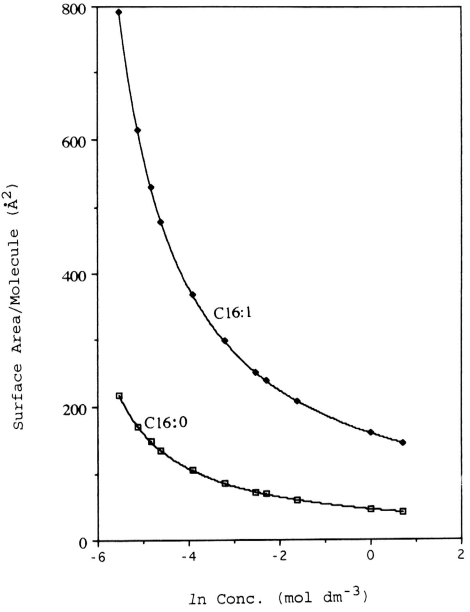


Fig. 15. Interfacial Tension of Palm Oil (NBDPO)/ Water Interface in the presence of Various Phospholipids Added to the Palm Oil (60°C).

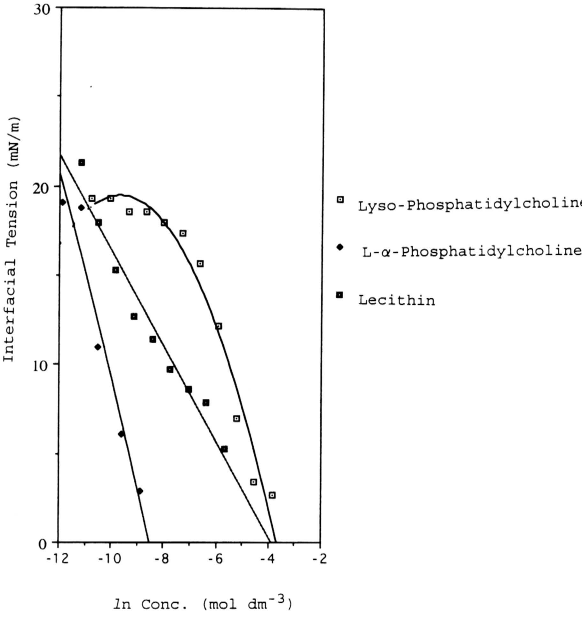
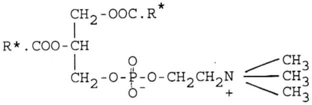
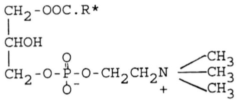


Fig. 16 : Molecular Structures of Phospholipids



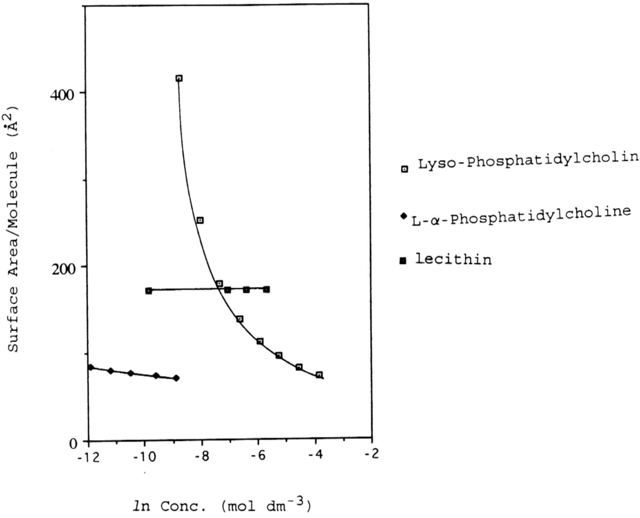
L-α-phosphatidylcholine



Lyso-phosphatidylcholine

R^* = C18/C16

Fig. 17. Influence of Various Phospholipids on the Molecular Area* of the Phospholipids at the Refined Palm Oil (NBDPO)/ Water Interface at 60°C



*In the calculation of the molecular area of the L-α-phosphatidylcholine, Lyso-phosphatidylcholine and lecithin, molecular weight of 469, 730 and 730 respectively were used.