Application of Membranes and Enzymes in Processing Vegetable Oils

A thesis submitted to the

University of Mysore

in fulfillment of the requirements for the degree of

Doctor of Philosophy

in

BIOTECHNOLOGY

by

S. Manjula

Under the supervision of

Dr. R. Subramanian

Department of Food Engineering

Central Food Technological Research Institute

Mysore-570020, INDIA

August 2009

S. Manjula
Senior Research Fellow
Department of Food Engineering
Central Food Technological Research Institute
Mysore-570020, India

Declaration

I hereby declare that the thesis entitled **"Application of membranes and enzymes in processing vegetable oils"** submitted for the degree of **Doctor of Philosophy** in **Biotechnology** to the **University of Mysore** is the result of the work carried out by me under the guidance of **Dr. R. Subramanian** in the Department of Food Engineering at Central Food Technological Research Institute, Mysore.

I further declare that the results of this work have not been submitted for the award of any other degree of any University.

Date: (S. Manjula)
Place: Mysore

Dr. R Subramanian
Scientist-F
Department of Food Engineering
Email: subbu@cftri.res.in
Tel: +91-821-2513910

Certificate

I hereby declare that the thesis entitled, **"Application of membranes and enzymes in processing vegetable oils"** submitted by **Ms. S. Manjula** for the degree of **Doctor of Philosophy** in **Biotechnology** to the **University of Mysore** is the result of the work carried out by her under my guidance in the Department of Food Engineering at Central Food Technological Research Institute, Mysore.

Date: (R. Subramanian)
Place: Mysore (Research Guide)

Dedicated to my beloved mother…..

Acknowledgements

With great pleasure and respect, I express my deepest gratitude to my research supervisor **Dr. R. Subramanian**, Scientist, Department of Food Engineering, Central Food Technological Research Institute (CFTRI), Mysore for his constant encouragement, valuable guidance, constructive criticism and untiring help throughout my doctoral program.

I am immensely grateful to Dr. S. Divakar, Head, Fermentation Technology and Bioengineering, CFTRI for his suggestions, constructive criticism and many help in my research work.

It is my immense pleasure to thank Dr. A.G. Gopala Krishna, Dr. L. Jaganmohan Rao, Dr. K. Udayasankar and Ms. T. N. Indira, CFTRI for their scientific help during the course of the research programme.

I am grateful to Prof. H. Nabetani at National Food Research Institute (NFRI), Tsukuba, Japan for providing the membranes, which were very essential for carrying out the research work. I also wish to thank Dr. I. Kobayashi, NFRI for his help in the surface tension measurements.

My profound thanks are due to Dr. Vasudeva Singh, Dr. H. Umesh Hebbar, Mr. B. Manohar, Dr. V. Baskaran, and Dr. Sakina Khatoon for permitting to use their research facilities needed for the work. I also wish to thank Mr. C. Gopal for his technical help in the preparation of crude vegetable oil samples for experimentation.

I wish to thank the staff members of Central Instrumentations Facility and Services Department for their technical help in analyzing the samples. I also thank the NMR Research Centre, Indian Institute of Science, Bangalore and NMR Facility Centre, Plantation Products, Spices and Flavour

Technology Department, CFTRI for recording NMR spectra presented in this work. My thanks are also due to Dr. M.S. Sethumadhav, Department of Studies in Geology, University of Mysore for the helpful discussion on elemental analysis.

I cordially thank my colleagues Mr. E. Nakkeeran, Ms. Anju Jose, Mrs. Chandini S. Kumar, Mr. K.H. Vishwanathan and other lab mates for their many help and support during my stay in CFTRI. My thanks are also due to Mr. M. Saravanan, for his timely help in providing the enzyme needed for the research work.

I am thankful to the Heads of the Department of Food Engineering and Human Resource Development and all the staff members of Food Engineering Department for their support during the course of my work. My special thanks are due to Mr. A. Chakkaravarthi, for his ready help whenever needed.

I am grateful to the Director, CFTRI, for providing me an opportunity to carry out my doctoral work at CFTRI.

I am deeply grateful to the Council of Scientific and Industrial Research, India for awarding Junior and Senior Research Fellowships which provided me the financial support during the tenure.

Finally I am indebted to my parents and brother for their patience and exceptional encouragement. Without their support this work would not have been a success.

S. Manjula

Abstract

The thesis was initiated through an exhaustive review of research conducted worldwide towards degumming, dewaxing, decolourizing and deacidifying edible oils using membrane technology. To begin with, the influence of phospholipid (PL) composition and solvent (hexane) medium on critical micelle concentration levels in crude oil and crude oil-hexane systems was investigated, which explained the dependency of ultrafiltration membranes on the initial PL concentration in their degumming performance and how the nonporous membranes achieved near complete degumming in vegetable oils under various conditions. Attempts made to enhance oil flux showed that the nonporous membrane did not reject triglycerides (TG) over a wide range of hexane dilution due to a positive flow coupling with hexane while improving the oil flux by an order of magnitude. Besides, the results revealed that the oil flux followed an inverse relationship with average molecular weights of TG despite their narrow range of existence in various vegetable oils, interestingly even under hexane-diluted conditions. Assessing the potential of nonporous membranes revealed its prospect as a single-step pretreatment process for simultaneous degumming, dewaxing and decolourizing crude rice bran oil besides eliminating the problem causing phosphoglycolipids. Enzymatic degumming employing phospholipase-A1 was found to be effective in oil system, however only to a moderate extent in hexane-oil system. Further, potential applications of membrane technology in nonaqueous systems were examined, specifically for enriching beneficial oryzanol in rice bran oil (RBO) and deoiling lecithin. Nonporous membrane exhibited moderate rejection of oryzanol (ferulate esters) in RBO owing to their hydrophilic nature, suggesting

a physical method for enriching oryzanol in RBO and thereby the possibility of producing a standard RBO with a guaranteed oryzanol content. The phenomenally high selectivity of nonporous membrane for PL was responsible for its high efficacy in deoiling lecithin in a favourable solvent (hexane) scoring over the industrially-practiced acetone-extraction method. Nonporous membranes possess the potential for being employed in various steps of vegetable oil processing, however the flux needs further improvement for industrial adoption. Thus present study has clearly brought out the effectiveness of nonporous membranes on their suitability in nonaqueous applications, principally in vegetable oil processing.

Table of Contents

List of Tables

List of Figures

Scheme

List of Abbreviations

AI	Acetone insoluble
$CDCl_3$	Deuterated chloroform
C_F	Contents of each component in the feed (kg/kg-oil)
C_P	Contents of each component in the processed oils (kg/kg-oil)
$C_{R,f}$	Final content of each component in the retentates (mg/kg-oil)
$C_{R,i}$	Initial content of each component in the retentates (mg/kg-oil)
CMC	Critical micelle concentration (mg/kg)
CNO	Coconut oil
CRBO	Crude rice bran oil
CSBO	Crude soybean oil
CSFO	Crude sunflower oil
DG	Diglycerides
DMSO-d_6	Deuterated dimethyl sulfoxide
ERBO	Enriched rice bran oil
FFA	Free fatty acids
GC	Gas chromatography
GNO	Groundnut oil
HOSF	High oleic sunflower oil
HPLC	High performance liquid chromatography
MEUF	Micelle-enhanced-ultrafiltration
MF	Microfiltration
MG	Monoglycerides
MO	Mustard oil
MW	Molecular weight
MWCO	Molecular weight cut off (Da)
NF	Nanofiltration
NHP	Nonhydratable phospholipids
NMR	Nuclear magnetic resonance
PA	Phosphatidic acid
PC	Phosphatidylcholine
PDMS	Polydimethylsiloxane
PE	Phosphatidylethanolamine

PES	Polyethersulfone
PGL	Phosphoglycolipids
PI	Phosphatidylinositol
PL	Phospholipids
PR	Percent reduction (%)
R^2	Correlation coefficient
R_o	Observed rejection (%)
RBO	Rice bran oil
RO	Reverse osmosis
SBO	Soybean oil
SCFE	Supercritical fluid extraction
SFO	Sunflower oil
SD	Standard deviation
SPVD	Short path vacuum distillation
TCNQ	7,7,8,8-tetracyanoquinodimethane
TG	Triglycerides
TMS	Tetramethylsilane
UF	Ultrafiltration
UV	Ultraviolet
VCR	Volume concentration ratio
v/v	Volume/Volume
W_f	Final weight of retentate (kg-oil)
W_i	Initial weight of retentate (kg-oil)
wt	Weight
w/v	Weight/Volume
w/w	Weight/Weight
^{1}H	Proton
2DHSQDCT	Two-dimensional Heteronuclear single quantum coherence transfer
^{13}C	Carbon-13
^{31}P	Phosphorus

Units

cm	Centimetre
cm^2	Square centimetre

cm^3	Cubic centimetre
Da	Dalton
g	Gram
G	Gauss
h	Hour
Hz	Hertz
J	Joule
kDa	Kilodalton
kg	Kilogram
kHz	Kilohertz
l	Litre
LMH	Litre per square metre per hour
m	Metre
M	Molar
m^2	Square metre
MHz	Megahertz
mg	Milligram
min	Minute
ml	Millilitre
MMT	Million metric ton
mPa	Millipascal
MPa	Megapascal
nm	Nanometre
ppm	Parts per million
rpm	Revolutions per minute
s	Second
V	Volt
°C	degree Centigrade
µl	Microlitre
µm	Micrometre
µmole	Micromole
%	Percent

1.1 Membrane technology in processing oils

Commercial sources of edible oils and fats include oilseeds, fruit pulp, animals and fish. Oilseeds are the major source for the production of edible oils. The total world production of major vegetable oils in 2007 was ~128 MMT (www.fas.usda.gov). The production of individual oils and the major countries producing these oils are presented in Table 1.1. The annual production of major vegetable oils in India is presented in Table 1.2. India ranks first in the production of RBO (www.fnbnews.com), and second in peanut and cottonseed oils, and third in rapeseed oil in the world (www.fas.usda.gov). The method chosen for oil extraction depends on the nature of raw material as well as the plant capacity. Pressing followed by solvent extraction is the method most widely employed for handling a wide variety of oilseeds (Young *et al.*, 1994), which contribute nearly 50% of the total vegetable oil produced in the world.

The main objective of refining is to remove, as much as possible, those contaminants that otherwise adversely affect the quality of the end product. The principal impurities in these oils are water, FFA, partial glycerides, phosphatides, oxidation products, pigments and trace elements such as copper, iron, sulfur and halogens (Young *et al.*, 1994). In the United States, the term 'refining' was originally applied only to the operations of pretreatment and deacidification or neutralization. In most other countries it meant the complete series of treatments, including bleaching and deodorization, to render the fat suitable for edible use. Now, many countries tend to speak a common language and US has accepted the word refining as being used by

other countries. Industrially the two most commonly used methods for refining are chemical and physical refining.

Table 1.1 World production of major vegetable oils and major producing countries (2007/08)

Vegetable oil	Production (MMT/year)	Major producing countries
Palm	41.305	Indonesia, Malaysia, Thailand, Colombia
Soybean	37.508	United States of America, China, Argentina, Brazil
Rapeseed	18.310	European Union, China, India, Canada
Sunflower	9.731	Russian Federation, European Union, Ukraine, Argentina
Cottonseed	5.004	China, India, United States of America, Turkey
Peanut	4.828	China, India, United States of America, European Union

Source: www.fas.usda.gov

In conventional chemical refining, the impurities are removed at various stages, namely, degumming, neutralizing, washing, drying, bleaching, filtering and deodorizing. This chemical process has many drawbacks, such as high energy demand, loss of neutral oil, need for large amounts of water and chemicals, loss of nutrients and disposal of highly polluted effluents. Dewaxing step is carried out only for certain types of oils such as rice bran and sunflower. In conventional physical refining, FFA are distilled off and this process offers many advantages over the chemical method such as improved product yield, elimination of soap stock and reduced effluent quantity. However, quality requirements of the pretreated crude oils are much more stringent, the most important being that the phosphorus and iron levels are

low. Solvent/miscella refining is another industrial method wherein hexane-oil miscella is mixed with sodium hydroxide solution for neutralization, reaction with phosphatides and decolourization. Certain limitations of this process are higher investment for explosion-proof equipment and solvent loss (Bhosle and Subramanian, 2005). Although physical refining offers several advantages over other methods, industries generally prefer to follow chemical refining as it offers a quality product from almost any type of crude oil.

Table 1.2 Annual production of major vegetable oils in India (2007/08)

Vegetable oil	Production (MMT/year)
Rapeseed	1.968
Peanut	1.625
Soybean	1.426
Cottonseed	1.085
Rice bran	0.800

Source: www.fas.usda.gov; www.seaofindia.com

Alternative approaches are needed to overcome the drawbacks of today's technology. In this context, membrane technology is being looked at as a potential alternative. A membrane process is remarkably simpler and offers many advantages over conventional processes, namely, low energy consumption, ambient temperature operation, no addition of chemicals and retention of all of the nutrients as well as other desirable components in the oil (Cheryan, 1998). Pressure-driven membrane processes are classified as RO, NF, UF and MF depending on the nature of particles or molecular sizes of

solutes to be separated. Commercial membrane devices are available in four major types, namely plate and frame, tubular, spiral-wound and hollow fiber.

Owing to the vast scope for energy savings as well as potential for improvement in oil quality, edible oil processing has become one of the prime areas for membrane applications. It has been reported that energy savings to the tune of ~50% could be achieved if deacidifying and bleaching steps in the conventional process are supplemented with membrane processes (Koseoglu and Engelau, 1990). Cheryan (1998) depicted the conceptual application of membranes in almost all stages of oil production and purification (Fig. 1.1). Raman *et al.* (1994) listed some of the potential applications of membrane technology in vegetable oil processing. Many of them have been evaluated at the laboratory or pilot plant scale; nevertheless, there are only few commercial membrane installations in the edible oil related industries, in spite of their vast potential and the considerable research efforts already put in.

Several researchers have attempted membrane processing of edible oils with and without solvents, by using porous as well as nonporous denser polymeric composite membranes. Earlier Snape and Nakajima (1996) and Cheryan (1998) reviewed the potential applications of membrane technology in the edible oil industry. Lot of research efforts has gone in to this field since then and recently we reviewed this subject again owing to its potential as an alternate technology for edible oil processing. Following a systematic approach, various attempts made towards degumming, dewaxing, decolourizing and deacidifying edible oils using membrane technology were classified into distinct categories based on the method of approach and the type of membrane used (Manjula and Subramanian, 2006).

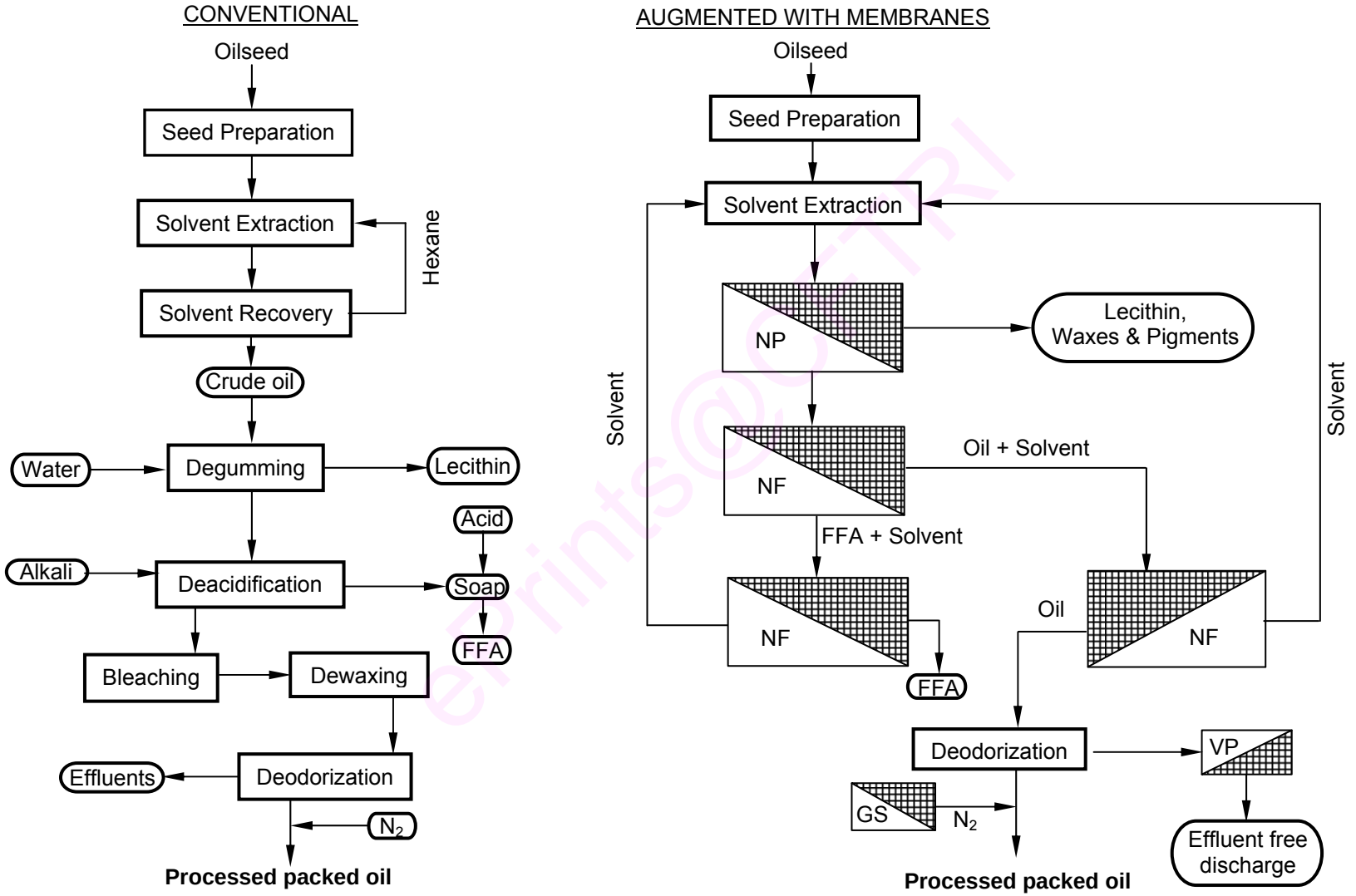


Fig. 1.1 Typical conventional processing of vegetable oils and processing augmented with membranes (Modified based on Cheryan, 1998)

1.1.1 Degumming

Degumming is the first step during refining of crude vegetable oils, wherein PL are removed, which otherwise would act as emulsifying agents leading to loss of neutral oil and finally resulting in low grade finished product (Young *et al.*, 1994). PL are classified as hydratable PL and NHP. The principal component of the hydratable PL is PC, whereas the NHP mainly consist of the calcium and magnesium salts of PA and PE (Young *et al.*, 1994).

In conventional processing, water or dilute acid is used during the degumming step. In the water-degumming process, PL are precipitated by hydration followed by agitation and removed by centrifugation. The PL content of an average quality of oil is reduced to a range between 1800 and 6000 mg/kg and the corresponding range of phosphorus content is 60 to 200 mg/kg (Segers and Sande, 1990). Acid degumming (where the hydratability of salts of PA is increased by addition of either phosphoric or citric acids) brings down the phosphorus content to ~50 mg/kg (Diosady *et al.*, 1982). However, this acid treatment may have an influence on the composition of the resulting PL affecting its quality as well as application (Ziegelitz, 1995). The super-degumming process, a patented process widely being used in the industries, produces an oil with a maximum phosphorus content of 30 mg/kg (Segers, 1982). The amount of acid used in the acid-degumming process varies between 0.05 and 0.2% of the oil weight and is even as high as 0.5% in oils containing an initial phosphorus content of 200 mg/kg and higher. Besides membrane degumming, enzymatic degumming, countercurrent extraction with supercritical CO_2 and ultrasonic degumming have also been reported (Young *et al.*, 1994).

TG constitute over 95% of crude vegetable oils. Oleic, linoleic and palmitic acids are the major fatty acids present in the common vegetable oils (Bockisch, 1998). Minor constituents, such as PL (600-800 Da), carotenoids (537-569 Da), FFA (256-282 Da) and tocopherols (402-472 Da), have lower molecular sizes than TG (600-1000 Da). Chlorophyll (892 Da) has a slightly higher molecular size than TG. Molecular size distribution of various oil constituents is shown in Fig. 1.2. The differences in molecular sizes among these various constituents are too small to use membrane alone for separation based on size exclusion.

The removal of PL from vegetable oil using membrane technique is the operation that has received the most attention. Several researchers have attempted membrane degumming of crude edible oils with and without solvents, by using porous as well as nonporous membranes. The various attempts made could be classified in to the following categories (Manjula and Subramanian, 2006).

i. Processing hexane-diluted oils with porous membranes (MF/UF)
ii. Processing undiluted oils with MF/UF membranes
iii. Processing undiluted oils using nonporous membranes
iv. Processing hexane-diluted oils using nonporous membranes
v. Processing undiluted/hexane-diluted oils with additives using MF/UF membranes

Among the various approaches listed above, processing undiluted oils using nonporous membranes showed excellent selectivity for separation of PL from various crude vegetable oils, but the highest oil flux achieved was only 0.8 LMH (Subramanian and Nakajima, 1997), which is very low for industrial

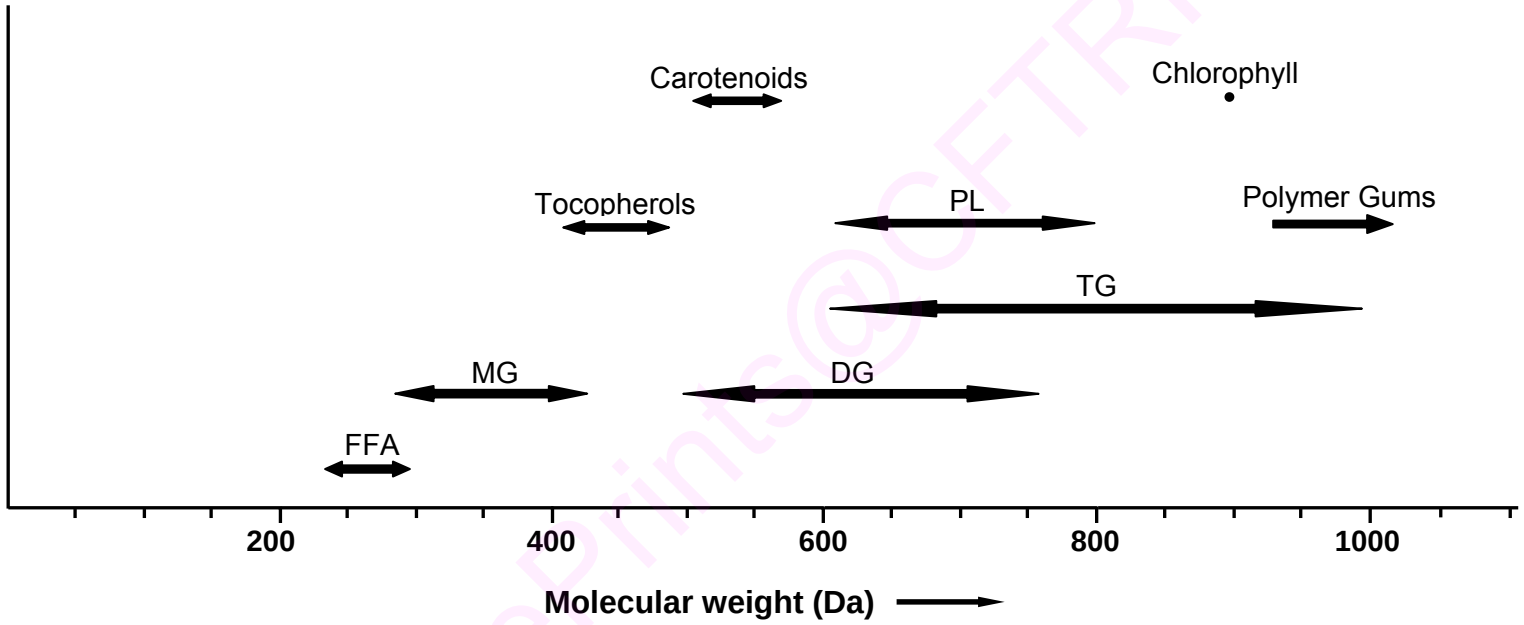


Fig. 1.2 Molecular size distribution of oil constituents

adoption. A laboratory made polysulfone UF membrane (Zhang *et al.*, 1996) gave higher flux (1.9 LMH) with undiluted vegetable oils compared to the nonporous membrane but with reduced selectivity (*PR* 93%). Processing hexane-oil miscella using nonporous membranes improved the oil flux by one order of magnitude while retaining the PL rejection (Saravanan *et al.*, 2006). Application of MEUF for processing hexane-oil miscella also resulted in very high selectivity for the separation of PL. A commercial polyimide membrane gave an oil flux of 14.4 LMH (Miki *et al.*, 1988), and another commercial PES membrane after solvent conditioning could achieve an oil flux of ~24.5 LMH (Garcia *et al.*, 2006). While a laboratory cast polyvinylidene flouride membrane gave a permeate oil flux of 20 LMH (Pagliero *et al.*, 2004) and another laboratory cast polyimide membrane gave a flux as high as 78 LMH (oil flux) however, with a comparatively lower (~90%) rejection (Kim *et al.*, 2002). Preconditioning polymeric MF membranes with solvents of varying polarity from more polar to less polar also resulted in high rejection of PL in vegetable oil miscella with a reasonable oil flux of 25 LMH (Jirjis *et al.*, 2001). Surfactant aided MF and alkali neutralization followed by membrane filtration are not essentially membrane-based techniques; rather membrane filtration is attempted as a substitute for the conventional centrifugal separation. MEUF of hexane-diluted oils appears to be the best approach in terms of PL rejection and permeate flux among the different membrane degumming processes discussed.

1.1.2 Dewaxing

Waxes are high-melting esters of fatty alcohols and fatty acids with low solubility in oils (Haraldsson, 1983). While wax usually does not negatively

affect the functionality of the products, the presence of wax affects the appearance of the product, which are often packaged in clear bottles (Anderson, 1996). Dewaxing is necessary only for certain types of oils such as corn, sunflower, canola and rice bran. The quantity of wax in crude oils varies from a few hundred mg/kg to over 2,000 mg/kg (Haraldsson, 1983). To get an oil with sufficient cold stability, the wax content has to be reduced to a level of ~10 mg/kg. In the integrated refinery, waxes are removed by a chilling, settling and separation process.

The traditional dewaxing process consists of a careful cooling of the deacidified oil followed by addition of a proportional amount of filter aid before crystallization of the wax, which then is removed by filtration in a pressure leaf filter (Haraldsson, 1983; Anderson, 1996). In order to get wax crystals of good filterability, the cooling has to be done slowly and under controlled conditions (Haraldsson, 1983). This traditional process works quite well for oils with wax content <500 mg/kg. More than a decade ago, an alternate process was developed using centrifuges to process high wax content oils. Different variants of this approach have been applied depending on the method of deacidification employed in the process and the size of plant operation. While centrifuge dewaxing is excellent for high-wax oils, residual wax content may be generally around 50 mg/kg, which may or may not provide clear oil (Anderson, 1996). Cold water washing after centrifuge dewaxing reduces the wax levels by another 10-20 mg/kg, which does not however ensure prevention of oil clouds. Adding a polish filtration step after centrifugal dewaxing assures very low wax contents (~10 mg/kg) and the oil almost always remain clear. Although there are varied approaches in placing

the polishing operation in the process, keeping the polish filtration as the last processing step appears to be the best option for the salad oil production.

Clogging of the filter media, entrapment of neutral oil in the filter earth and disposal costs associated with the process are some of the unfavourable factors associated with the conventional dewaxing method (Cheryan, 1998). Although employing centrifugal method reduces some of these problems associated with pressure leaf filters, the necessity for a polish filtration step is not eliminated. Membranes could overcome some of these problems and there are some attempts on dewaxing edible oils with and without solvents and additives, mainly using porous membranes. The various attempts made could be classified in to the following categories (Manjula and Subramanian, 2006).

i. Processing undiluted oils with MF membranes
ii. Processing hexane-diluted oils with UF membranes
iii. Processing undiluted and hexane-diluted oils with additives using MF/UF membranes

All the three approaches discussed above have shown their efficacy for dewaxing vegetable oils. MF membranes (120-500 nm) were very effective for dewaxing undiluted SFO but with adequate precooling. Muralidhara *et al.,* (1996), demonstrated that crash cooling and adequate maturation of oil facilitated crystallization of wax and completely eliminated wax in decolourized SFO during membrane processing. This method could be examined for high wax content oils. UF of hexane-oil miscella for achieving simultaneous degumming and dewaxing is more attractive and deserves greater attention as it is achieved in a single-step without precooling the oil. Although

approaches made with chemical additives were effective and attractive as a single-step process for simultaneous dewaxing, degumming and deacidification, it is not an exclusive membrane-based approach. Among these approaches, the performance of UF membrane with hexane-oil miscella could be termed very good as indicated by the cold test results of the processed oil. However, reduction in wax content has not been quantified. The majority of the research work on dewaxing has been carried out with SFO and it may be desirable to focus on other oils such as RBO where dewaxing is critical.

1.1.3 Deacidification

The removal of FFA from crude oil represents the most delicate and difficult stage in the refining cycle, since it determines the quality of the final product. Chemical, physical and miscella deacidification methods have been used industrially for deacidification. During chemical deacidification process, there are always considerable losses of neutral oils, sterols, tocopherols and vitamins. Furthermore, disposal and utilization of resulting soap stock may create problems of environmental pollution. On the other hand, practical experience with physical deacidification has shown that it leads to acceptable results only when good quality starting oils are used. Besides, incomplete removal of undesirable components during the pretreatment of oil has to be compensated for by an increased use of bleaching earth. The two-stage solvent removal system and the associated higher cost of installing a totally enclosed and explosion-proof equipment, for adequate safety, limit the application of miscella deacidification. There are several drawbacks to

today's technology and so alternative approaches are needed to overcome these drawbacks. Bhosle and Subramanian (2005) reviewed the various newer approaches attempted by researchers, namely biological deacidification, solvent extraction, reesterification, supercritical fluid extraction and membrane processing.

The molecular weight of fatty acids are <300 Da and that of TG are >600 Da. The ideal process would use a hydrophobic membrane with pores so precise that they could effectively separate the FFA from the TG (Raman *et al.*, 1994). Several researchers have attempted the deacidification of vegetable oils with and without solvents, by using porous as well as nonporous membranes. Different attempts made could be broadly classified into the following three categories (Manjula and Subramanian, 2006).

i. Direct deacidification

   Processing undiluted and hexane-diluted oils using nonporous membranes

   Processing undiluted and solvent-diluted oils using NF/UF/MF membranes

ii. Deacidification of oils with pretreatment

   Pretreatment followed by filtration using MF/UF membranes

   Pretreatment followed by selective separation using porous membranes

iii. Deacidification using solvent extraction coupled with membrane separation

   Membrane extraction

   Solvent extraction followed by membrane separation

Among the various approaches, direct deacidification is the most desirable one. However, the differences in the molecular weights of TG and

FFA are too small to use any porous membrane for the separation in undiluted oils. Attempts made with nonporous membranes showed low selectivity and poor flux, unsuitable for practical application. When oil was dissolved in an organic solvent (hexane, ethanol and acetone), NF membranes showed a good selectivity for FFA, the best being with acetone which gave a selectivity as high as 7-14, but the oil throughput was unacceptably low (Bhosle *et al.*, 2005; Zwijnenberg *et al.*, 1999). A reasonable combination of high selectivity and oil throughput was not observed in any of these organic solvents and membranes used for direct deacidification. Pretreatment with alkali prior to filtration resulted in very high reduction (>90%) of FFA in the processed oils (Raman *et al.*, 1996a; Krishna Kumar *et al.*, 1996). However, this approach is essentially similar to the conventional chemical deacidification process wherein soap separation has been attempted with a porous membrane in place of conventional centrifugal separation. In another approach (Keurentjes, 1991), soap separation was attempted by selective separation using a combination of hydrophobic and hydrophilic porous membranes, which remained as an academic interest. Membrane extraction technique (Keurentjes, 1992) is of great interest but high mass transfer resistance and consequent vast membrane area requirement makes it unattractive for industrial adoption. While solvent extraction followed by membrane separation (Raman *et al.*, 1996b; Kale *et al.*, 1999) seemed to be technically feasible, it is not attractive with two solvents in the process. Despite all the above research efforts, there is no breakthrough in identifying a potential membrane-based approach suitable for developing a successful technology in deacidification.

1.1.4 Decolourization

The removal of colour from edible oil is necessary to provide an acceptable finished product to the consumer. Primarily the colour pigments are extracted along with the oil and mainly consist of carotenoids, chlorophyll, gossypol and related compounds depending on the type of source. Carotenoids and chlorophyll are the two common colour pigments present in most vegetable oils. Gossypol is present only in cottonseed oil and consequently makes the oil unsuitable for physical refining due to its heat sensitive nature. Chlorophyll being a sensitizer of photo-oxygenation can promote oxidation in the presence of light and decrease the oxidative stability of oils to a great extent. They also act as catalyst poisons by blocking the active sites of nickel and impair the hydrogenation process. Carotenoids are beneficial compounds and their removal from oil may not be absolutely required. However, process conditions aimed at removing undesirable compounds usually remove these beneficial compounds as well. Besides, consumers over the years are used to colourless and bland oil. It may be welcome if a refining process could be developed that could remove undesirable impurities from crude oil while retaining the beneficial compounds such as tocopherols and carotenoids to a larger extent.

In the conventional refining process, the colour compounds are removed at various steps and maximum reduction occurs during bleaching (Subramanian *et al.*, 1998a). In the subsequent deodorization step, all the thermally degradable colour compounds are removed. Bleaching is usually achieved by heating the oil to about 100°C and passing through a bed of activated earth, activated carbon or amorphous silica (Young *et al.*, 1994).

The small amounts of adsorbent carried along by oil are removed by filtration. Bleaching basically involves adsorption that removes not only colour compounds but also other minor impurities. The residual soaps are removed and peroxides are decomposed into aldehydes and ketones due to further oxidation. The decomposed products are also adsorbed to the bleaching agent thereby improving stability and flavour of the oil. Therefore TOTOX value (the sum of the p-anisidine value and twice the peroxide value), an Index of oil quality and stability is used as one of the parameters to evaluate the bleaching operation (Hodgson, 1996).

The conventional bleaching process using adsorbents has several disadvantages. Commercial bleaching commonly produces 0.1-0.2% of conjugated fatty acids in the glycerides through isomerization of nonconjugated fatty acids (Hodgson, 1996). Such conjugated dienes and trienes would predispose the oils to further oxidative changes. Such isomerization could be reduced by maintaining prior oxidation to a minimum by deaerating the oil before bleaching and by carrying out bleaching under vacuum (Hodgson, 1996). During bleaching operation, tocopherol contents are reduced rendering the oil less stable (Young *et al.*, 1994). Bleaching earths retain oil to the extent of 30-70% of its weight used in the process (Cheryan, 1998), which cannot easily be recovered. Bleaching earth is used at 2% level and is a major cost apart from the associated disposal problem.

Molecular weight of carotenoids are <570 Da, chlorophyll is 892 Da and TG are 600-1000 Da. The differences in the molecular sizes between colour pigments and TG are too small to use membrane (NF) alone for separation based on size exclusion. Some researchers have attempted

decolourization of vegetable oils using porous as well as nonporous membranes and their attempts could be classified in to the following categories (Manjula and Subramanian, 2006).

i. Processing undiluted oils using MF/UF membranes
ii. Processing hexane-diluted oils using MF/UF membranes
iii. Processing undiluted oils using nonporous membranes
iv. Processing hexane-diluted oils using nonporous membranes

The various approaches discussed above have shown that decolourization approach needs to be specific to individual oils depending on the nature of colouring compounds present. An UF membrane showed reduction in gossypol (71-82%) in hexane-diluted cottonseed oil (Koseoglu *et al.*, 1990). Chlorophyll reduction was somewhat consistent (41-67%) with cottonseed, soybean, rapeseed, peanut and meadowfoam oils. However, carotenoids rejection and Lovibond colour reduction was not consistent and varied drastically between oils. UF membranes showed reduction in all forms of colour measurement only with cottonseed and peanut oils. Nonporous membranes showed consistently good colour reduction with various undiluted crude vegetable oils in terms of colour measurements, namely, visible spectra and Lovibond, as well as estimations of colour compounds, namely, carotenoids (xanthophylls) and chlorophyll (Subramanian *et al.*, 2004). Interestingly, the nonporous membrane did not show any selectivity for carotenes (Sarita Arora *et al.*, 2006). Hexane dilution improved the oil flux by one order of magnitude and did not significantly affect the rejection of colour compounds (xanthophylls, chlorophyll and Maillard browning products) by the nonporous membrane (Saravanan *et al.*, 2006; Kondal Reddy *et al.*, 2001).

Employing nonporous membranes for processing hexane-diluted oils holds some promise for practical application and worth investigating for its colour reduction capabilities with other common vegetable oils.

Performance of UF membranes

UF membranes exhibited excellent PL rejection performance under undiluted as well as hexane-diluted conditions. Besides, it gave higher oil flux with hexane dilution suitable for industrial adoption. UF of hexane-oil miscella was also proved to be effective for dewaxing oils without a precooling step. This approach appears to be the best for degumming application and also suitable for simultaneous dewaxing of oils. However, carotenoids rejection and Lovibond colour reduction was not consistent and varied drastically between oils with UF membrane.

Performance of nonporous membranes

For degumming crude vegetable oils, hydrophobic nonporous membranes showed a near total rejection of PL that was consistent with various vegetable oils under undiluted conditions, but the oil flux was low. Besides these membranes showed consistently good colour reduction with various undiluted crude vegetable oils in terms of colour measurements, namely, visible spectra and Lovibond, as well as estimations of colour compounds, namely, carotenoids (xanthophylls) and chlorophyll. Interestingly, the nonporous membrane did not show any selectivity for carotenes. Hexane dilution improved the oil flux by one order of magnitude and did not significantly affect the rejection performance.

Nonporous membranes offer several advantages over UF membranes for processing hexane-oil miscella, in terms of higher rejection of PL,

carotenoids and chlorophyll. The membrane process will become economically attractive if it is even partially effective for colour removal in addition to PL reduction, considering the amount of clay used in the conventional bleaching process and its associated problems. These membranes may be effective for wax removal as well. Therefore, nonporous membrane processing may be worth investigating as an alternate processing method for degumming, decolourizing and dewaxing steps.

1.2 Characterization of PL reverse micelles

PL are surfactants having both hydrophobic and hydrophilic groups. They form reverse micelles in nonaqueous systems such as vegetable oils and hexane-oil miscella. During processing undiluted crude vegetable oils, nonporous membranes exhibited greater/almost complete rejection of PL (Subramanian *et al.*, 2004). The rejection of PL by these membranes could have been due to the formation of PL reverse micelles, swollen in the presence of small quantities of water and having an affinity for some of the other impurities, such as colour compounds, which were then rejected by size exclusion. Another possibility for the observed behavior could have been a solution-diffusion effect, that is, interactions between individual solutes as well as their interactions with the silicon layer of the membrane. Characterization of PL in model and real systems confirmed that PL reverse micelles are formed in CSBO when the PL content exceeds CMC (1020 mg/kg) and the size of PC reverse micelles was estimated to be in the range of 3.56-4.70 nm (Subramanian *et al.*, 2001a). The nonporous membranes were effective in rejecting PL even when the PL content is low as in the case of expelled GNO

(690 mg/kg) and SFO (120 mg/kg). This suggested that the mechanism of transport was the solution-diffusion effect and the rejection of PL was attributed to low solubility in the membrane material. Size exclusion may provide a synergistic effect in the rejection when the PL content is above the CMC (Subramanian *et al.*, 2001a).

The average molecular weight of PL is around 600-800 Da and that of neutral TG is around 600-1000 Da. In SBO-hexane system, Gupta (1986) reported that the size of the PL mixed micelles is between 18-200 nm, which is much larger than the size (1.5 nm) of TG (Segers and Sande, 1990) and size (~4 nm) of PC reverse micelles (Subramanian *et al.*, 2001a). Hexane is the common solvent used for the extraction of oil from oilseeds and pressed cakes. After the extraction, hexane is stripped off, leaving the crude oil for further processing. In membrane processing of vegetable oils, hexane dilution is being practiced for the improvement of productivity/flux through the membrane. In the case of hexane-diluted crude oil systems, the size of PL reverse micelles (18-200 nm) formed is much larger (Gupta, 1986) and many researchers have employed UF membrane typically with a MWCO of 20 kDa as the PL size corresponds to 40-400 kDa, for successful degumming of crude oils (Miki *et al*., 1988; Lin *et al*., 1997; Pagliero *et al*., 2004). The CMC of soy PL in hexane system was reported to be 250 mg/l (Ichikawa *et al.*, 2000). Hancer *et al.* (2002) reported that the CMC level of hydratable PL (70 mg/kg) was lower than that of NHP (180 mg/kg) using model hexane-SBO systems containing sodium and calcium salts of dipalmitoyl PA, respectively and also demonstrated the influence of water content on CMC of the system.

1.3 Enhancement of oil flux in nonporous membrane

Studies were initiated by researchers at National Food Research Institute, Japan and Central Food Technological Research Institute, India, on membrane processing of vegetable oils without any pretreatment or dilution with organic solvent considering the fact that a large quantity of vegetable oil produced in the world is by expression. The differences in the molecular sizes among the various oil constituents are too small (Fig. 1.2) to use a porous membrane (UF/NF) for separation based on size exclusion. Hence, nonporous membranes were employed to assess whether hydrophobic nonporous membranes exhibit the required selectivity for the desired separation. Processing undiluted oils using nonporous membranes showed excellent selectivity for separation of PL from various crude vegetable oils, but the highest oil flux of 0.8 LMH achieved is very low for industrial adoption (Subramanian *et al.*, 2004).

The important aspects of membrane processing that affect the application and commercialization are high membrane cost and low permeate flux (Zhu *et al.*, 1999). Feed properties such as concentration and viscosity, and operating parameters, namely, transmembrane pressure, operating temperature and cross flow velocity influence permeate flux in a membrane process. Although operating pressure and temperature had significant effect on the permeation rate of TG (Subramanian *et al.*, 2003), the scope for flux improvement was rather limited within the permissible limits of these operating conditions. Further, the mode of operation of membrane processing system would also influence the permeate flux. Miscella flux obtained in a cross-flow mode was 3-5 fold greater than the flux obtained in the dead-end filtration

mode using an UF membrane (Pagliero *et al.*, 2001; 2004). However, such an improvement in flux is not expected with undiluted oils in a nonporous membrane wherein permeability is controlled by solution-diffusion effect.

Assisted filtration approaches such as application of electric and ultrasonic fields have been attempted in aqueous systems to reduce fouling in MF membranes (Tarleton, 1988). Sarkar *et al.* (2009) reported a 33% increase in permeate flux while assessing the rejection of methylene blue in sodium dodecyl sulfate solution by a UF membrane (10 kDa) in the presence of an electric field (direct current 1000 V/m). Chen *et al.* (2002) reported 2 fold improvement of permeate flux with silica solution of 0.5 g/l applying an ultrasonic field (20 kHz) to a MF ceramic membrane system, while there was no such improvement in pure water flux under similar conditions. The results indicated that ultrasonic field could be applied for reducing the concentration polarization and membrane fouling effects and thereby improve the process flux. However, the improvements achieved with such approaches in nonaqueous systems would not be adequate to meet the industrial requirements.

While membrane applications in aqueous processing are witnessing a spectacular growth, applications in nonaqueous processing seemed to be still waiting for the breakthrough. The main hindrances for the adoption of membrane technology for edible oil processing are related to selectivity, productivity and longevity of membranes. The high viscosity of the feed during processing vegetable oils mainly affects the flux in the membrane process. To overcome this constraint, solvent dilution of feed had been practiced by the earlier researchers working on MEUF towards oil degumming

for maintaining high flux through the membrane. Hexane has been the natural choice since it is the common solvent used for the extraction of oil from oilseeds and pressed cakes. de Moura *et al.* (2005) reported a oil flux of 5 LMH with undiluted SBO using a laboratory cast UF membrane from PES (MWCO ~100 kDa) in a stirred batch cell which improved to 15.3 LMH when oil was diluted with hexane (25% miscella). The selectivity for PL was also very high during MEUF of hexane-oil miscella, besides higher oil flux. A commercial PES membrane after solvent conditioning could achieve an oil flux of 24.5 LMH (Garcia *et al.*, 2006).

Earlier studies from this laboratory with undiluted oils revealed the potential of nonporous membranes for simultaneous degumming and decolourization of crude vegetable oils (Subramanian *et al.*, 2004). To address the low permeate flux obtained in the process, processing hexane-oil miscella was examined as an alternate approach and evaluated the efficacy of these nonporous membranes for degumming and colour reduction under hexane-diluted conditions. These attempts showed that oil flux could be improved by one order of magnitude without significantly affecting the membrane selectivity by hexane dilution and without exceeding the solvent ratio (oil content ~25 to 30%) generally used for oil extraction in the industrial solvent extraction plants (Sarita Arora *et al.*, 2006; Saravanan *et al.*, 2006).

1.4 Importance and challenges posed by RBO

RBO has gained special importance because of its balanced fatty acid profile and due to the presence of minor constituents with proven nutritional benefits such as γ-oryzanol, tocotrienols in addition to tocopherols and squalene and

better oxidation stability arising from these factors (Kaimal *et al.*, 2002). India is the largest producer of RBO with a potential of 1.2 MMT per year. According to the estimates, the annual production of RBO in 2006 was 0.75 MMT, out of which 0.73 MMT was utilized for edible purpose and the remaining for non-edible purposes (Solvent Extractors Association Report, 2008). RBO consumption towards the edible purpose increased over the years from 78% to 97% of total production during 2001 to 2006 (Codex Alimentarius Commission, 2003; Solvent Extractors Association Report, 2008) owing to the awareness of its health benefits and increased use in hydrogenated fats as well as in blended oils. The unusually high content of waxes, FFA, unsaponifiable constituents, PL and glycolipids as well as the dark colour makes the refining process difficult (Kaimal *et al.*, 2002). Improvements in the refining process could increase the use of RBO for direct edible purposes.

Physical refining is advantageous over chemical refining as this process could considerably reduce the neutral oil losses while preserving the bioactive compounds such as oryzanol in the processed RBO. However, the prerequisite for this process is a very low phosphorus (<10 mg/kg) and iron (preferably <0.2 mg/kg) contents in the pretreated oil (Cleenerwerck and Dijkstra, 1992). Colour of the final product also gets affected if these requirements are not met. Presence of high amounts of FFA in RBO also favours physical refining which necessitates adoption of efficient pretreatment techniques.

RBO is a somewhat variable feedstock in composition and quality and selection of an appropriate process is essential for efficient degumming.

Many alternative approaches such as solvent refining, biorefining, simultaneous degumming and dewaxing using $CaCl_2$ treatment, enzymatic degumming and membrane technology have been attempted by the researchers to overcome the difficulties in the RBO processing. Considering the drawbacks associated with solvent refining and biorefining, Rajam *et al.* (2005) developed a process for simultaneous degumming and dewaxing using $CaCl_2$ treatment and demonstrated it on an industrial scale. Enzymatic degumming using phospholipase is reported to be successful in reducing the phosphorus content to a level of 5 mg/kg (Roy *et al.*, 2002).

Pretreatment of RBO using ceramic as well as polymeric membranes was attempted by various researchers. Lin *et al.* (1997) reported 98.7% PL reduction with RBO miscella using 1 kDa MWCO polymeric membrane. Ceramic membranes offer several advantages over polymeric membranes that include resistance to abrasion and chemical attack, tolerance towards high temperatures over a wide range of pH, ease of cleaning by a high-temperature treatment and also tolerance to cleaning with alkali and acid. Earlier attempts using ceramic membranes (pore size 60-350 nm) could achieve only ~69% gum removal in RBO (De *et al.*, 1998). In a more recent study, Subramanyam *et al.* (2006) achieved 95% reduction in phosphorus along with simultaneous reduction in colour (42-62%) while processing CRBO miscella (30% and 20% oil concentration) using ceramic membranes (MWCO 1, 15 and 300 kDa). Studies from this laboratory reported a very low phosphorus content in membrane-processed RBO in the range of 18-31 mg/kg using nonporous membranes (Saravanan *et al.*, 2006). Membrane

technology appears to be a potential technology to meet the stringent pretreatment requirements of physical refining for processing RBO.

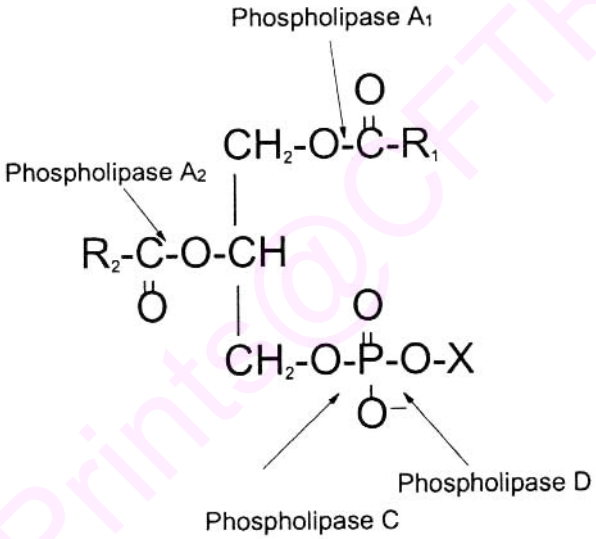
Kaimal *et al.* (2002) reported for the first time the presence of novel phosphorus-containing glycolipids as the root causing problem in RBO processing. RBO contains high content of glycolipids (~6%) which was found to be a central problem and their removal appeared crucial for successful processing of the oil (Kaimal *et al.*, 2002). The conventional degumming pretreatment is not efficient due to these PGL that further affects the oil colour in the subsequent processing steps making it difficult to produce a final product of acceptable quality.

1.5 Enzymatic degumming of RBO

Oil degumming process plays a critical role in the physical refining of edible oil. For successful physical refining, the phosphorus content in the pretreated oil should be below 10 mg/kg (Cleenerwerck and Dijkstra, 1992). Acid degumming is a widely followed method in the industry, by which the phosphorus content after degumming is reduced to ~15-80 mg/kg depending on the source and quality of oil. Though acid degumming is a very simple method, it is not sufficiently reliable for all types of oil (Yang *et al.*, 2008). Many alternative approaches have been attempted. Biotechnological approaches led to the development of enzymatic degumming employing phospholipase which has received the attention from the processors. Phospholipase-mediated degumming is a unique process quite distinct from the well known acid degumming variations, since both hydratable and NHP present in the oil are hydrolyzed to the corresponding lysophospholipids,

which migrate to the aqueous phase under the conditions employed (Clausen, 2001), facilitating their easy removal by centrifugation, yielding a low phosphorus content degummed oil.

Phospholipases constitute a class of hydrolytic enzymes, which can hydrolyse the ester bonds of PL. The main phospholipase types are A_1, A_2, C and D with their target sites as shown (Scheme 1.1). In connection with enzymatic oil-degumming mainly phospholipases of type A_1 or A_2 are of relevance (Clausen, 2001).



Scheme 1.1 A general representation of a PL indicating the position of cleavage of different phospholipases, where X = H, choline, ethanolamine, serine, inositol etc. (Clausen, 2001)

Enzymatic degumming processes have been explored by other researchers with phospholipases from different sources (Ulbrich-Hoffman, 2000; Grunwald, 2000). However, only three enzymes, a phospholipase A_2 from porcine pancreas and two kinds of microbial phospholipases A_1 from *Fusarium oxysporum* and *Thermomyces lanuginosus*, have been reported for successful industrial scale degumming applications (Yang *et al.*, 2008).

Enzymatic oil degumming was first introduced as an industrial process by the German Lurgi Company as the 'EnzyMax Process' in the 1990s with phospholipase A_2 from porcine pancreas (Lecitase-10L). Subsequently, Novozymes substituted Lecitase-10L with Lecitase-Nova, a phospholipase of microbial origin owing to limited enzyme source (porcine pancreas), high optimal pH and non-compliance of kosher and halal specifications (Clausen, 2001). Lecitase-Novo is a phospholipase A_1 from *Aspergillus oryzae* and proved to be superior to Lecitase-10L in laboratory degumming tests (Clausen, 2001). Subsequently, Novozymes brought out a third generation phospholipase A_1, Lecitase-Ultra from *Thermomyces lanuginosus/Fusarium oxysporum* produced by submerged fermentation of a genetically modified *Aspergillus oryzae*. It exhibited a high specificity for hydrolysis of PL in oil when the reaction temperature is above 40°C (Yang *et al.*, 2006a). The characteristics of these three enzymes from Novozymes are listed in Table 1.3.

Clausen (2001) conducted a comparative study on the performance of Lecitase-10L and Lecitase-Novo for degumming rapeseed oil in the laboratory. Lecitase-Novo was suitable for degumming different oil qualities ranging from water-degummed oil to crude oil. Lecitase-10L preferentially hydrolyzed PL in aqueous phase (hydrophilic) while Lecitase-Novo preferentially hydrolyzed PL in oil phase (lipophilic). The hydrolyzed lysophospholipids were found only in aqueous phase. A high water content in the crude oil is required for degumming with Lecitase-10L which could lead to separation problems with gums as they may not form a paste. In contrast, Lecitase-Novo could perform oil degumming at very low water content.

Table 1.3 Characteristics of commercial phospholipases used in degumming oils

Sl. no.	Details	Lecitase-10L	Lecitase-Novo	Lecitase-Ultra
1*	Source	Porcine pancreas	*Fusarium oxysporum*	*Thermomyces lanuginosus/ Fusarium oxysporum*
2*	Specificity	A2	A1	A1
3*	Molecular weight, kDa	12-14	~28	~35
4*	Ca^{2+} dependence	Yes	No	No
5*	Td (DSC), °C	70-80	50	60
6*	Optimum temp. (degumming), °C	65-70	45	50
7*	Optimum pH (degumming)	5.5	4.5	4.5
8*	Effective with 1% water	No	Yes	Yes
9*	Kosher/Halal compliance	No	Yes	Yes
10	Published literature	Rapeseed oil - Clausen, 2001	Rapeseed oil - Clausen, 2001; RBO - Roy *et al.*, 2002; Sheelu *et al.*, 2008	SBO and rapeseed oil - Yang *et al.*, 2006a,b; 2008

*Source: www.novozymes.com

Consequently the first oil mill (Cereol, Germany) which introduced enzymatic degumming with Lecitase-10L in 1994 switched over to Lecitase-Novo in mid 2000 and reported that the water content for oil degumming could be reduced from 5% to 1.5%. Enzymatic degumming of CRBO was first attempted by Roy *et al.* (2002) using Lecitase-Novo. The results indicated that phospholipase A_1 mediated degumming followed by bleaching is a very mild protocol for pretreating RBO to reduce the phosphorus levels to <5 mg/kg suitable for physical refining.

Yang *et al.* (2006b) characterized Lecitase-Ultra and applied for degumming rapeseed oil and SBO. Lecitase-Ultra is an acidic lipase, which exhibits maximal activity at pH 5.0, and it has inherent activity towards both PL and TG structures. When the temperature is over 40°C, the phospholipase activity predominates, and the lipase activity is partly suppressed. In the oil degumming process the enzyme was able to identify only the PL as substrate owing to specificity to hydrolyze them, and the yield loss due to TG hydrolysis was only marginal. PL in the oil were easily converted by enzyme catalyzed hydrolysis and the phosphorus level reduced to <10 mg/kg within 5-6 h at 50°C with an enzyme addition of 30 mg/kg (oil), whereas the residual phosphorus was ~20 mg/kg if no enzyme was added in the process (Yang *et al.*, 2006a). These researchers also optimized the process conditions using response surface methodology. The optimal set of variables was reported to be an enzyme dosage of 39.6 mg/kg, a temperature of 48.3°C and a pH of 4.9. They performed an enzymatic degumming plant trial on a 400 tons/day oil production line using Lecitase-Ultra on rapeseed oil.

The pH showed a strong impact on the degumming performance and the phosphorus content could be reduced from 120.5 (crude oil) to <10 mg/kg by lowering the pH in the range of 4.6-5.1 (Yang *et al.*, 2006a). Similar degumming performance was achieved with CSBO in the optimum pH range of 4.8-5.1 (Yang *et al.*, 2008). Through analysis of PL compounds in the gum by Electrospray ionization-mass spectrometer and phosphorus content, it could be seen that both glycerophospholipids and lysophospholipids existed with contents of 45.7 and 54.3%, respectively. The emulsifying capacity of lysophospholipids is higher than PL for oil/water emulsions, while glycerophospholipids are more hydrophilic and this may explain the reason why the oil and gum phases can be separated easily in the enzymatic process with a lesser oil loss (Yang *et al.*, 2008). Clausen (2001) earlier reported the catalyzed mechanism of the enzyme only with the production of lysophospholipids but Yang *et al*., (2008) were the first one to document the production and role of glycerophospholipids. Successful enzymatic degumming seems to be related to the production of glycerophospholipids.

Enzymatic degumming is a suitable process for the physical refining, offering several advantages. Apart from the reduction in the amount of acid, alkali and waste water during the refining process, an enhancement in product yield and a reduction in operating costs can also be observed (Klaus *et al.*, 1998). A case study on enzymatic degumming of rapeseed oil revealed that there is a saving of 1.27 US$ per ton of oil over conventional physical refining process and a much larger saving of 10.86 US$ per ton of oil over conventional chemical refining process (www.novozymes.com).

As described above, the earlier research work on enzymatic degumming had been rather focused on rapeseed oil and SBO. Roy *et al.* (2002) made the first attempt with CRBO using Lecitase-Novo. Subsequently attempts were made to recycle phospholipase-A_1 (Lecitase) in the process. Lecitase immobilized on hydrogel (containing gelatin cross linked with glutaraldehyde) was employed for degumming CRBO in a spinning basket bioreactor for 6 cycles. Phosphorus content in the degummed oil did not reduce below 60-70 mg/kg from an initial value of 400 mg/kg (Sheelu *et al.*, 2008). Although there was no loss of enzyme activity, immobilization of Lecitase actually reduced its degumming efficiency compared to free enzyme, hence alternate methods for enzyme recycle need to be explored.

1.6 Other potential applications of membranes in oil processing

1.6.1 Enrichment of oryzanol in RBO

The major source of oryzanol is rice bran and it is extracted into oil fraction during the extraction process. Oryzanol is a group of compounds containing ferulate (4-hydroxy-3-methoxycinnamic acid) esters of triterpene alcohols and plant sterols (ferulic acid esterified to cycloartenol, 24-methylene cycloartanol, β-sitosterol and campesterol). CRBO typically contains ~15,000 mg/kg of γ-oryzanol. These ferulic acid esters also possess antioxidant properties similar to that of tocopherols. Various physiological functions have been associated with oryzanol intake including a decrease in plasma cholesterol and decreased platelet aggregation, hepatic cholesterol synthesis and cholesterol absorption (Orthoefer, 1996).

The method of refining crude oil affects the oryzanol content in refined oil. Alkali refining resulted typically in a decrease in total oryzanol content from 16,000 to 2,000 mg/kg, while physical refining retained ~66% of the oryzanol in the oil (Orthoefer, 1996). During chemical refining, most of the oryzanol are removed along with soapstock, a byproduct containing 1.3-3.1% oryzanol (Das *et al.*, 1998). Therefore, soapstock of RBO obtained from chemical refining process is generally used as the primary source for recovering oryzanol. Over the decades, the methods of oryzanol isolation from soap stock have been improved, and selected methods have been summarized by Narayan *et al.* (2006). There were also attempts to isolate oryzanol directly from rice bran (Xu and Godber, 2000) by SCFE and CRBO employing either column chromatography-based techniques (Singh *et al.*, 2000) or SCFE (Dunford and King, 2000).

Oryzanol is used in various applications in pharmaceutical, therapeutic and dietary preparations. Besides, it has also been proposed to add back oryzanol to RBO (Hitotsumatsu and Takeshita, 1994). However, it may be desirable to enrich oryzanol present in the oil rather than isolating it and then adding it back to the oil. The following are few reports on the enrichment of oryzanol in RBO.

Cherukuri *et al.* (1999) described an extraction procedure using lower aliphatic alcohols for obtaining ERBO having enhanced anti-oxidant content (tocols and oryzanol) from CRBO. The yield of ERBO was in the range of 31-45% with an oryzanol enhancement of ~80-90%. Karan (1998) reported that dimethylformamide extraction of CRBO after hexane dissolution was superior to alcohol extraction. The oryzanol content in RBO could be enriched by a

factor of 7.1 with a yield of ~4% ERBO. Cherukuri *et al.* (1999) also reported that SPVD (0.002 mm Hg and 235°C) of refined RBO could enhance the oryzanol content from 0.18% to 1.7% with a distillate percentage of 6.3%. Dunford and King (2000) evaluated SCFE technique for reducing the FFA content in RBO and reported enrichment of phytosterol in the deacidified RBO fraction. When CRBO with 7% FFA and 1.3% oryzanol content was subjected to fractionation at 13.6 MPa, 45°C and 1.2 l/min CO_2 flow rate, there was a decrease in FFA content (<1%) and increase in oryzanol content (1.78%) in the raffinate fraction. The methods proposed above are associated with some limitations such as employing either organic solvent for extraction or capital intensive equipment with lower throughput or severe conditions for separation in a capital intensive process.

Although a large number of attempts have been made towards processing edible oils using membrane technology, there is no membrane-based process approach reported for the separation/concentration of oryzanol. The differences in the molecular sizes among the oryzanol mixture (~600 Da) and that of TG (>600 Da) are too small to use a porous membrane (NF) for separation based on size exclusion, whereas in nonporous membranes, the separation is due to solution-diffusion effect and such membranes may possess the required selectivity for the concentration of oryzanol.

1.6.2 Deoiling of lecithin

Lecithin is an important coproduct of edible oil processing. Lecithin refers to a complex mixture of PL, TG and other substances such as glycolipids, FFA

and carbohydrates. Lecithin is obtained by water degumming crude vegetable oils and separating and drying the hydrated gums. It is the PL portion of lecithin that is mainly responsible for giving form and function to lecithin (Sipos and Szuhaj, 1996a). Lipids that are composed of two fatty acids and a phosphorus-containing region joined by ester linkages to a glycerol backbone are called PL. They are found in all living cells of animals and plants. Although the highest concentrations of PL occur in animal products, the major commercial source is the soybean, which contains 0.3-0.6% (Sipos and Szuhaj, 1996b). Nevertheless, PL from other vegetable sources, i.e., corn, cottonseed, linseed, peanut, rapeseed, rice bran, safflower and sunflower have also been studied and used. Lecithin is widely used in the food, pharmaceuticals and cosmetic industries.

Deoiling of crude lecithin is a prerequisite in making high-purity lecithin products. Acetone has been the solvent of choice and currently used in the industry for the separation of glycerides and PL, based on the insolubility of PL and glycolipids in acetone. Eliminating solvent processing could maintain 'relatively natural' characteristics in its products and avoid formation of condensation products such as mesityl oxide reported in the acetone-extraction process (Sipos and Szuhaj, 1996a). An alternative to acetone-extraction is the treatment of lipid mixtures with supercritical gases or mixtures (Schneider, 1989). For deoiling of crude soy lecithin with pure CO_2, pressures as high as 60-100 MPa were required (Eggers and Wagner, 1993). Besides, increasing viscosity of the lecithin during the deoiling process prevented the complete removal of the oil. The operating pressure was reduced to 8 MPa in the temperature range of 40-55°C when propane (80 wt%) was used as an

entrainer along with CO_2 (20 wt%) to maintain the lecithin in a liquid state during extraction and made it possible to obtain an oil-free product. Deoiling was also demonstrated using supercritical CO_2 in the presence of a co-solvent such as ethanol and acetone at moderate pressures (17 and 20 MPa) at 62°C (Teberikler *et al.*, 2001). However, SCFE involves capital intensive equipment owing to high pressure processing.

Deoiling of lecithin employing UF has been also reported (Gupta, 1977; Hutton and Guymon, 2000). Commercial lecithin containing 40% TG was dissolved to 10% solution in hexane and passed through a UF membrane (MWCO 20 kDa) in recycle mode to get deoiled lecithin containing 6% TG content. Processing in diafiltration mode (25% lecithin in hexane as initial feed) with a lower MWCO (10 kDa) UF membrane could reduce TG content to 3% in deoiled lecithin (Gupta, 1977). Another research group made a similar attempt with UF for deoiling lecithin followed by few more additional processing steps like bleaching and addition of tocopherols and tocotrienols in solvent medium before subjecting the retentate fraction to desolventization. Polyvinylidene fluoride membrane having a MWCO of 10-50 kDa was employed in the process and the deoiled lecithin obtained from the process contained 90% AI (Hutton and Guymon, 2000).

The separation of a mixture of constituents in porous membranes is mainly based on size exclusion. While in the case of nonporous membranes, the separation is due to the solution-diffusion effect. Earlier studies from this laboratory showed that hydrophobic nonporous membranes possess very high selectivity to separate PL from undiluted as well as hexane-diluted oils (Subramanian *et al.*, 2004; Saravanan *et al.*, 2006; Sarita Arora *et al.*, 2006).

The nonporous membranes tend to offer greater yield of PL compared to UF based membrane processes.

1.7 Scope of the present investigation

An exhaustive review of the research carried out on edible oil processing using membrane technology (Manjula and Subramanian, 2006) forms a prelude to the present investigation, which suggested nonporous membranes as a better choice for achieving simultaneous degumming, dewaxing and decolourizing crude vegetable oils. Considering the importance and challenges posed during processing, RBO has been given the importance among the various vegetable oils used in the study.

The PL content of crude oil plays an important role in the degumming performance of membrane-based processes since these surfactant molecules form reverse micelles in vegetable oils and hexane-oil miscella. In this study the influence of PL composition and solvent (hexane) medium on the CMC levels in undiluted and hexane-diluted crude vegetable oils has been investigated to evaluate the degumming performance of UF and nonporous membranes with oil systems varying in PL contents (below and above CMC).

Permeate flux is an important factor for the practical application of membrane processing. Oil flux is generally low during membrane processing owing to its viscous nature and there are attempts to improve the oil flux using solvent dilution. In the present work, the influence of hexane dilution and transmembrane pressure on the permeate flux has been studied in a nonporous membrane with various vegetable oils (MO, GNO, RBO, SFO and CNO) for achieving greater permeate flux.

The unusually high content of waxes, FFA, unsaponifiable constituents, PL and glycolipids as well as the dark colour in CRBO makes the refining process difficult. Besides, the presence of phosphorus-containing glycolipids, PGL causes severe processing problems. Improvements in the refining process could increase the use of RBO for direct edible purposes. Therefore, efficacy of nonporous membrane towards simultaneous degumming, dewaxing and decolourization of CRBO has been studied while also assessing its capability for specific removal of PGL.

Phospholipase-mediated degumming is a unique process quite distinct from the well known acid degumming variations and construed as a suitable process for the physical refining. However, there are only few attempts on CRBO despite the challenges posed by it during processing. The efficacy of enzymatic degumming of CRBO has been studied using a third generation microbial phospholipase A_1 in oil as well as hexane medium.

The physiologically beneficial ferulate esters (oryzanol) present in CRBO are lost to a greater extent during conventional refining of CRBO depleting their content in the final product. With a view to enhance the oryzanol content in the processed oil, its enrichment in RBO has been attempted using nonporous polymeric membranes under undiluted as well as hexane-diluted conditions with crude, refined and model oil systems.

Deoiling of lecithin is a prerequisite in making high-purity products. The UF has been proposed as an alternative to acetone-extraction for the separation of glycerides and PL, the industrial practice that affects the 'relatively natural' characteristics of lecithin products. The nonporous membranes tend to offer greater yield of PL compared to UF owing to its

excellent selectivity towards PL. In the present investigation, deoiling of lecithin using nonporous membrane has been examined in hexane medium with soy and rice bran lecithins.

2.1 Materials

2.1.1 Raw materials

Four lots of CRBO were used in the study. Two lots of CRBO were obtained from the industry: M/s M.K. Agrotech (lot-1), Srirangapatna, India and M/s Habib Agro Industries (lot-2), Mandya, India. Another two lots of CRBO were extracted in the laboratory from parboiled (lot-3) and raw (lot-4) rice bran obtained from the local market. CSFO was laboratory-extracted using a powered ghani (Khadi and Village Industries commission, Ahmadabad, India). Commercial (totally refined) MO, GNO, RBO, SFO, CNO and SBO were purchased from the local market.

Laboratory grade soy lecithin (lot-1) was obtained from M/s Wako Pure Chemical Industries, Osaka, Japan and commercial grade soy lecithin (lot-2) was obtained from M/s Sakthi Soya Ltd., Coimbatore, India. Rice bran lecithin was prepared in the laboratory according to Pragasam *et al.* (2002).

2.1.2 Chemicals and solvents

Oryzanol used as a reference in the analyses and in the preparation of model oil systems was of analytical grade (purity ~98%) and obtained from M/s Wako Pure Chemical Industries, Osaka, Japan. PC, PI and PE standards were obtained from Sigma Chemical Company, St Louis, USA. HPLC-grade solvents such as acetonitrile, methanol, chloroform, dichloromethane were obtained from either Ranbaxy Fine Chemicals (New Delhi, India) or Qualigens Fine Chemicals (Mumbai, India).

Hexane used in the experiments was of laboratory grade and purchased from M/s S.d. Fine Chem. Ltd, (Mumbai, India) and M/s Ranbaxy

Fine Chemicals (New Delhi, India). Oleic acid was of analytical grade and procured from Loba Chemie Pvt. Ltd. (Mumbai, India). All other laboratory and analytical grade chemicals and solvents were procured from reputed manufacturers in the country.

2.1.3 Enzymes

Lecitase-Ultra was kindly supplied by M/s Novozymes (Bagsvaerd, Denmark), Bangalore, India.

2.1.4 Membranes

Two nonporous polymeric composite hydrophobic membranes with PDMS as active layer and polyimide as support layer, NTGS-2200 (~3 μm active layer thickness) and NTGS-2100 (Nitto Denko, Kusatsu, Japan) and one polymeric composite hydrophilic UF membrane (MWCO: 20 kDa), SelRO MPF-U20-S (Koch Membrane Systems, Massachusetts, USA) were used in the study. The membranes were cut into circular discs (7.5 cm diameter with 32 cm^2 effective area) and fitted in the membrane cell in such a way that active surface comes into contact with the feed material.

2.2 Membrane apparatus

Experimental runs were conducted in a self-stirred flat membrane test cell (Model: C40-B, Nitto Denko) under nitrogen atmosphere (Fig. 2.1). The membrane cell was placed on a magnetic stirrer, and the magnetic spin bar fitted into the cell provided the agitation. Stirring was employed to minimize concentration polarization effect, if any and the speed was maintained at 800

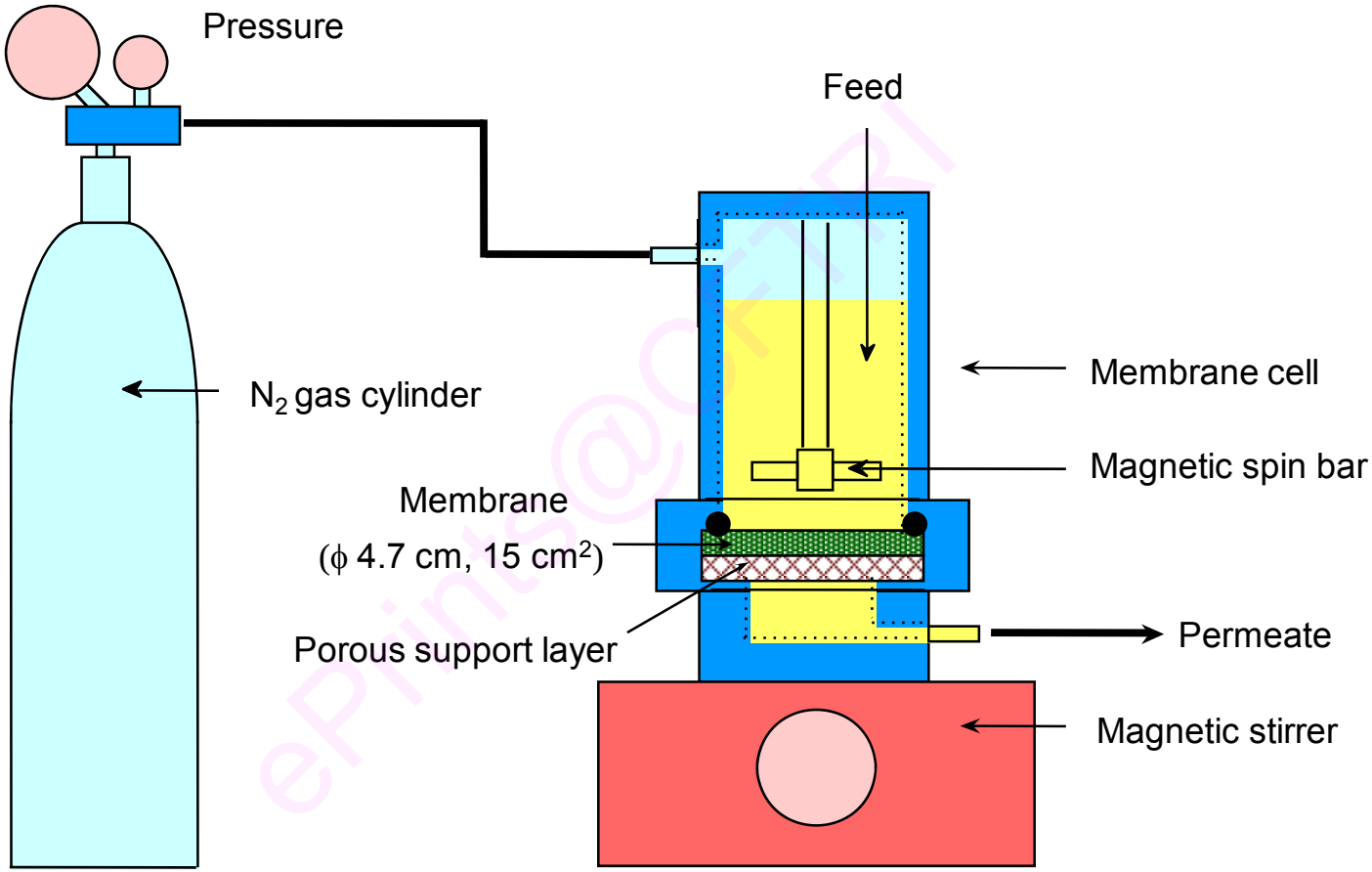


Fig. 2.1 Self-stirred flat membrane test cell

rpm. Experiments using nonporous membranes were carried out at different pressures with undiluted (2-4 MPa) and hexane-diluted (0.5-4 MPa) oil samples by adjusting the pressure regulator of the nitrogen cylinder. UF experiments were operated at 0.7 MPa. Experimental runs with refined oils (MO, GNO, RBO and SFO) during oil flux enhancement studies were conducted at 24°C. All other experimental runs were carried out at room temperature (28-30°C). New membrane was used for each set of experimentation. The unit was operated in batch mode by charging the cell with 80 g oil or 180-200 g hexane-diluted oil, and the run was terminated when a predetermined quantity of permeate had permeated.

2.2.1 Diafiltration

The unit was operated in three modes of operation by charging the cell with a predecided quantity of lecithin-hexane mixture, namely, (a) batch operation (b) discontinuous diafiltration and (c) simulated continuous diafiltration (Fig. 2.2). In batch operation, the lecithin-hexane mixture was processed until a desired VCR was reached.

In the first step of the discontinuous diafiltration mode, the lecithin-hexane mixture was processed until a desired VCR was reached and stopped the run. Then the retentate fraction was diluted to its original volume with hexane and the experimental run was continued as before. This was continued until the desired number of steps of diafiltration was achieved.

During continuous diafiltration mode, the feed volume is maintained constant at any given time by compensating the permeation with a continuous dilution of the feed. Considering the practical difficulties in carrying out a

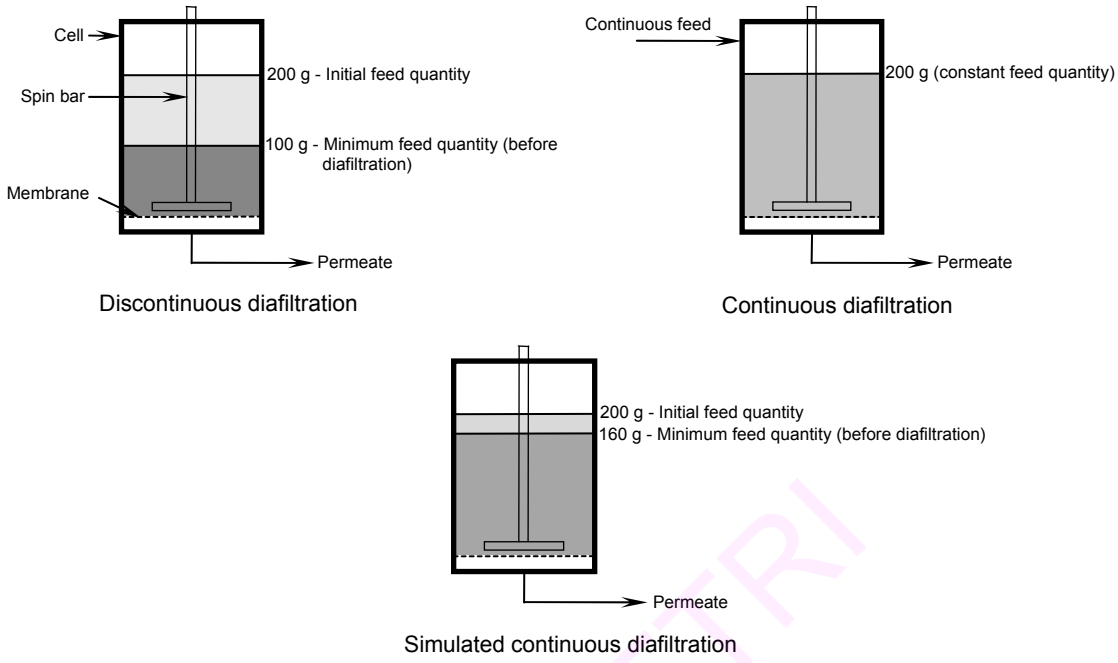


Fig. 2.2 Diafiltration in batch membrane cell

continuous diafiltration during batch processing, a simulated run was conducted by stopping the run between small intervals (after 20% permeation) and adding equivalent amount of fresh hexane to the retentate before restarting the run. The experimental run was thus continued until the desired level of diafiltration was achieved.

2.2.2 Membrane cleaning

The membrane was cleaned using hexane after each run by dipping the membrane in hexane for few minutes and the surface was wiped with a soft tissue paper. In the case of lecithin experiments, secondary layer formation was significant due to higher PL concentration in the feed and the cleaned membrane was reused only after recovering the original hexane flux.

2.3 CMC measurements

2.3.1 TCNQ solubilization

Procedure described by Kanamoto *et al.* (1981) was generally followed. Lecithin or deoiled lecithin (PL) or PC was dissolved in SBO (totally refined) and hexane by mixing for 12 h and 15 min, respectively to obtain their respective stock solutions. Samples containing different levels of PL/PC were obtained by various desired dilutions with refined SBO/hexane. In the case of CRBO, samples with different PL concentrations were obtained by various desired dilutions of CRBO with refined RBO. About 10 mg of TCNQ were added to 5 g of oil sample in a small conical flask and the mixture was agitated for 5 h at room temperature. In the case of hexane-diluted systems, 2 h of reaction time was sufficient. After sedimentation of excess TCNQ by centrifugation at 800 x g for 20 min, absorbance measurements at 480 nm were made in a UV-Visible spectrophotometer (Model UV-160A, Shimadzu, Kyoto, Japan) with an aliquot of respective reaction mixture without TCNQ addition as reference fluid. The CMC was taken as the change in slope in the curve between absorbance and PL concentrations on a semi-log plot.

2.3.2 Surface tension

Measurements were based on the principle of Wilhelmy method using an automatic Interfacial Tensiometer (Model: CBVP-Z, Kyowa Interface Science Company, Asaka, Japan). Determination of CMC of PL was carried out by measuring surface tension of oil/hexane-oil samples containing varying but known PL concentrations prepared using CRBO, refined RBO and hexane. The CMC corresponds to the PL concentration where there is an abrupt

change in surface tension and obtained from a simple plot between these two parameters.

2.4 Enzymatic degumming

RBO (100 g) was placed into a 250-mL conical flask fitted with stopper. The oil was heated to about 70-80°C in a water bath, and a citric acid solution (0.166 mL of 45% citric acid) was added. The mixture was allowed to condition for 30 min at 70-80°C under stirring (~500 rpm). Then the temperature of the oil was decreased to about 50°C. It was followed by the addition of NaOH solution (0.732 ml of 4% NaOH), deionized water (2% of oil weight) and predecided quantity of Lecitase enzyme solution. Then the flask was placed in a water bath at 50°C with stirring at ~500 rpm. Samples were drawn for phosphorus and fatty acid analyses at the end of 5 h as well as during intermittent periods.

Enzymatic degumming experiments in solvent-phase were conducted as above but using a two-necked round bottomed flask fitted with a reflux condenser and placed on a magnetic stirrer. Solvent (hexane or petroleum ether) was added along with oil in the ratio of 1:1 (w/w). The water addition varied in the range of 2-20% to the total weight of oil and hexane.

2.5 Analyses

Feed, permeate and retentate samples of each experimental run were analyzed for various specific constituents. In the experiments conducted with hexane-oil and hexane-lecithin systems, the samples were analyzed after

evaporating the hexane under vacuum using a flash evaporator (at 40°C for 40 min followed by 5 min flushing with nitrogen).

2.5.1 Chemical analyses

2.5.1.1 PL content in oil and lecithin samples

Phosphorous content of the oil samples was measured by the standard molybdenum blue method as per the AOCS method, Ca 12-55 (AOCS, 1998). In the case of lecithin samples, phosphorous content of the samples was measured after mixing with a known quantity of refined SBO or RBO as the case may be. The PL equivalent was calculated by multiplying the phosphorous content by a factor of 30 for SFO, SBO and soy lecithin, and 31 for RBO and rice bran lecithin samples.

2.5.1.2 AI content

AI content in crude and deoiled lecithin samples were determined using AOCS method Ja 4-46 (AOCS, 1998).

2.5.1.3 Wax content

Wax content in RBO samples was determined in terms of AI as proposed by Ramaswamy *et al.* (1980). Chilled (5-7°C) acetone was added to 5 ml of oil sample (1:1, v/v) and centrifuged at 5000 rpm for 20 min. Supernatant oil was decanted carefully and insoluble portion (wax) was washed with 5 ml of chilled acetone and centrifuged. The wax obtained was then dried in vacuum oven and weighed.

2.5.1.4 FFA content

FFA content in the oil samples was determined using AOCS method, Ca 5a-40 (AOCS, 1998).

2.5.1.5 Spectrophotometric analysis of oryzanol

Oryzanol content in the oil samples was determined by spectrophotometric method at a specific wave length of 315 nm (Seetharamaiah and Prabhakar, 1986) using dichloromethane as solvent.

2.5.1.6 Moisture

Estimation of moisture content was done using coulometric Karl Fisher titrator (Model DL32, Mettler Toledo, Greifensee, Switzerland).

2.5.2 Physical measurements

2.5.2.1 Colour measurement

Oil samples. Colour was measured by two different methods, Lovibond method (Cc 13e-92) of AOCS (AOCS, 1998) and area under absorption spectra in the visible range (Boekenoogen, 1968; Subramanian *et al.*, 1998a).

The Lovibond colour of the oil samples was determined in a Lovibond Tintometer (Model F, The Tintometer Ltd., Salisbury, United Kingdom) using a glass cell with an optical path length of 25.4 mm. The colour was matched using colour racks of red (R) and yellow (Y) and it was expressed as (5*R* + *Y*).

The total spectrometric colour of the samples was estimated from the area under the absorption spectra between 350 and 800 nm. The

spectroscopic data were recorded using the spectrophotometer using a 10 mm cuvette with distilled water as blank.

Lecithin samples. The total colour of the samples was estimated from the area under the absorption spectra between 350 and 800 nm (Subramanian *et al.*, 1998a). The spectroscopic data were recorded using the spectrophotometer. Absorbance in the visible range was measured after dissolving the lecithin samples in hexane (5%, w/v) and using a 10 mm cuvette with hexane as blank.

2.5.2.2 Viscosity

Measurement of viscosity (apparent) of samples was carried out using disc spindle measuring system in a digital viscometer (Model DV-II+, version 2.0; Brookfield Engineering Laboratories, Stoughton, USA) at room temperature (28-30^{o}C).

2.5.3 Enzyme assay

2.5.3.1 Determination of phospholipase activity

Phospholipase assay was performed with PL emulsion. One unit of phospholipase is the amount of enzyme which releases 1 µmol of titratable FFA per minute under the described conditions. Substrate solution: 25% PL (deoiled soy lecithin) and 4% polyvinyl alcohol solution were emulsified at a volume ratio of 1:4. Analysis conditions: 4 ml of PL emulsion, 5 ml of 0.01 M citric acid buffer and 1 ml of enzyme solution were mixed and incubated at 37°C for 10 min. The reaction was terminated with the addition of 95% ethanol (15 ml) after incubation, and the liberated FFA were titrated with 0.05

M NaOH. Blanks were measured with a heat-inactivated enzyme sample. For this purpose an enzyme stock solution was kept at 100°C for 15 min for inactivation, cooled to ambient temperature and used in the assay as described for the active enzyme sample.

2.5.3.2 Determination of lipase activity

Lipase activity was determined according to the phospholipase method as described above but using refined RBO as a substrate.

2.5.4 GC and HPLC analyses

2.5.4.1 Fatty acid composition

Analysis of fatty acid composition was done by GC (Model: GC-15A, Shimadzu) as per the AOCS method, Ce 1-62 (AOCS, 1998). Fatty acids present in oil were first converted to fatty acid methyl esters before injecting in to GC column to obtain the fatty acid profile. GC details: packed column, 4.8 x 3000 mm (chromosorb W(HP) 80-100 mesh, precoated with 15% diethylene glycol succinate). Measurement conditions: column oven - 180°C, injection block - 220°C and detection block - 230°C (isothermal operation); hydrogen and nitrogen flow 40 ml/min and air flow 300 ml/min.

2.5.4.2 Oryzanol analysis

Oryzanol contents in oil samples were determined in the reversed-phase HPLC by the method followed by Xu and Godber (2000) with slight modification. The mobile phase consisted of methanol, acetonitrile, dichloromethane and acetic acid (50:44:3:3, v/v). HPLC details: column C-18,

2.5 x 250 mm with 5 µm particles (Merck, Darmstadt, Germany); LC-10AT HPLC pump, SCL-10A system controller and SPD-10A UV-VIS detector (Shimadzu). Measurement conditions: absorbance 330 nm, column temperature 30°C, flow 1.4 ml/min and analysis time 40 min. Sample preparation: 0.5 g of oil sample was dissolved in 3 g of dichloromethane and then diluted with an equal amount of mobile phase before analysis.

2.5.4.3 Individual PL analysis

Individual PL (PC, PI and PE) in lecithin samples were analyzed by normal-phase HPLC using acetonitrile, methanol and phosphoric acid (780:10:9, v/v) as mobile phase (Hurst and Martin, 1984). HPLC details: column Partisil, 4.6 x 250 mm with 5 µm silica (Whatman International Ltd., Maidstone, England); LC-10AT HPLC pump, SCL-10A system controller and SPD-10A UV-VIS detector (Shimadzu). Measurement conditions: absorbance 205 nm, column temperature 25°C, flow 1 ml/min and analysis time 40 min. Lecithin samples were dissolved in known amount of chloroform before analysis. The quantification of the individual PL was based on the standard curves obtained with HPLC standards of PC, PI and PE.

2.5.5 NMR spectroscopy of PGL

PGL were Isolated as per the procedure followed by Vali *et al.* (2004). CRBO and process stream samples were treated with boiling water (5% of sample weight) under stirring for 60 min and centrifuged for 30 min at 8000 rpm. The decanted oil phase was given a second treatment with boiling water (10% of sample weight) under stirring for 45 min and centrifuged as above. The first

and second sludge obtained above were combined, dispersed in hexane and centrifuged. The oil-free sludge obtained was dried in a lyophilizer (Model: FD3, Heto-Holten, Allerad, Denmark). The above mentioned dried fractions of the feed, permeate, retentate were subjected to NMR analysis for identification and confirmation of removal of PGL.

^{1}H and ^{13}C NMR analyses were carried out on a Brüker Avance 500 NMR spectrometer operating at 500.18 MHz for ^{1}H and 125.77 MHz for ^{13}C under ambient conditions. About 40 mg of the samples was dissolved in a mixture of $CDCl_3$ and DMSO-d_6 (1:1, v/v) for recording the spectra. A region from 0 to 10 ppm for ^{1}H and 0 to 220 ppm for ^{13}C was scanned. For proton spectra, typically 16 scans and for carbon spectra, 2048 scans were accumulated to get a good spectrum. The samples were referenced to TMS to within ±0.01 ppm for ^{1}H and ±0.1 ppm for ^{13}C. In the case of carbon spectra, proton-noise decoupled spectra were obtained. 2DHSQCT spectra were recorded in magnitude mode with the sinusoidal state z gradients of strength 25.7, 15.42 and 20.56 G/cm in 5:3:4 ratio applied for 1 ms duration each with a gradient recovery delay of 100 μs to defocus unwanted coherences. Incrementation was in 256 steps with a 4K size computer memory. Spectra were processed using the unshifted and $\pi/4$ shifted sine bell windows function in F_1 and F_2 dimension, respectively.

^{31}P NMR spectra were recorded on a Brüker Avance 400 instrument operating at 162.001 MHz for phosphorus at a probe temperature of 20ºC. Proton decoupled spectra were obtained for feed, permeate and retentates after dissolving about 50 mg sample in a mixture of $CDCl_3$ and DMSO-d_6 (1:1, v/v). About 20-32 scans were accumulated for each sample. A spectral width

of 64000 Hz was employed. A line broadening function of 1 Hz was used and all the signals were referenced to 85% phosphoric acid external reference to within ±0.05 ppm.

2.6 Performance parameters

The performance of the membrane process was expressed in terms of rejection, and reduction of individual components, selectivity and permeate flux.

The percentage rejection (R_o) was determined assuming that it was constant during each batch of the experimental run using the following equation.

$$R_o = \frac{100\ln\left(C_{R,f} / C_{R,i}\right)}{\ln\left(W_i / W_f\right)} \quad (1)$$

where $C_{R,i}$ and $C_{R,f}$ are the initial and final contents of each component in the retentates (mg/kg-oil), and W_i and W_f are the initial (feed) and final weights of retentate (kg-oil), respectively.

The percent reduction (PR) was calculated using the following equation.

$$PR = \frac{100\left(C_F - C_P\right)}{C_F} \quad (2)$$

where C_F and C_P are the contents of each component in the crude and processed oils (kg/kg-oil), respectively.

Selectivity (degree of separation) was calculated using the following equation

$$\text{Selectivity} = \frac{\text{Weight ratio between two specific oil constituents in permeate}}{\text{Weight ratio between the same two oil constituents in feed}} \quad (3)$$

Enrichment is the ratio of the individual oil constituent (oryzanol) in the processed oil (retentate) to the feed.

$$\text{Enrichment (fold)} = \frac{\text{Oryzanol content in the retentate (mg/kg)}}{\text{Oryzanol content in the feed (mg/kg)}} \qquad (4)$$

Flux measurement was done by weighing the permeate collected for every hour and the flux reported was the average of hourly measurements and expressed in kg/(m^2·h). Oil flux in hexane-diluted samples was calculated after evaporating hexane from the permeate. Hexane was evaporated from the retentate fractions also to check the material balance of oil for each run.

PL, AI, wax, moisture, oryzanol, viscosity, phospholipase and lipase assay, GC and HPLC analyses were done in duplicates/triplicates and suitably expressed as mean ± SD wherever applicable.

3.0 Significance and focus of the work

PL are surfactant molecules which form reverse micelles in nonaqueous systems when the PL content exceeds CMC. Therefore the PL content of crude oil plays an important role in the degumming performance of membrane-based processes. Hexane is the common solvent used for oil extraction and had been the natural choice for oil dilution in the improvement of oil flux during membrane processing, which could also influence the reverse micelle formation due to its hydrophobic nature. In the present work, the influence of PL composition and solvent (hexane) medium on the CMC levels in undiluted and hexane-diluted crude vegetable oils was investigated to assess the degumming performance of UF and nonporous membranes and to evaluate the rejection mechanism in oil systems varying in PL contents (below and above CMC).

3.1 CMC of PL in undiluted oil systems

Soy lecithin used in the study contained ~58% of PL and the rest being oil. The absorbance values of TCNQ solubilized in undiluted model system (lecithin in refined SBO) were plotted as a logarithmic function of PL concentration (Fig. 3.1) and the CMC of PL mixed micelles in oil was estimated to be 850 mg/kg (Table 3.1). Employing the same technique, Subramanian *et al.* (2001a) reported the CMC of PC in refined HOSF to be 440 mg/kg. HPLC analysis of lecithin showed that individual PL, namely PC, PI and PE were 13.6%, 8.5% and 13.7% of total PL, respectively. CMC of PL depends on their degree of hydration; higher the hydration rate, the lower is the CMC. The hydration index of different PL, PC, PI, PE and PA, were

reported to be in the magnitude of 100, 44, 16 and 8.5, respectively on an arbitrary scale of 100 (Segers and Sande, 1990). Therefore, the CMC of oil system containing only PC (440 mg/kg) was apparently much lower compared to that of mixed PL system (850 mg/kg). Further, it is obvious from the hydration index that less hydrating PLs could significantly affect the formation of mixed micelles and thereby increase the CMC value of the system.

Table 3.1 CMC values of PC/mixed PL in model and real systems

Description	CMC of PL (mg/kg)	
	TCNQ solubilization	Surface tension
Undiluted systems		
Lecithin + SBO	850	-
CSBO*	1020	1350
CRBO (lot-1)	900	1000
PC + HOSF oil*	440	530
Hexane-diluted systems		
Lecithin + hexane	520	-
Deoiled lecithin + hexane	460	-
PC + hexane	70	-
CRBO + hexane (30:70) (lot-1)	-	260

* Values reported in earlier studies (Subramanian *et al.*, 2001a).

The CMC of mixed PL in the CSBO system was reported to be 1020 mg/kg (Subramanian *et al.* 2001a). However in the analogous model system (soy lecithin in refined SBO) studied, the CMC of mixed PL (850 mg/kg) was lower than the corresponding real system. The PC content in total PL was

23.6% in the model oil system while it was 5.7% in the real CSBO system (Subramanian *et al.* 2001a) which could be the probable reason for the lower CMC of mixed PL in the model oil system.

Rice bran is another rich source of lecithin besides the common commercial source soybean. The CMC of mixed PL in CRBO was lower than CSBO (Table 3.1). As can be seen from the hydration index, the actual composition of the PL in the crude oils would influence the mixed micelle formation. The HPLC analysis showed that PC and PE contents of CRBO to be 750 and 240 mg/kg, respectively. The higher ratio of PC to total PL (PC content-9.7%) in CRBO could have resulted in lower CMC compared to CSBO.

Physical properties of a solution containing surfactant, such as surface tension, electrical conductivity, turbidity and osmotic pressure change abruptly when the CMC is exceeded (McClements, 1999). This is due to the fact that properties of a solution depend upon whether the surfactant molecules are dispersed as monomers or micellar aggregates. The plot between surface tension and concentration of mixed PL in CRBO gave the CMC as 1000 mg/kg exploiting the dependency of the surface tension on surfactant concentration (Fig. 3.2). The CMC value of CRBO obtained by surface tension method was similar to the value obtained by TCNQ solubilization technique (Table 3.1). However, considering the narrow range of surface pressure existing in the oil systems, the TCNQ solubilization technique should be preferred for determining the CMC (Subramanian *et al.*, 2001a).

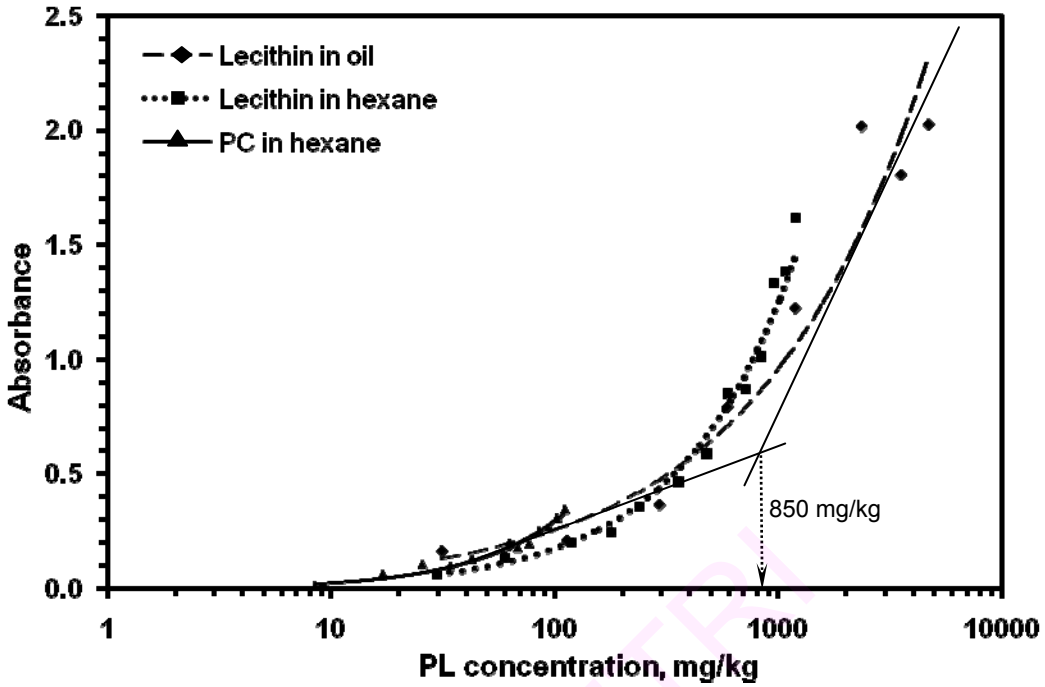


Fig. 3.1 CMC of PC/PL in oil and hexane

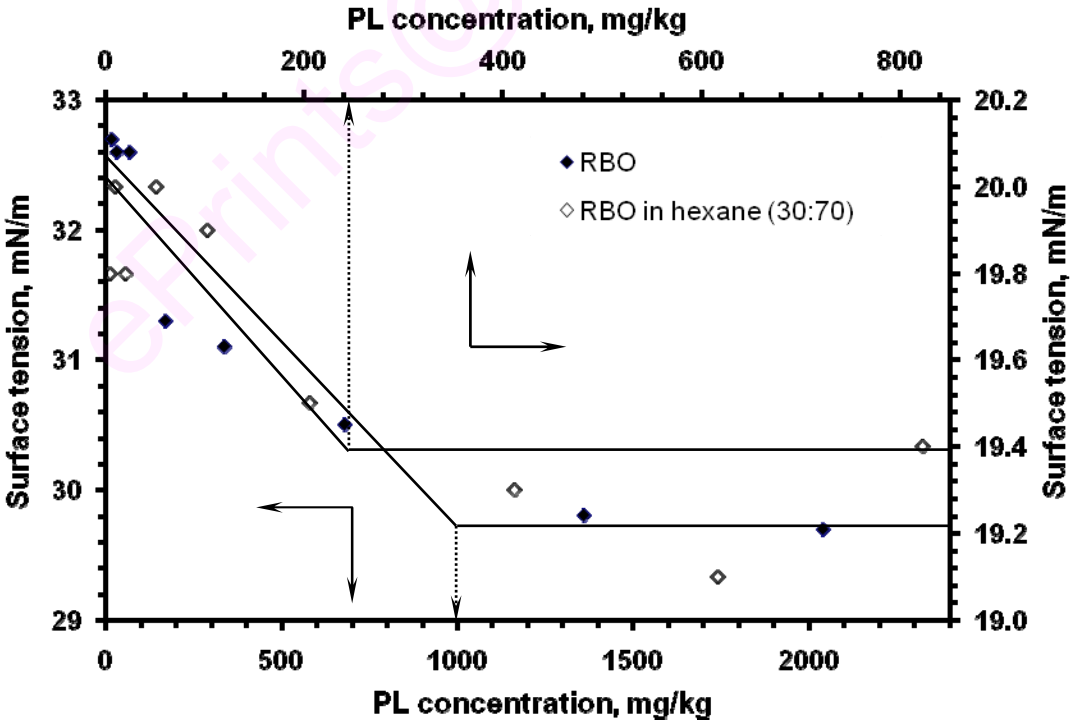


Fig. 3.2 Surface tension of CRBO and CRBO-hexane at various PL concentrations

3.2 CMC of PC/PL in hexane-diluted systems

The CMC of mixed PL in hexane system was 520 mg/kg (~350 mg/l), which was much lower than the CMC of mixed PL in oil system (~1020 mg/kg). One of the major driving forces to form reversed micelles is hydrophobic interactions between amphiphilic molecules and solvent molecules. Hexane is more hydrophobic than the oil constituents (TG). Therefore, hydrophobic repulsion between organic solvents and hydrophilic molecule is stronger in the homogeneous solution. This leads to the formation of reversed micelles at a lower amphiphilic molecule concentration (Ichikawa *et al.*, 2000). Similarly the CMC of PC was much lower in hexane system (70 mg/kg) when compared to the undiluted oil system (440 mg/kg).

When deoiled lecithin was dissolved in hexane and used in the CMC determination of mixed PL (Fig. 3.3), the value was slightly lower (460 mg/kg) than the system containing normal lecithin without deoiling (520 mg/kg). The oil content in the normal lecithin made the solvent system less nonpolar compared to hexane system containing deoiled lecithin, consequently responsible for a slightly higher CMC value.

The CMC of mixed PL in CRBO-hexane miscella (oil:hexane::30:70, w/w) was estimated to be 260 mg/kg by surface tension method (Fig. 3.2), which was much lower than undiluted CRBO system (1000 mg/kg) owing to greater hydrophobic nature of hexane as explained earlier. Although surface tension method is difficult to work when the measurement range is very narrow, it is a quick method to differentiate systems having appreciable difference.

3.3 Effect of moisture content on CMC of mixed PL

The effect of moisture content on the CMC of mixed PL was studied by varying the water content in the hexane system (Fig. 3.4). The CMC value decreased from 520 mg/kg to 430 mg/kg with increase in the moisture content from 100 to 800 mg/kg in the system (Table 3.2). In lecithin-oil-hexane system, Hancer *et al.* (2002) reported a large decrease in CMC value of mixed PL from 330 to 110 mg/kg when the addition of water was increased from 100 to 1800 mg/kg. There was not such a drastic change in CMC values observed in the present study as the systems were different in terms of range of oil and moisture contents studied. Besides, water addition was kept under control so as to avoid total hydration of PL present in the system leading to their precipitation. When the water content is increased the amphiphilic PL molecules becomes more hydrophilic, consequently the hydrophobic repulsive forces between these amphiphilic PL molecules and hydrophobic solvent would be stronger due to which formation of micelles would happen at a lower concentration. This is similar to the phenomenon occurring in aqueous system wherein a small amount of oil lowers the CMC.

3.4 Membrane processing of vegetable oils

The PL content in crude vegetable oil depends on the type of oil source as well as method of oil extraction. Pressing followed by solvent extraction is the method most widely employed for handling a variety of oilseeds. However, low oil bearing materials such as rice bran and soybean are directly solvent-extracted without any pre-pressing step. The PL content of major vegetable oils, namely soybean, rapeseed, sunflower, peanut and rice bran is in the

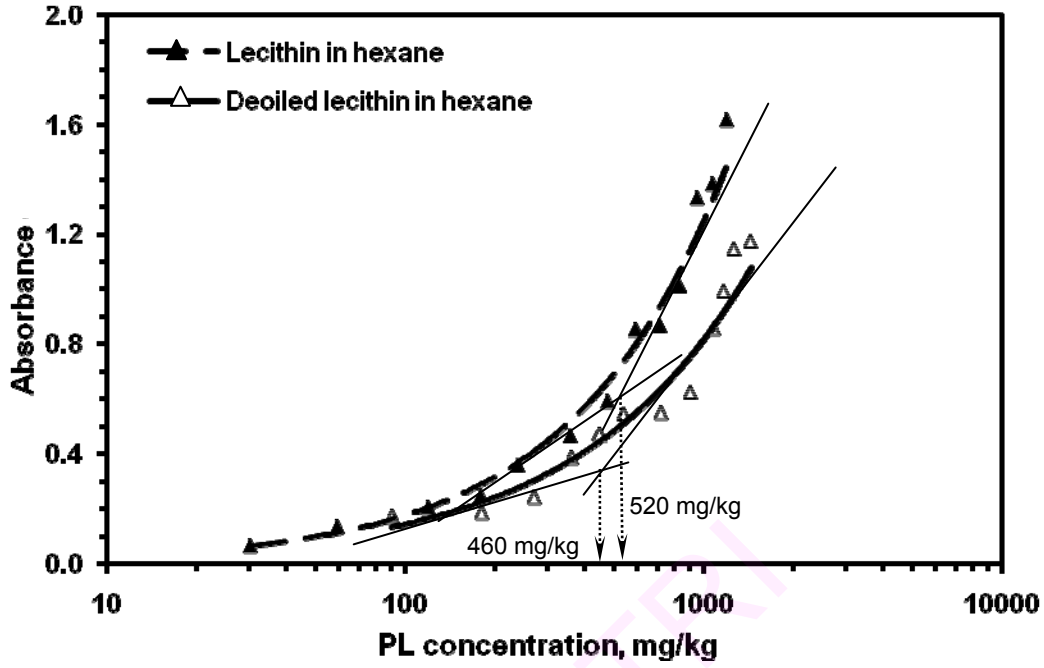


Fig. 3.3 CMC of PL (lecithin and deoiled lecithin) in hexane

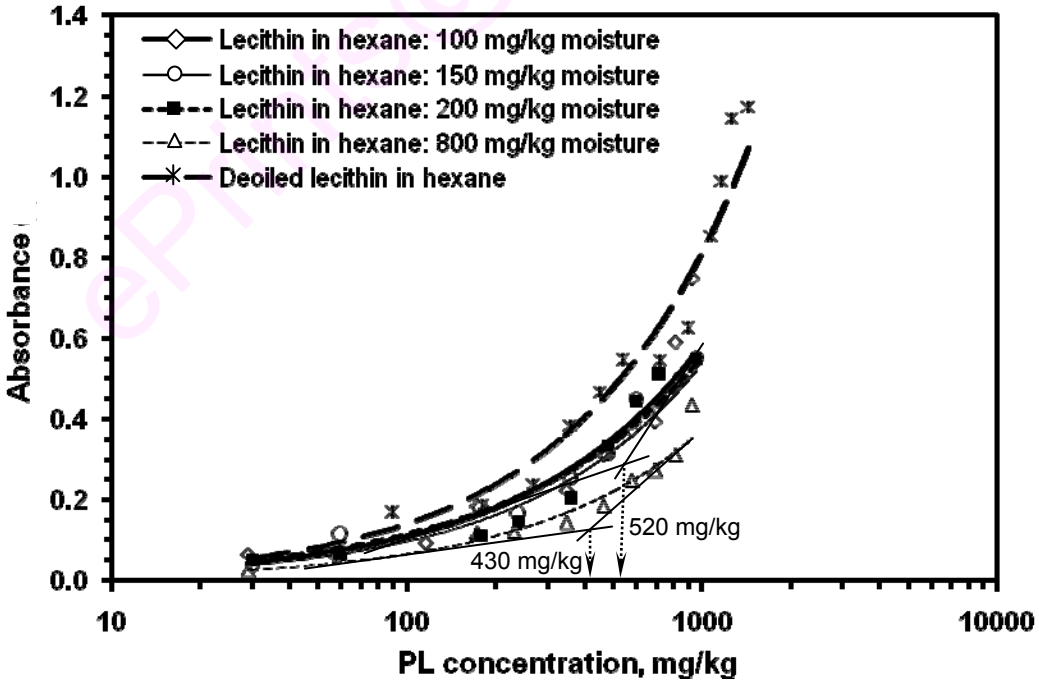


Fig. 3.4 CMC of PL in hexane at various moisture levels

Table 3.2 Effect of moisture content on CMC value of PL (lecithin) in hexane system

Moisture content[a] (mg/kg)	CMC of PL by TCNQ solubilization (mg/kg)
100.4±8.8	520
155.0±11.1	470
203.0±17.4	470
799.6±10.1	430

[a] Values are expressed as mean ± SD of triplicate (n=3) measurements.

range of 1.5-2.1%, 1.0-1.5%, 0.1-1.0%, ~0.35% and 4-5%, respectively (Sipos and Szuhaj, 1996b; Eskin *et al.*, 1996; Davidson *et al.*, 1996; Young, 1996; Orthoefer, 1996). Prepressed oils contain relatively less amounts of PL compared to solvent extracted oils (Subramanian *et al.*, 1998b). These amphiphilic surfactant molecules tend to form reverse micelles in non-aqueous systems such as vegetable oils and hexane-oil miscella. The hydrophilic polar heads are oriented inward in the reverse micelles and interact with other polar compounds. Depending upon the concentration and composition of PL, they may exist as reverse micelles or monomers in the oil/hexane system. As the CMC is the PL concentration at which micelle formation occurs, the PL content of crude oil plays an important role in the degumming performance of the membrane. Hence, while selecting a membrane for degumming the initial PL content of the crude oil has to be considered for achieving the desired result.

3.4.1 Processing model and real oil systems using UF membrane

Model systems containing PL in hexane solution below and above CMC (520 mg/kg) levels were processed using a 20 kDa UF membrane. The performance of the membrane towards PL rejection is shown in Table 3.3. The membrane expectedly showed complete rejection of PL based on size exclusion when the PL concentration was above the CMC. When the concentration of PL (400 mg/kg) in feed was below CMC, they would exist as monomers. Nevertheless, the PL rejection was nearly complete (98.9%) which could be explained as follows. During membrane processing, the concentration of PL at/near the membrane surface would be higher than the bulk concentration, leading to the formation of the micelles owing to the increase in concentration facilitating their rejection. Even when the concentration of PL was very low (100 mg/kg) well below CMC, the membrane did not show larger permeation (very low rejection) of PL and the PL rejection was as high as 81.8% owing to concentration polarization leading to formation of reverse micelle, as explained above. It may also be noted that in the former case (initial PL content in feed 400 mg/kg), the bulk concentration of PL on the feed side actually reached a level greater than CMC during processing.

The rejection performance of real systems behaved similar to the model systems. The UF membrane showed greater rejection while processing hexane-diluted CRBO as the PL content was well above the CMC level. In the case of pressed SFO, the PL content was 1030 mg/kg and its CMC is expected to be higher than the CMC of SBO (1020 mg/kg) based on the composition of individual PL, mainly the PC/PL ratio owing to the fact that

Table 3.3 Performance of 20 kDa UF membrane at various feed concentrations of PL in oil and hexane systems

Description	CMC (mg/kg)	PL content (mg/kg)		PL rejection (%)	Flux [kg/(m^2·h)]	
		Feed	Retentate		Total	Oil
Model systems						
Lecithin + hexane	520	700	1050	~100	0.51	-
Lecithin + hexane	520	400	830	98.6	0.72	-
Lecithin + hexane	520	100	180	81.8	0.62	-
Real systems						
CRBO + hexane (lot-2)	260	7580	24100	99.9	0.20	0.10
CSFO (pressed)	>1020	1030	1190	39.7	0.05	0.05

PC content in SFO is the lowest among various vegetable oils (Sipos and Szuhaj, 1996a). The rejection of PL in this undiluted system was only moderate (39.7%) when compared to the model hexane systems (82-99%) with PL contents below CMC. It may also be noted that the bulk concentration of PL in the retentate/feed was always below CMC during processing SFO. The lower PL rejection in undiluted SFO system could be due to lower oil flux (0.05 kg/m^2·h) compared to the higher permeate flux (0.62-0.72 kg/m^2·h) experienced in hexane systems consequently leading to higher rejection. Concentration polarization depends mainly on the solute content in the feed and also influenced by the permeate (solvent) flux; both these factors had a direct impact on its effect.

3.4.2 Processing undiluted and hexane-diluted crude oil systems using nonporous membrane

The performance of nonporous membranes in rejecting PL in real crude oil systems is shown in Table 3.4. The PL content in the feed was well above CMC in RBO. Literature values of individual PL content showed that PC content is the highest in GNO among SBO, GNO, RBO and SFO (Sipos and Szuhaj, 1996a). Hence, CMC of PL in GNO is expected to be lower than the CMC of RBO (900 mg/kg) based on the composition of individual PL composition, mainly the PC/PL ratio. The nonporous membranes were very effective in rejecting PL whether the PL content in the oils were below or above CMC. Even in hexane-diluted conditions, the PL rejection by the membrane was nearly as effective as that of undiluted systems. The performance was generally in accordance with the earlier observation in our laboratory with nonporous membranes from NTGS series with various crude oils (Subramanian *et al*., 2004; Sarvanan *et al*., 2006; Sarita Arora *et al*., 2006).

The 20 kDa UF membrane exhibited very high rejection in both undiluted and hexane-diluted vegetable oils systems when the PL content is above CMC level. UF was also effective when the PL content in the feed was slightly below CMC. However, complete elimination of PL was not achieved and cannot be guaranteed when the PL content is far lower than CMC (Table 3.3). On the other hand, nonporous membranes exhibited excellent selectivity in rejecting PL under all conditions (Table 3.4). Although not very high oil flux was achieved in the present experiments with UF membrane (owing to its hydrophilic nature) much higher flux values have been reported

Table 3.4 Performance of nonporous membrane (NTGS-2200) with different crude vegetable oils varying in PL content

Description	CMC (mg/kg)	PL content (mg/kg)		PL rejection (%)	Flux [kg/(m^2·h)]	
		Feed	Retentate		Total	Oil
Undiluted systems						
GNO (pressed)*	<900	690	-	100	0.08	0.08
CRBO (solvent-extracted) (lot-3)	900	4325	5900	99.6	0.03	0.03
CRBO (solvent-extracted) (lot-2)	900	14830	19530	99.8	0.03	0.03
Hexane-diluted systems						
CRBO + hexane (lot-3)	260	2162	3352	97.7	0.47	0.24
CRBO + hexane (lot-2)	260	3700	21350	98.8	2.04	0.51

* Values reported in earlier studies (Subramanian *et al.*, 2001a).

employing hydrophobic UF membranes by the earlier workers (Miki *et al*., 1988; Garcia *et al*., 2006). Taking in to consideration the productivity factor, it is desirable to promote UF membranes for degumming with strategies to overcome the deficiency in handling crude oils containing PL content below CMC. From the present studies, it is apparent that this could be achieved by raising the level of PL content in the feed above CMC by mere addition of supplementary PL to the system.

While the above proposal of UF may be suitable for most of the crude vegetable oils, difficult to process oils such as RBO should be dealt with caution. The presence of PGL has been reported to be the root causing problem in processing RBO (Kaimal *et al.*, 2002) and to ensure its complete elimination it may be desirable to use nonporous membranes for degumming which may offer other benefits including partial dewaxing and decolourizing of crude oils.

Higher the PC content greater is the efficiency of water degumming process due to its higher hydration rate and encapsulating ability to other less hydrating PL (Subramanian *et al.*, 1999). On the other hand in the membrane degumming process, lower the ratio of PC/PL, greater might be the CMC of PL mixed micelles and so also its corresponding size enabling greater rejection in a porous membrane working with size exclusion as the predominant rejection phenomenon. Accordingly, less hydrating PL shall be used to increase the level of PL content above CMC in low PL content oils for greater rejection towards complete elimination of PL by the porous membranes.

3.5 Conclusions

The fast hydrating PC had an influence on CMC of mixed PL in oil and hexane-oil systems. The systems containing higher PC to PL ratio gave lower CMC values and vice versa. Hexane as a solvent in the system showed greater influence on reverse micelle formation due to its hydrophobic nature. The CMC of PL is lower in hexane-diluted oil systems compared to undiluted oil systems due to greater hydrophobic forces between the solvent and amphiphilic PL. The initial PL content played a crucial role in the rejection performance of UF membrane. The rejection by UF membrane was almost total when PL content was above CMC, however when it was low, rejection was dependent on concentration polarization effect controlled by solute concentration and flux. On the other hand, nonporous membranes showed almost complete degumming in vegetable oils irrespective of initial PL content and whether the oil system was undiluted or hexane-diluted.

4.0 Significance and focus of the work

Permeate flux is an important performance parameter in a membrane process which has a direct bearing on the process economics. Oil flux is generally low during membrane processing owing to its viscous nature and hexane dilution of feed stock has been practiced to improve the oil flux in UF processes. With a similar process approach, NF membranes rejected TG (Stafie *et al*., 2004) while nonporous membranes did not display any selectivity between TG and hexane (Kondal Reddy *et al*., 2001). In the present work, the influence of hexane dilution and transmembrane pressure on the permeate flux was studied in a nonporous membrane with various vegetable oils (MO, GNO, RBO, SFO and CNO) for achieving greater permeate flux.

4.1 Processing undiluted oils

The flux of CNO (0.2 kg/m^2·h) was nearly three times higher than GNO, RBO and SFO (~0.065 kg/m^2·h) in a nonporous membrane (Table 4.1) while MO gave the lowest flux (0.035 kg/m^2·h). The effect of transmembrane pressure on oil flux is shown in Fig. 4.1. The permeate flux increased with applied pressure with all the oils and a linear relationship seemed to exist between them in the range of pressure studied. In the case of CNO that exhibited high flux, the flux increased and reached a plateau with much decreased influence of pressure thereafter.

Transport of oil constituents through a nonporous membrane is mainly controlled by solution diffusion effect (Subramanian *et al.*, 2004). The direct role of viscosity in the hydraulic flow in porous membranes is well established

and viscosity could influence the permeation through nonporous membranes as well. MO was the most viscous and CNO was the least viscous and they showed the lowest and highest permeate flux, respectively; the flux of GNO, RBO and SFO fell in the order of their viscosity (Table 4.1). The flux of various oils as a function of their viscosity is shown in Fig. 4.2. According to the Wilke and Chang equation, diffusivity is inversely proportional to the viscosity of the dilute solutions of nonelectrolyte solutes (Treybal, 1981). Although this relation is not directly applicable in the present situation where membrane exists in the solid form, the general trend showed that higher the viscosity, lower the permeate flux and vice versa. A better correlation was seen when linearity was tested (R^2 = 0.858) indicating a proportional role of inverse viscosity on flux within the specific range of vegetable oils studied.

Table 4.1 Oil permeate flux of undiluted vegetable oils

Type of oil	Major fatty acids	Viscosity[a] (mPa.s)	Flux [kg/(m^2·h)]
MO	Erusic, Linoleic	70.9±1.7	0.035
GNO	Oleic, Linoleic	58.3±2.6	0.064
RBO	Oleic, Linoleic	58.0±2.3	0.067
SFO	Oleic, Linoleic	57.0±3.9	0.069
CNO	Lauric, Myristic	47.8±4.7	0.190

[a]Values are expressed as mean ± SD of triplicate (n=3) measurements.

Besides viscosity, actual composition of oils also plays a significant role in the transport in terms of their solubility and diffusivity through the nonporous membrane. The decreasing order of relative preferential

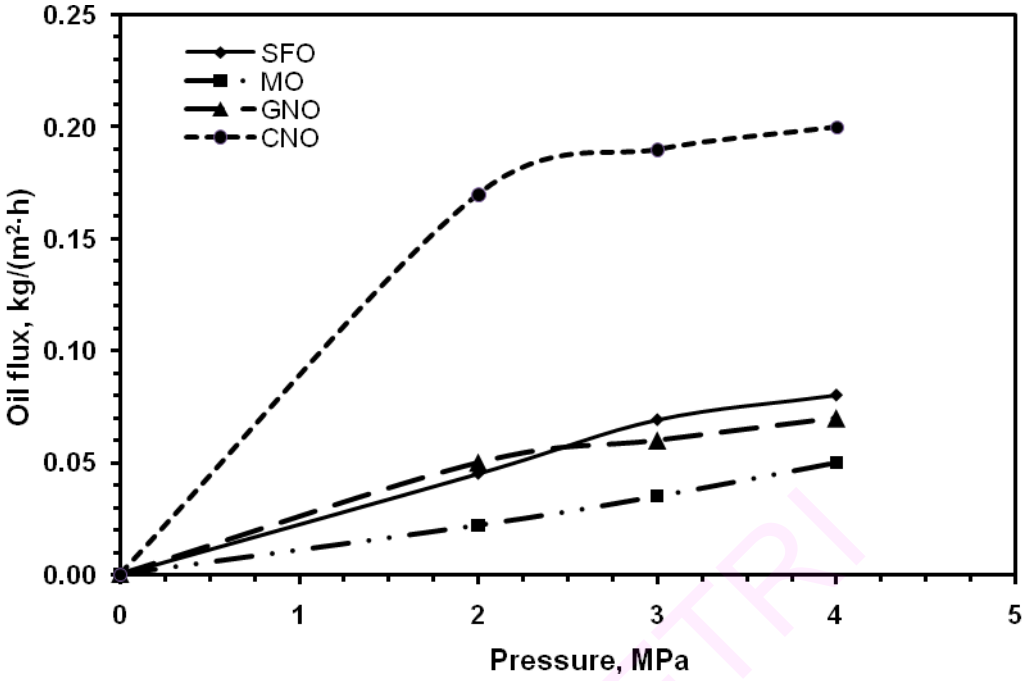


Fig. 4.1 Effect of pressure on oil flux of undiluted vegetable oils

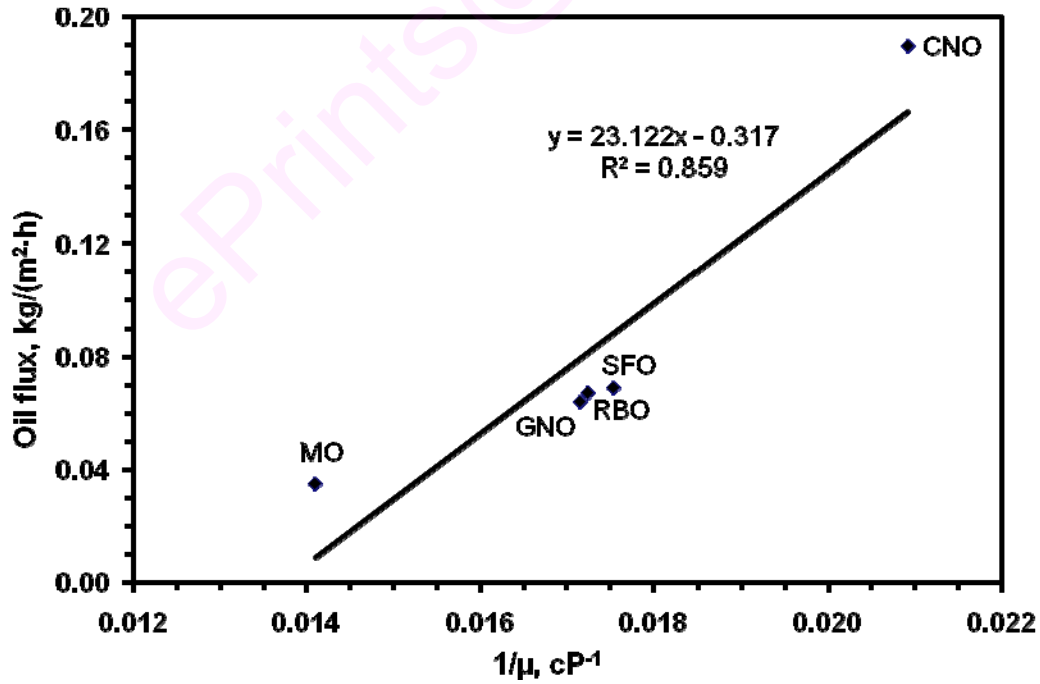


Fig. 4.2 Oil flux as a function of oil viscosity

permeation in the nonporous membranes is expected to be FFA, tocopherols, TG, aldehydes, peroxides, colour pigments and PL (Subramanian *et al.,* 2001c). In totally refined vegetable oils, TG content is above 99% and could be considered only as a mixture of various TG without the impurities. The fatty acid composition of the oils used in the study is presented in Table 4.2. The major fatty acids present in MO, GNO, RBO, SFO and CNO are erusic, oleic, oleic, linoleic and lauric acids, respectively. In vegetable oils, these fatty acids are esterified to the glycerol molecule and present as TG. Based on the relative fatty acid composition, the average carbon chain length of fatty acids and molecular weight of TG were calculated for the individual oils (Table 4.2). The inverse relationship between oil flux and average molecular weight of TG is shown in Fig. 4.3 which indicated that as the average carbon chain length increases the flux decreases and vice versa. Although the molecular size difference among various types of TG present in these oils is only 291 Da, the inverse relation between flux and molecular size gave a very high correlation. Diffusivity is inversely proportional to molecular weight and therefore the small difference in the molecular size among these TG would not be responsible for the 5 fold difference observed in flux values between CNO and MO. This suggests that the difference in permeability must have arisen from the difference in solubility rather than diffusivity. The extent of solubility of FFA in a solvent varies depending on the carbon number; the solubility of saturated C10 and C18 fatty acids in acetone were reported to be 4070 and 15.4 g/l, respectively (www.cyberlipid.org/index). Similarly, it could be expected that the solubility of low molecular weight TG consisting of short chain carbon atoms would be higher in membrane material than TG

Table 4.2 Fatty acid composition and average molecular weight of various vegetable oils

Sample description	Caprylic C8:0 (%)	Capric C10:0 (%)	Lauric C12:0 (%)	Myristic C14:0 (%)	Palmitic C16:0 (%)	Stearic C18:0 (%)	Oleic C18:1 (%)	Linoleic C18:2 (%)	Linolenic C18:3 (%)	Arachidic C20:0 (%)	Erusic C22:1 (%)	Avg. chain length of FA	Avg. MW of TG
MO	-[a]	-	-	-	1.9	1.1	11.4	15.5	12.8	7.0	47.3	19.5	961
GNO	-	-	-	-	10.6	3.0	59.3	24.6		1.4	-	17.6	868
RBO	-	-	-	-	19.7	0.9	41.5	34.5	1.5	0.81	-	17.4	859
SFO	-	-	-	-	7.0	4.4	25.2	62.8			-	17.8	868
CNO	8.4	5.7	46.6	18.8	7.9	2.5	6.9	2.4	-	-	-	12.8	670

[a] Below detectable level.

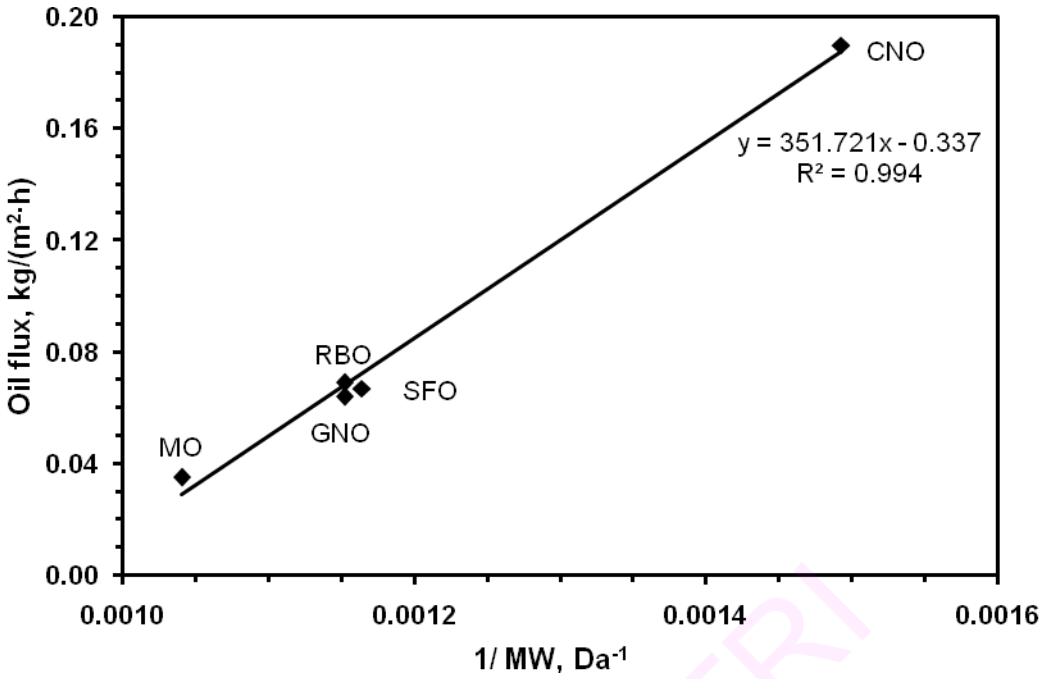


Fig. 4.3 Relation between oil flux and average molecular weight of TG of various vegetable oils

consisting of long chain carbon atoms Although, analysis of role of viscosity and molecular size of TG on flux improved our understanding on the permeability through a nonporous membrane, the oil flux obtained with undiluted oils was very low and subsequent experiments were conducted to improve the oil flux by dilution with hexane.

4.2 Processing hexane-diluted oils

4.2.1 Effect of hexane dilution on flux in SFO

Studies were conducted with SFO as model oil under hexane-diluted conditions at various oil concentrations from 5-90% at 1 and 2 MPa to improve the oil flux and assess the selectivity of the nonporous membrane between TG and hexane. The effect of hexane dilution on total flux, oil flux and selectivity for SFO at various oil concentrations is shown in Fig. 4.4. The

total flux increased with increase in dilution and the increase was higher at higher operating pressure. Correspondingly, there was increase in oil flux but only up to a certain dilution beyond which it actually started decreasing (Fig. 4.4). The maximum oil flux (1.243 kg/(m^2·h)) was achieved at 20% oil concentration and 2 MPa pressure without the membrane showing any selectivity between TG and hexane. The membrane did not show any selectivity over a wide range of hexane dilution indicating that unit positive coupling between TG and hexane remained unaffected except at very high oil concentration (90% oil concentration) where the coupling seemed to be faintly getting affected (selectivity ~1.2). The decline in oil flux beyond certain point suggests that there is a critical limit of hexane dilution beyond which the oil flux does not show an incremental improvement although it results in an increase in total flux. This critical limit of hexane dilution depended on operating pressure; 20% oil concentration at 2 MPa and 15% oil concentration at 1 MPa in the case of SFO. Keeping this critical factor of dilution in view, the process conditions need to be optimized for any individual oil.

The effect of transmembrane pressure on total and oil flux was studied over a wide range of hexane-dilution and the data obtained at 15% and 20% oil concentration are shown in Fig. 4.5. The total flux increased with applied pressure at all dilutions up to a certain limit and there was no appreciable increase in flux with further increase in applied pressure. In the case of 15% oil concentration, the limiting pressure was 2.5 MPa. The oil flux also generally increased with increase in pressure. However, the oil flux was much higher at 10% oil concentration than at 5% concentration in the entire

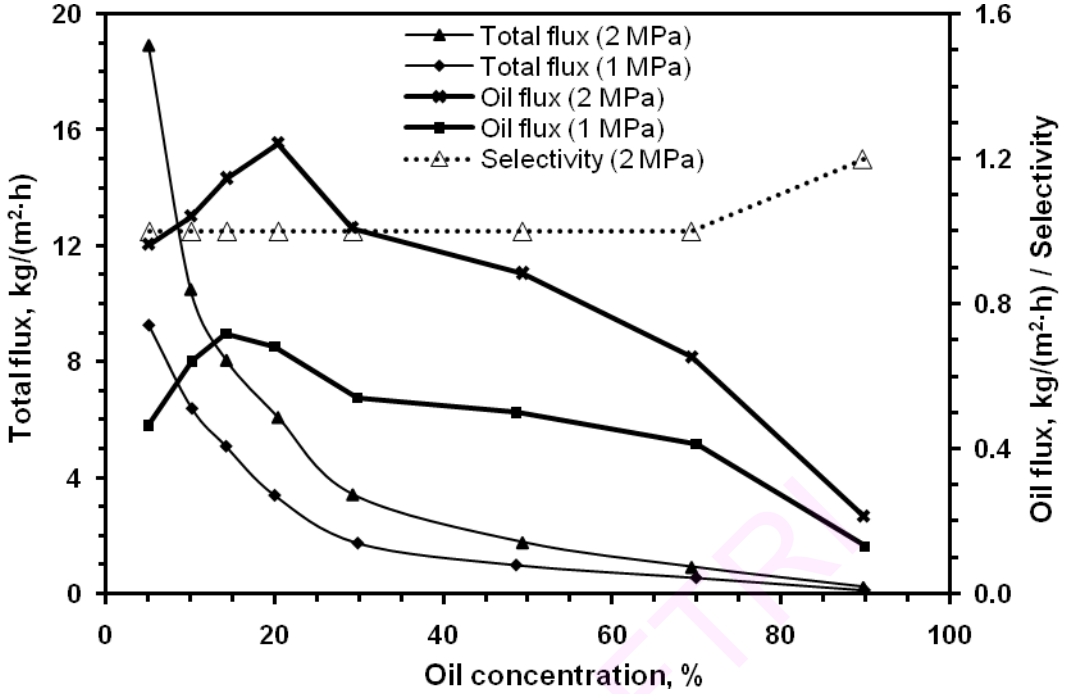


Fig. 4.4 Effect of dilution on selectivity and total and oil flux of SFO at two different pressures

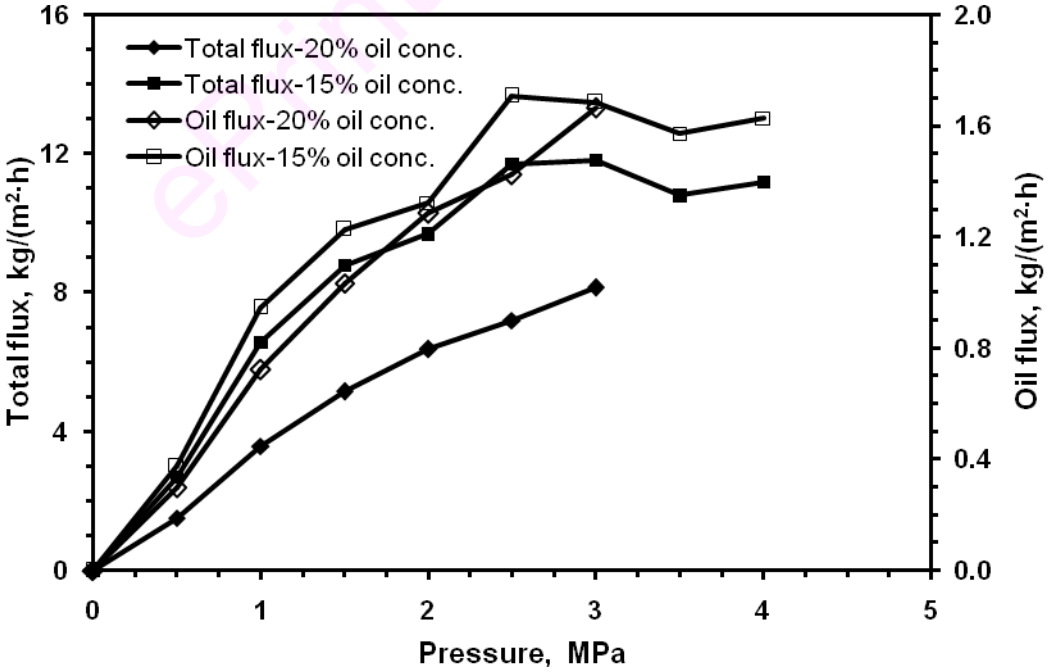


Fig. 4.5 Effect of pressure on total and oil flux of SFO at various dilutions

range of operating pressure suggesting once again the existence of a critical limit of hexane dilution that could affect the improvement in oil flux when exceeded.

4.2.2 Effect of hexane dilution on flux in other oils

Hexane dilution improved the permeate oil flux in all the vegetable oils studied by at least one order of magnitude and applied pressure increased the total flux as well as oil flux. In the case of high viscous MO, increasing the hexane dilution from 50% to 20% oil concentration in miscella increased the oil flux by 19 to 30 fold at 2 MPa and 30 to 40 fold at 3 MPa with respect to undiluted oil flux. Increase in pressure from 1 to 3 MPa resulted in ~1.3 fold increase in oil flux at lower hexane dilution (50% oil concentration) which increased to ~1.8 fold at higher dilution (25% oil concentration). Under similar conditions, the increase in oil flux in GNO, RBO and CNO were 1.8, 1.5 and 1.5 fold at lower dilution and 2.6, 1.9 and 1.8 fold at higher dilution, respectively.

The oil flux obtained with MO, GNO, RBO, SFO and CNO at the highest dilution (20% oil concentration) and pressure (3 MPa) employed were 1.0, 1.4, 1.6, 1.7 and 2.9 kg/(m^2·h), respectively. The flux data obtained at select conditions without exceeding the practical limit of oil concentration handled in industrial miscella are presented in Table 4.3. The effect of hexane dilution on oil flux was more prominent in MO (29 fold increase) while it was less prominent in the case of CNO (15 fold increase). Flux of low viscous oils was relatively high which improved further with hexane dilution. On the other hand, the oil flux of high viscous oils were low which improved many folds with hexane dilution but could not reach the flux values obtained

with low viscous oils, indicating the basic composition of individual oils playing a major role.

Table 4.3 Improved permeate oil flux of various vegetable oils under hexane-diluted conditions

	Undiluted oils	Oil flux of hexane-diluted oils [kg/(m^2·h)]			
	Flux	25% miscella		33% miscella	
Type of oil	[kg/(m^2·h)]	2 MPa	3 MPa	2 MPa	3 MPa
MO	0.035	0.89	1.03	0.86	0.94
GNO	0.064	1.12	1.51	1.12	1.42
RBO	0.067	0.98	1.22	0.90	1.02
SFO	0.069	1.41	1.55	1.33	1.35
CNO	0.190	2.40	2.80	2.40	2.60

The influence of feed viscosity on total flux of MO, GNO, RBO, SFO and CNO at various dilutions is shown in Fig. 4.6. All these oils exhibited an inverse relationship between viscosity and total flux with undiluted and various levels of hexane-diluted conditions. A higher correlation obtained with each one of these oils (0.890 to 0.988) considering both undiluted as well as hexane-diluted conditions, revealed the role of viscosity of oil systems during membrane processing.

The relation between oil flux and average molecular weight of TG in hexane-diluted oils is shown in Fig. 4.7. As in the case of undiluted oil system, the oil flux of hexane-diluted oils at various dilutions followed an inverse relationship with the average molecular weights of TG. It is interesting to note that the molecular weight of TG plays a prominent role in

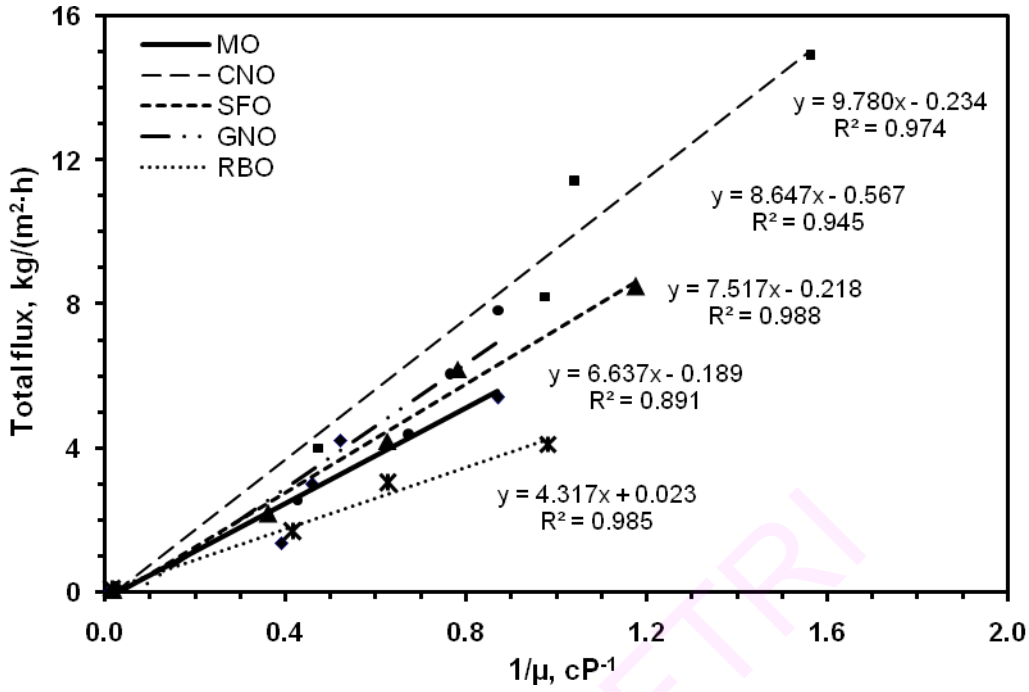


Fig. 4.6 Influence of feed viscosity on total flux of undiluted and hexane-diluted oils

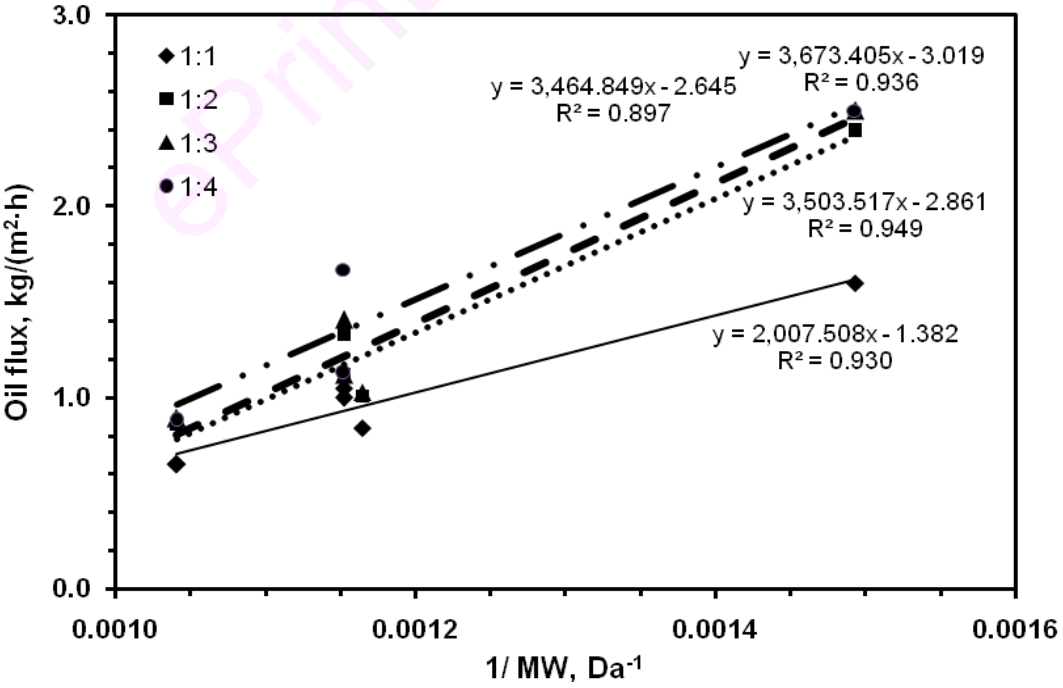


Fig. 4.7 Relation between oil flux and average molecular weight of TG under hexane-diluted conditions

their permeation even under hexane-diluted conditions. This distinctive feature played by carbon chain length of fatty acids could probably be well exploited in characterizing the nonporous membranes similar to the use of salts and sugars in characterizing RO membranes.

4.2.3 Selectivity between TG and hexane in nonporous and NF membranes

The nonporous membrane used in the study (NTGS-2200) did not show any selectivity over a wide range of hexane dilution (5-80% oil concentration) and operating pressure (0.5-4 MPa) indicating that unit positive coupling between TG and hexane remained unaffected (Fig. 4.4) except at very high oil concentration (90% oil concentration) where the coupling seemed to be faintly getting affected (selectivity ~1.2). A similar observation of unit selectivity was made with another nonporous membrane (NTGS-2100) from the same series with hexane-oil system (Kondal Reddy *et al.*, 1996). In this liquid mixture system permeability of oil and hexane are interdependent and oil flux was due to a positive flow coupling with hexane.

There are a few studies reported on hexane-oil systems employing NF membranes. Stamatialis's group conducted studies with hexane-oil miscella using a laboratory cast NF membrane with PDMS as active layer. The system behaved like a true solute solvent system with the NF membrane; increase in pressure resulted in higher hexane flux accompanied with increased TG retention while high oil concentration decreased hexane transport resulting in lower retention of TG (Stafie *et al.*, 2004). The membrane exhibited a TG rejection of ~83-93% in the range of oil concentration (8 and 19%) and pressure (0.5-3 MPa) studied (Stamatialis *et*

al., 2006). In another study on deacidifying vegetable oils, a commercial PDMS NF membrane (MPF-50) showed a TG rejection of >90% while processing a mixture of model oil (SBO and FFA) and hexane (Raman *et al.*, 1996a). Zwijnenberg *et al.* (1999) reported 81-90% and 92-98% TG rejection using PEBAX [poly(amide-b-ether)copolymer)] and cellulose membranes, respectively over a oil concentration range of 9-11% while deacidifying vegetable oil in acetone medium.

Molecular weight of hexane is lower (86.18 Da) and its size is reported to be 0.75 nm (Wu and Lee, 1999). Nonporous membrane used in the study is a commercial membrane (NTGS-2200) with an active layer thickness of 3 μm while the thickness of PDMS active layer in the laboratory cast NF membrane was 1 μm (Stafie *et al.*, 2004). However, the normalized hexane flux of nonporous membrane (0.7 LMH) was greater than the flux reported for NF membrane (0.4 LMH) which could be due to greater porosity in commercial membranes. Earlier studies from this laboratory on direct deacidification showed that these nonporous membranes displayed a selectivity of ~2 between TG and FFA which was completely lost with the addition of hexane in the system (Bhosle *et al.*, 2005). The selectivity between FFA and TG in undiluted conditions indicated that the membrane used in the present study is a denser membrane, probably with smaller micropores in the polymer network compared to NF membranes.

Membrane-solvent interactions can be expected to vary with changes in solvent properties such as dielectric constant, molecular size, dipole moment and solubility parameter (Machado *et al.*, 1999). The solubility parameters of PDMS, hexane and oil (TG) are 15.5, 14.9 and 16.0 $(J/cm^3)^{1/2}$,

respectively (Stafie *et al.*, 2004). Interaction parameter gives a qualitative estimation of the interactions between the polymer and penetrant taking in to consideration of both entropy and enthalpy contributions. The difference in solubility parameters determine the enthalpy contribution which was small (<1) between hexane and PDMS, and oil and PDMS. Stafie *et al.* (2004) reported that the interaction parameter for PDMS/oil system (2.11) is higher than PDMS/hexane system (0.56) and cited this difference as the reason for high sorption of hexane than oil in PDMS. Again the small interaction between oil and PDMS was considered as the reason for the rejection of TG. In contrast the nonporous membrane from the same membrane material did not show any rejection in hexane-oil system which could be due to unit positive coupling (solvent induced solute dragging) between hexane and oil.

Considering these two contrasting permeation behavior of nonporous and NF membranes with similar system (Hexane-TG) and membrane polymer (PDMS), we tend to think that the membrane structure could play a vital role in deciding the transport phenomenon, typically the swelling nature and subsequent pore size formed. Smaller the pore size in the swelled polymer network (nonporous membrane), greater is the possibility for solute dragging as well as positive coupling. On the other hand, pore size in the swelled membrane could be large enough (NF) for the solvent but smaller for the solute, leading to the rejection of TG (1.5 nm) while allowing the permeation of hexane (0.75 nm). However, further in-depth investigation using membranes with varying pore size and thickness is required to understand the actual differences in the transport phenomenon.

4.3 Conclusions

Hexane dilution improved the permeate oil flux in all the vegetable oils by at least one order of magnitude and applied pressure increased the total flux as well as oil flux. The effect of hexane dilution on oil flux was more pronounced in viscous oils. All the oils exhibited an inverse relationship between viscosity and total flux under undiluted and various levels of hexane-diluted conditions. The oil flux of undiluted as well as hexane-diluted oils interestingly followed an inverse relationship with the average molecular weights of TG, despite their narrow range (670-961 Da) of existence. The nonporous membrane did not show any selectivity over a wide range of hexane dilution (5-80% oil concentration) and operating pressure (0.5-4 MPa) indicating unit positive coupling between TG and hexane, endorsing hexane dilution as an effective approach for enhancing oil flux.

5.0 Significance and focus of the work

Nonporous membranes offer several advantages over UF membranes for processing hexane-oil miscella, in terms of higher rejection of PL, carotenoids and chlorophyll (Subramanian *et al*., 2004). The membrane process will become economically attractive if it is even partially effective for colour removal in addition to PL reduction, considering the problems associated with the use of clay in the conventional bleaching process. The unusually high content of waxes, FFA, unsaponifiable constituents, PL and glycolipids as well as the dark colour in CRBO makes the refining process difficult. The presence of PGL was reported to be the root causing problem in RBO processing (Kaimal *et al.,* 2002). Improvements in the refining process could increase the use of RBO for direct edible purposes. In the present investigation, efficacy of a nonporous membrane was assessed for simultaneous degumming, dewaxing and decolourizing CRBO.

5.1 Characteristics of CRBO

The average PL content of CRBO is in the range of 4-5% and wax content varies from less than 1% to more than 4%, depending on the origin of the rice bran and method of extraction (Orthoefer, 1996). The PL content of the CRBO samples was only between 0.4% and 1.5% (Table 5.1). The lower values could be attributed to settling down of hydrated PL during bulk storage in industrial samples (lots 1 and 2) and mild extraction conditions employed in laboratory samples (lots 3 and 4). In the CRBO used in the study, the wax content of lot-3 and lot-4 measured as AI was only 1.02% and 0.66%, respectively (Table 5.1).

Table 5.1 Characteristics of different experimental lots of CRBO

Sample description	PL content[a] (mg/kg)	Wax content[a] (%)	Lovibond colour			Area under spectra
			R	*Y*	(5*R* + *Y*)	
Lot-1[b]	4460±156	-[c]	5.0	20.0	45.0	12.6
Lot-2[b]	14820±219	-	7.0	30.0	65.2	14.6
Lot-3[d]	7720±110	1.02±0.17	5.1	21.0	46.5	14.8
Lot-4[e]	5290±138	0.66±0.04	7.4	4.5	41.5	22.3

[a] Values are expressed as mean ± SD of duplicate (n=2) measurements.

[b] CRBO-industry samples.

[c] Not measured.

[d] CRBO-laboratory extracted sample from parboiled rice bran.

[e] CRBO-laboratory extracted sample from raw rice bran.

Carotenoids and chlorophyll are the two common colour pigments present in most of the vegetable oils. Besides these compounds, Maillard browning products are also present in RBO. The colour of CRBO is dark greenish brown to light yellow, depending on the condition of the bran, extraction method and composition of bran (Orthoefer, 1996). Lovibond method of colour measurement is the common method practiced in the oil industries. The Lovibond colour values (5*R* + *Y*) of CRBO varied between 41.5 and 65.2 (Table 5.1). The absorption spectra between 350 and 750 nm for four different lots of CRBO used in the study is shown in Fig. 5.1. Parboiled-CRBO is generally darker in colour than oil from raw rice bran (Orthoefer, 1996). Lovibond colour value of CRBO obtained from parboiled rice bran used in the study was higher than the raw-CRBO. But the spectrum of raw-CRBO (lot-4) was more intense than the parboiled-CRBO (lot-3). The characteristic peak (at ~454 nm) of carotenoids was not found in the spectra of CRBO, which was not a surprise considering the nature of oil-bearing

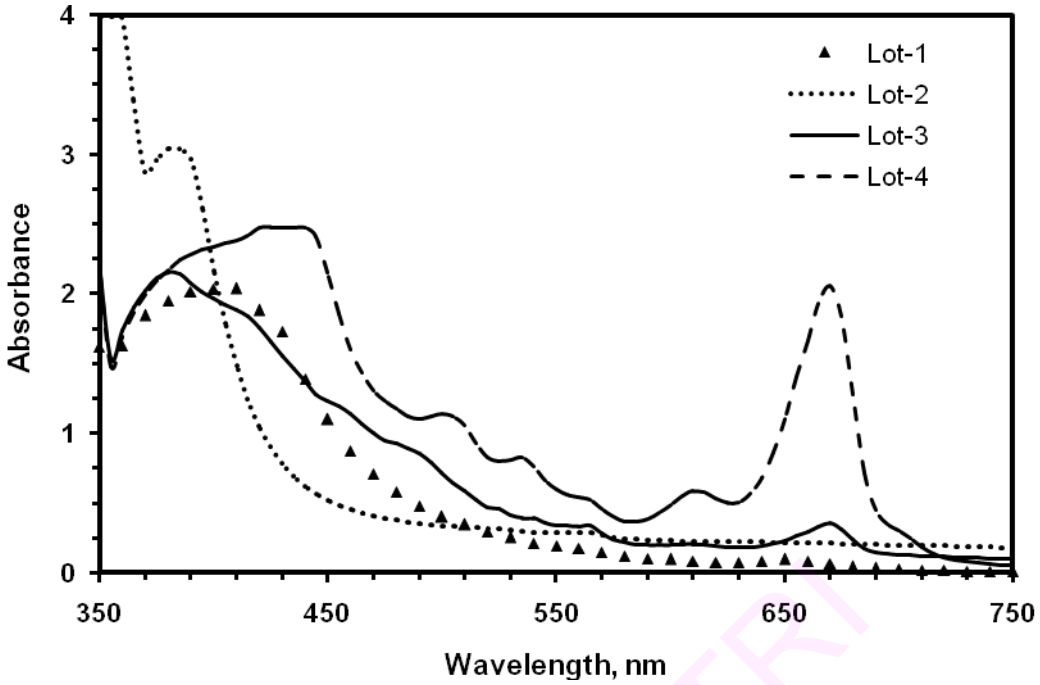


Fig. 5.1 Visible spectra of different experimental lots of CRBO

material. The chlorophyll pigment (absorbance maximum ~640-660 nm) was present to a greater extent in the raw-CRBO (lot-4) and only to a lesser extent in parboiled-CRBO (lot-3) that could be due to the hydrothermal treatment the paddy had undergone during parboiling. However, the characteristic peak of chlorophyll completely vanished in industrial samples owing to greater exposure to light during storage.

5.2 Degumming

The phosphorus content of membrane-processed RBO and rejection of PL by the nonporous membranes are presented in Table 5.2. Both NTGS-2100 and NTGS-2200 membranes showed very high reduction (above 99%) of PL content in undiluted oils. The hydrophobic nonporous membranes were effective in reducing PL, colour compounds and oxidation products while retaining beneficial compounds in undiluted oils systems (Subramanian *et al.*,

2004). However, the oil flux was only 0.03 kg/m^2·h which is very low for industrial adoption. Therefore attempts were made using nonporous membranes for processing oils under hexane-diluted conditions.

Table 5.2 Phosphorus content of membrane-processed CRBO

	Phosphorus content[a] (mg/kg)		R_o	Flux [kg/(m^2·h)]	
Hexane dilution	Feed	Permeate	(%)	Total	Oil
NTGS-2200 membrane; crude lot-1					
Undiluted	143.9±5.0	0.9±0.1	99.6	0.03	0.03
1:1	143.9±5.0	1.4±0.6	97.7	0.47	0.24
1:2	141.5±6.4	7.0±0.7	96.2	0.86	0.29
1:3	137.0±4.2	8.0±2.1	95.1	1.38	0.35
NTGS-2200 membrane; crude lot-2					
Undiluted	478.5±6.4	1.1±0.7	99.8	0.03	0.03
1:3	478.5±6.4	11.5±0.7	98.8	2.04	0.51
NTGS-2100 membrane; crude lot-1					
Undiluted	142.5±6.4	2.0±0.2	99.1	0.03	0.03

[a] Values are expressed as mean ± SD of duplicate (n=2) measurements.

Rejection of PL by the nonporous membrane was not significantly affected by hexane dilution. The percentage rejection of PL varied from 95.1% to 98.8%, which indicated that the membrane possess a very high selectivity for PL even under hexane-diluted conditions. The oil flux increased by nearly 17 fold at 1:3 dilution as compared with oil flux obtained with undiluted RBO (Table 5.2). The PL rejection remained almost unaffected (97.7-98.8%) in lot-2 at various levels of dilution, while a marginal decrease in PL rejection from 97.7% to 95.1% was observed in lot-1 with increase in hexane dilution. This could be attributed to the composition of individual PL in

the oil. PC has the highest hydration rate among the various PL followed by PI, PE and PA besides having the ability to encapsulate other PL (Subramanian *et al.*, 2001a) forming reverse micelles in nonaqueous systems. When the concentration of hydratable PL is low, most of the less hydrating PL including salts of PE and PA could exist as monomers facilitating their permeation through the membrane, especially so in the presence of hexane. In the case of nonporous membranes, separation/permeation of components depends on their own solubility and diffusivity in the membrane material. In addition, it also depends on the coupling effect as well as solubility of individual components in other permeating components including the solvent being used (Bhosle *et al.*, 2005). The greater solubility of PL in hexane could be the probable reason for their reduced rejection by the membrane in hexane-diluted system, especially when they exist as monomers.

Rejection performance of the nonporous membrane observed in the present study was in agreement with the performance observed earlier with various vegetable oils (Subramanian *et al*., 2004, Sarvanan *et al*., 2006; Sarita Arora *et al*., 2006). The phosphorus content in the permeates of the membrane-processed oils was generally ~10 mg/kg (Table 5.2), which adequately meets the prerequisite for physical refining. The near complete removal of PL in the oil indicates that both hydratable as well as NHP were effectively rejected by the membrane. This also suggests that the PGL were probably also removed during the membrane process. However, it is desirable to ensure that these phosphorus-containing glycolipids which pose difficulties during processing RBO were actually eliminated by the membrane.

5.2.1 Elimination of PGL

RBO contains relatively high amounts (~6%) of glycolipids which includes PGL that are responsible for inefficient PL removal during conventional degumming processes. Besides, high surface activity of these compounds lead to high losses in alkali refining process (Kaimal *et al.*, 2002). The probable structure of phosphorus-containing glycolipids was elucidated as 1,2-diacyl-3-*O*-phosphate-*O*-(6-*O*-acyl-α-D-galacto-pyranosyl)-*sn*-glycerol by the above researchers (Vali *et al.*, 2004). Identification of these compounds was confirmed using NMR spectroscopy in our laboratory.

5.2.2 NMR spectroscopic analysis

2DHSQCT NMR analyses of the feed, permeate and retentate were carried out to identify the membrane rejected components unequivocally. NMR data of the feed, retentate and permeate are shown in Figs. 5.2, 5.3A and 5.3B, and 5.4, respectively and in Table 5.3. Various species of the PGL are found to be present in the feed namely 1,2-diacyl-3-*O*-phospho-*O*-(6-*O*-acyl α/β-D-galactopyranosyl)-*sn*-glycerol, 1,2-diacyl-3-*O*-phospho-*O*-*sn*-glycerol (PA) and 6-*O*-acyl-α/β-D-galactopyranoside (Scheme 5.1).

The presence of multiple carbon signals between 171 and 173 ppm and olefinic CH=CH signals clearly indicated the presence of long chain acyl groups of oleic and linoleic acids nature. The signals present in different intensities indicate that such components are of different proportions. Based on the chemical shift values of the carbohydrate signals in the 60-106 ppm range, the nature of the carbohydrate molecule was deduced to be the D-galactose. C1-α signal of D-galactose at 101.2 ppm and that of C1-β at 106

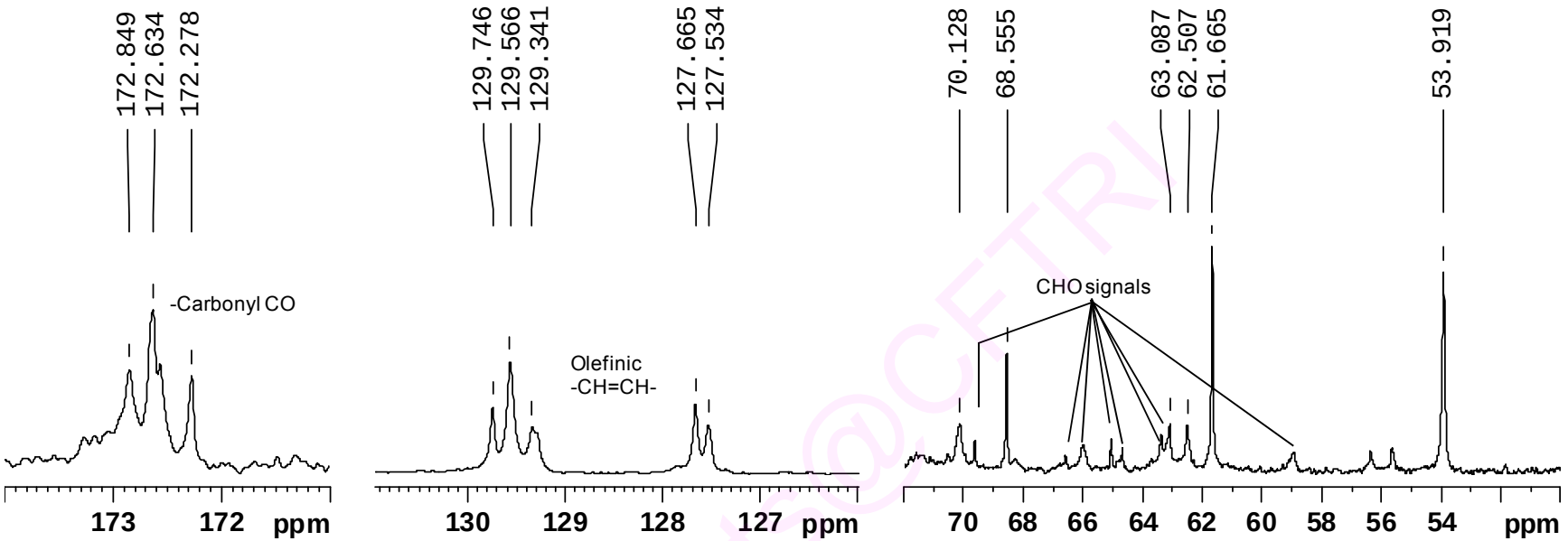


Fig. 5.2 ^{13}C NMR spectrum of the CRBO feed. ~40 mg dissolved in $CDCl_3$-DMSO-d_6 mixture was employed for recording the spectrum at 125.8 Hz. Other conditions are mentioned in the text. CHO - Carbohydrate signals.

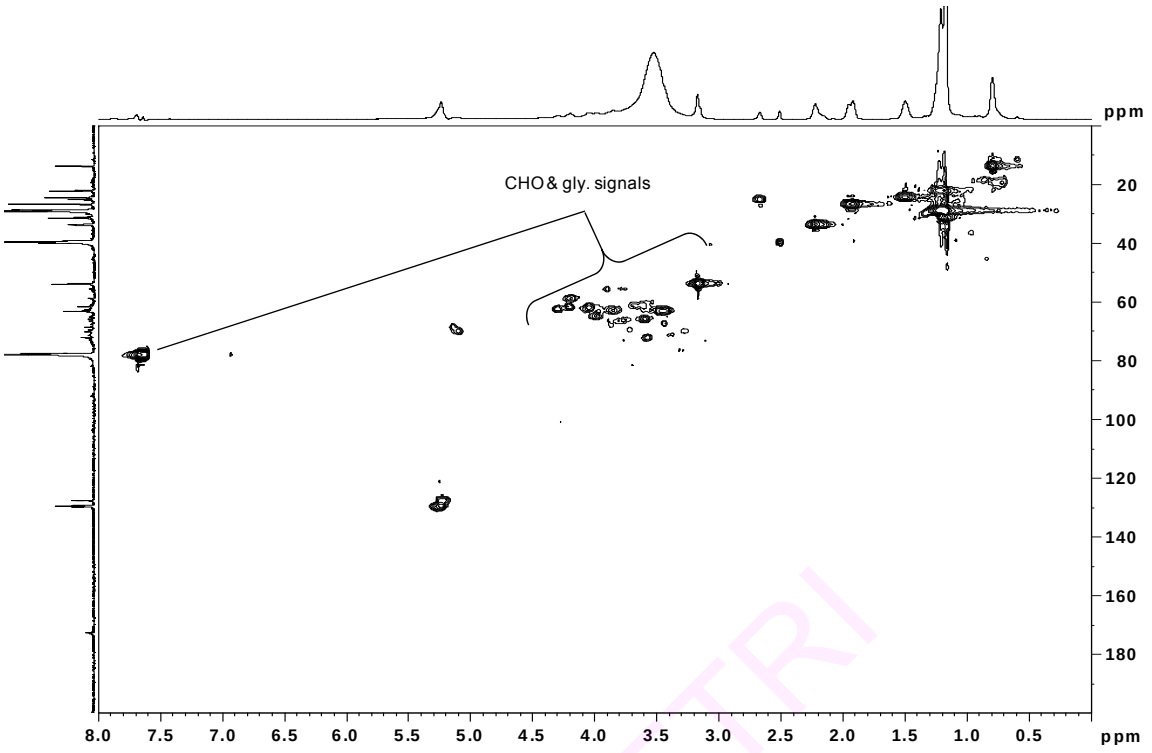


Fig. 5.3A 2DHSQCT spectrum of the retentate. Gly.-Glyceryl signals.

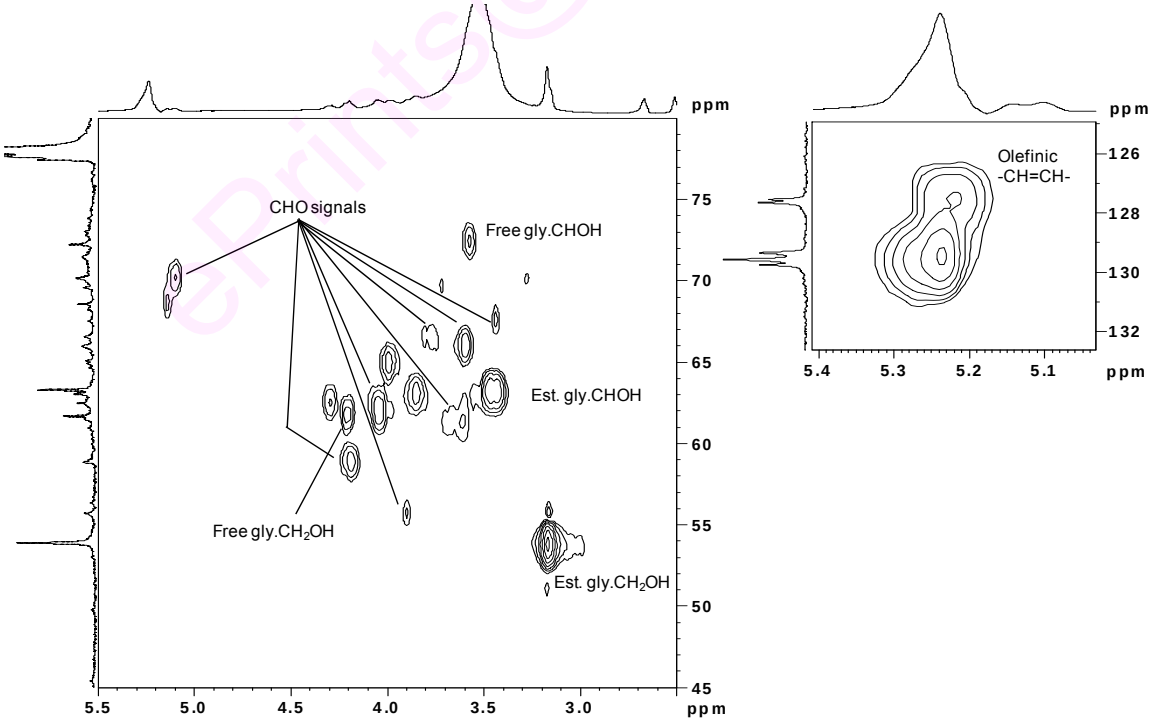


Fig. 5.3B Expanded region for 3.7-5.5 ppm of corresponding to the CHO–carbohydrate signals of the spectrum. Est.-Esterified glycerol.

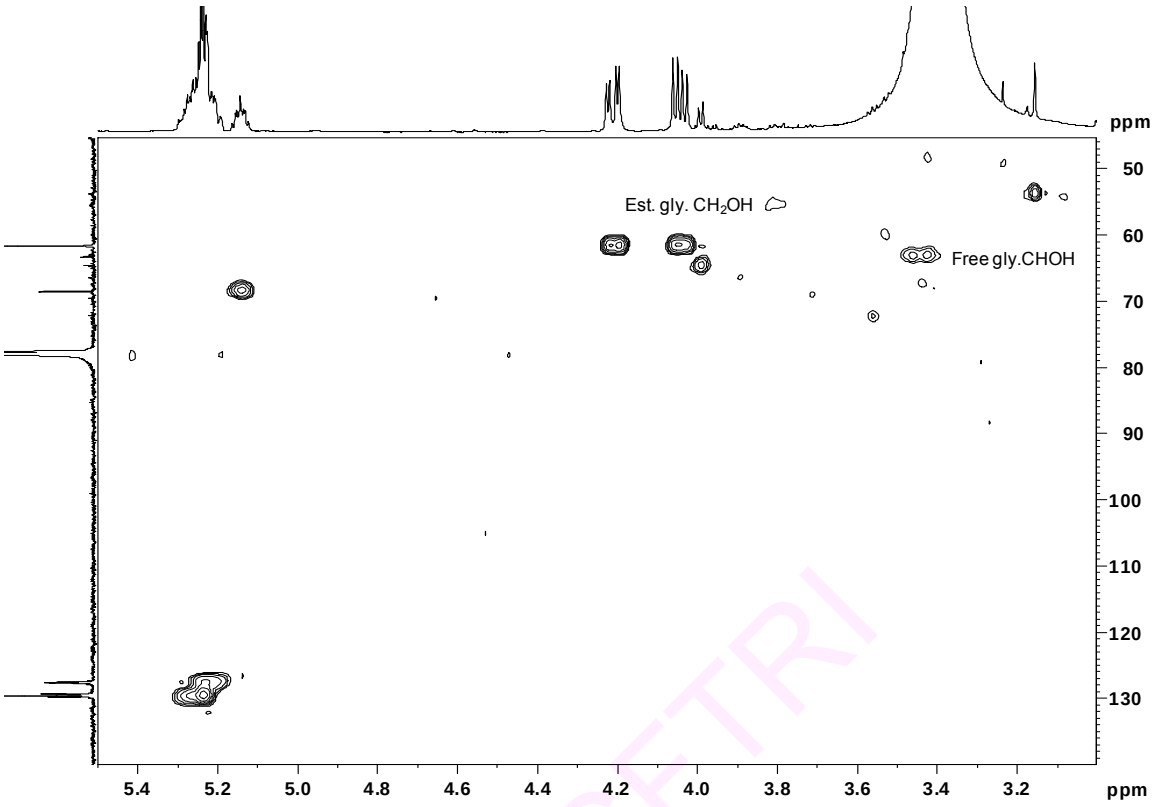


Fig. 5.4 Esterified glycerol portion of the spectrum for 3.1-5.5 ppm of the permeate

ppm also showed that both α and β anomers of D-galactose were present. The olefinic carbon signals between 127.6-129.6 ppm also pointed to the olefinic nature of the acyl groups from oleic and linoleic acids. The glyceryl –CH–O– and $–CH_2–O$ signals were quite prominent between 45.4 and 53.6 ppm corresponding to the esterified glyceryl alcoholic groups. However, free glyceryl carbon signals between 61.7 and 68.7 ppm with significant intensity showed that hydrolyzed products of glycolipids also were present. While the feed and retentate fractions indicated the presence of the above mentioned compounds, the permeate appeared to be significantly devoid of them. Less intense signals in the permeate indicated that the PGL in RBO were retained by the membrane to a larger extent.

Table 5.3 NMR data of the PGL of RBO[a]

Groups	Chemical shifts	
	$^{1}H_{(\delta ppm)}$	$^{13}C_{(\delta ppm)}$
-CO 1	-	172.4
-CO 1'	-	172.7
-CO 1''	-	171.8
$-CH_2-$ 2, 2', 2''	-	-
$-CH_2-$ 3, 3', 3''	1.50	24.0
$-CH_2-$ 4, 4', 4''	-	27.9
$-CH_2-$ 5, 5', 5''	-	28.7
$-CH_2-$ 6, 6', 6''	1.20	-
$-CH_2-$ 7, 7', 7''	1.38	26.8
$-CH_2-$ 8, 8', 8''	2.00	33.6
-CH= 9, 9', 9''	5.22	129.6
-CH= 10, 10', 10''	5.25	127.8
$-CH_2-$ 11, 11', 11''	1.93	33.9
-HC= 12, 12', 12''	5.21	127.6
-HC=13, 13', 13''	5.30	129.5
$-CH_2-$ 14, 14', 14''	1.33	33.3
$-CH_2-$ 15, 15', 15''	1.36	31.1
$-CH_2-$ 16, 16', 16''	1.32	34.6
$-CH_2-$ 17, 17', 17''	1.19	22.2
$-CH_3-$ 18, 18', 18''	0.82	13.9
$-CH_2$-O 1'''	4.10	49.8
-CH-O 2'''	5.14	53.6
$-CH_2$-O 3'''	4.24	45.4
$-CH_2$-O 1''' Free	4.02	61.7

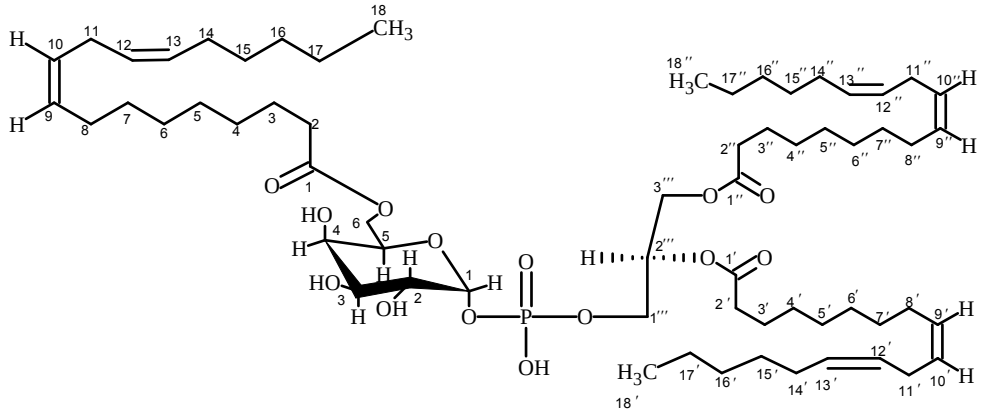
Continued in the next page

Groups	Chemical shifts	
	$^{1}H_{(\delta ppm)}$	$^{13}C_{(\delta ppm)}$
-CH_2-O 3''' Free	4.21	61.7
-CH-O 2''' Free	-	68.7
C1-α	4.15	101.2
C2-α	3.38	69.1
C3-α	3.39	69.5
C4-α	3.4	70.2
C5-α	3.37	70.3
C6-α	3.36	62.5
C1-β	4.52	106.0
C2-β	-	71.8
C3-β	3.15	-
C4-β	3.12	72.4
C5-β	-	-
C6-β	3.34	62.9

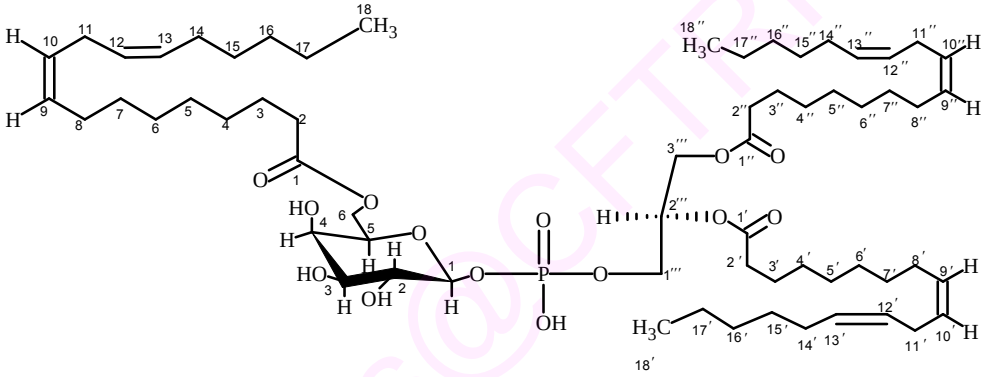
[a] Some of the assignments are interchangeable. Errors in chemical shifts: ± 0.01 for protons, ± 0.1 for carbon.

The ^{1}H NMR data was found to be complementary to the ^{13}C data in identifying the above mentioned compounds. The acyl group signals between 0.82 and 3.0 ppm comprising –CH_3 and –CH_2 signals and olefinic –CH=HC– signals between 5.21 and 5.3 ppm were confirmative of long chain oleic and linoleic acids constituting lipid moieties of the molecules. Free and esterified glyceryl proton signals were observed between 4.02 and 4.21 and 4.1 and 5.14 ppm, respectively.

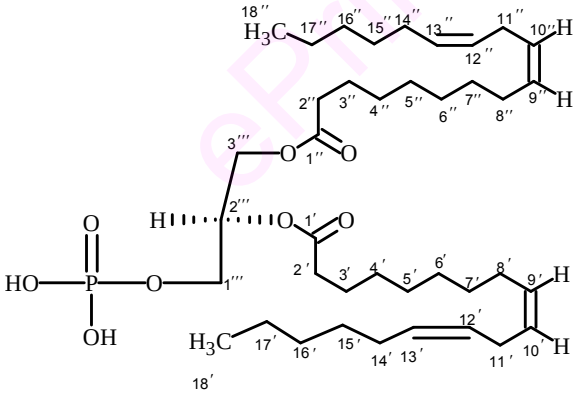
The ^{31}P NMR data clearly showed the absence of any phosphate signal in the permeate, which once again confirmed the absence of phosphorus compounds in the permeate. Here, also the 1,2-diacyl-3-phospho-*O*-*sn*-glycerol and lyso-acyl PA signals were observed between 3.5and 4.05 ppm (with respect to 85% phosphoric acid), along with 1,2-diacyl-



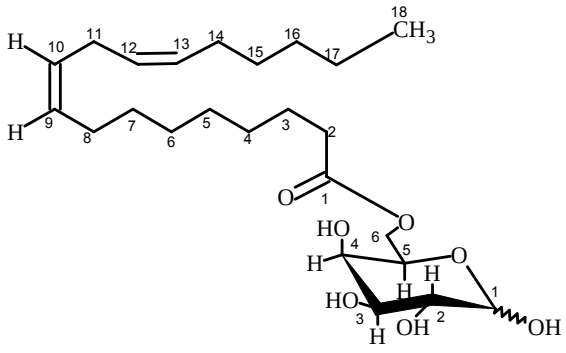
1,2-Diacyl-3-*O*-phospho-*O*-(6-*O*-acyl-α-D-galactopyranosyl)-*sn*-glycerol



1,2-Diacyl-3-*O*-phospho-*O*-(6-*O*-acyl-β-D-galactopyranosyl)-*sn*-glycerol



1,2-Diacyl-3-*O*-phospho-*sn*-glycerol



6-*O*-Acyl-D-galactopyranoside

Scheme 5.1 PGL components detected by ^{1}H, ^{13}C and ^{31}P NMR spectroscopic technique

3-*O*-phospho-*O*-(6-*O*-acyl-α/β-D-galactopyranosyl)-*sn*-glycerol and 1,2-diacyl-3-*O*-phospho-*O*-α/β-galactopyranoside signals between 5.12 and 5.7 ppm in both the feed and retentate, indicating that these compounds were retained by the nonporous membrane. A large number of less intense carbohydrate signals in the regions 3.36-4.15 ppm and 3.12-4.52 ppm also indicated that the α and β anomers of esterified D-galactose were present. Thus, the NMR study was quite useful in not only identifying the compounds but also in bringing out the efficacy of the NTGS-2200 membrane in the separation process.

Many researchers attempted MEUF for degumming hexane-oil miscella and reported to be the best for degumming application in terms of PL rejection and permeate flux. Iwama (1987) had shown that MEUF could actually be employed for simultaneous degumming and dewaxing of SBO and rapeseed oil without any additives. However, the efficiency of rejection of colour compounds by the ultrafiltration membranes was not consistent and varied between membranes and between oils (Koseoglu *et al.*, 1990). The nonporous membranes used in the present study exhibited an excellent selectivity towards eliminating PL as well as PGL. Therefore, these nonporous membranes were evaluated further whether they could offer a single-step process for simultaneous degumming, dewaxing and decolourizing crude vegetable oils.

5.3 Decolourization

The colour removal poses greater difficulty while processing CRBO compared to any other vegetable oil owing to inefficient pretreatment steps, and

parboiling compounds the problem further, both leading to colour fixation in oil. The important prerequisite for successful physical refining is to reduce the phosphorus content in the oil to <10 ppm, as phosphorus-containing components cause colour fixation in the final oil during exposure to the higher temperatures of physical refining (Roy *et al.*, 2002). The colour values (area under the visible spectra and Lovibond) of membrane-processed RBO are presented in Table 5.4. From the spectra (Fig. 5.1), it was noticed that RBO contains Maillard browning products that are mainly responsible for its colour rather than carotenoids and chlorophyll. The area under the visible spectra showed a good reduction of colour (48-55%) in undiluted as well as in hexane-diluted oils (Table 5.4; Fig. 5.5). Chlorophylls were present in laboratory prepared CRBO samples and were eliminated to an extent of 72% in raw-CRBO (lot-4) during membrane processing. Low absorption of chlorophyll in the membrane material appears to be the reason for its high retention. Chlorophyll being a sensitizer of photo-oxygenation can promote oxidation in the presence of light and decrease the oxidative stability of oils to a great extent. Earlier studies showed that rejection of chlorophyll was affected to a lesser extent with hexane dilution while rejection of carotenoids was affected to a much larger extent (Kondal Reddy *et al.*, 2001). In the present study, the major colour compounds (browning products) were rejected reasonably well by the membranes even under hexane-diluted conditions (Fig. 5.5). The efficiency of rejection of colour compounds by the nonporous membranes was consistent and did not vary between membranes (NTGS-2200 and NTGS-2100) and between the three lots of oils studied. The spectra of membrane-processed oils were similar to the spectra obtained

for commercially refined RBO indicating the suitability of membrane process for colour removal (Fig. 5.5).

Table 5.4 Colour values of membrane-processed CRBO

Sample (Oil:Hexane)	Lovibond			*PR*[a] (%)	Area under spectra	*PR*[a] (%)
	R	*Y*	(*5R+Y)*			
NTGS-2200 membrane						
Feed (lot-1)	5.0	20.0	45.0		12.6	
Permeate (1:0)	-[b]	-	-	-	5.9	52.9
Permeate (1:1)	1.1	1.6	7.1	84.2	6.3	49.6
Permeate (1:2)	-	-	-	-	6.3	49.6
Permeate (1:3)	-	-	-	-	6.6	47.5
Feed (lot-3)	5.1	21.0	46.5		14.8	
Permeate (1:0)	4.4	12.3	34.3	26.2	7.1	52.1
Feed (lot-4)	7.4	4.5	41.5		22.3	
Permeate (1:0)	3.1	10.1	25.6	38.3	10.0	55.3
NTGS-2100 membrane - crude lot-1						
Permeate (1:0)	1.0	1.9	6.9	84.6	6.3	50.0

[a] Reduction in processed oil.

[b] Not measured.

Lovibond colour measurement is widely practiced by the industry. A substantial reduction in colour as high as 84% was achieved even under hexane-diluted conditions while processing lot-1. However, colour reduction (26-85%) by the same membrane (NTGS-2200) varied greatly between three different lots of CRBO under undiluted conditions; despite 5*R* + *Y* values in their feed (41.5-46.5) did not vary much. Parboiled-CRBO is usually darker in colour than oil from raw rice bran. The Lovibond colour (5*R* + *Y*) values of

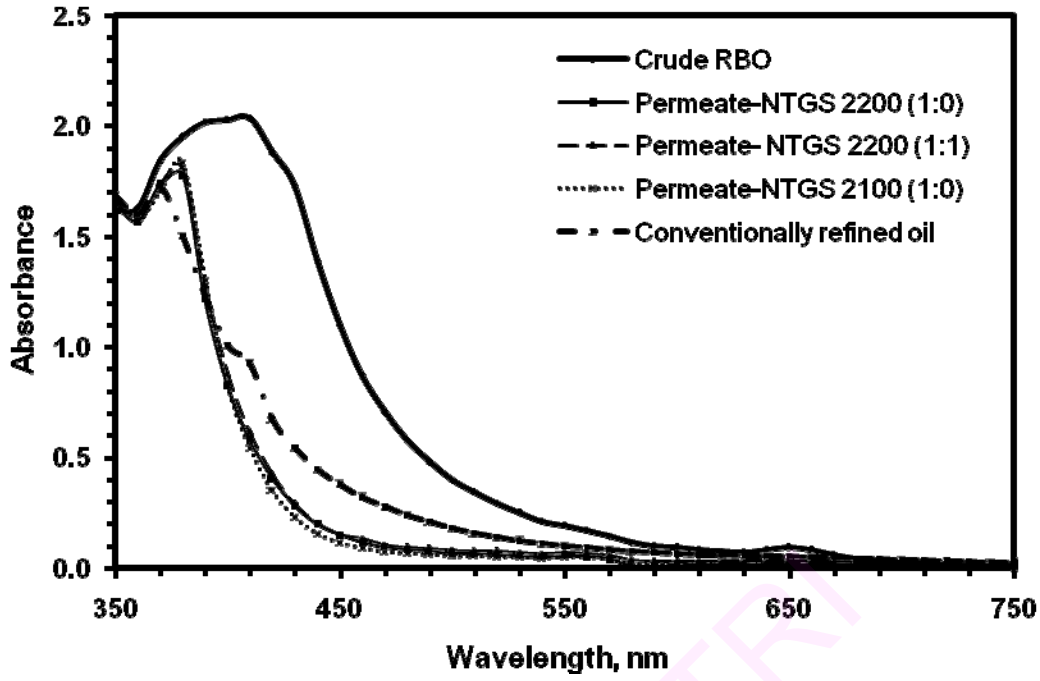


Fig. 5.5 Visible spectra of membrane-processed CRBO (lot-1)

raw and parboiled-CRBO were 41.5 and 46.5, respectively and corresponding reduction in colour during membrane processing were 38.3% and 26.2% (Table 5.4). The lower reduction in colour in parboiled-CRBO could be attributed to the hydrothermal treatment received during parboiling and probably to its consequential effect on colour. The complexities associated with the measurement of colour will be resolved only by characterization of colour compounds present in CRBO which merits further investigation.

5.4 Dewaxing

Dewaxing is necessary only for certain types of oils such as corn, sunflower, canola and rice bran. In the integrated commercial refinery, waxes are removed by a chilling, settling and a separation process. The wax reduction in the membrane-processed oil measured as AI was to the extent of 39-51% under undiluted conditions (Table 5.5). The selectivity of the nonporous

Table 5.5 Simultaneous degumming, dewaxing and decolourization of CRBO using NTGS-2200 membrane

Description	Phosphorus[a] (mg/kg)	Wax[a] (%)	Colour (area under spectra)
Feed (lot-3)	249.0±3.5	1.02±0.17	14.2
Permeate	12.0±1.4	0.50±0.03	6.8
PR (%)	95.2	51.0	52.1
Feed (lot-4)	170.6±4.4	0.66±0.04	16.8
Permeate	1.2±0.3	0.40±0.03	7.5
PR (%)	99.3	39.4	55.3

[a] Values are expressed as mean ± SD of duplicate (n=2) measurements.

membrane for wax was not as high as was observed for PL. Wax rejections were moderate and its content in the processed oil was in the range of 4000-5000 mg/kg. Many earlier researchers have attempted dewaxing edible oils with and without solvents and additives mainly using MF membranes (Mutoh *et al*., 1985; De *et al*., 1998). A polymeric MF membrane (pore size 120 nm) reduced the wax content in undiluted decolourized sunflower oil by 15% without cooling which improved to 99% when the feed was precooled to 5°C before processing (Mutoh *et al.*, 1985). Approaches made with chemical additives (phosphoric acid and sodium hydroxide) using MF (Mutoh *et al.*, 1985) were effective as a single-step process for simultaneous dewaxing, degumming and deacidifying oils but not devoid of chemicals used in the conventional process. The membrane processing of CRBO in the present study was carried out at room temperature and probably adequate precooling could increase the dewaxing efficiency. Nevertheless, exclusive membrane-based approaches without precooling and additives may offer several

advantages. Adding a polish filtration step at the end of the proposed membrane process may be desirable to ensure very low wax contents in oil and high cold stability. The unfavourable factors associated with the conventional dewaxing method such as clogging of the filter media, entrapment of neutral oil in the filter earth, and disposal costs are reduced even with partial reduction of wax in the membrane process.

5.5 Fatty acid composition

Palmitic, oleic and linoleic fatty acids constitute more than 90% of the fatty acid portion of the glycerides in RBO (Orthoefer, 1996). The fatty acid profile of permeates and retentates obtained during membrane processing of undiluted and hexane-diluted RBO are given in Table 5.6. There was no appreciable difference in the fatty acid composition between the permeate and retentate indicating that the formidable fatty acid balance of RBO is unaffected during the process. The major molecular species of rice bran TG are palmitic–linoleic–oleic, oleic–linoleic–palmitic, palmitic–linoleic–linoleic, linoleic–linoleic–palmitic and triolein (Orthoefer, 1996). The difference in the molecular sizes among these species is only ~30 Da. The differences in the molecular sizes and probably in the polarity among these TG are not sufficient enough to induce selectivity in the membrane.

5.6 Simultaneous degumming, dewaxing and decolourization

Conceptually, membranes could be used in almost all stages of oil refining. Although membrane technology has been extensively investigated for a long time (~30 years) all around the world, only a few commercial applications

Table 5.6 Fatty acid profile of membrane-processed CRBO

	Fatty acid composition[a] (relative %)					Selectivity[b]		
Fraction	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Palmitic	Oleic	Linoleic
Undiluted system								
Feed (lot-1)	21.70±0.28	1.20±0.14	43.05±0.21	29.90±1.27	2.10±0.14			
Permeate	21.10±0.14	1.75±0.31	44.15±0.35	29.65±1.06	1.20±0.28	0.97	1.03	0.99
Retentate	21.40±0.28	1.85±0.07	45.05±1.48	28.45±0.49	1.20±0.14			
Diluted system (1:1)								
Feed (lot-2)	23.65±0.07	2.05±0.01	44.24±0.08	28.68±0.25	1.25±0.07			
Permeate	23.85±0.07	2.07±0.00	44.16±0.06	28.23±0.01	1.42±0.00	1.01	1.00	0.99
Retentate	21.57±0.26	1.73±0.02	43.22±0.13	31.93±0.14	1.57±0.02			

[a] Values are expressed as mean ± SD of duplicate (n=2) measurements.

[b] Selectivity between palmitic/oleic/linoleic and total fatty acids.

have been reported. In this context, a single-step pretreatment process devoid of chemicals would be an attractive preposition.

Nonporous membranes used in the study showed excellent selectivity for PL in undiluted as well as hexane-diluted conditions (Tables 5.2 and 5.5). The membrane process was also effective in eliminating PGL which are reported to cause severe problems during processing CRBO. The wax content reduced to the extent of 39-51% (Table 5.5). Reduction of total colour compounds by the membrane was ~50% (Table 5.4) with a greater selectivity for the effective removal of chlorophyll pigments which would otherwise affect the oxidative stability of oils. The membrane process will become economically attractive if it is even partially effective for colour and wax removal in addition to PL reduction, considering the associated problems such as amount of clay used in the conventional bleaching process, entrapment of neutral oil in the filter earth during conventional dewaxing process and their disposal costs. Efficient pretreatment followed by physical refining seems to be a better approach to overcome the difficulties in processing RBO.

5.7 Conclusions

The nonporous membranes were able to reduce the phosphorus content in RBO below 10 mg/kg along with removal of PGL, which are identified as the potential source for the colour development during processing RBO. ^{1}H, ^{13}C and ^{31}P NMR analyses also clearly aided in arriving at the structural nature of the PGL in the retentate separated by the nonporous membrane. Besides effective degumming, membranes could achieve partial reduction in wax content and substantial reduction in colour that would definitely ease the difficulties of subsequent processing steps of physical refining.

6.0 Significance and focus of the work

Biotechnological approaches led to the development of enzymatic degumming employing phospholipase. Enzymatic degumming is construed as a suitable process for the physical refining, offering several advantages. However, there are only few attempts on CRBO despite the challenges posed by it during processing. The efficacy of enzymatic degumming of CRBO was studied using the third generation microbial phospholipase A_1. Attempts were also made on enzymatic degumming of CRBO in hexane medium considering the associated advantages.

6.1 Enzymatic degumming of CRBO

The phosphorus content of CRBO was 390 mg/kg. Water degumming and acid degumming of CRBO reduced the phosphorus content to 128 mg/kg and 85 mg/kg, respectively. The phospholipase activity of Lecitase-Ultra used in the study was estimated to be ~4500 Units/ml with PL (soy lecithin) as substrate while the lipase activity was ~5500 Units/ml with TG (refined RBO) as substrate.

Degumming of CRBO with varying enzyme (Lecitase-Ultra) dosage was studied and the results are presented in Table 6.1. In the first attempt reported on the enzymatic degumming of CRBO, Roy *et al.* (2002) employed Lecitase-Novo with an enzyme dosage of 800 Units/kg-oil and reported reduction in phosphorus content from 400 mg/kg in CRBO to 18 mg/kg in enzyme degummed oil, while immobilized Lecitase employed for degumming CRBO reduced the phosphorus content in the degummed oil only to the extent of 60-70 mg/kg (Sheelu *et al.*, 2008). In the present experiments, 1250

Units/kg-oil enzyme dosage (Lecitase-Ultra) achieved 67 mg/kg of phosphorus in the degummed oil from an initial level of 390 mg/kg in CRBO. When the dosage was increased to 2500 Units/kg-oil, the phosphorus content reduced to 10 mg/kg whereas the residual phosphorus was 250 mg/kg without the enzyme addition. Although the experimental conditions of control run (without the enzyme addition) resembled the acid degumming process, the reduction in phosphorus content achieved was far less. Further increase in enzyme dosage beyond 2500 Units/kg-oil, did not improve the degumming efficiency. The reduction of phosphorus to 10 mg/kg in the degummed oil meets the prerequisites of physical refining.

Table 6.1 Enzymatic degumming of CRBO with varying enzyme dosage using Lecitase-Ultra[a]

Enzyme dosage (Units/kg-oil)	Final phosphorus content (mg/kg)	Reduction (%)
Control	250	37.3
1250	67	83.2
2500	10	97.5
6300	10	97.5

[a] Initial phosphorus content in CRBO 390 mg/kg; water addition 2%; incubation period 5 h.

Yang *et al.* (2006b) had earlier reported in their characterization study on Lecitase-Ultra that when the incubation temperature was over 40°C, the phospholipase activity dominated, and the lipase activity greatly suppressed. Further it was observed that the yield loss due to TG hydrolysis was only marginal during degumming of rapeseed oil and SBO and the specificity of enzyme hydrolysis was impressively only towards PL. However, in the

present experiments with CRBO, enzyme utilization towards hydrolysis of PL and TG was found to be 80% and 20%, respectively (Table 6.2). During enzymatic degumming process, phospholipase A1 attacks the Sn-1 position of PL molecules (Scheme 1.1) releasing an equivalent number of fatty acid molecules. An increase of FFA from 7.1% to 7.6% was observed during degumming CRBO at an enzyme dosage of 2500 Units/kg-oil. Out of 0.5% net increase of FFA, ~0.4% could be attributed to PL hydrolysis as evidenced by the reduction in phosphorus content from 390 to 10 mg/kg while the rest of the FFA formed could be attributed to the hydrolysis of TG (~20%). This proportion of hydrolysis of PL and TG was similar even at 1250 Units/kg-oil enzyme dosage. Although Lecitase-Ultra exhibited independently higher lipase activity than phospholipase activity under assay conditions, the hydrolysis of PL was favourably higher than TG under the conditions employed for oil degumming.

Table 6.2 Enzyme utilization towards hydrolysis of PL and TG

Sample description	Enzyme dosage (Units/kg-oil)	FFA content (%)	Enzyme utilization (%)	
			Hydrolysis-PL	Hydrolysis-TG
CRBO		7.1		
Enzyme-degummed RBO	1250	7.5	82	18
Enzyme-degummed RBO	2500	7.6	80	20

Enzymatic degumming reaction of CRBO was studied as a function of time and is presented in Fig. 6.1. The phosphorus content of CRBO was reduced to ~6 mg/kg in 2 h of incubation period and remained at that level up to 5 h of reaction period. Although, this suggested that 2 h of incubation

period may be sufficient for enzymatic degumming, an incubation period of 5 h was followed in all the subsequent experimental runs to ensure effective end result while handling different qualities of oil and PL present in oil.

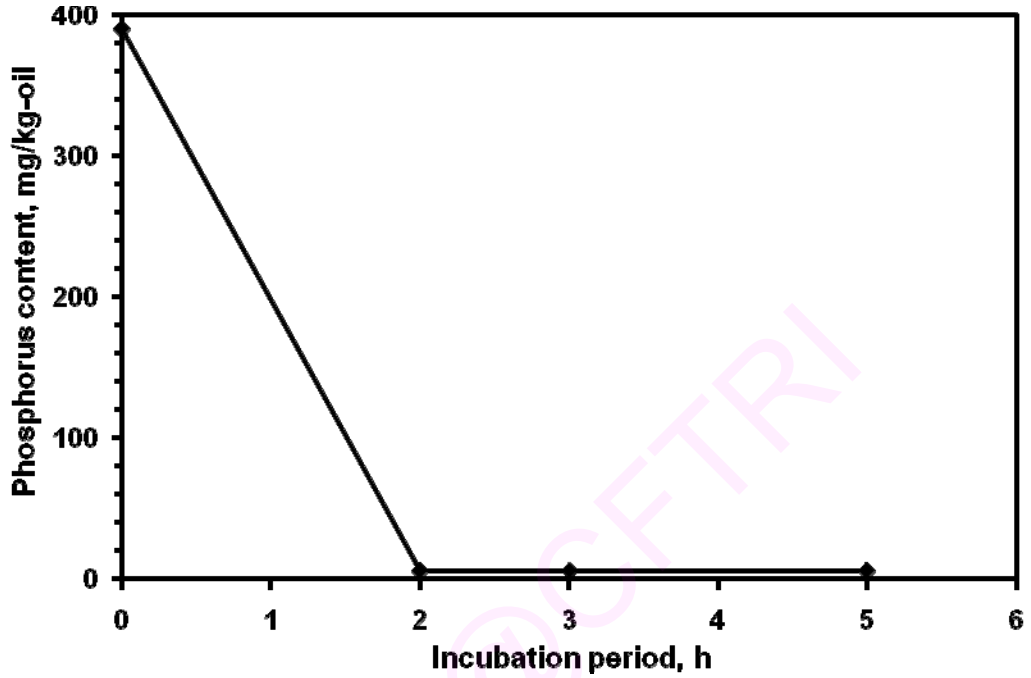


Fig. 6.1 Reduction in phosphorus content in CRBO as a function of incubation time during enzymatic degumming (water addition 2%; enzyme dosage 2520 Units/kg-oil)

6.2 Enzymatic degumming of different qualities of RBO

Degumming experiments were also conducted with water and acid-degummed RBO. The results are shown in Table 6.3. The phosphorus level in water-degummed RBO reduced from 128 mg/kg to 10 mg/kg after enzymatic degumming, similar to the phosphorus level achieved with CRBO. However, phosphorus content in the acid-degummed oil could be reduced only to the extent of 31 mg/kg with enzymatic degumming. The residual phosphoric acid trapped in NHP could be an effective inhibitor for phospholipase reducing the substrate binding and hence the extent of

hydrolysis of substrate. The phosphorus content in the corresponding control sample was 35 mg/kg close to the enzyme treated sample (31 mg/kg) which supports the above explanation why enzymatic hydrolysis is not effective with acid degummed oils.

Based on the studies with crude and water degummed rapeseed oils, Clausen (2001) recommended adoption of enzymatic degumming directly to crude oils since normal water-degumming step could be omitted. While our studies with Lecitase-Ultra on CRBO supported the above view, it also revealed that acid-degummed oil is not suitable for enzymatic degumming. Omission of normal water-degumming step in the process would lead to reduced operating costs and reduced oil loss.

Table 6.3 Enzymatic degumming of different quality of RBO varying in phosphorus content using Lecitase-Ultra[a]

	Phosphorus content (mg/kg)		
Sample description	Initial	Final (enzyme)	Final (control)
CRBO	390	10.1	60.0
Water-degummed RBO	128	8.9	16.4
Acid-degummed RBO	85	31	35

[a] Enzyme dosage 2520 Units/kg-oil; water addition 2%; incubation period 5 h.

6.3 Enzymatic degumming of CRBO in solvent phase

Hexane is the common solvent used for the extraction of oil from oilseeds and pressed cakes. Hence, it would be advantageous if hexane-oil miscella is directly used in enzymatic degumming as in the case of 'miscella refining' that would probably offer possibilities to minimize the oil losses and increase the yield. Therefore, in the present study the efficacy of enzymatic degumming of

RBO using Lecitase-Ultra was studied in solvent phase. Hexane was used in the preliminary experiments and subsequently switched over to petroleum ether (40-60°C) in order to maintain the required reaction temperature constant (50°C). The results of solvent-phase enzymatic degumming are presented in Table 6.4. The phosphorus content in degummed RBO as a function of incubation period during enzymatic degumming is shown in Fig. 6.2.

Table 6.4 Enzymatic degumming of different grades of RBO in solvent phase

Sample description	Solvent	Water addition (%)	Enzyme dosage (Units/kg-oil)	Incubation time (h)	Phosphorus content (mg/kg)	
					Initial	Final
Crude RBO	Hexane	2	2520	5	390	380
Crude RBO	Petroleum ether	5	2520	6	390	285
Crude RBO	Petroleum ether	10	2520	4	390	120
Crude RBO	Petroleum ether	20	5040	4	390	71
Water-degummed RBO	Petroleum ether	10	5040	4	128	63

The hexane-oil system with 2% water addition and 2520 Units/kg-oil enzyme dosage practically did not show any reduction of PL in the oil even after 5 h of incubation period (Fig. 6.2). This could be due to the poor contact between the highly nonpolar solvent and the enzyme (polar) which resulted in almost no reduction in PL in the oil. Hence subsequent experiments were conducted with increased water content keeping the enzyme dosage constant to improve be reduced from 390 mg/kg to 285 mg/kg with 5% water addition. By increasing the water addition to 10%, the phosphorus content could be

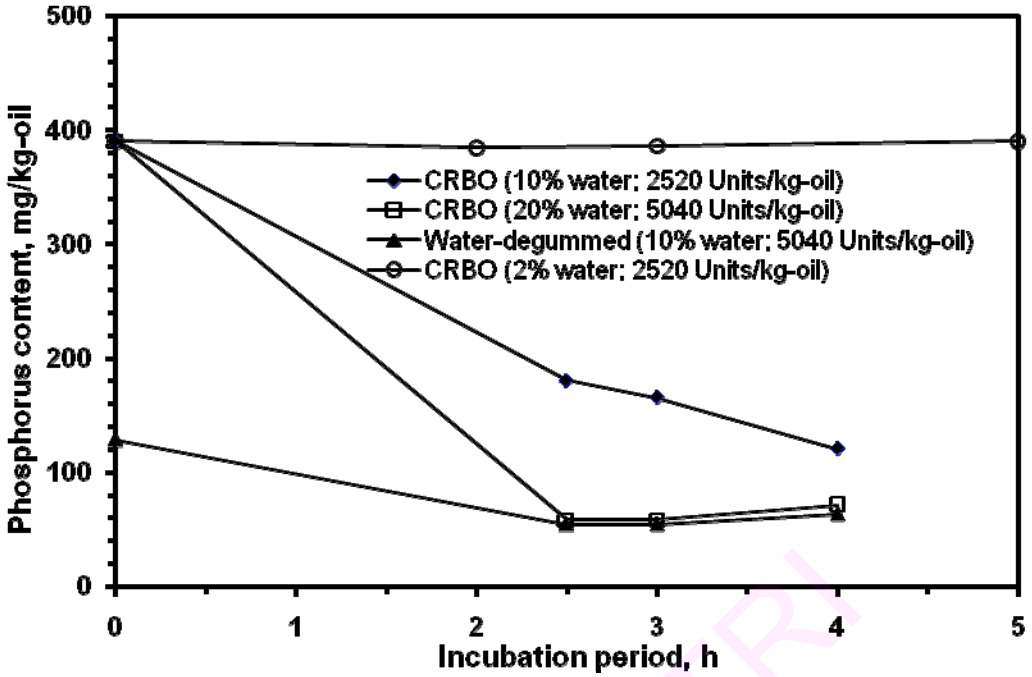


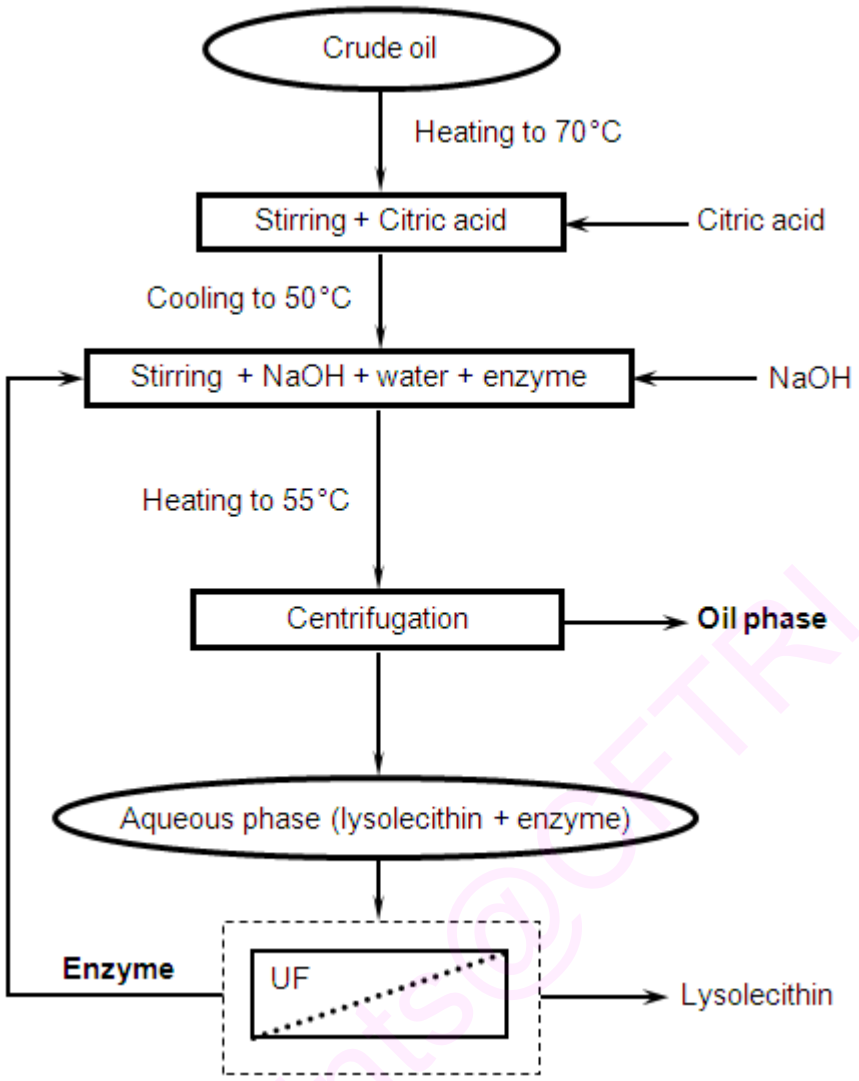
Fig. 6.2 Reduction in phosphorus content in CRBO as a function of incubation time during enzymatic degumming in solvent phase (hexane/petroleum ether)

reduced to 180 mg/kg in 2.5 h that reduced further to 120 mg/kg by extending the incubation period to 4 h (Fig. 6.2). In the subsequent run, water content was increased to 20% while simultaneously increasing the enzyme dosage (5040 Units/kg-oil) that resulted in reduction of phosphorus level to 71 mg/kg. With similar enzyme dosage, the phosphorus content in water-degummed oil could be reduced from 128 mg/kg to 63 mg/kg with only 10% water addition. However in these two cases, there was no further decrease in phosphorus content beyond 2.5 h of incubation period (Fig. 6.2). Phosphorus levels in the degummed oil obtained in solvent phase degumming was similar to the values obtained with immobilized enzyme in oil phase degumming (Sheelu *et al.*, 2008). However, the phosphorus levels achieved does not meet the industrial standards for adoption in physical refining process. Besides, high level of water addition in the order of 10-20% is not feasible for practical applications

unless a scheme for enzyme recycle from aqueous phase is introduced in the process. Earlier attempts on lipase-catalyzed hydrolysis were not as successful as interesterification of fats for similar reasons (Valivety *et al.*, 1991, 1993). Water plays a significant role in enzyme-catalyzed hydrolysis and esterification reactions (Hahn-Hagardal, 1986; Gayot *et al.*, 2003). While a critical amount of water is necessary for maintaining the active conformation of the enzyme, excess water facilitates hydrolysis (Halling, 1989, 1994; Zaks and Klibanov, 1985, 1988). However, because of the high nonpolar nature of the medium, hydrolysis also does not occur with as much ease as in the absence of the solvent due to variation in the water activity, hydration shell and dielectric characteristics of the solvent-water medium. Considering the benefits of solvent phase enzymatic degumming of crude vegetable oils, it is desirable to make wider attempts including other sources of phospholipases.

6.4 Proposed scheme for enzyme recycling

The use of enzyme is often hampered by high cost and many methods of recycling have been resorted to for solving this problem, enzyme immobilization being the most common approach. Membrane technology has contributed significantly in the field of bioprocessing owing to its inherent advantages over the conventional processes. Exploiting this mild processing technology, an integrated approach combining enzymatic treatment and membrane separation has been proposed to explore the possibility of recycling/reusing the enzyme in the process (Scheme 6.1A). The phospholipase enzymes could be reused in the process after completing its process duty. However, considering the thermal stability of the enzyme, care

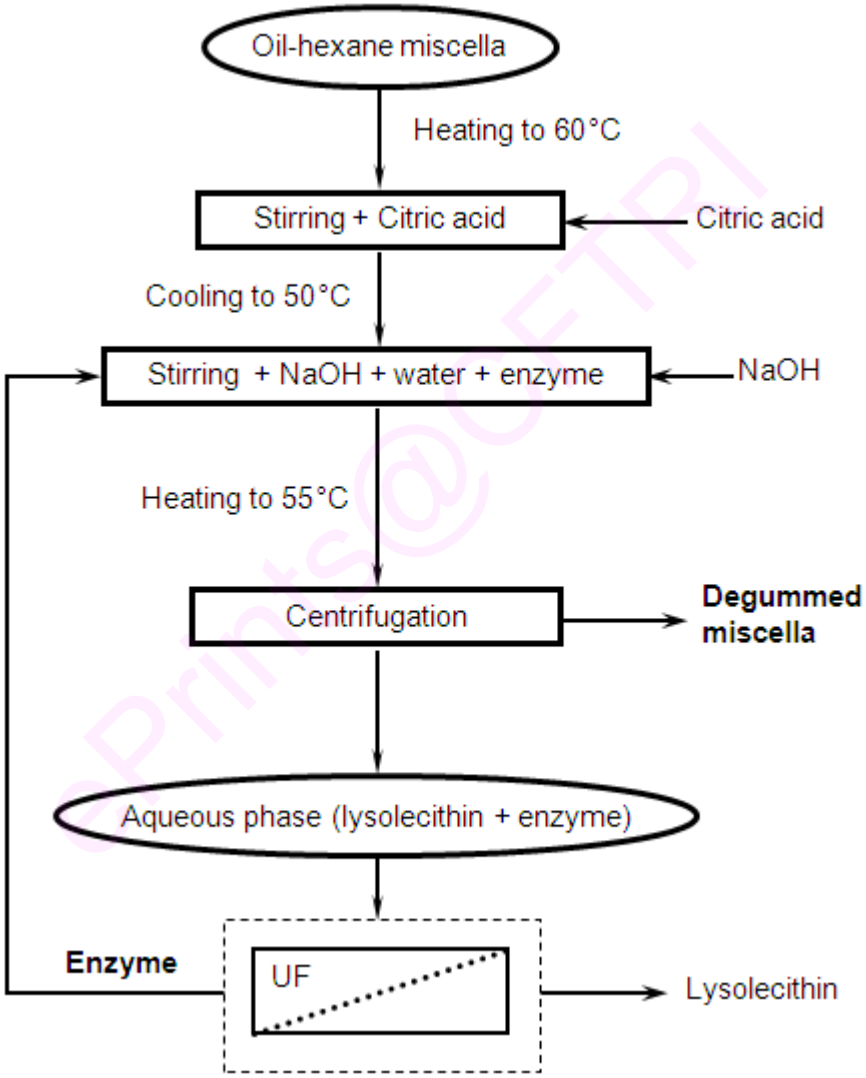


Scheme 6.1A Recycling of phospholipase in enzymatic degumming process

must be taken not to subject the enzyme above 60°C temperature at any point in the process. Accordingly, the centrifugal separation meant for separating the aqueous and nonaqueous phases should be managed with other process parameters, centrifugal force and residence time to compensate any efficiency loss owing to slightly lower operating temperature. A membrane process employing a hydrophilic UF membrane (low MWCO typically ~5 kDa) shall be introduced to recover or separate the enzyme from the aqueous sludge obtained from the centrifugation step after suitable dilution. The enzyme retained by the membrane could be reused in the process while permeate (lysolecithin) could be discarded. The aqueous phase in the

process contributes only to the extent of 2-3%; therefore a smaller membrane unit would be adequate even for a commercial processing plant.

In the case of solvent phase enzymatic degumming the efficiency of centrifugal separation would be naturally higher; however, greater degumming efficiency should be accomplished to achieve low phosphorus levels in the oil. The scheme proposed (Scheme 6.1B) for enzyme recycle would allow even



Scheme 6.1B Recycling of phospholipase in solvent phase enzymatic degumming process

higher levels of water addition during enzymatic degumming step. However, it is necessary to examine these integrated approaches on a pilot scale operation to establish the process feasibility.

6.5 Conclusions

Lecitase-Ultra employed for enzymatic degumming of CRBO was effective and reduced the phosphorus content in the oil to less than 10 mg/kg from an initial level of 390 mg/kg after 2 h of incubation period. When enzymatic degumming was attempted in hexane phase, there was practically no PL reduction at lower water content (2%) even after 5 h of incubation period due to the poor contact between the highly nonpolar solvent and enzyme (polar). Increasing the water addition to 20% reduced the phosphorus level in the degummed-oil to 71 mg/kg which is still insufficient to meet the prerequisites of physical refining. It may be desirable to make wider attempts considering the benefits of integrated processing approach.

7.0 Significance and focus of the work

Oryzanol present in rice bran is associated with various physiological functions. However, these beneficial ferulate esters are lost to a greater extent during conventional refining of CRBO depleting their content in the final product. In the present investigation, oryzanol enrichment in RBO was attempted using nonporous polymeric membranes under undiluted as well as hexane-diluted conditions with different (crude, refined and model oil) systems with a view to enhance the oryzanol content in the processed oil.

7.1 Selectivity of nonporous membranes for oryzanol in real and model oil systems

Studies were carried out using two nonporous membranes NTGS-2100 and NTGS-2200 to evaluate their selectivity for oryzanol in CRBO and various other model oil systems. The oryzanol content in the feed, observed rejection and permeate flux during membrane processing are presented in Table 7.1. The rejection of oryzanol in CRBO was 33.4% and 41.3% with NTGS-2100 and NTGS-2200 membranes, respectively. In the case of nonporous membranes, separation/permeation of components depends on their own solubility and diffusivity in the membrane material. In addition, it also depends on the coupling effect as well as solubility of individual components in other permeating components including the solvent being used (Bhosle *et al.*, 2005). All the above factors are also concentration-dependent besides solubility being greatly influenced by the polarity of individual components (Barton, 1991). Oryzanol is composed of 25% to 50% of campesteryl, 10% to 40% of 2,4-methylene cycloartanyl, 15% to 30% of cycloartenyl and 15% to

Table 7.1 Rejection of oryzanol in RBO systems by nonporous membranes[a]

	Feed			NTGS-2100		NTGS-2200	
	Oryzanol[b]	Oleic acid[b]	Oryzanol[c]	R_o	Flux	R_o	Flux
Type of system	(%)	(%)	(mg/kg)	(%)	[kg/(m^2·h)]	(%)	[kg/(m^2·h)]
Real system							
CRBO	-	-	15200±424	33.4	0.03	41.3	0.03
Model systems							
TG-oryzanol	1	-	13600±1004	29.7	0.08	30.9	0.05
TG-oryzanol	2	-	23600±156	26.7	0.07	-	-
TG-oleic acid-oryzanol	1	49.5	12400±707	22.2	0.14	32.3	0.09
TG-oleic acid-oryzanol	2	49.0	21500±721	-	-	30.7	0.10
Oleic acid-oryzanol	1	99.0	10300±453	27.7	0.51	45.8	0.37
CRBO-TG (1:1)	-	-	9600±863	39.0	0.03	44.1	0.03

[a] CRBO (lot-1); 20% permeation of feed; operating pressure 3 MPa.

[b] Addition.

[c] Estimated value. Values are expressed as mean ± SD of duplicate (n=2) measurements.

26% of β-sitosteryl ferulates. The molecular weight of this mixture of four compounds varying in composition is calculated as ~600 Da. The alcohol group present in the ferulate moiety of components of oryzanol gives rise to a relatively high polarity (Xu and Godber, 2000). This may explain the moderate rejection of oryzanol in spite of its lower molecular weight (~600 Da) compared to TG (600-1000 Da), since the active surface (PDMS) of the nonporous denser membranes used in the study is hydrophobic in nature. In an earlier study from this laboratory on the rejection of carotenoids (Subramanian *et al.*, 2001b), rejection of xanthophylls (oxygenated carotenoids) and permeation of β-carotene (hydrocarbon carotenoids) in a nonporous hydrophobic membrane revealed the influence of polarity of compounds on their transport.

The above studies were extended to different model oil systems for assessing the membrane selectivity (Table 7.1). The rejections of oryzanol in TG (refined RBO)-oryzanol system with NTGS-2100 and NTGS-2200 membranes were 29.7% and 30.9%, respectively. The lower rejections observed with the above model system as compared to CRBO could be attributed to the basic differences between the real and model systems in terms of their composition. Inclusion of oleic acid in the model oil system increased the permeation rate, and the highest permeation rate was observed in oleic acid-oryzanol system (Table 7.1). Oleic acid exhibited higher differential permeability over TG in a nonporous denser membrane owing to the cumulative effect of its higher solubility as well as diffusivity in the membrane material (Subramanian *et al.*, 2001c). However, it appears that the permeability of the solute (oryzanol) was not influenced much by its solubility

in oleic acid resulting in its higher rejection in oleic acid-oryzanol system. In this system, cumulative effect of higher molecular weight and relatively higher polarity of oryzanol seemed to be responsible for their rejection. Earlier studies from this laboratory on degumming showed that these two membranes possess excellent selectivity in near-complete elimination of PL in crude vegetable and model oils (Subramanian *et al.*, 2004). Although the rejection of oryzanol was not as high as that of PL, the moderate rejection of oryzanol by these membranes evoked interest. NTGS-2200 membrane exhibited higher selectivity for oryzanol compared to NTGS-2100 in crude as well as model oil systems. Although these two membranes were prepared from the same membrane materials (active and support layers), the difference in their observed rejection values were significant which could be probably attributed to the differences in the thickness of their active (PDMS) layers. Relatively lower flux values obtained with NTGS-2200 compared to NTGS-2100 membrane (Table 7.1) also suggested differences in their active layer thickness. Considering the higher selectivity for oryzanol, NTGS-2200 membrane was used in further experiments.

7.2 Processing undiluted crude and model oil systems

The performance of NTGS-2200 membrane was assessed at different levels of permeation during batch processing of CRBO and TG-oryzanol model system. Oryzanol content in the process streams and enrichment fold at various stages along with the overall observed rejection and permeate flux for CRBO and TG-oryzanol system are presented in Tables 7.2 and 7.3, respectively. Palmitic, oleic and linoleic are the major fatty acids in RBO, and

Table 7.2 Enrichment of oryzanol in CRBO at various levels of permeation[a]

Fraction no.	Permeation	Oryzanol content[b] (mg/kg)		R_o	Enrichment	Permeate flux
		Permeate	Retentate	(%)	(Fold)	[kg/(m^2·h)]
1	0-25%	16100±170	19800±849	-	1.13	-
2	25-44%	13430±622	21900±565	-	1.11 (1.24)[c]	-
3	44-58%	13900±438	24500±933	-	1.12 (1.39)[c]	-
4	58-68%	13930±608	27300±580	-	1.11 (1.55)[c]	-
5	0-68%	-	27300±580	36.9	1.55	0.028

[a] Crude (lot-2); oryzanol content in feed 17600±919 mg/kg; feed viscosity 62 mPa.s; operating pressure 4 MPa.

[b] Values are expressed as mean ± SD of duplicate (n=2) measurements.

[c] Cumulative enrichment.

their contents in CRBO and model oils used in the present study were 23.6%, their contents in CRBO and model oils used in the present study were 23.6%, 44.2% and 28.7%; and 20.6%, 41.1% and 33.0%, respectively. The oryzanol content in the final retentate (ERBO) increased to a level of 27,300 mg/kg from an initial value of 17,600 mg/kg in CRBO. The oryzanol content increased during the batch process and its enrichment steadily increased at various levels of permeation, in spite of the increased solute diffusion owing to its increased concentration in the feed. The overall rejection of oryzanol by the membrane after 68% permeation was 36.9% with an enrichment fold of 1.55.

As in the case of CRBO, the oryzanol enrichment steadily increased, and its concentration reached a level of 30,300 mg/kg from an initial value of 20,400 mg/kg in the model system as well. The improvement in oryzanol content in the model oil was 1.49 fold after 85% permeation. The overall rejection of oryzanol was 19.9%, which was lower than the rejection observed

Table 7.3 Enrichment of oryzanol in a model oil system at various levels of permeation[a]

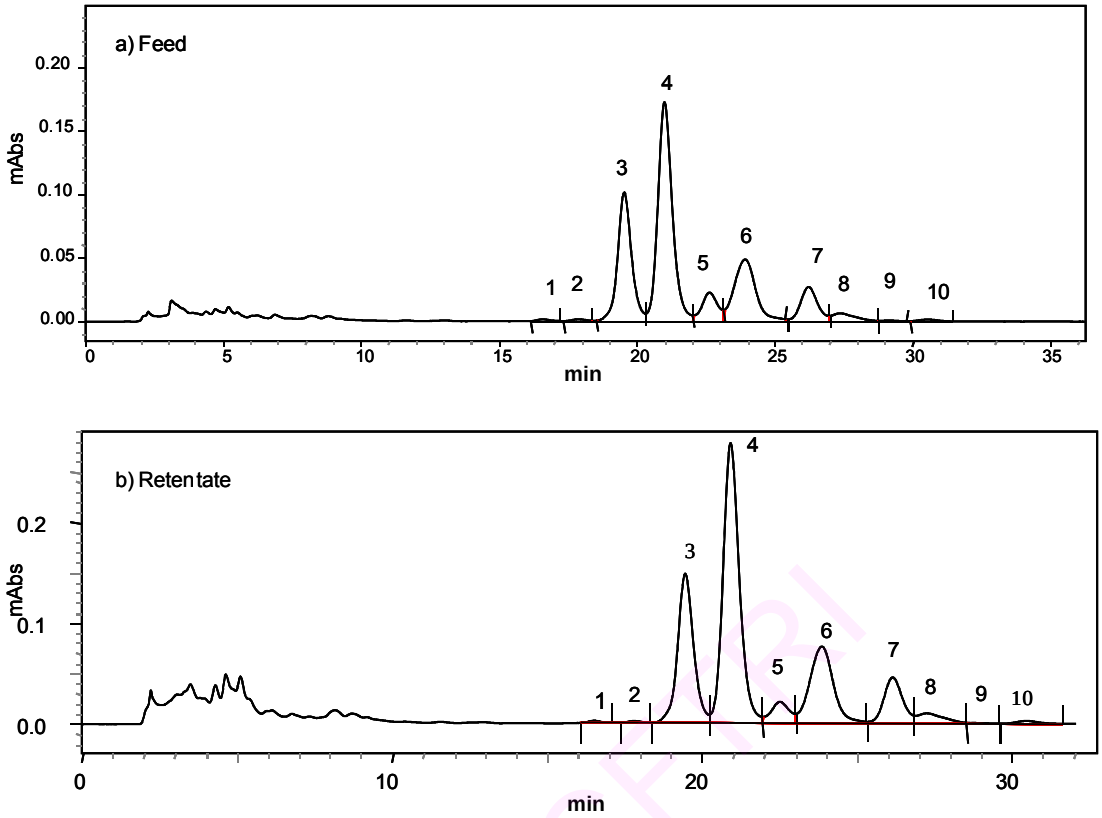
Fraction no.	Permeation	Oryzanol content[b] (mg/kg)		R_o (%)	Enrichment (Fold)	Permeate flux [kg/(m^2·h)]
		Permeate	Retentate			
1	0-17%	12500±1061	22000±1414	-	1.08	-
2	17-38%	16900±141	23600±1032	-	1.07 (1.16)[c]	-
3	38-57%	19300±608	25600±424	-	1.08 (1.25)[c]	-
4	57-85%	22300±862	30300±580	-	1.18 (1.49)[c]	-
5	0-85%	-	30300±580	19.9	1.49	0.046

[a] Oryzanol content in feed 20400±990 mg/kg; feed viscosity 61 mPa.s; operating pressure 2 MPa.

[b] Values are expressed as mean ± SD of duplicate (n=2) measurements.

[c] Cumulative enrichment.

with crude oil (36.9%). This could be due to the basic differences between the real and model systems as discussed earlier. The average PL content of CRBO is in the range of 4-5% (Orthoefer, 1996). From our study on characterization of PL reverse micelles (Chapter 3), it can be construed that the PL content in CRBO is above CMC. The hydrophilic polar heads of PL are inward in the reverse micelles formed in the system, and these inner polar regions would have affinity for other polar components such as oryzanol present in the system resulting in their increased rejection. The model oil (TG-oryzanol) did not contain PL which could be the probable reason for the lower rejection of oryzanol. The HPLC analysis of the process streams of model oil system showed all the ten components of oryzanol in their chromatograms (Fig. 7.1). The four major peaks were identified as ferulate esters of cycloartenol, 24-methylenecycloartanol, campesterol and β-sitostenol. These major components were rejected by the membrane to the



Peak no.	Compound	Retention time (min)	Area percent (relative) Feed	Retentate
1	Stigmastenyl ferulate	16.5	0.27	0.27
2	Stigmasteryl ferulate	17.8	0.31	0.27
3	Cycloartenyl ferulate	19.5	23.67	22.89
4	24-Methylene cycloartanyl ferulate	20.9	41.77	43.84
5	Campestenyl ferulate	22.5	5.69	3.78
6	Campesteryl ferulate	23.8	17.60	17.42
7	Sitostenyl ferulate	26.1	7.58	8.31
8	Sitosteryl ferulate	27.2	2.44	2.56
9	Campestanyl ferulate	29.1	0.20	0.11
10	Sitostanyl ferulate	30.4	0.47	0.55
	Total area		15279417	23235472

Fig. 7.1 HPLC Chromatogram of membrane-processed TG-oryzanol system. Column C-18; Mobile phase methanol, acetonitrile, dichloromethane and acetic acid (50:44:3:3, v/v); Flow rate 1.4 ml/min; UV Detector at 330 nm.

extent of 19.4%, 23.7%, 20.5% and 25.4%, respectively, without deviating much from the rejection of total oryzanol estimated based on HPLC (20.0%) and spectrophotometric (19.9%) analyses. The permeate flux obtained with crude oil system was lower than that of model oil system which could be due

to the higher viscosity as well as the greater effect of concentration polarization owing to the presence of various other impurities in the crude oil.

7.3 Processing hexane-diluted crude oil system

Studies from this laboratory (Saravanan *et al.*, 2006; Sarita Arora *et al.*, 2006) showed that significant improvement in oil flux could be achieved in nonporous denser membranes by hexane dilution of crude vegetable oils without practically affecting the rejection of PL. The same approach was examined to assess the membrane selectivity for oryzanol under hexane-diluted conditions and to improve the oil flux, keeping in view the process economics. The permeate flux increased by nearly 10 fold at 1:1 dilution and 18 fold at 1:3 dilution while processing hexane-diluted CRBO (Table 7.4). However, the rejection of oryzanol decreased to 17.9% in hexane-diluted system (1:1 dilution) compared to undiluted system (39.6%), and the rejection decreased further with increase in hexane dilution. Similar behavior was observed in the rejection of colour compounds in hexane-diluted crude vegetable oils (Saravanan *et al.*, 2006; Sarita Arora *et al.*, 2006; Kondal Reddy *et al.*, 2001). The rejection in a multi-component system is controlled by several factors. Besides membrane parameters, the interaction between the components in the feed also plays a significant role. Further, it appears that hexane seemed to have positive coupling with other oil constituents that are soluble in hexane during its transport across the nonporous denser membrane increasing their permeation or in other words lowering their rejection. Although hexane dilution affected the rejection of oryzanol, this could be the practical approach considering the oil flux improvement.

Table 7.4 Enrichment of oryzanol in hexane-diluted CRBO[a]

Oil-solvent ratio	Feed viscosity (mPa.s)	Oryzanol content[b] (mg/kg)		R_o (%)	Enrichment (Fold)	Yield (%)	Oil flux [kg/(m^2·h)]
		Permeate	Retentate				
1:1	3.2	15400±580	24100±297	17.9	1.37	17	0.32
1:3	1.9	16600±911	21900±1295	12.5	1.24	17	0.51

[a] Crude (lot-2); oryzanol content in feed 17600±919 mg/kg; 90% permeation; operating pressure 2 MPa.

[b] Hexane free basis. Values are expressed as mean ± SD of duplicate (n=2) measurements.

7.4 Processing undiluted and hexane-diluted refined oil system

The ability of nonporous membranes was examined for producing refined RBO with guaranteed oryzanol content that would conform to the efficacy levels of oryzanol in dietary and therapeutic applications. The oryzanol content in chemically refined RBO was 2,420 mg/kg, which improved to 7,340 mg/kg after membrane processing under undiluted conditions. The overall rejection of oryzanol by the membrane after 90% permeation was 44.2% with an enrichment fold of 3.03 (Table 7.5). Similar to the observations made with crude oil, hexane dilution of refined oil improved the oil flux (15 to 18 fold) but affected the selectivity of the membrane as evidenced from the lower rejection values (Table 7.5). The results indicated that it may be possible to use membrane processing in conjunction with conventional refining process to guarantee a minimum oryzanol content in the finished product.

7.5 Conclusions

The results revealed that the nonporous hydrophobic membrane possesses a reasonable selectivity towards enriching oryzanol in RBO due to the nature of

Table 7.5 Enrichment of oryzanol in undiluted and hexane-diluted refined RBO[a]

Oil-solvent ratio	Feed viscosity (mPa.s)	Oryzanol content[b] (mg/kg)		R_o (%)	Enrichment (Fold)	Yield (%)	Oil flux [kg/(m^2·h)]
		Permeate	Retentate				
1:0	58.0	1880±141	7340±42	44.2	3.03	10	0.068
1:1	1.53	2150±141	4480±170	26.0	1.85	9.4	1.03
1:2	1.25	2220±164	4940±113	26.1	2.04	6.5	1.24

[a] Oryzanol content in feed 2420±85 mg/kg; 90% permeation; operating pressure 2 MPa.

[b] Hexane free basis. Values are expressed as mean ± SD of duplicate (n=2) measurements.

the ferulic esters leading to their moderate rejection. Owing to the preferential permeation of FFA, the enriched oryzanol fraction (retentate) obtained in the process would have reduced FFA content. Although hexane dilution affected the selectivity while improving the oil flux, this approach still appears to be potential which would gain utility with the development of high selectivity-flux membranes. Membrane processing could be used for concentration/enrichment of oryzanol present in RBO by physical means under mild process conditions, to produce a standard RBO with guaranteed oryzanol content.

8.0 Significance and focus of the work

Deoiling of lecithin is a prerequisite in making high-purity products including PC. Acetone has been the solvent of choice of the industries for the separation of glycerides and PL, although it was affecting the 'relatively natural' characteristics of lecithin products. As an alternative to acetone-extraction, deoiling of lecithin using a nonporous membrane was examined in a favourable solvent (hexane) medium with soy and rice bran lecithins.

8.1 Efficacy of nonporous membrane for deoiling lecithin

Preliminary studies conducted to evaluate the efficacy of a nonporous membrane for deoiling lecithin revealed that PL were rejected to the extent of 97.5%. Consequently, AI content in soy lecithin increased by 28% from an initial level of 63.2 to 81.0% in a single-step batch operation (Table 8.1). The nonporous membranes from the same series used in the earlier studies of this laboratory achieved almost complete degumming while processing crude vegetable oils in undiluted as well as hexane-diluted systems (Subramanian *et al.*, 2004; Saravanan *et al.*, 2006; Sarita Arora *et al.*, 2006). In the present study with a lecithin-hexane system, the increase in AI content in the retentate fraction implied that the membrane possessed the required selectivity for the intended application.

Earlier studies on processing hexane-oil miscella also showed that there is no selectivity between oil and hexane over a wide range of oil concentration in the miscella (Kondal Reddy *et al.*, 2001). Accordingly, in the present batch system the maximum removal of oil from the feed would be proportional to the amount of permeation. The level of AI content in the

Table 8.1 Membrane deoiling of soy lecithin[a]

Experimental description	Lecithin-solvent ratio (w/w)	VCR	AI[b] (%)	PL[b] (%)
Primary run	1:9	5.7	81.0±0.3	-
Secondary run	1:9	5.1	89.6±1.1	-
Ternary run	1:37	5.2	95.7±0.9	89.7±1.1

[a] lot-1; AI content 63.2%±0.7; PL content 57.7%±1.3.

[b] In the retentate (hexane free basis). Values are expressed as mean ± SD of duplicate (n=2) measurements.

deoiled lecithin obtained in a single-step processing was not very high. Lecithin with higher AI content (~95%) would be preferred in most of the applications owing to their greater purity and lower oil content. In order to improve the performance of the membrane process, a secondary run was conducted with the deoiled lecithin obtained from the primary run after suitable hexane dilution. As a consequence, the AI content improved from 81.0 to 89.6%, which improved further to 95.7% after a ternary run at a higher dilution (Table 8.1). AI measurement is a simple and rough measure of PL but also includes other minor substances such as waxes. When the AI content increased from 63.2 to 95.7%, the corresponding increase in PL content was from 57.7 to 89.7% in soy lecithin after three steps of processing (Table 8.1). The results revealed that diafiltration could be employed in the process. Diafiltration is a common method used to improve the yield as well as purity of target compounds in a membrane process. Therefore, subsequent studies were carried out employing diafiltration in order to achieve greater removal of oil from lecithin as well as to improve the PL content in the deoiled lecithin.

8.2 Deoiling of lecithin during discontinuous diafiltration

The AI and PL contents of soy and rice bran lecithin samples during discontinuous diafiltration using nonporous membrane are presented in Table 8.2. As discussed in the earlier section, understandably oil removal increased with diafiltration and the quantity of oil removed was somewhat proportional to the ratio of permeate to feed in each step of diafiltration. During processing soy lecithin, the PL content improved from an initial level of 56.5 to 85.7% in the retentate fraction after four steps of diafiltration (200% dilution to feed). In the case of rice bran lecithin, the same level of PL (85.5%) could be achieved with three steps of diafiltration (150% dilution to feed). Further, the increase in PL content in rice bran lecithin was 2.6 fold while it was only 1.5 fold in soy lecithin in spite of a higher amount of hexane used during diafiltration. Initial

Table 8.2 Membrane deoiling of soy and rice bran lecithins during discontinuous diafiltration[a]

Sample description	AI[b] (%)	PL[b] (%)	PL improvement (Fold)
Soy lecithin (lot-2)			
Feed	59.6±1.0	56.5±1.0	
Final retentate[c]	89.1±1.4	85.7±1.3	1.5
Rice bran lecithin			
Feed	-	33.3±0.3	
Final retentate[d]	-	85.5±1.4	2.6

[a] Feed: 20 g of lecithin dissolved in 180 g of hexane.

[b] Hexane free basis. Values are expressed as means ± SD of duplicate (n=2) measurements.

[c] Retentate (25 g) obtained after four steps of diafiltration with 100 g of hexane addition in each step.

[d] Retentate (25 g) obtained after three steps of diafiltration with 100 g of hexane addition in each step.

PL content in the lecithin as well as the amount of solvent used in the process could affect the PL and oil contents in the deoiled lecithin. It is possible to increase the PL content further (above 90%) with a corresponding decrease in the oil content in deoiled lecithin by increasing the solvent volume during diafiltration. Continuous diafiltration is preferred to discontinuous diafiltration in many instances during plant operations. However, considering the practical difficulties in carrying out continuous diafiltration in a batch membrane cell, a simulated run was subsequently attempted.

8.3 Deoiling of lecithin during simulated continuous diafiltration

The AI and PL contents of soy lecithin samples during simulated continuous diafiltration are presented in Table 8.3. During the simulated run, the AI content in soy lecithin improved from an initial level of 63.2 to 95.7% in the retentate fraction after ten steps of diafiltration (200% dilution to feed) and the corresponding increase in the PL content was 91.3% from 57.7%. Continuous diafiltration resulted in higher PL content in the deoiled lecithin (91.3%) compared to discontinuous diafiltration (85.7%) for an equal amount of hexane dilution in the process (Table 8.2). Besides, continuous diafiltration of the lecithin mixture helped in reducing the viscosity of the feed sample right from the start of the run and enabled a higher permeation rate (1.5 LMH) when compared to discontinuous diafiltration run (0.6 LMH), thus reducing the processing time.

8.4 Composition of individual PL

The PL content in CSBO and CRBO reported to be in the range of 1.5-2.1% (Sipos and Szuhaj, 1996b) and 4-5% (Orthoefer, 1996), respectively. The

Table 8.3 Membrane deoiling of soy lecithin in a simulated continuous diafiltration run

Sample description	Weight (g)	Hexane addition (g)	AI[a] (%)	PL[a] (%)
Feed[b]	200	180	63.2±0.7	57.7±1.3
Intermediate feed	~190	~40[c]	-	-
Final retentate[d]	~30	0	95.7±1.3	91.3±1.4

[a] Hexane free basis. Values are expressed as means ± SD of duplicate (n=2) measurements.

[b] 20 g of lecithin (lot-1) dissolved in 180 g of hexane.

[c] Hexane added during each diafiltration step.

[d] Retentate obtained after ten stages of diafiltration.

typical composition of individual PL in soy lecithin, PC, PI, PE and others are in the ratio of 28:25:17:30 (Sipos and Szuhaj, 1996a). The individual PL in soy and rice bran lecithins before and after membrane deoiling are presented in Table 8.4. PC, PI and PE together contributed to 57.3% (lot-1) and 30.8% (lot-2) of total PL in the soy lecithin and their composition was not altered much during membrane processing. In the case of rice bran lecithin, the contribution of PC, PI and PE was only 4.9% indicating the greater presence of other PL. Besides, the composition of PL in the processed rice bran lecithin altered significantly during processing and the contents of PC, PI and PE together increased to 7.9%. PC has the highest hydration rate among the various PL followed by PI, PE and PA besides having the ability to encapsulate other PL (Segers and Sande, 1990). In the case of rice bran lecithin used in the present study, PC content was too low (<3%). Therefore most of the less hydrating PL such as salts of PE and PA could exist as monomers, facilitating their permeation and consequently leading to the

increase in the concentration of PC, PI and PE existing as reverse micelles, in the retentate fraction (deoiled lecithin).

Table 8.4 Individual PL contents in lecithin samples[a]

Sample description	PL (%)	Individual PL (mg/kg)			PC+PI+PE (%)
		PC	PI	PE	
Soy lecithin (lot-1)[b]					
Feed	57.7±1.3	130000	68400	132000	33.0±1.5
Deoiled	91.3±1.4	220000	124000	220000	56.4±1.6
Soy lecithin (lot-2)[c]					
Feed	56.5±1.0	82000	23300	68700	17.4±0.4
Deoiled	85.7±1.3	124000	32500	91000	24.8±0.2
Rice bran lecithin[c]					
Feed	33.3±0.3	9150	5220	2090	1.6±0.1
Deoiled	85.5±1.4	42500	14000	10800	6.7±0.8

[a] Values are expressed as means ± SD of duplicate (n=2) measurements.

[b] Samples of simulated continuous diafiltration run.

[c] Samples of discontinuous diafiltration run.

8.5 Colour of the deoiled lecithin

The visible spectra of soy lecithin samples (feed, permeate and deoiled lecithin) are shown in Fig. 8.1. The area under the absorbance spectra between 350 and 550 nm gives a rough measure of all pigments that exhibit absorption in the red-yellow region. The colour of the lecithin improved after membrane processing as revealed by the reduction in the area of the spectra (~60%). The spectra of the permeate of soy lecithin was similar to the typical spectra of crude SBO (Subramanian *et al.*, 1998a). Carotenoids

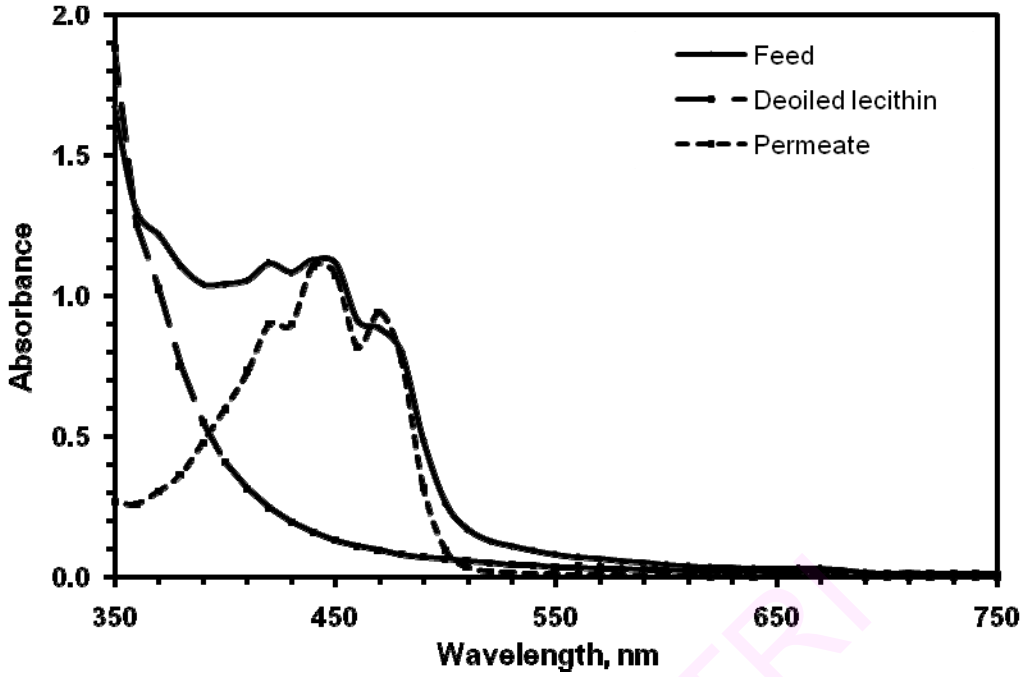


Fig. 8.1 Colour reduction in soy lecithin during membrane deoiling process (simulated continuous diafiltration)

(xanthophylls) are the predominant red/yellow pigments in CSBO and have a characteristic absorption around 450 nm in the visible range. Earlier studies from this laboratory revealed that the nonporous hydrophobic membrane rejected oxygenated carotenoids (xanthophylls-lutein) to a greater extent while largely allowing the permeation of hydrocarbon carotenoids (ß-carotene) in undiluted model systems owing to the nature of their polarity (Subramanian *et al.*, 2001b). Another study revealed that the rejection of carotenoids in SBO reduced with increased hexane dilution of oil (Kondal Reddy *et al.*, 2001). In the present study, carotenoids largely permeated through the membrane owing to the greater extent of hexane dilution involved in the process and as a result, the spectra of the deoiled lecithin did not show the presence of these pigments. In such systems, the solubility of individual compounds in the solvent is also important in addition to their solubility in the

membrane material. Probably, the spectra of the retentate could be used as an indirect indicator of the oil present in the lecithin and a measure of performance of deoiling process. The colour of the membrane deoiled soy lecithin was lighter while the conventionally processed lecithin by acetone-extraction is generally darker without bleaching.

In RBO Maillard browning products are also present, besides carotenoids and chlorophyll (Orthoefer, 1996). During membrane processing the colour reduction in RBO ranged between 43 and 53% for hexane-diluted samples whereas it was 74% in the undiluted samples (Saravanan *et al.*, 2006). The colour compounds present in RBO may contribute for the colour of its lecithin besides other degradation products formed during the process and storage. The visible spectra of rice bran lecithin indicated that carotenoids did not significantly contribute to its colour (Fig. 8.2). Further, comparison of the spectra of feed and deoiled lecithin showed that the

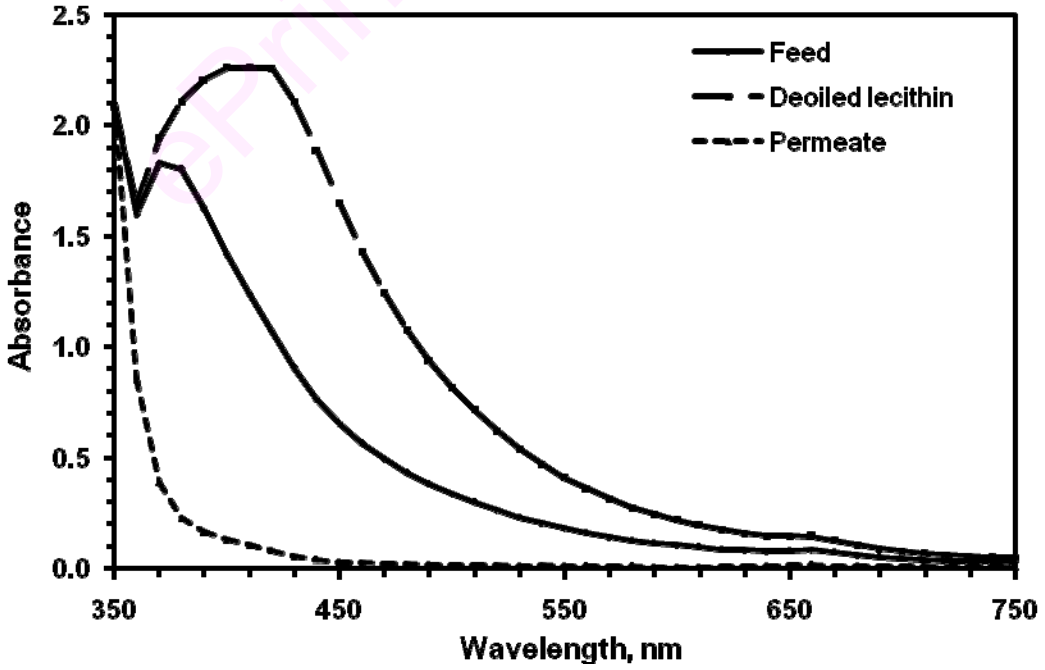


Fig. 8.2 Visible spectra of rice bran lecithin during the membrane deoiling process (discontinuous diafiltration)

majority of the degradation products contributing to the colour were retained by the membrane even under hexane-diluted conditions. This is also reflected in the greater reduction in colour of the permeate fraction. The results suggest that it may be necessary to include a conventional bleaching step in the processing of rice bran lecithin.

8.6 Improvement in PL content in lecithin by nonporous membrane

In all the experimental runs on deoiling lecithin described above, the AI content and so also the PL content in lecithin samples increased to a greater extent depending on their initial levels as well as the conditions employed during membrane processing. PL are amphiphilic molecules containing hydrophilic polar heads and hydrophobic non-polar tails with an average molecular weight of around 600-800 Da. These surfactant molecules form reverse micelles in nonaqueous systems and it has been reported that the size of the mixed micelles in SBO-hexane system is between 18 and 200 nm (Gupta, 1986). High rejection of PL by nonporous membranes in undiluted (Subramanian *et al.*, 2004) and hexane-diluted oils (Saravanan *et al.*, 2006; Sarita Arora *et al.*, 2006) was primarily attributed to its low solubility in the membrane material. In the case of hexane- oil miscella systems with PL content above CMC, size exclusion may provide a synergistic effect as the size of reverse micelles would be much larger (Sarita Arora *et al.*, 2006). Although the major constituents of crude oil and lecithin are the same, their concentrations are quite different in these two systems. In crude oils, the PL content varies from 1 to 5%, whereas PL is the major portion (~55-60%) in lecithin while the oil predominantly accounts for the rest. In the case of

nonporous membranes, separation/permeation of components depends on their own solubility and diffusivity in the membrane material. In addition, it also depends on the coupling effect as well as the solubility of individual components in other permeating components including the solvent being used. All these factors are dependent on the concentration of individual components. In spite of the vast difference in the concentrations of major constituents, the nonporous membrane showed excellent selectivity for PL in lecithin-hexane system as in the case of oil-hexane system studied earlier (Saravanan *et al.*, 2006).

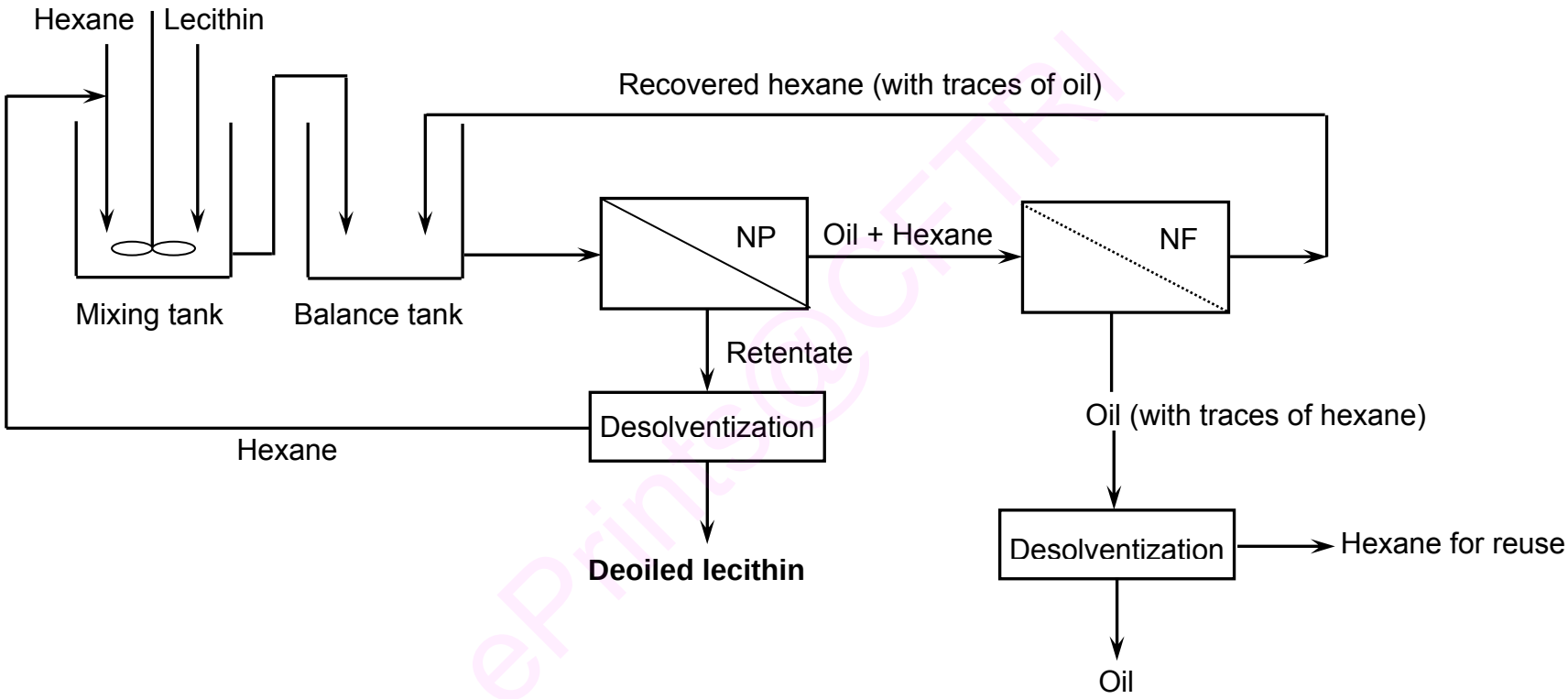
8.7 Proposed scheme

The proposed process for deoiling lecithin involves dissolving lecithin in hexane and processing through a nonporous membrane in diafiltration mode of operation, followed by desolventization of the retentate fraction by thermal evaporation of hexane under vacuum. A large amount of hexane (~44 L hexane/1 kg lecithin) is used in the process as dilution and diafiltration steps are involved in the process to obtain deoiled lecithin with a higher PL/AI content. The hexane need to be recovered and reused in the process. The energy requirement in the conventional thermal desolventization is very high as it involves a phase change and thus has a high impact on the process economics. NF membranes could be used for hexane recovery and Raman *et al.* (1996a) reported 50% reduction in the energy required for evaporation of hexane. NF membranes exhibited ~85-90% rejection of TG in hexane-oil mixtures (Stafie *et al.*, 2004) which may be reasonable enough for employing NF in the process. Therefore, a NF step has been incorporated for the

recovery of hexane from the permeate stream of the nonporous membrane (Scheme 8.1) and the recovered hexane is recycled back in the nonporous membrane section for continuous diafiltration. The integrated membrane process as illustrated in Scheme 8.1 would be economically more attractive.

8.8 Conclusions

Processing lecithin by physical means using nonporous polymeric membranes under mild process conditions resulted in a product which is practically free from oil and the proposed process offers certain advantages over acetone- extraction (industrially-practiced) and SCFE (proposed alternative) processes. Nonporous membranes tend to offer greater yield compared to the UF based membrane process owing to their greater retention of PL including probably their monomers. These membranes showed substantial reduction in colour in soy lecithin, but increased intensity of colour in rice bran lecithin suggests the necessity of including a conventional bleaching step.



Scheme 8.1 Proposed scheme for deoiling lecithin

An exhaustive review of the research attempts made towards degumming, dewaxing, decolourizing and deacidifying edible oils using membrane technology suggested UF for simultaneous degumming and dewaxing of oils. However, carotenoids rejection and Lovibond colour reduction was not consistent and varied drastically between oils with UF membranes. Nonporous membranes offer several advantages over UF membranes for processing hexane-oil miscella, in terms of higher rejection of PL, carotenoids and chlorophyll. The membrane process will become economically attractive if it is even partially effective for colour removal in addition to PL reduction. Besides, the nonporous membranes seemed to posses the ability for wax removal as well. Accordingly nonporous membranes were investigated for degumming, dewaxing and decolourizing CRBO which posed many challenges in processing.

Characterization of PL reverse micelles

The influence of PL composition and solvent (hexane) medium on the CMC levels in undiluted and hexane-diluted crude vegetable oils was investigated to evaluate the PL rejection mechanism during membrane degumming of oil systems varying in PL contents (below and above CMC) as well as to assess the degumming performance of UF and nonporous membranes. The fast hydrating PC had an influence on CMC of mixed PL in oil and hexane-oil systems. CMC levels in real and model systems indicated that higher PC to PL ratio lowers the CMC value and vice versa. Hexane as a solvent in the system showed greater influence on reverse micelle formation due to its hydrophobic nature. Accordingly, the CMC of PL was lower in hexane-diluted

systems (520 mg/kg for lecithin in hexane) when compared to undiluted oil systems (850 mg/kg for lecithin in SBO) owing to the greater hydrophobic-repulsive forces between hexane and hydrophilic polar heads of PL. The initial PL content played a crucial role in the rejection performance of UF membrane. The PL rejection of UF membrane was near complete when the PL content of system was above CMC. Among the systems with lower PL contents (<CMC), rejection was greater in hexane-diluted systems (82-99% for lecithin in hexane system) than in undiluted oil systems (~40% for sunflower oil system) owing to greater concentration polarization effect responsible for reverse micelle formation at the membrane surface leading to their subsequent rejection. UF membranes are more suitable for industrial adoption owing to higher productivity and their rejection performance could be kept high by careful manipulation of initial PL content. On the other hand, nonporous membranes showed almost complete degumming in vegetable oils irrespective of initial PL content and the type of system (undiluted and hexane-diluted).

Enhancement of oil flux in nonporous membrane with hexane dilution

Permeate flux plays an important role in determining the process economics. As the oil flux in nonporous membranes was very low, the factors influencing the permeate flux was studied with various oils (MO, GNO, RBO, SFO and CNO) for achieving greater permeate flux. Hexane dilution improved the permeate oil flux in all the vegetable oils by at least one order of magnitude and applied pressure increased the total flux as well as oil flux. Operating pressure of 2-3 MPa and 2-4 fold hexane dilution were adequate to achieve

the maximum permeate oil flux among the various oils studied. The effect of hexane dilution on oil flux was more prominent in the most viscous MO (29 fold increase) while it was less prominent in the case of least viscous CNO (15 fold increase). However, there is a critical limit of hexane dilution beyond which the oil flux did not show an incremental improvement although it resulted in an increase in total flux. All the oils exhibited an inverse relationship between viscosity and total flux under undiluted and various levels of hexane-diluted conditions. The oil flux of undiluted as well as hexane-diluted oils interestingly followed an inverse relationship with the average molecular weights of TG, despite their narrow range (670-961 Da) of existence. The nonporous membrane did not show any selectivity over a wide range of hexane dilution (5-80% oil concentration) and operating pressure (0.5-4 MPa) indicating unit positive coupling between TG and hexane, which was in contrast to TG rejection behavior reported for NF membranes from the same membrane material (PDMS) with a similar nonaqueous system. This suggests that membrane structure could play a vital role in deciding the transport phenomenon, typically the swelling nature and subsequent pore size formed in the polymer network.

Simultaneous degumming, dewaxing and decolourizing CRBO

The efficacy of nonporous membranes was evaluated for simultaneous degumming, decolourizing and dewaxing CRBO. Membrane processing of CRBO showed that PL were almost completely rejected (>99%) by nonporous membranes in both industrial and laboratory prepared samples. Phosphorus levels in the membrane-processed RBO samples were very low (<10 mg/kg)

which indicated that both hydratable as well as nonhydratable PL were effectively rejected by the membrane. NMR spectroscopy confirmed the elimination of PGL in the membrane process, which is known to cause problems in the subsequent processing steps affecting the colour of the processed oil. Further, ^{1}H, ^{13}C and ^{31}P NMR analyses clearly aided in arriving at the structural nature of the PGL present in CRBO. The colour reduction measured in terms of area under the visible spectra (350-550 nm) was to the extent of 50-55%. The membrane process also reduced the wax content to the extent of 40-50%. Hexane dilution improved the oil flux by one order of magnitude without affecting the selectivity to a greater extent. Simultaneous membrane degumming, dewaxing and decolourization appears to be a potential process meeting the specific pretreatment challenges posed by CRBO and to realize the benefits of physical refining in terms of better quality final product as well as higher yield.

Enzymatic degumming

The efficacy of enzymatic degumming was assessed using the third generation phospholipase-A1 (Lecitase-Ultra from *Thermomyces lanuginosus/Fusarium oxysporum* with higher thermal stability up to 60°C) with CRBO. Lecitase-Ultra was effective and reduced the phosphorus content in the oil to less than 10 mg/kg from an initial level of 390 mg/kg after 2 h of incubation period at 50°C. Although Lecitase-Ultra exhibited independently higher lipase activity than phospholipase activity under assay conditions, the hydrolysis of PL (80%) was favourably higher than TG (20%) under the conditions employed for oil degumming. The efficacy of enzymatic

degumming of RBO using this microbial phospholipase was also studied in solvent phase for its wider applicability. However, practically there was no PL reduction at lower water content (2%) even after 5 h of incubation period due to the poor contact between the highly nonpolar solvent and enzyme (polar). Increasing the water addition to 20% reduced the phosphorus level in the degummed-oil to 71 mg/kg but did not match the performance of oil-phase degumming. A scheme for enzyme recycle employing membrane separation technique has been proposed which would offer several process benefits.

Enriching oryzanol in RBO

Oryzanol is associated with various physiological functions; however, these beneficial ferulate esters are lost to a greater extent during conventional refining of CRBO depleting their content in the processed oil. In the present investigation, oryzanol enrichment in RBO was attempted using nonporous polymeric membranes under undiluted as well as hexane-diluted conditions with different (crude, refined and model oil) systems varying widely in their oryzanol content. During membrane processing, oryzanol content in the refined RBO increased from 2,420 to 7,340 mg/kg (~3 fold enrichment). Processing crude oil and model oil systems, the oryzanol content in the oil improved from 17,600 to 27,300 mg/kg and 20,400 to 30,300 mg/kg, respectively. The enrichment of oryzanol was due to its moderate rejection by the nonporous hydrophobic membrane owing to the hydrophilic nature of the ferulic esters. Hexane dilution improved the oil flux by one order of magnitude but reduced the selectivity. Membrane processing could be used for concentration/enrichment of oryzanol present in RBO by physical means

under mild process conditions thus offering advantages over the methods proposed earlier (solvent extraction, SCFE and SPVD). ERBO may find wider applications in the pharmaceutical, therapeutic and dietary preparations as well as in producing standard cooking oil with guaranteed oryzanol content.

Deoiling of lecithin

Deoiling of lecithin using a nonporous membrane was examined in a favourable solvent (hexane) medium with soy and rice bran lecithins. During the membrane process, AI content of soy lecithin increased from 63.2 to 81.0% in a single-step batch operation. The membrane exhibited an excellent selectivity since PL reverse micelles formed in the system were rejected almost completely due to low solubility probably aided synergistically by size exclusion. Diafiltration achieved greater oil removal from lecithin as reflected in terms of higher AI and PL contents in the deoiled lecithin. In discontinuous diafiltration, the PL content increased from 33.3 to 85.5% in rice bran lecithin (150% dilution to feed) and 56.6 to 85.7% in soy lecithin (200% dilution), respectively. The simulated continuous diafiltration run showed slightly greater PL content in soy lecithin (91.3%) compared to discontinuous diafiltration (89.7%) besides offering higher productivity. The membrane showed a colour reduction of ~60% in soy lecithin but there was no improvement in rice bran lecithin due to the retention of degradation products which suggests the necessity of including a conventional bleaching step. Processing lecithin by physical means using nonporous polymeric membranes under mild process conditions resulted in a product which is practically free from oil and offers certain advantages over the currently

practiced and proposed processes. The proposed integrated membrane process with nonporous (deoiling) and NF (solvent recovery) membranes could be an attractive preposition besides being an acetone free process.

Our research efforts employing membrane technology revealed its prospects as a single step pretreatment process for simultaneous degumming, dewaxing and decolourization of crude vegetable oils besides its potential applications towards enrichment of oryzanol in RBO and deoiling lecithin. In addition to high selectivity, productivity and longevity of membranes are important factors for industrial adoption. In this regard, development of suitable hexane-resistant membranes offering higher flux is necessary.

The results obtained in this study would also be useful in extending the application of nonporous denser membranes for the separation/concentration of various constituents in other nonaqueous systems. We hope that this knowledge will enhance the efforts towards industrialization of membrane technology for various nonaqueous applications including the production of good quality of edible oil.

Anderson, D., A Primer on Oils Processing Technology, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons Inc., New York, 1996, Vol. 4, pp. 1-60.

AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th edn., edited by Firestone, D., AOCS Press, Champaign, 1998, Methods Ca 12-55, Cc 13e-92, Ce 1-62 and Ja 4-46.

Barton, A.F.M., *Handbook of Solubility Parameters and Other Cohesion Parameters*, 2nd edn., CRC Press, Boca Raton, 1991.

Bhosle, B.M. and R. Subramanian, New Approaches in Deacidification of Edible Oils - A Review, *J. Food Eng.,* **69**:481-494 (2005).

Bhosle, B.M., K. Ebert and R. Subramanian, Deacidifying Vegetable Oils Using Polymeric Membranes, *Eur. J. Lipid Sci. Technol.,* **107**:746-753 (2005).

Bockisch, M., *Fats and Oils Handbook,* AOCS Press, Champaign, 1998, pp. 53-120.

Boekenoogen, H.A., *Analysis and Characterization of Oils, Fats and Fat Products*, John Wiley & Sons Inc., London, 1968, pp. 59-67.

Chen, D., K.W. Linda and W.W. Harold, Using Ultrasound to Reduce Ceramic Membrane Fouling by Silica Particles, in *Proceedings of American Chemical Society,* Florida, 2002, pp. 166-169.

Cherukuri, R.S.V. and C. Rukmini, Process for Obtaining Micronutrient Enriched Rice Bran Oil, U.S. Patent 5,985,344 (1999).

Cheryan, M., *Ultrafiltration and Microfiltration Handbook,* 2nd edn., Technomic, Lancaster, 1998, pp. 293-298, 406-413.

Cleenerwerck, B. and A.J. Dijkstra, The Total Degumming Process – Theory and Industrial Application in Refining and Hydrogenation, *Fat Sci. Technol*., **94**:317-322 (1992).

Clausen, K., Enzymatic Oil-Degumming by a Novel Microbial Phospholipase, *Eur. J. Lipid Sci. Technol.,* **103**:333-340 (2001).

Codex Alimantarius Commission report, Joint FAO/WHO Foods Standards Programme, Codex Committee on Fats and Oils, 18th session, 3-7 February, London, 2003.

Das, P.K., A. Chaudhuri, T.N.B. Kaimal and U.T. Bhalerao, Isolation of γ-Oryzanol Through Calcium Ion Induced Precipitation of Anionic Micellar Aggregates, *J. Agric. Food Chem.*, **46**:3073-3080 (1998).

Davidson, H.F., E.J. Campbell, R.J. Bell and R.A. Pritchard, Sunflower Oil, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons Inc., New York, 1996, Vol. 2, pp. 603-689.

de Moura, J.M.L.N., L.A.G. Goncalves, J.C.C. Petrus and L.A. Viotto, Degumming of Vegetable Oil by Microporous Membrane, *J. Food Eng.,* **70**:473-478 (2005).

De, B.K., R. Das, B.K. Dutta and D.K. Bhattacharyya, Membrane Degumming and Dewaxing of Rice Bran Oil and Its Refining, *Fett/Lipid*, **100**:416-421 (1998).

Diosady, L.L., P. Sleggs and T. Kaji, Chemical Degumming of Canola Oils, *J. Am. Oil Chem. Soc.,* **59**:313-316 (1982).

Dunford, N.T. and J.W. King, Phytosterol Enrichment of Rice Bran Oil by a Supercritical Carbon Dioxide Fractionation Technique, *J. Food Sci.,* **65**: 1395-1399 (2000).

Eggers, R. and H. Wagner, Extraction Device for High Viscous Media in a High-Turbulent Two Phase Flow with Supercritical Fluid Extraction with Supercritical Carbon Dioxide, *J. Supercrit. Fluids,* **6**:31-37 (1993).

Eskin, N.A.M., B.E. Mcdonald, R. Przybylski, L.J. Malcolmson, R. Scarth, T. Mag, K. Ward and D. Adolph, Canola Oil, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons Inc., New York, 1996, Vol. 2, pp. 1-95.

Garcia, A., S. Alvarez, F. Riera, R. Alvarez and J. Coca, Sunflower Oil Miscella Degumming with Polyethersulfone Membranes-Effect of Process Conditions and MWCO on Fluxes and Rejections, *J. Food Eng.,* **74**:516-522 (2006).

Gayot, S., X. Santerelli and D. Coulson, Modification of Flavonoid Using Lipase in Non-Conventional Media: Effect of the Water Content, *J. Biotechnol*., **101**:29-36 (2003).

Grunwald, P., Preparation and Application of Immobilized Phospholipases, in *Enzymes in Lipid Modification*, edited by Bornscheuer, John Wiley & Sons Inc., Wienheim, 2000, pp. 263-291.

Gupta, A.K.S., Novel Developments in Refining of Edible Oils, *Fette Seifen Anstrichm.,* **88**:79-86 (1986).

Gupta, A.K.S., Process for Refining Crude Glyceride Oils by Membrane Filtration, U.S. Patent 4,062,882 (1977).

Hahn-Hagardal, B., Water Activity: A Possible External Regulator in Biotechnical Processes, *Enzyme Microb. Technol.*, **8**:322-327 (1986).

Halling, P.J., Organic Liquids and Biocatalysts: Theory and Practice, *Trends Biotechnol*., **7**:50-52 (1989).

Halling, P.J., Thermodynamic Predictions for Biocatalysis in Non-Conventional Media: Theory, Dynamics Predictions and Recommendations for Experimental Design and Analysis, *Enzyme Microb. Technol.*, **16**:178-206 (1994).

Hancer, M., A. Patist, R.T. Kean and H.S. Muralidhara, Micelletization and Adsorption of Phospholipids and Soybean Oil into Hydrophilic and Hydrophobic Surfaces in Nonaqueous Media, *Colliods Surf., A*, **204**:31-41 (2002).

Haraldsson, G., Degumming, Dewaxing and Refining, *J. Am. Oil Chem. Soc.,* **60**:251-256 (1983).

Hitotsumatsu, H. and Y. Takeshita, Process for Producing Rice Bran Oil, U.S. Patent 5,290,579 (1994).

Hodgson, A.S., Refining and Bleaching, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons Inc., New York, 1996, Vol. 4, pp. 157-212.

Hurst, W.J. and R.A. Martin, The Analysis of Phospholipids in Soy Lecithin by HPLC, *J. Am. Oil Chem. Soc*., **61**:1462-1463 (1984).

Hutton, K.J. and J.S. Guymon, Process for Producing Deoiled Phosphatides, U.S. Patent 6,140,519 (2000).

Ichikawa, S., S. Sugiura, M. Nakajima, Y. Sano, M. Seki and S. Furusaki, Formation of Biocompatible Reversed Micellar Systems Using Phospholipids, *Biochem. Eng. J.*, **6**:193-199 (2000).

Iwama, A., New Process for Purifying Soybean Oil by Membrane Separation and Economical Evaluation of the Process, *J. Am. Oil Chem. Soc*., **64**:1258 (1987).

Jirjis, B., H.S. Muralidhara and D.D. Otten, Method for Removing Phospholipid from Vegetable Oil Miscella, Method for Conditioning a Polymeric Ultrafiltration Membrane, and Membrane, U.S. Patent 6,207,209 (2001).

Kaimal, T.N.B., S.R. Vali, B.V.S.K. Rao, P.P. Chakrabarthy, P. Vijayalakshmi, V. Kale, K.N.P. Rani, O. Rajamma, P.S. Bhaskar and T.C. Rao, Origin of Problems Encountered in Rice Bran Oil Processing, *Eur. J. Lipid Sci. Technol.*, **104**:203-211 (2002).

Kale, V., S.P.R. Katikaneni and M. Cheryan, Deacidifying Rice Bran Oil by Solvent Extraction and Membrane Technology, *J. Am. Oil Chem. Soc*., **76**:723-727 (1999).

Kanamoto, R., Y. Wada, G. Miyajima and M. Kito, Phospholipid-Phospholipid Interaction in Soybean Oil, *J. Am. Oil Chem. Soc.,* **58**:1050-1053 (1981).

Karan, A., Separation of Oryzanol from Crude Rice Bran Oil, M.S. Thesis, Toronto University, Canada, 1998.

Keurentjes, J.T.F., G.I. Doornbusch and K. van't Riet, The Removal of Fatty Acids from Edible oil: Removal of the Dispersed Phase of Water-in-Oil Dispersion by a Hydrophilic Membrane, *Sep. Sci. Technol*., **26**:409-423 (1991).

Keurentjes, J.T.F., J.T.M. Sluijs, R.J.H. Franssen and K. van't Riet, Extraction and Fractionation of Fatty Acids from Oil Using an Ultrafiltration Membrane, *Ind. Eng. Chem. Res*., **31**:581-587 (1992).

Kim, I.C., J.H. Kim, K.H. Lee and T.M. Tak, Phospholipid Separation (Degumming) from Crude Vegetable Oil by Polyamide Ultrafiltration Membrane, *J. Membr. Sci.,* **205**:113-123 (2002).

Klaus, D., An Enzymatic Process for the Physical Refining of Seed Oils, *Chem. Eng. Technol*., **21**:3-6 (1998).

Kondal Reddy, K., M. Nakajima, J.B. Snape, T. Kawakastu, S. Ichikawa and H. Nabetani, Membrane Decolorization of Crude Oil, in *Proceedings of International Congress on Membranes and Membrane Processes (ICOM '96)*, Yokohama, Japan, 1996, pp. 894-895.

Kondal Reddy, K., R. Subramanian, T. Kawakatsu and M. Nakajima, Decolorization of Vegetable Oils by Membrane Processing, *Eur. Food Res. Technol.,* **213**:212-218 (2001).

Koseoglu, S.S. and D.E. Engelgau, Membrane Applications and Research in the Edible Oil Industry: An Assessment, *J. Am. Oil Chem. Soc*., **67**:239-249 (1990).

Koseoglu, S.S., K.C. Rhee and E.W. Lusas, Membrane Processing of Crude Vegetable Oils: Laboratory Scale Membrane Degumming, Refining and Bleaching, in *Proceedings of World Conference on Edible Fats and Oils Processing: Basic Principles and Modern Practices*, edited by D.R. Erickson, American Oil Chemists' Society, Champaign, IL, 1990, pp.182-188.

Krishna Kumar, N.S. and D.N. Bhowmick, Separation of Fatty Acids/Triacylglycerol by Membranes, *J. Am. Oil Chem. Soc*., **73**:399-401 (1996).

Lin, N., K.C. Rhee and S.S. Koseoglu, Bench-Scale Membrane Degumming of Crude Vegetable Oil: Process Optimization, *J. Membr. Sci.,* **134**:101-108 (1997).

Machado, D.R., D. Hasson and R. Semiat, Effect of Solvent Properties on Permeate Flow Through Nanofiltration Membranes. Part I: Investigation of Parameters Affecting Solvent Flux, *J. Membr. Sci.,* **163**:93-102 (1999).

Manjula, S. and R. Subramanian, Membrane Technology in Degumming, Dewaxing, Deacidifying, and Decolorizing Edible Oils, *Crit. Rev. Food Sci. Nutr.,* **46**:569-592 (2006).

McClements, D.J., *Food Emulsions – Principles, Practice and Techniques,* CRC Press, Boca Raton, 1999, pp. 83-159.

Miki, S., M. Kusuda, A. Iwama and T. Ochiai, Application of UF membrane process for purifying soybean oil by plate-type module having thin channels, in *Proceedings of Sessions Lectures and Scientific Presentations on ISF-JOCS World Congress,* Japan Oil Chemists' Society, 1988, Vol. 1, pp. 95-101.

Muralidhara, H.S., B.F. Jirjis and G.F. Seymour, Process for Removing Vegetable Oil Waxes by Fast Cooling Vegetable Oil and Using a Porous Non-Metallic Inorganic Filter, U.S. Patent 5, 482, 633 (1996).

Mutoh, Y., K. Matsuda, M. Ohshima and H. Ohuchi, Method of Dewaxing a Vegetable Oil, U.S. Patent 4,545,940 (1985).

Narayan, A.V., R.S. Bharate and K.S.M.S. Raghavarao, Extraction and Purification of Oryzanol from Rice Bran Oil and Rice Bran Oil Soapstock, *J. Am. Oil Chem. Soc*., **83**:1-8 (2006).

Orthoefer, F.T., Rice Bran Oil, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons Inc., New York, 1996, Vol. 2, pp. 393-409.

Pagliero, C., N. Ochoa, J. Marchese and M. Mattea, Degumming of Crude Soybean Oil by Ultrafiltration using Polymeric Membranes, *J. Am. Oil Chem. Soc.,* **78**:793-796 (2001).

Pagliero, C., N. Ochoa, J. Marchese and M. Mattea, Vegetable Oil Degumming with Polyimide and Polyvinylideneflouride Ultrafiltration Membranes, *J. Chem. Technol. Biotechnol*., **79**:148-152 (2004).

Pragasam, A., T.N. Indira and A.G. Gopala Krishna, Preparation and Physico Chemical Characteristics Evaluation of Rice Bran Lecithin in Relation to Soya Lecithin, *Bev. Food World,* **12**:19-22 (2002).

Rajam, L., D.R. Soban Kumar, A. Sunderesan and C. Armugahan, A Novel Process for Physically Refining Rice Bran Oil Through Simultaneous Degumming and Dewaxing*, J. Am. Oil Chem. Soc.*, **82**:213-220 (2005).

Raman, L.P., M. Cheryan and N. Rajagopalan, Solvent Recovery and Partial Deacidification of Vegetable Oils by Membrane Technology, *Lipid,* **98**:10-14 (1996a).

Raman, L.P., M. Cheryan and N. Rajagopalan, Deacidification of Soybean Oil by Membrane Technology, *J. Am. Oil Chem. Soc.,* **73**:219-224 (1996b).

Raman, L.P., N. Rajagopalan and M. Cheryan, Membrane Technology (in Vegetable Oil Processing), *Oils Fats Int.,* **10**:28-38 (1994).

Ramaswamy, K.G., A.G. Gopalakrishna and D.P. Sen, Refining of Rice Bran Oil, *J. Oil Technol. Assoc*. *India*, Jan/Mar:16-19 (1980).

Roy, S.K., B.V.S.K. Rao and R.B.N. Prasad, Enzymatic Degumming of Rice Bran Oil, *J. Am. Oil Chem. Soc*., **79**:845-846 (2002).

Saravanan, M., B.M. Bhosle and R. Subramanian, Processing Hexane-Oil Miscella using Nonporous Polymeric Composite Membranes, *J. Food Eng*., **74**:529-535 (2006).

Sarita Arora, S. Manjula, A.G. Gopala Krishna and R. Subramanian, Membrane Processing of Crude Palm Oil, *Desalination,* **191**:454-466 (2006).

Sarkar, B., S. Das Gupta and S. De, Application of External Electric Field to Enhance the Permeate Flux During Micellar Enhanced Ultrafiltration, *Sep. Purif. Technol*., **66**:263-272 (2009).

Schneider, M., Fractionation and Purification of Phospholipids, in *Lecithins: Sources, Manufacture and Uses*, edited by B.F. Szuhaj, AOCS Press, Champaign, 1989, pp. 109-130.

Seetharamaiah, G.S. and J.V. Prabhakar, Oryzanol Content of Indian Rice Bran Oil and Its Extraction from Soap Stock, *J. Food Sci. Technol.,* **23**:270-273 (1986).

Segers, J.C. and R.L.K.M. van der Sande, Degumming - Theory and Practice, in *Proceedings of the World Conference on Edible Fats and Oils*

Processing: Basic Principles and Modern Practices, American Oil Chemists' Society, Champaign, IL, 1990, pp. 88-93.

Segers, J.C., Superdegumming, a New Degumming Process and its Effect on the Effluent Problems of Edible Oil Refining, *Fette Seifen Anstrichm.,* **84**:543-546 (1982).

Sheelu, G., G. Kavitha, W. Nitin and W. Fadnavis, Efficient Immobilization of Lecitase in Gelatin Hydrogel and Degumming of Rice Bran Oil Using a Spinning Basket Reactor, *J. Am. Oil Chem. Soc.,* **85**:739-748 (2008).

Singh, R.P., R.K. Khanna, A. Mathur and R. Srivastava, Isolation of Oryzanol Concentrate from Rice Bran Oil, *J. Oil Technol. Assoc. India,* **32**:55-58 (2000).

Sipos, E.F. and B.F. Szuhaj, Lecithins, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons Inc., New York, 1996a, Vol. 1, pp. 311-395.

Sipos, E.F. and B.F. Szuhaj, Soybean Oil, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons Inc., New York, 1996b, Vol. 2, pp. 497-601.

Snape, J.B. and M. Nakajima, Processing of Agricultural Fats and Oils Using Membrane Technology, *J. Food Eng.*, **30**:1-41 (1996).

Solvent Extractors Association of India, 29th All India Seminar on Rabi Oilseeds, Oil Trade and Industry, Agra, India, 2nd March, 2008.

Stafie, N., D.F. Stamatialis and M. Wessling, Insight into the Transport of Hexane-Solute Systems Through Tailor-Made Compostite Membranes, *J. Membr. Sci.,* **228**:103-116 (2004).

Stamatialis, D.F., N. Stafie, K. Buadua, M. Hempenius and M. Wessling, Observations on the Permeation Performance of Solvent Resistant Nanofiltration Membranes, *J. Membr. Sci*., **279**:424-433 (2006).

Subramanian, R. and M. Nakajima, Membrane Degumming of Crude Soybean and Rapeseed Oils, *J. Am. Oil Chem. Soc.,* **74**:971-975 (1997).

Subramanian, R., H. Nabetani, M. Nakajima., S. Ichikawa, T. Kimura and T. Maekawa, Rejection of Carotenoids in Oil Systems by a Nonporous Polymeric Composite Membrane, *J. Am. Oil Chem. Soc.,* **78**:803-807 (2001b).

Subramanian, R., K.S.M.S. Raghavarao, H. Nabetani, M. Nakajima, T. Kimura and T. Maekawa, Differential Permeation of Oil Constituents in Nonporous Denser Polymeric Membranes, *J. Membr. Sci*., **187**:57-69 (2001c).

Subramanian, R., K.S.M.S. Raghavarao, M. Nakajima, H. Nabetani, T. Yamaguchi and T. Kimura, Application of Dense Membrane Theory for Differential Permeation of Vegetable Oil Constituents, *J. Food Eng.,* **60**:249-256 (2003).

Subramanian, R., M. Nakajima and T. Kawakatsu, Processing of Vegetable Oils Using Polymeric Composite Membranes, *J. Food Eng.*, **38**:41-56 (1998a).

Subramanian, R., M. Nakajima, A. Yasui, H. Nabetani, T. Kimura and T. Maekawa, Evaluation of Surfactant-Aided Degumming of Vegetables Oils by Membrane Technology, *J. Am. Oil Chem. Soc.,* **76**:1247-1253 (1999).

Subramanian, R., M. Nakajima, K.S.M.S. Raghavarao and T. Kimura, Processing Vegetable Oils Using Nonporous Denser Polymeric Composite Membranes, *J. Am. Oil Chem. Soc.,* **81**:313-322 (2004).

Subramanian, R., M. Nakajima, T. Kimura and T. Maekawa, Membrane Process for Premium Quality Expeller-Pressed Vegetable Oils, *Food Res. Int.,* **31**:587-593 (1998b).

Subramanian, R., S. Ichikawa, M. Nakajima., T. Kimura and T. Maekawa, Characterization of Phospholipid Reverse Micelles in Relation to Membrane Processing of Vegetable Oils, *Eur. J. Lipid Sci. Technol.,* **103**:93-97 (2001a).

Subramanyam, C.V., M. Venkateswara Rao, V. Balasubrahmanyam and D.N. Bhowmick, Membrane Degumming of Crude Rice Bran Oil; Pilot plant Study, *Eur. J. Lipid Sci. Technol*. **108**:746-752 (2006).

Tarleton, E.S., How Electric and Ultrasonic Fields Assist Membrane Filtration, in *Proceedings of Filtration and Separation,* Filtration Society, 1988, pp. 402-406.

Teberikler, L., S. Koseoglu and A. Akgerman, Deoiling of Crude Lecithin Using Supercritical Carbon Dioxide in the Presence of Co-solvents, *J. Food Sci.,* **66**:850-853 (2001).

Treybal, R. E., *Mass Transfer Operations*, 3rd edn, McGraw Hill, New York, 1981, pp. 21-44.

Ulbrich-Hoffman, R., Phospholipases used in Lipid Transformations, in *Enzymes in Lipid Modification*, edited by Bornscheuer, John Wiley & Sons Inc., Wienheim, 2000, pp. 219-262.

Vali, S.R., P.P. Chakrabarti and T.N.B. Kaimal, Characterization of a Novel Phosphoglycolipid from Rice Bran Oil, *J. Oil Technol. Assoc. India*, **36**:3-14 (2004).

Valivety, R.H., G.A. Johnston, C.J. Suckling and P.J. Halling, Solvent Effect on Biocatalysis in Organic Systems: Equilibrium Position and Rates of Lipase Catalyzed Esterification, *Biotechnol. Bioeng.*, **38**:1137-1143 (1991).

Valivety, R.H., P.J. Halling and A.R. Macrae, Water as a Competitive Inhibitor of Lipase-Catalyzed Esterification in Organic Media, *Biotechnol. Lett*., **15**:1133-1138 (1993).

Wu, J.C.S. and E.N. Lee, Ultrafiltration of Soybean Oil/Hexane Extract by Porous Ceramic Membranes, *J. Membr. Sci*., **154**:251-259 (1999).

Xu, Z. and J.S. Godber, Comparison of Supercritical Fluid and Solvent Extraction Methods in Extracting γ-Oryzanol from Rice Bran, *J. Am. Oil Chem. Soc.,* **77**:547-551 (2000).

Yang, B., R. Zhou, J.G. Yang, Y.H. Wang and W.F. Wang, Insight into the Enzymatic Degumming Process of Soybean Oil, *J. Am. Oil Chem. Soc.,* **85**:421-425 (2008).

Yang, B., Y.H. Wang and J.G. Yang, Optimization of Enzymatic Degumming Process for Rapeseed Oil, *J. Am. Oil Chem. Soc.,* **83**:653-658 (2006a).

Yang, J.G., Y.H. Wang, B. Yang, G. Mainda and Y. Guo, Degumming of Vegetable Oil by a New Microbial Lipase, *Food Technol. Biotechnol*., **44**:101-104 (2006b).

Young, C.T., Peanut Oil, in *Bailey's Industrial Oil and Fat Products*, 5th edn., edited by Y.H. Hui, John Wiley & Sons Inc., New York, 1996, Vol. 2, pp. 377-392.

Young, F.V.K., C. Poot, E. Biernoth, N. Krog, N.G.J. Davidson and F.D. Gunstone, Processing of Fats and Oils, in *The Lipid Handbook*, 2nd edn.,

edited by F.D. Gunstone, J.L. Harwood and F.B. Padley, Chapman and Hall, London, 1994, pp. 249-276.

Zaks, A. and A.M. Klibanov, Enzyme Catalysis in Monophasic Organic Solvents, *J. Biol. Chem.*, **263**:3194-3201 (1988).

Zaks, A. and A.M. Klibanov, The Effect of Water on Enzyme Activity in Organic Media, *Proc. Natl. Acad. Sci. U.S.A.*, **82**:3192-3196 (1985).

Zhang, S.Q., O. Kutowy and A. Kumar, Application of Ultrafiltration Membranes in Refining of Crude Vegetable Oils, in *Proceedings of the 8th Annual Meeting of North American Membrane Society*, Ottawa, Canada, 1996, p. 95.

Zhu, C., G.L. Liu, C.S. Cheung, C.W. Leung and Z.C. Zhu, Ultrasonic Stimulation on Enhancement of Air Gap Membrane Distillation, *J. Membr. Sci.,* **161**:85-93 (1999).

Ziegelitz, R., Lecithin Processing Possibilities, *Inform*, **6**:1224-1230 (1995).

Zwijnenberg, H.J., A.M. Krosse, K. Ebert, K.V. Peinemann and F.P. Cuperus, Acetone-Stable Nanofiltration Membranes in Deacidifying Vegetable Oil, *J. Am. Oil Chem. Soc*., **76**:83-87 (1999).

Websites

www.cyberlipid.org/index

www.fas.usda.gov

www.fnbnews.com

www.novozymes.com

www.seaofindia.com

Publications from thesis

1. **Manjula, S.** and R. Subramanian, Membrane Technology in Degumming, Dewaxing, Deacidifying, and Decolorizing Edible Oils, *Crit. Rev. Food Sci. Nutr.,* **46**:569-592 (2006).

2. **Manjula, S.** and R. Subramanian, Laboratory Studies on Deoiling of Lecithin, *J. Am. Oil Chem. Soc.,* **85**:574-580 (2008).

3. **Manjula, S.** and R. Subramanian, Enriching Oryzanol in Rice Bran Oil Using Membranes, *J. Appl. Biochem. Biotechnol.*, **151**:629-637 (2008).

4. **Manjula, S.** and R. Subramanian, Simultaneous Degumming, Dewaxing and Decolorizing Crude Rice Bran Oil Using Nonporous Membranes, *Sep. Purif. Technol.*, **66**:223-228 (2009).

5. **Manjula, S.**, S. Divakar and R. Subramanian, Evaluation of Phosphoglycolipids Elimination from Rice Bran Oil by Nonporous Membrane using NMR Spectroscopy, *Eur. J. Lipid Sci. Technol.* (In press; DOI 10.1002/ejlt.200800287).

Presentations at national and international conferences

1. **Manjula, S.,** Sarita Arora and R. Subramanian, Improvement of Oil Flux in a Nonporous Membrane with Hexane Dilution. In *Proceedings* of International Congress on Membranes and Membrane Processes, ICOM-2005, 21-26 August, Seoul.

2. **Manjula, S.** and R. Subramanian, Optimization of Hexane Dilution in Enhancing Oil Flux in a Nonporous Membrane. In *Proceedings* of Indian Convention of Food Scientists and Technologists, ICFOST-2005, 9-10 December, Bangalore**.**

3. **Manjula, S.** and R. Subramanian, Pretreatment of Rice Bran Oil Using Membranes. In *Proceedings* of Indian Convention of Food Scientists and Technologists, ICFOST-2006, 16-17 November, Hyderabad.

4. **Manjula, S.** and R. Subramanian, Enriching Oryzanol in Rice Bran Oil Using Membranes. In *Proceedings* of New Horizons in Biotechnology, NHBT-2007, 26-27 November, Trivandrum (***Awarded the best paper***, *Subject area: Food and Agricultural Biotechnology*).

5. **Manjula, S.**, S. Divakar and R. Subramanian, Evaluation of Nonporous Membranes for the Removal of Phosphoglycolipids in Rice Bran Oil. In *Proceedings* of International Food Convention, IFCON-2008, 15-19 December, Mysore (***Awarded the best paper***, *Subject area: Fats and Oils*).