STUDIES ON PHYSICAL, THERMAL AND HYDRODYNAMIC PROPERTIES OF INTERESTERIFIED OILS

A Thesis Submitted to the

University of Mysore

For the award of the Degree of

Doctor of Philosophy

In

Biotechnology

By

Sukumar Debnath, M.Tech.

Department of Lipid Science and Traditional Foods
Central Food Technological Research Institute
(*Council of Scientific & Industrial Research*)
Mysore - 570 020, INDIA

December 2010

Certificate

I, Sukumar Debnath, certify that this thesis is the result of research work done by me under the supervision of Dr. B. R. Lokesh at Department of Lipid Science and Traditional Foods, Central Food Technological Research Institute, Mysore - 570 020. I am submitting this thesis for possible award of Doctor of Philosophy (Ph.D.) degree in Biotechnology of the University of Mysore.

I further certify that this thesis has not been submitted by me for award of any other degree/diploma of this or any other University.

Sukumar Debnath

Signature of Doctoral candidate
Signed by me on 23.12.2010

BRLokesh

Signature of Guide

Date: 23.12.2010

Date: 23.12.2010

Counter signed by

BRLokesh

Signature of Chairperson/
Head of Department/
Institution with name and official seal

प्रधान / Head
लिपिड विज्ञान एवं पारंपरिक खाद्य विभाग
Department of Lipid Science & Traditional Foods
केन्द्रीय खाद्य प्रौद्योगिक अनुसंधान संस्थान
Central Food Technological Research Institute
मैसूर / MYSORE-570 020

वेब साईट / Website : http://www.cftri.com

फोन / ☎: 0821-2516802, 2514760, 2514306 फैक्स / Fax: 0821-2517233

केन्द्रीय खाद्य प्रौद्योगिक अनुसंधान संस्थान, मैसूर - 570 020, भारत
Central Food Technological Research Institute
Mysore - 570 020, INDIA
cftri

Dr. B. R. Lokesh, Ph.D.
Scientist-G & Head
Department of Lipid Science and Traditional Foods
Central Food Technological Research Institute
Mysore - 570 020, Karnataka, INDIA

Date: 23.12.2010

Certificate

I, hereby certify that this Ph.D. thesis entitled, "Studies on physical, thermal and hydrodynamic properties of interesterified oils" submitted by Sukumar Debnath for the award of the degree of Doctor of Philosophy in Biotechnology to the University of Mysore, is the result of the research work carried out by him in the Department of Lipid Science and Traditional Foods, Central Food Technological Research Institute, Mysore, under my guidance and supervision during the period of 2004-2010.

Date: 23.12.2010
Place: CFTRI, Mysore

Dr. B. R. Lokesh
Guide & Research Supervisor

Dedicated To....

My Parents and Teachers

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Date: 23.12.2010
Place: CFTRI, Mysore *Sukumar Debnath*

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Central Food Technological Research Institute
Mysore – 570020, India

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Oils and fats are vital constituents in our daily diet, which provide energy, essential fatty acids, and serve as a carrier of fat soluble vitamins. In India, about 80% of the edible oils such as ground nut, sunflower, safflower, mustard, rice bran and coconut oil as well as hydrogenated fats are used for cooking and frying. During frying several undesirable physical and chemical changes occur in oils/fats such as hydrolysis, oxidation as well as thermal decomposition and thermal polymerization. Extensive research has been reported in literature to describe the chemical changes in frying oils, but very few have attempted to relate these chemical changes to the physical and thermal properties of the oil. Physical properties of oil influence its heat transfer characteristics during immersion frying. There is an awareness regarding the utilisation of blended oil for culinary uses. The blended oil was reported to show higher oxidative stability, less free fatty acid and less polar compound formation as well as higher heat stability during frying operations. However, biotransformation of edible oil through enzymatic interesterification holds further promise as such modifications produce clear, transparent, homogeneous, highly stable oils with improved physical and/or thermal characteristics. These various aspects is addressed in this investigation.

The following sections in brief furnish the organization of the matter covered in the thesis.

Introduction

In this section a brief literature review of the existing information on effect of frying on health and nutrition, physico-chemical, thermal properties of oils, modification of edible oils and fats through blending as well as enzymatic interesterification reactions, frying studies of Indian traditional foods using native and modified oils are presented.

Materials and Methods

The source of enzyme and chemicals and experimental methodology used in the present investigation is presented in detail.

Chapter-1: Evaluation of Edible Oils
Effect of Blending and Enzymatic Interesterification on
-Section A: Physico-chemical Properties &
-Section B: Thermal Properties

This chapter deals with the evaluation of selected unmodified oils for initial screening of oils required for blending as well as interesterification. This chapter highlighted also blending of two oils selected after initial screening and enzymatic interesterification of the oil blends for physico-chemical and thermal properties. Based on initial study using seven locally available oils such as coconut oil (CNO), sunflower oil (SFO), sesame oil (SESO), ground nut oil (GNO), mustard oil (MO), rice bran oil (RBO) and refined, bleached, deodorized, palm olein (RBDPO), two oils namely RBO and RBDPO were selected for further studies. These oils contain endogenous antioxidants such as oryzanol, tocotrienol in RBO and β-carotene in RBDPO. The flow behavior, the apparent viscosity, thermo-analytical properties, triacylglycerol molecular structure and thermo-oxidative stability of RBO and RBDPO and their blends were evaluated. These values were compared with interesterified oil blends prepared by subjecting blended oils to lipase treatment. The quality parameters namely, free fatty acid, peroxide value, diene value, triene value, polar material, colour unit were compared between fresh and oils used for frying of food materials.

The blending of oils shifted peak melting point of triacylglycerols towards a lower temperature range from 10.36 ˚C in RBDPO to 2.97 ˚C in blended oil (BL) (5:5 ratio of RBO:RBDPO), which further decreased to – 4.76 ˚C in the enzyme interesterified oil blend (5:5 EI). Trisaturated triacylglycerols decreased from 4% in RBDPO to 0.6% in EI (5:5 ratio of RBO:RBDPO). It was also found that the first step of thermal decomposition started at 190, 212.7, 220.8 and 238.9°C for RBDPO, RBO, BL and EI, with corresponding mass loss of 34.7, 28.7, 55.5 and 41.4 %, respectively.

These oils were used also for frying of *poori* in a continuous mode up to 10 frying cycles. It was observed that kinematic viscosity increased significantly during continuous frying and correlated well (r= 0.97) with changes in polar material.

The interesterified oils were more resistant to degradation during frying in terms of the formation of colour, diene, triene and total polar materials compared to native or blended oils. The interesterified oil was found to have more thermo-oxidative stability compared to native and blended oils as measured by Thermogravimetric (TG) analysis.

The sensory evaluation of the *poori* fried in these oils revealed no significant (p>0.05) differences in various attributes of the fried product. Odour profiles of RBO and RBDPO was not altered significantly (p>0.05) after continuous frying for 10 cycles. The blends and interesterified oil of blends show desirable (fresh oil note) odour profiles. However, in heated oil note increased on increased frying cycles. The product (*poori*) fried using blended and interesterified oils showed good consumer acceptance score.

Chapter-2: Hydrodynamic and Heat Transfer Properties of Enzyme Interesterified Oils

This chapter deals with the effect of blending of oils and enzymatic interesterification of oil blends on hydrodynamic properties (kinematic viscosity) and heat transfer characteristics. The methodology for determination of kinematic viscosity and convective heat transfer coefficients of unmodified oils and their selected blends and interesterified counterparts were tested at elevated temperatures (over a range of 170-190°C) for kinematic viscosity (ν) and convective heat transfer coefficient (h). Cannon^{TM} Ubbelhode calibrated capillary viscometer in a custom designed convective air bath heater and a highly conductive cylindrical metal were used for generating ν and h data respectively. The value of ν decreases and h increases with increasing the temperature for which oils were subjected. A good correlation between ν ($R^2 > 0.996$) or h ($R^2 > 0.987$) of these oils and their selected binary blends at the above temperatures range were observed.

The study also indicated that interesterified oils exhibited low levels of hydrodynamic property (3.61×10^{-6} to 2.91×10^{-6} m^2s^{-1}) and higher levels of heat transfer (221.0 to 258.0 Wm^{-2} $^{\circ}C^{-1}$) properties. The significant ($p<0.05$) decrease in kinematic viscosity and an increase in heat transfer coefficient make these oils thermo-physically better suited for frying of foods. The effect of different parameters (one at a time) responsible for enzymatic interesterification reaction, such as level of individual oil in the oil blend, reaction temperature, enzyme concentration, reaction time on hydrodynamic (kinematic viscosity) and heat transfer properties of oils are also addressed. Blended and interesterified oils provided desired kinematic viscosity and higher levels of heat transfer properties than unmodified oils and therefore, are physical and thermally better suited for frying. Therefore, by blending of two selected oils in appropriate proportions and by interesterification, oils with different ranges of viscosities and heat transfer coefficient was achieved. Since the kinematic viscosity and heat transfer coefficient were changing with the conditions employed, the reaction conditions were optimised following Response Surface Methodology (RSM).

Chapter-3: Optimization of Enzymatic Interesterification Process by Response Surface Methodology

This chapter deals with the optimization of enzymatic interesterification reaction conditions by Response Surface Methodology to obtain interesterified oil blend with desired properties. The response surface methodology was adapted to develop interesterified oil having minimum kinematic viscosity and maximum heat transfer coefficient. The enzymatic interesterification reaction of RBO and RBDPO blend was carried out using immobilized 1,3-specific lipase. Four variables, namely RBO (20-80 %) in RBO-RBDPO blend, reaction temperature (25-65 °C), enzyme concentration (1-13 %, w/w) and reaction time (1-13 h) were selected and optimized using RSM coupled with Central Composite Rotatable Design (CCRD) techniques. Simultaneous optimization results predicted that optimum reaction conditions for enzyme interesterified oil, having minimum kinematic viscosity ($2.63 \times 10^{-6} m^2 s^{-1}$) and maximum heat transfer coefficient ($262.0\ Wm^{-2}\ K^{-1}$) were at 62% RBO in the blend, reaction temperature 65°C, enzyme concentration 10% (w/w) and reaction time 6.4 h. The predicted values were validated experimentally and corroborated with DSC melting profile and triacylglycerol molecular species data. The modified oils obtained after optimization, were evaluated for the frying performances by measuring the heat transfer and mass transfer properties. To understand the mass transfer phenomenon during deep fat frying, oil partitioning was studied during frying of potato slices using RBDPO.

Chapter-4: Mass Transfer Study of a Deep-Fat Frying Process

This chapter addresses the mass transfer study of a frying process. Holding of product at frying temperature immediately after deep-fat frying resulted in significant mass transfer reduction from surface to core, thereby making oil available on the surface. This surface oil can be removed by blotting with absorbent paper. The sensory analysis of fried product indicated that the removal of surface oil did not cause any significant changes in the overall quality. The kinetics of partitioning of oil on the surface as well as in the structure during holding of fried potato slices fried in RBDPO at elevated temperatures (100, 120, 140, 160 and 180°C) was compared with that at 25°C (control). Oil partition coefficient, defined as the ratio of oil present on the surface to the structure, during holding of samples at 180°C was found to be 3.06 in products fried in RBDPO, whereas the oil partition coefficient of sample held at ambient temperature was 0.53, which indicated the availability of oil on the surface of fried product held at elevated temperature. The mass transfer coefficient of oil corroborated with the above finding. The holding of deep-fat fried product at 180°C followed by the removal of oil from surface using absorbent paper reduced the oil content of the final product from 0.44 to 0.33 kg of oil/kg dry solids. During deep-fat frying most of the oil remained on the surface but migrates into the core during holding after frying. The present work thus widens the scope for development of the suitable and alternative method for obtaining low fat fried product for health conscious individuals without sacrificing overall quality of the product.

However, in the next chapter the modified oil obtained after optimization of interesterification study using RSM, was evaluated for the frying performances by measuring the heat transfer and mass transfer properties.

Chapter-5: Frying Quality of Enzyme Interesterified Oils

This chapter deals with the effect of frying time on heat and mass transfer properties of oil obtained after optimization study. The effect of frying cycles on physical, chemical and heat transfer qualities of the oil blend, enzyme interesterified oil containing RBO and RBDPO during deep-fat frying of *poori* was studied. The frying was carried out in intermittent mode (5 batches each batch consisting of 6 *pooris*, each batch for 3 min in a day without any time lag between the batches, as per the frying practice at Indian homes) and it was repeated for 6 consecutive days, which was referred as six frying cycles. Hydrodynamic properties (kinematic viscosity, density, surface tension), physical property (colour), chemical properties (free fatty acid value, peroxide value, total polar materials content, fatty acid composition, radical scavenging activity and oryzanol content), moisture and oil uptake and thermal properties (heat transfer coefficient) of RBO, RBDPO, blended and interesterified oil were studied. It was observed that free fatty acid content, peroxide value and total polar materials increased marginally in first two cycles in RBO and in the interesterified oil while radical scavenging activity decreased slightly in these oils. Radical scavenging activity decreased significantly in RBDPO with an increase in frying cycles. There was no significant ($p>0.05$) change in oryzanol content in RBO after heating and frying experiments with an increase in frying cycles. Relative amounts of total saturated fatty acids increased marginally in RBDPO due to decrease in polyunsaturated fatty acids content *per se* during deep-fat frying cycles. The convective heat transfer coefficient was found to decrease marginally in RBO and interesterified oil with an increase in frying cycle due to insignificant increase in kinematic viscosity of these oils for every frying cycle. Fried oil causes health hazards after reaching its total polar material to 24%, it needs to be discarded. Poori was fried in the intermittent mode till the fried oil reaches to its discarding point. It was observed that during the frying of poori, the total polar material reached to a discarding point of 24% for RBO, RBDPO, BLO and EIO after 15, 21, 23 and 25 frying cycles, respectively. However, despite above marginal changes in physical and chemical properties, there was no significant ($p>0.05$) difference in the sensory data of *poori* fried in oil subjected to 6 frying cycles.

General Discussion and Summary

The overall findings relating to present investigation relevant to the stated objectives with emphasis on basic understanding about structure function relationship of modified oils on physical, thermal and hydrodynamic properties and its utility as frying oil is presented in the concluding section of the thesis. A brief summary of the major findings is also enclosed at the end of the general discussion. In conclusion, the present study highlighted the development of the enzyme interesterified oil with improved physical, hydrodynamic and thermal properties and thermo-oxidative stabilities. The above study has thus given an insight into the use of lipase catalyzed interesterification reaction as a means for obtaining cooking oil suitable for frying, cooking operations.

Bibliography

Bibliography section covered relevant references from literature.

Sukumar Debnath
(Candidate)

Date: 23 December, 2010

Dr. B. R. Lokesh
(Guide & Research supervisor)

Date: 23 December, 2010

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General Introduction

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Symbols, Abbreviations and Units Used

ν = kinematic viscosity, m^2s^{-1}

ν_0 = frequency factor, (-)

h = convective heat transfer coefficient, $Wm^{-2}\,{}^{\circ}C^{-1}$

t = time, s

T= absolute temperature, K

T_t = transducer temperature, °C

T_A = average oil temperature, °C

T_i = initial transducer temperature, °C

$T_{\circ}$ = °Temperature of the medium (oil) (°C)

A = area of transducer, m^2

ρ = density of aluminum transducer, kgm^{-3}

c_p = specific heat of the transducer, $Jkg^{-1}\,{}^{\circ}C^{-1}$

E_a = activation energy, $Jmol^{-1}$

R- universal gas constant, $Jmol^{-1}K^{-1}$

r = radius of the cylinder, m

D = Diameter of the cylinder, m

V = Volume of the transducer, m^3

A_0= constant,

L = linear effect, Q = quadratic effect

T* - Temperature of thermal degradation, Dm*- Mass loss

DAS – Data acquisition system

M - myristic, P - palmitic, S - stearic, O - oleic, L – linoleic,

U-unsaturated triacylglycerol,

S-saturated triacylglycerol

K_o -kinetic constant for oil uptake, (s^{-1})

CNO - Coconut Oil

GNO - Ground nut Oil

MO - Mustard Oil

SESO - Sesame Oil

SFO - Sunflower Oil

RBO - Rice bran Oil

FFA – free fatty acids (%)

PV- Peroxide value ($meqO_2kg^{-1}$)

TPM-total polar material (%)

General Introduction

India is an agriculture-based country. It is a major contributor for the Indian economy. About 66% of the Indian population earns their livelihood from agriculture related activities. Oil seeds occupy an important place in the Indian economy. The major oilseeds grown in India are groundnut, rapeseed/mustard, soybean, sunflower, niger seed, castor seed, sesame, linseed and safflower. The major states which grow oilseeds are Andhra Pradesh, Gujarat, Haryana, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal. These states cover nearly 92.47 % of the total area as well as 96.58 % of the total production in the country as a whole (http://www.economywatch.com/indianeconomy). The total annual production of oil seeds in 2007-2008 is 297.55 Lakh tones and net availability of edible oils from all domestic sources was 86.54 lakh tones. But the consumption of edible oils (from domestic and import sources) is 142.62 lakh tonnes (*Source: (i) Production of oilseeds: Ministry of Agriculture, ii) Net availability and consumption of edible oils: Directorate of Vanaspati, Vegetable Oils & Fat*). Among the total edible oil imports (82 lakh tones), palm oil constitutes the largest share with as much as 50-55 lakh tonnes of total consumption. So there is a need for an alternate source of oils other than oil extracted from major oilseeds. Therefore, oil from sources other than major oilseeds such as rice bran oil, palm oil are considered to fulfill the gaps. These oils are now available in Indian market and used for culinary purposes. Not much is known about their properties when used for frying purposes. So the present work will address this issue.

OILS AND FATS

Oils and fats are essential component of our daily diet, which provides desirable sensory, physical, nutritional and biological functions. These serve as a source of energy and provide essential fatty acids such as linoleic and linolenic acids . They also help in absorption and transport of fat-soluble vitamins (A, D, E, and K) and the carotenoids and enhance bioavailability of phytochemicals (Spector and York, 1985; Yang *et al*., 2004). Fat regulates cholesterol metabolism and also provides precursor substrates for prostaglandins, responsible for regulating many physiological functions. Fats and oils vary in their physical, thermal and nutritional properties depending upon the type of fatty acids attached to the glycerol backbone. Each triglyceride molecule is composed of saturated fatty acids (SFA) such as, lauric, myristic, palmitic and stearic, which are in solid state at the ambient temperature, and have higher melting point, monounsaturated fatty acids (MUFA) such as oleic acid and polyunsaturated fatty acids (PUFA), such as linoleic, linolenic acid, which are in liquid state at ambient temperature. Fatty acids are generally classified as short chain, medium chain and long chain fatty acids based on their chain lengths and as saturated, monounsaturated and PUFA based on the presence and number of double bonds. The source of fatty acids is furnished in the Fig. 1.

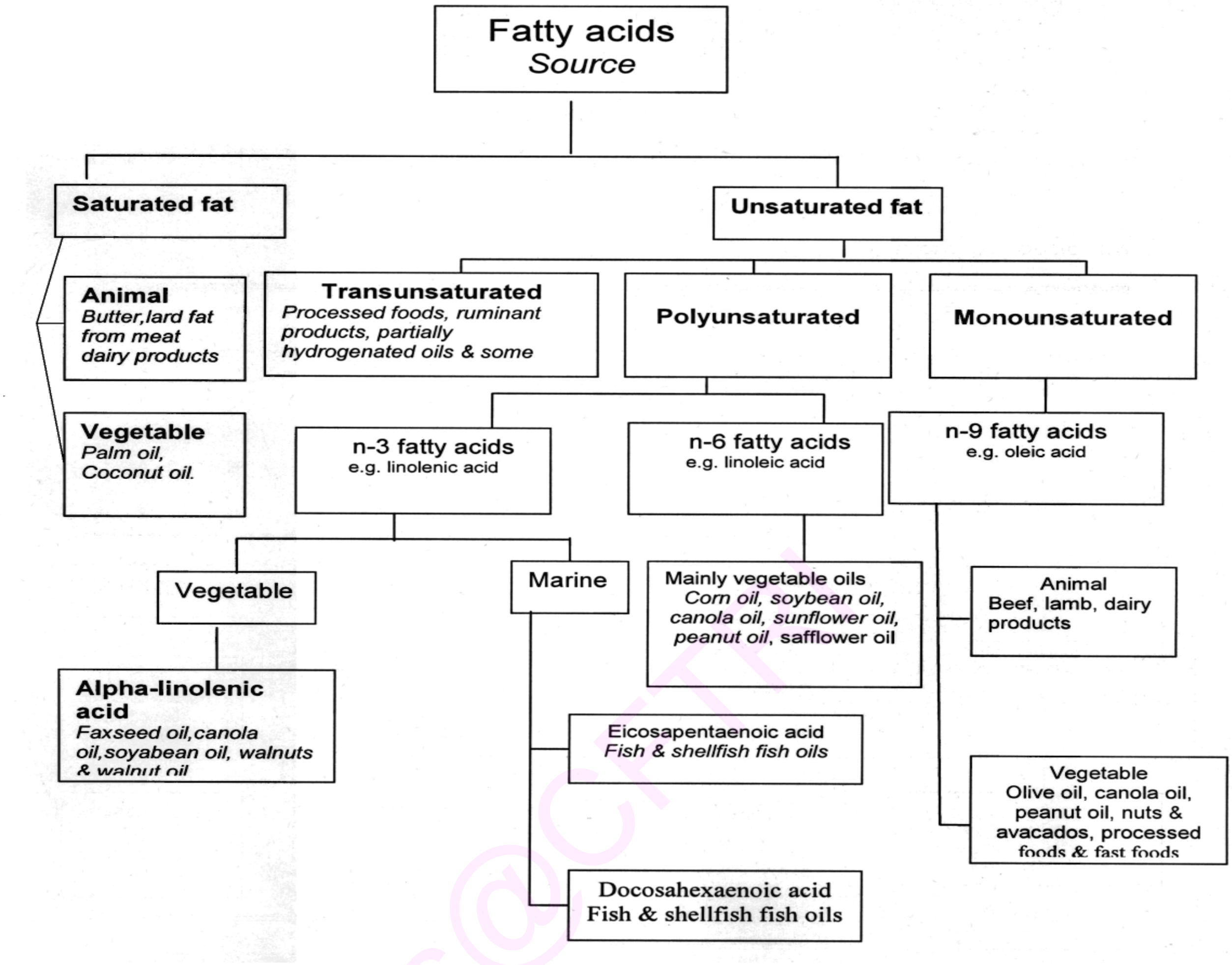


Fig. 1 Classification of Fatty acids (Chong *et al*., 2006)

Saturated fatty acids

The fatty acids with no double bond are known as saturated fatty acids. They range from butyric acid (C4:0) to stearic acid (C18:0). Triglycerides containing saturated fatty acids with 8 to12 carbon atoms are called medium chain triglycerides (MCT). They are found in kernel oils and lauric fats. They are odourless and stabile to oxidation. Their melting points are lower as compared to long chain saturated fatty acids and are in liquid or semi-solid state at room temperatures.

Monounsaturated fatty acids (MUFA)

The long chain fatty acids with a single double bond are known as monounsaturated fatty acids. The predominant monounsaturated fatty acids (MUFA) are palmitoleic acid, (16:1) oleic acid (18:1, cis), vaccenic acid (18:1, trans), cetoleic acid (20:1) and erucic acid (22:1).

Polyunsaturated fatty acids (PUFA)

The unsaturated fatty acids with more than one double bond are classified as polyunsaturated fatty acids (PUFA). The predominant polyunsaturated fatty acids (PUFA) are linoleic acid, (18:2), linolenic acid (18:3), eicosapentaenoic acid (20:5), docosahexaenoic acid (22:6).

VEGETABLE OILS AND FATS

Different varieties of edible vegetable and animal oils and fats are available in the market for human consumption. Many specific oils are traditionally used in Indian cooking for frying. However, their thermo-oxidative stability is not fully addressed. In recent times, the use of highly stable oils and fats for frying or cooking is receiving greater attention. Vegetable oils, differ in their fatty acid composition (Table 1). Most of the vegetable oils used in India contain high levels of oleic and /or linoleic acid and palmitic acid as the major saturated fatty acid. The fatty acid profiles of edible oils play an important role in physical, thermal properties and which influences their stability during storage and frying.

Table 1. Fatty acid composition (%) of some common vegetable oils

Fatty acids	Coconut oil	Palm kernel oil	Palm oil	Oilve oil	Peanut oil	Sunflower oil	Soybean oil	Linseed oil	Rapeseed oil
8:0	8.0	3.9	-	-	-	-	-	-	-
10:0	6.4	4.0	-	-	-	-	-	-	-
12:0	48.5	49.6	0.3	-	-	-	-	-	-
14:0	17.6	16.0	1.1	-	-	-	-	-	-
16:0	8.4	8.0	45.1	13.7	11.6	11.0	11.0	4.8	2.8
18:0	2.5	2.4	4.7	2.5	3.1	4.7	4.0	4.7	1.3
18:1	6.5	13.7	38.8	71.1	46.5	18.6	23.4	19.9	23.8
18:2	1.5	2.0	9.4	10.0	31.4	68.2	53.2	15.9	14.6
18:3	-	-	-	0.6	-	0.5	7.8	52.7	7.3
20:0	-	-	-	0.9	1.5	0.4	0.3	-	0.7
20:1	-	-	-	-	1.4	-	-	-	12.1
20:2	-	-	-	-	-	-	-	-	0.6
22:0	-	-	-	-	3.0				0.4
22:1	-	-	-	-	-	-	-	-	34.8
24:0	-	-	-	-	1.0	-	-	-	1.0

Source: Chow (1992)

FAT INTAKE IN INDIA

Regional variation in dietary habits is obsered in India. The choice of edible oils used for cooking purposes varies from one region to the other. Ground nut oil are mostly used by the people in Western and Southern India. Coconut oil is used in Kerala, safflower oil in parts of North Karnataka and South Maharastra, rapeseed oil/mustard oil in Northern Punjab to Eastern West Bengal. The refined palm olein in consumed in some parts of the Southern India. Sesame oil is mainly used in Tamil Nadu. Rice bran oil is making an entry use in India and is promoted for its health benefits. Therefore, the consumption pattern of different edible oils varies depending on the region. Vegetable oils are mostly used for cooking and frying operations in India.

RICE BRAN OIL

Rice bran oil (RBO) is an edible oil produced in several countries such as India, Korea, Japan, China and Indonesia as cooking oil (Gopala Krishna, 2002). India is the major producer of RBO in the world amounting to about 8, 00,000 tons per annum. It is prefered for cooking, frying due to its high smoke point and subtle flavour. The nutritional qualities and health benefits of rice bran oil are established by different researchers. The oil has an unique fatty acids composition for a stable oil with minor components such as oryzanol, phytosterol, tocotrienol, tocopherol and squalene which show cholesterol-lowering and skin improving effects in addition to antioxidant effects. Presence of oryzanol and higher amounts of unsaponifiable matter (such as phytosterols) are unique to RBO. This oil has been studied as a frying oil (Gopala Krishna *et al*., 2005). A US patent has been filed on the use of RBO as an antioxidant in high linoleic oils such as soyabean and rapeseed oils (Taylor *et al.,* 1996). RBO contains 22% saturated, 43% monounsaturated and 35% polyunsaturated fatty acids.

PALM OIL

India imports palm oil from Indonesia and Malaysia, for its use as cooking oil. Palm oil supplements traditional edible oils in India. It is reported to have many nutritional and health benefits.

REFINED, BLEACHED, DEODORIZED, PALM OLEIN

Refined, bleached, deodorized, palm olein (RBDPO) is a refined fraction of red palm oil and is commonly known as refined palm olein. It is used as a cooking and deep fat frying oil in India. RBDPO is rich in antioxidants such as β-carotene, tocotrienol, tocopherol. RBDPO contains 48% saturated, 42% monounsaturated and 10% polyunsaturated fatty acids.

DEEP FAT FRYING

More than 80% of edible oils in India are used for frying the dishes. Deep-fat frying is commonly used for preparing fried foods. It is the most common unit operation in snack food preparation. Frying involves simultaneous heat and mass transfer. Heat is transferred from a moving fluid (oil) to the solid (food material). Heat transfer is measured by convective heat transfer coefficient. Mass transfer is considered by the oil uptake/oil inflow by/to the product and the moisture outflow from the product. The mass transfer is measured by mass transfer coefficient. It is important to understand the factors involved during frying to improve efficiency in processing of fried foods. In open frying, oil is heated to high temperatures such as 160-180°C. During frying the oil is exposed to air for a specific period of time. The process involved in deep-fat frying process is shown in Fig. 2.

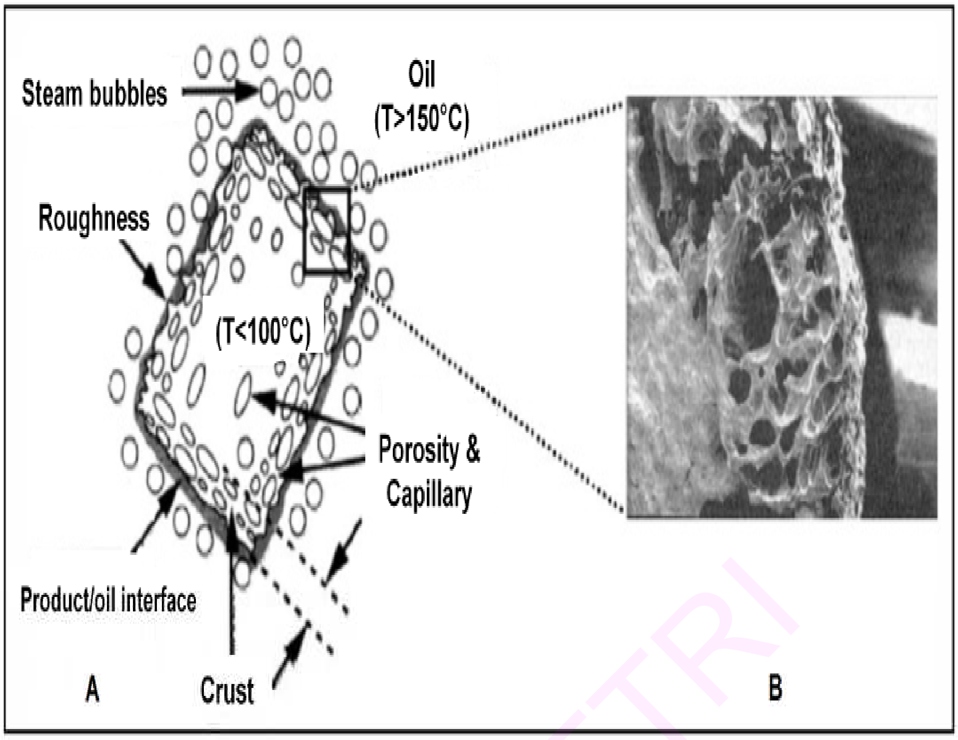


Fig. 2 **A**: Schematic presentation of a cross-section of a piece of food during deep fat frying
B: Scanning electron microscope image of a cross-section of the crust of a fried potato
(Source: Mellema, 2003)

Heat is transferred from the oil to the food. This results in evaporation of water and absorption of oil by the food. The evaporation of water leads to shrinkage of the food material and leads to the formation of surface porosity and roughness. As the product undergoes frying, the moisture inside the food is converted to steam, creating a pressure gradient as the surface dries out and causes oil to adhere to product’s surface. During frying of food for a long period of time the moisture content in the crust slowly disappears and the steam bubbles vanish. According to Bouchon *et al.* (2003) oil absorption during frying can occur by three different mechanisms. These are surface oil, the amount of oil that remains on the surface, penetrated surface oil, the oil that penetrates into the food during cooling and structural oil, the oil that is absorbed during frying (Fig. 3).

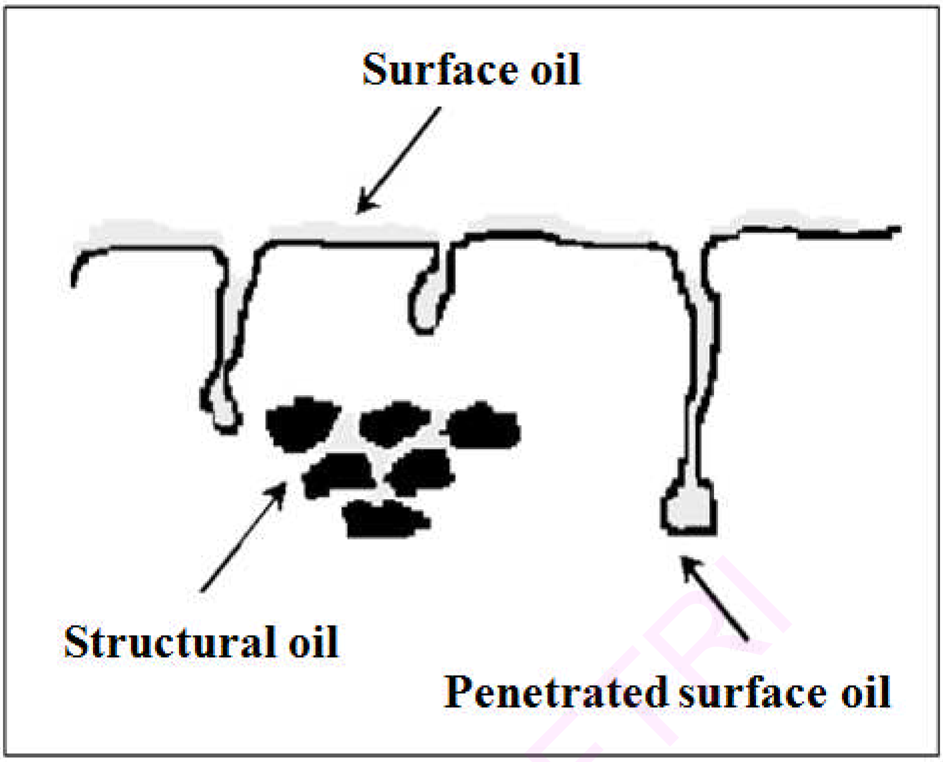


Fig. 3 Three locations of oil in the product microstructure after frying process (Bouchon, 2003)

Most of the oil enters the product from the surface during cooling (Bouchon *et al.,* 2003). Various factors affect the oil absorption during deep fat frying. These are the quality of the oil in which the food is being fried, composition of food, frying temperature, time and shape of the product, initial moisture content, initial interfacial tension and porosity. The relationship between oil temperature, viscosity and surface heat transfer coefficient of oil is important in determining the rate of frying. Studies on physical properties and heat and mass transfer of oils, oil absorption, and processing rates associated with deep-fat or immersion frying are necessary for getting fried foods with proper texture and colour.

The Indian snack market is one of the largest snack markets in the Asia-Pacific region of worth of $ 459 million in 2006. It accounts for 3% of the total Asia-Pacific snack market revenue. Among the various ready-to-eat snack foods, such as samosa, poori, namkeen, chips preferred by Indians, potato chips occupy the largest product category, which holds the 85% share of the total market revenue (http://www.ezinearticles.com/?Find-Out-the-Truth-About-Indian-Snack-Industry&id=3477773). Since there is a rising demand for ready-to-eat snack foods, the snack industry is going to grow further in the future.

Different types of oils are used in food industries for making fried products depending on the availability and cost of oils. Blended oils have been used for decades in restaurants, delis, industrial manufacturing units. They are gaining popularity worldwide due to added advantages they offer such as improved thermal stability, nutritional benefits and ability to impart the desired properties. They are economical also compared to many selected vegetable oils. The main advantages of using blended oils are their versatility, flavor profile, low price and improved physical, chemical and thermal properties such as smoke point, viscosity, and surface heat transfer coefficient. Physical and thermal properties of blended oils are different from the native oils.

However, the consumers are becoming aware of implications of taking fried foods. The consumption of saturated fat is recognized as one of the major concern because it plays an important role in raising the risk factors for cardiovascular diseases, cancer, diabetes and hypertension (Weisburger, 2000).

Oil inflow/uptake

Deep-fat frying is the immersion of food material into the oil at frying temperature and its subsequent cooking with the result of fried food having specific color, flavor, crust, texture and palatibilty. Frying of food material is a complex phenomenon and believed to be first started around the sixth century BC (Morton, 1998). It is a simultaneous heat and mass transfer process, which involves interaction between food material and frying oil. It is fundamentally a dehydration (Saguy and Pinthus, 1995; Saguy *et al.*, 1998) in which the temperature of the product does not rise beyond 100°C. Uptake of oil is an exchange process between oil and evaporated moisture after frying. Oil uptake depends on several factors, such as oil quality (Dobarganes *et al.*, 2000), oil composition, surface active compounds, (Blumenthal, 1991; Blumenthal, 1996; Stier, 2000), interfacial tension between oil and food materials (Pinthus and Saguy, 1994), crust development (Saguy *et al.*, 1997; Aguilera and Gloria, 2000), higher surface area and roughness (Rubnov and Saguy, 1997), coating with batter or breading (Khalil, 1999; Rayner *et al.*, 2000; Shih *et al.*, 2001) frying temperatures (Dobarganes *et al.*, 2000).

Moisture outflow

During frying the cellular structure (Pedreschi *et al.*, 2001) of the food material changes, the water is evaporated through capillaries and channels of the cellular structure, due to formation of steams and forms voids. The oil present on the surface penetrates into the product. The oil uptake occurs mainly during the cooling of the fried product (Ufheil and Escher, 1996).

Quality of oil

Deep-fat frying is generally carried out at higher temperatures (160 to 180°C) in presence of air. During this process, three major reactions such as hydrolysis, oxidation and thermal polymerization occur (Paul and Mittal, 1997, Nawar, 2000). These reactions change physical (color, viscosity) and chemical (fatty acid composition, polar materials) properties of oils. The amount of moisture present in the product, oxygen, high temperature and duration of frying are responsible for these changes. During frying, hydrolysis occurs due to cleavage of bonds between glycerol and fatty acids owing to the release of vapour from the food. This results in the formation of mono and diglycerides, free fatty acids and glycerol (O'Brien, 1998). Oxidation of oil may occur in three stages, which affects aroma, color and flavour of the oil. The primary oxidation occurs when an oil is heated at higher temperature in presence of air. This leads to the formation of hydroperoxides and can be monitored by determining peroxide value of oil. The secondary oxidation leads to the formation of alcohols, acids and carbonyl compounds, volatile and non-volatile compounds. The tertiary oxidation leads to the polymerization of secondary oxidation products, resulting in the increase of oil viscosity and darkening of oil colour. The rate of oxidation reaction depends upon the level unsaturation, polyunsaturated fatty acids, presence of oxygen and free radicals. Oxidation is important factor and related to organoleptic properties of the product due to formation of rancid odours and other oxidation products that may cause health hazards.

Thermal polymerization

During frying at higher temperatures, when the oxygen supply is less, oil is covered by steam evaporated from the product; the thermal polymerization occurs (Gertz *et al.*, 2000). Thermal polymerization leads to the formation of cyclic monomers, dimers and polymers (Sebedio and Chardigny, 1996, Gertz *et al.*, 2000).

Effect of frying on nutrients

Deep-fat frying is considered as an inexpensive, fast and efficient method of cooking (Dana and Saguy, 2001). It has some advantages over other cooking methods, such as, the temperature within the product remains at 100°C and duration of frying is restricted to few seconds to minutes. So, less deterioration of heat-sensitive vitamins occur in case of frying process as compared to operations involving baking or boiling. However, deep fat frying causes loss of vitamin A as compared to stir-frying methods due to solubility of vitamin A in oil. The fried foods absorb significant amounts of vitamin E from the oil (Holland *et al.*, 1991). It was reported also that deep fat frying of French fries decreases digestible starch concentration and increases the dietary fiber content. This contributes to fiber uptake while consumption of French-fries (Thed and Phillips, 1995).

Regulations of quality of frying oil

Most of the European countries are concerned about the quality of frying oil. They stipulate that the total polar materials (TPM) as the marker of oil quality should be within 25% of TPM. It was recommended (http://www.dgett.de/news/recomm.htm) that the frying oil should be replaced after reaching its total polar materials and polymer content to 24 and 12%, respectively. In Israel, Ministry of Health recommended the level of total polar materials of frying oil should be maintained below 25%. The Japanese Ministry of Health and Welfare mandated that the frying oil should meet smoking point, acid value and carbonyl value of more than 170°C, less than 2.5 mg/g and less than 50, respectively.

Frying practices in industry, restaurant and fast-food outlets

Frying operations, at industrial level, in restaurants and in fast-food outlets, expose the oil to high temperatures for long periods of time. In industrial level frying practices, the turnover time of oil is usually maintained at lesser than below 8 h by continuously replenishing the oils used for frying with the fresh one. This facilitates them to continued used of oils for prolonged frying (Romero *et al.*, 1999). But in case of restaurants and in fast-food outlets the turn over time is comparatively longer because of the batch process and the fryer is kept idle at higher temperature. This affects the quality of the oil, resulting in darkening of colour, increase in viscosity and foam formation and crossing the permitted level of total polar materials.

Frying and Health

During frying, food material undergoes some changes, which include improvement of colour, texture, flavour and palatability. Frying the foods with fresh oil results in desirable coloured fried product but increases oil absorption by fried product was also observed (Blumenthal, 1991). On the contrary, prolonged frying leads to the poor sensory characteristics imparted to the product, cause nutritional losses and may cause health hazards by lowering digestibility (Paul and Mittal, 1997).

Trans fatty acid

To improve the stability, frying oil is subjected to partial hydrogenation, which increases its trans fatty acid content (Aro *et al.*, 1998). Trans fatty acids present in the oil increases risk of cardiovascular diseases (Willett and Ascherio, 1994) by affecting serum lipoproteins (Katan *et al.*, 1984). In Denmark, the regulatory body allows the maximum trans fatty acid concentration of 15% in unused frying oil (Fox, 2001). During prolonged frying at higher temperature, trans fatty acids may increase. As increase in trans fatty acid concentration is reported for sunflower oil subjected to continuous frying condition (Aro *et al.*, 1998).

Cyclic fatty acid monomers

When a vegetable oil is heated at a temperature of 200°C during frying or refining, linoleic and linolenic acids in the oil are transformed into cyclic fatty acid monomers. The food containing cyclic fatty acid monomers are absorbed and transported via lymphatic systems after consumption. The absorption of higher amounts of cyclic fatty acid monomers are detrimental to health (Paul and Mittal, 1997; Romero *et al*., 2006).

Frying, mutagens and carcinogens

During frying oxidation of oil occurs which generates volatile compounds. Volatile oxidation products are reported to be mutagenic when vapours are inhaled during frying process (Marquez-Ruiz and Dobarganes, 1996). During extensive exposure of oil, specially at restaurants, at higher temperatue forms polar materials are formed, of which contains malonaldehyde (MDA). MDA shows mutagenic activity (Esterbauer, 1993; Hageman *et al.*, 1988; Basu and Marnett, 1983; Shamberger *et al.*, 1974). It is reported that MDA at higher concentration causes skin cancer (Mukai and Goldstein, 1976). Therefore, monitoring of the formation of MDA during frying is essential.

Hydrodynamics, physical and thermal properties of oils

Hydrodynamics is a study of liquids in motion, which deals with the dynamics of fluid especially incompressible fluids. This property covers kinematic viscosity, specific gravity and surface tension. During deep fat frying process the oil gets exposed to higher temperature. During this operation, the kinematic viscosity as well as specific gravity of the oil increases whereas the surface tension decreases. During frying temperature the thermal properties of the oil also changes. The heat transfer coefficient of the oil gradually decreases with prolong frying operations (Da Silva and Singh, 1995; Tseng *et al.,* 1996). Te physical property such as colour also gradually darkens. Hence, it is required to modify and develop the oil suitable for frying with improved hydrodynamic, physical and thermal properties. Colour and homogeneity (Sreerama *et al.,* 2005) of the oil has specific role to play as far as the consumer acceptance is concerned. The modified/processed oil should have acceptable colour. It should also have stability for storage and during frying for reasonable duration.

Need for the development of special or modified oils

The above-mentioned survey/reports underscore the need for development of special oils having oxidative and thermal stability. The fatty acid composition and the degree of unsaturation in the fatty acids play key role in stability of oils. The types of fatty acids such as saturated, monounsaturated and polyunsaturated fatty acids play important role in oil stability. Saturated fatty acids (e.g. palmitic acid) are highly stable, monounsaturated fatty acids (e.g. oleic acid) are relatively stable and polyunsaturated fatty acids (e.g. linoleic, linoleic acid), although they are nutritionally good, but unstable during frying and cause the generation of degradation products (Brinkmann, 2000). The presence of natural antioxidants such as tocopherol, tocotrienol, oryzanol in oil is an important factor for its stability during frying. Although saturated fatty acids have higher oxidative stability, at higher temperatures they decompose and form carboxylic acids, aldehydes and ketones. Consumption of satutrated fats leads to heath risks, such as heatrt diseases, cancer, diabetes and hypertension. Therefore, there is a need for the development of modified oils. Developments of high-oleic canola and high-oleic soybean oil using crop breeding technologies are successful attempts for the production of stable oils (Moreira *et al.*, 1999; Hazebroek, 2000). These approaches are expensive and time consuming. The modifications of oil can be achieved economically by either fractionation, physical blending or enzymatic interesterification processes. The development of the special/modified oil will be of great importance to academia as well as to the industry.

MODIFICATION OF OILS

The characteristics required for specific end use of an oil or fat depends upon its triglyceride structure and specific distribution of fatty acids. This influences its physical, chemical and thermal properties. To obtain a fat or oil with specific melting point, solid fat content, viscosity, heat transfer, it often becomes necessary to modify them, which cannot be obtained by simple physical mixing of different oils. This can be achieved by restructuring the oils/fats by following different means, such as (a) hydrogenation, (b) fractionation, (c) blending and (d) interesterification.

(A) Hydrogenation

It is a chemical means in which hydrogen, in presence of catalyst, e.g., nickel, converts unsaturated fatty acids into saturated fatty acids. This changes the physical and thermal properties of oils. It also produces trans fatty acids, which cause health hazards (Mozaffarian and Willett, 2007).

(B) Fractionation

It is a thermochemical process, in which a mixture is separated into two or more fractions having specific physical and thermal properties. This process is followed for fractionation of fats and oils such as palm oil (Trailter and Dieffenbacher, 1985) lard, tallow (Grompone, 1989) and milk fat (De greyt and Huyghebaert, 1993).

(C) Blending and interesterification

Physical mixing of two or more unmodified oils can change the physical and thermal properties of oils. The modification of oils by rearrangement of their fatty acids within the triglycerides moiety by interesterification (Fig. 4) can alter the physical, thermal and nutritional properties in a desirable manner.

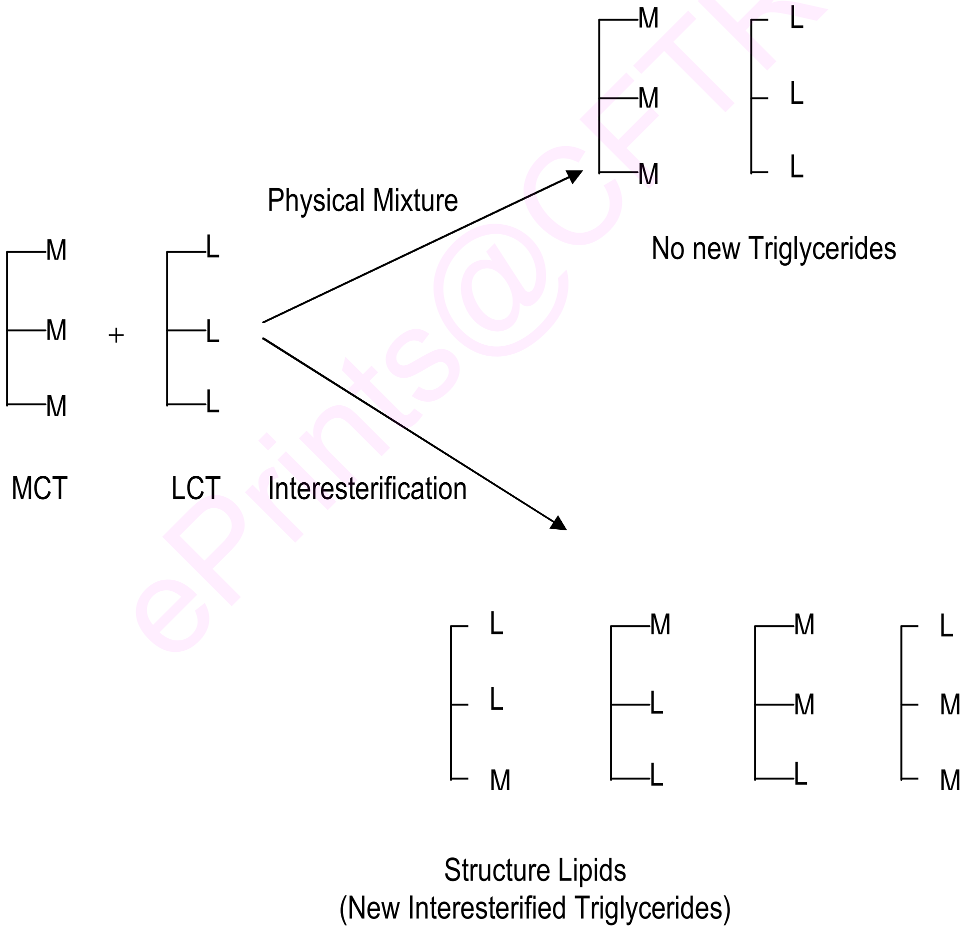


Fig. 4 Difference between a physical mixture of medium chain (M) and long chain triglycerides (L) and structured lipids containing randomized, medium and long chain fatty acids in the same molecule (*source: Akoh, 1995*)

ADVANTAGES OF BLENDED OILS

(i) Versatility

Blended oil can be used for cooking, frying, salad dressings. A food manufacturer can take advantage of blended oil's versatility. Most blended oils available in the market contain vegetable oils, such as olive oil, soybean oil, rice bran oil, canola oil, sunflower oil, palm olein. Blended oils provide a balance between fatty acids for promoting health.

(ii) Flavour

Blended oils are often used for a specific flavor profile. Blended oil does not have domination of a specific flavor of the individual oils from which it is made. For example, introducing olive oil flavor for a particular recipe or dish without olive oil dominating the taste buds. Peanut oil is used for making mayonnaise, with just a little olive oil added for flavor (www.deliaonline.com/ingredients/oil).

(D) Interesterification

Interesterification is a reaction in which a fatty acid is esterified to a glycerol backbone which may react with fatty acids (acidolysis) or alcohols (alcoholysis) or esters (transesterification). The major aim of the modification by interesterification is to produce new triacylglycerol (TAG) species from native fats or oils which provides required physical, thermal, chemical, desired functional and nutritional properties (Laning, 1985). Interesterification can be accomplished by means of chemical and enzymatic methods.

(i) Chemical interesterification

Interesterification by chemical method had been used in food industries since 1940 for improving spredability and baking properties of lard (Weiss *et al*., 1961). There are some chemically interesterified products available in the market such as Olestra, Salatrim. In this method interesterification is catalysed by alkali metals or alkali metal alkylates. The reaction requires high temperature and anhydrous environment. The chemical interesterification produces desired amount of randomised TAG molecular species as well as a number of undesired products which may not be easy to separate.

Drawbacks of chemical interestrification

(a) It is non-specific intereseterification process, which results in the formation of many reaction products, b) the procedural steps are tedious, c) it employs toxic catalyst or solvents and d) strong reaction conditions, such as high temperature, and pressure are employed.

ii) Enzymatic interesterification

Modification of oils and fats by enzyme had been practiced because of its versatility. This technology started in early eighties for manufacturing of cocoa butter equivalents, which, later on was patented by Uniliver (Colman and Macrae, 1980) and Fugi oil (Matsuo *et al.*, 1980). Enzyme interesterification of fats and/or oils are carried out by using lipase enzyme produced from different microorganisms such as, *Aspergillus niger, Candida* *cylindracea*, *Mucor miehei*, *Rhizopus delemar, Rhizopus arrhizus, Staphylococcus aureus*. The reaction matrix/system contains a water immiscible phase (oils or fats) and an aqueous phase (lipase). Lipase catalyses the hydrolysis of oil in presence of water and esterification occurs under limited water condition (Macrae, 1985; Jaeger *et al.,* 1994). In acidolysis reaction, an acyl group is transferred between an acid and an ester while incorporating new fatty acids into triglycerides. In alcoholysis, the reaction occurs between an alcohol and triacylglycerols. In transesterification reaction, acyl group gets exchanged between two esters such as two triglycerides or within a triglyceride and a fatty acid ester. This type of reaction changes physical properties of the native fats and oils or oil blends by changing the positional distribution of fatty acids in the triglyceride moiety (Fig. 5).

Lipases can act as a catalyst in wide number of bioconversion processes, such as hydrolysis, interesterification, esterification, alcoholysis, acidolysis and aminolysis. Lipase catalysed reactions has many food applicatons such as oils and fats, dairy and bakery and also in pharmaceutical industries. (Aravindan *et al.,* 2007). In general, the enzyme, lipase catalyses the hydrolysis of triglycerides and forms free fatty acids, glycerol, monoacylglycerol and diacyglycerol. Under controlled conditions, the lipase can also form acylglycerol by catalysing reverse reaction between free fatty acids and glycerol. The triglycerides are insoluble in water and lipases catalyse the hydrolysis of ester bonds at the interphase between substrate (triglyceride) and aqueous phase. Lipases are highly selective and specific and show positional and substrate specificity. There are different classes of lipases (Camp *et al.,* 1998) which carry out reactions as follows: (i) Lipase hydrolyses TAG releasing fatty acids, e.g., *Candida cylindracea, Staphylococcus aureus*, (ii) 1, 3-specific lipase catalyses the reaction and targets sn-1 and sn-3 positions of TAG molecular species, e.g., *Mucor miehei, Rhizopus delemar, Rhizopus arrhizus*, (iii) Lipases which hydrolyses mono, di and triacylglycerol with different rates, e.g. lipases preset in rats and humans tissues (Jensen *et al.,* 1983), (iv) Lipases which catalyse exchange of specific types of fatty acids in the triacylglycerol, e.g. lipase from *Geotrichum candidum* (Macrea, 1985) and (v) Stereo-specific lipases which show a faster rate of hydrolysis for fatty acids located on sn-1 (sn-3) position or sn-3 (sn-1) position, e.g., lipase from milk, adipose tissue etc. The selectivity and specificity of lipase depend upon the condition of the reactions, such as time, temperature, pH, water activity etc.

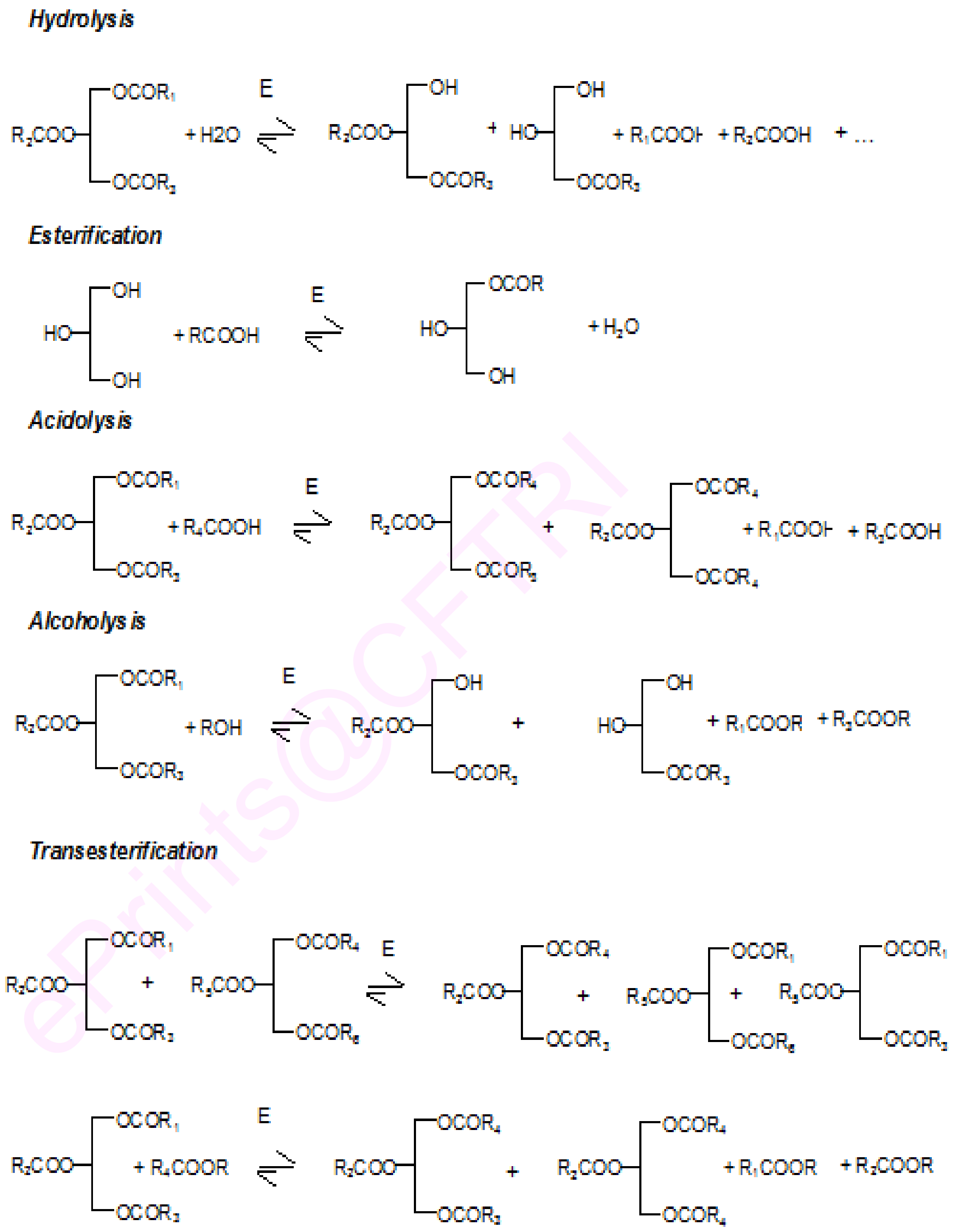


Fig. 5 Principal lipase catalysed reactions in oil and fat modifications
(*Source:* Villeneuve and Foglia, 1997)

STABILITY AND REUSABILITY OF LIPASES

The stability and reusability of the enzyme are important requirements for industrial production of high quality of interesterified oil. Immobilisation of lipase can change its stability due to active form of ternary structure of the enzyme. Immobilisation of lipases facilitates to enhance the thermostability of enzyme by terminating the reaction after its physical removal from the substrate (Fig. 6). The lipase from Rhizomucor miehei is a commercially available enzyme. It is highly active (Rodrigues and Fernandez-Lafuente, 2010) and stabile. The main uses of this enzyme is modification of oils and fats by esterification, transesterification, acidolysis and interesterification.

GENETIC MODIFICATION

The genetic modification of oil seeds is required to develop cultivars to produce oils of desirable qualities such as higher oxidative stability, the predetermined fatty acid composition, improved nutrient components such as tocopherol, phytosterol. The fatty acid composition of vegetable oils differ from one species to the other (Gunstone *et al*., 1986). Plant breeders have developed cultivars for producing oils, which can provide health benefits (Corner, 1983). It is reported in the literature that genetic modification of cotton seed oil help the food industry in providing high-oleic acid and high stearic acid cotton seed oil which is useful for making stable frying oil and hard stock in margarine production (Liu *et al*., 2000).

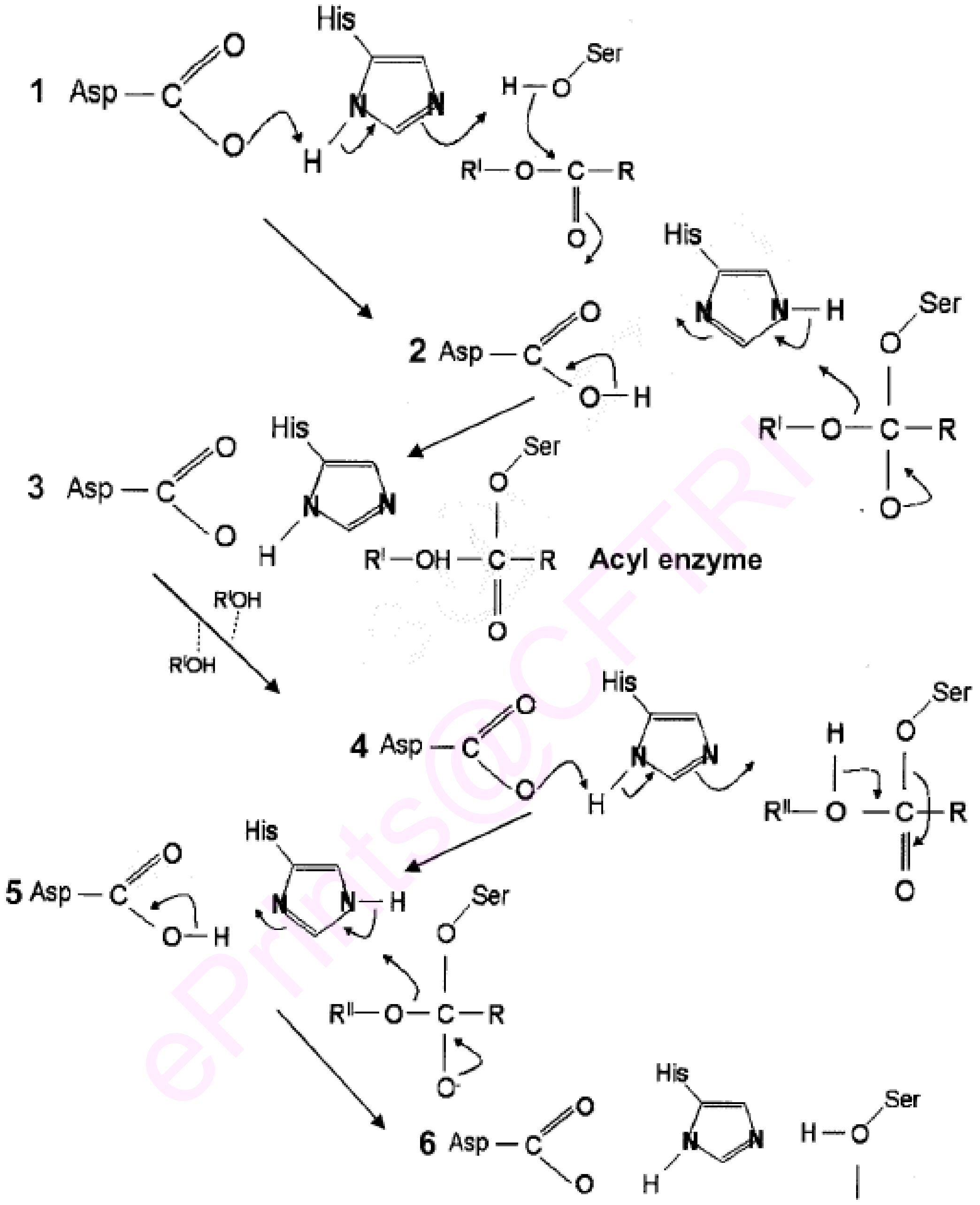


Fig. 6 Mode of action of lipases (catalytic mechanism) (Marangoni and Rousseau, 1995)

The genetic modification approach allows one to create genetically modified plants that produce ‘designer oils’, in which fatty acid profile is specifically altered for precise end uses (Lands, 1986). In recent times many food industries are paying attention to the composition of the triacylglycerols as well as development of ‘structured lipids’, in which composition of oils are modified to suit end product requirements (Norris, 1990). It is also reported that the content and composition of tocopherols, sterols and phospholipids and fatty acids in soybean oil can be altered by genetic modification of soybean at the molecular level (Mounts *et al*., 1996). Another application of genetic modification is the development of Canola-quality Indian mustard (*Brassica juncea*) for cultivation in hot and low-rainfall areas by imparting heat heat and drought tolerance, disease resistance (Woods *et al*., 1991, Burton, *et al*., 2004) and higher oxidative stability (Wijesundera *et al*., 2008) than canola (*Brassica napus*).

These examples give an insight into the need for modification of oils and fats for specific use. This research work proposes to examine how the blending as well as enzyme interesterification influences the physical, thermal, and hydrodynamic properties of the selected oils.

The objectives of this work are to:

1. (a) develop a protocol based on enzyme treatment of selected vegetable oils for the production of oil blend with desirable physical, thermal and hydrodynamic properties,

2. study the heat and mass transfer phenomena in an optimized model oil blend for a specific product (e.g., Indian traditional fried food) during immersion frying and

3. study changes in the heat and mass transfer aspects of the oil blend during prolonged frying.

The proposed studies includes, (i) the selection and standardization of suitable oil model system based on locally available vegetable oils, protocol for the enzyme treatment for producing interesterified oils. (ii) studies on (a) physical, thermal and hydrodynamic properties of the optimized oil blends, (b) heat and mass transfer in the prepared oil blend during immersion frying and (c) changes in heat and mass transfer aspects of the oil blend during intermittent and continuous frying.

AIM AND SCOPE OF THE PRESENT WORK

In food processing, properties of oils such as viscosity, heat transfer play important role for arriving at process parameters, needed for design, and fabrication of equipments used for frying operations.

Understanding the frying characteristics of oil which is partitioning between surface and structure of fried foods is important to develop healthy snack foods. Exposure of oils to continuous frying conditions may induce oxidation. Monitoring of lipid oxidation, polymerization are essential criteria to evaluate the stability of oil. Monitoring of degradation of oils is important for storage and frying stability of oil.

In order to get a stable oil for frying operations biotechnological tools, such as, lipase catalyzed interesterification using immobilized 1, 3- specific lipase from *Rhizomucor miehei* was adapted to modify triglycerides in oil in desired manner. Enzymatic interesterification reactions were controlled by variables such as substrate concentration/level of oil in the blend, enzyme concentration, reaction temperature and reaction time. These parameters were optimized for developing of modified oils which has low viscosity and high heat transfer coefficient. The physical and thermal properties of these oils were evaluated. A custom designed air heater was used for generating data on kinematic viscosities. The physico-chemical and thermal properties such as hydrodynamic property in terms of kinematic viscosity, triglycerides composition, melting profile, heat transfer coefficient of interesterified oils were studied. These studies would help in understanding the changes in the physico-chemical and thermal properties of the blended and enzyme interesterified modified oils in comparison to that of reference oils. The reference oils used in this study were rice bran oil and refined palm olein.

The kinetics of oil partitioning between surface and structure during frying was studied to understand the oil distribution in the product. The mass transfer in terms of moisture outflow and oil absorption during prolonged frying of *poori* (an Indian traditional fried food) were also studied using reference, blended and interesterified oils to assess the degradation of oil during frying conditions. The results of these studies provide us an insight into the use of modified oils with better physical, thermal and hydrodynamic properties in frying operations.

Materials and Methods

MATERIALS

Raw materials

Edible vegetable oils

Commercially available Rice bran oil (RBO) and refined palm olein (RBDPO) were procured from Habib Agro Industries, Mandya, Karnataka, and Mysore Mercantile Co. Ltd., Bangalore, Karnataka, India, respectively. Coconut oil (CNO), Ground nut oil (GNO), Mustard oil (MO), Sunflower oil (SFO) and Sesame oil (SESO) were procured from a local super market.

Different edible oils, viz., coconut oil (90.5% saturated, 7.0% monounsaturated and 2.5% polyunsaturated fatty acids), groundnut oil (18.4% saturated, 42.8% monounsaturated and 33.4% polyunsaturated fatty acids), rice bran oil (21.8 % saturated, 43% monouturated and 35.3% polyunsaturated fatty acids), refined palm olein (RBDPO) (47.7% saturated, 41.5% monounsaturated and 10.8% polyunsaturated fatty acids), mustard oil (3.0% saturated, 68.9% monounsaturated and 26.3% polyunsaturated fatty acids), sunflower oil (9.6% saturated, 47.4% monounsaturated and 42.1% polyunsaturated fatty acids) and sesame oil (9.8% saturated, 48.9% monounsaturated and 40.2% polyunsaturated fatty acids) were chosen for the initial experiment for selection of oils as these oils represent broad spectra of oils used in the snack food industries.

Enzyme and Chemicals

Lipase enzyme, Lypozyme® RM IM is a Gift from Novozymes, Denmark. All the standards for fatty acids, standards of triglycerides, 2, 2 diphenyl 1-picrylhydrazyl (DPPH) were obtained from Sigma–Aldrich Co., St. Louis, MO, USA. Hydrochloric acid, potassium hydroxide, potassium iodide, chloroform, petroleum ether, ethyl alcohol, phenolphthalein, sodium thiosulphate are procured from Loba Chemie, Mumbai. HPLC grade hexane, methanol, acetone, acetonitrile were procured from Sigma–Aldrich Co., St. Louis, MO, USA.

Potatoes

Potatoes (*Solanum tuberosum*) were procured from a local super market. Cylindrical potato fries (dia 1.0 cm x length 4.0 cm) were cut using a mechanical potato slicer.

Wheat flour

Wheat flour was procured from a local super market. Moisture, fat, protein, carbohydrate, ash content of the wheat flour were 12.9 2.7, 10.5, 73.4 and 0.5%, respectively.

METHODS

Selection of oils

Coconut oil (CNO), Ground nut oil (GNO), Mustard oil (MO), Palm olein (RBDPO), Rice bran oil (RBO), Sunflower oil (SFO) and Sesame oil (SESO) are extensively used for frying and cooking purposes in India. For selection of suitable oils required for this work an initial study was conducted using these oils for frying of *poori* (a traditional Indian breakfast and snack food, made of wheat flour). *Poori* (70-80 mm dia and 2-3 mm thickness) was deep fat fried using the above oils at 173±1^{0}C for 3 min with a batch of 6 *pooris* at a time. The frying was repeated for continuously for 10 cycles with same number of *pooris* in each cycle. The fried p*ooris* were evaluated based on sensory odour profiles of control and fried oils. The physic-chemical properties of oils used for frying were analysed. Sensory odour profiles of the selected blended and interesterified oils were studied. Consumer accepetance study of fried *poori* using blended as well as enzyme interesterified oils was also carried out.

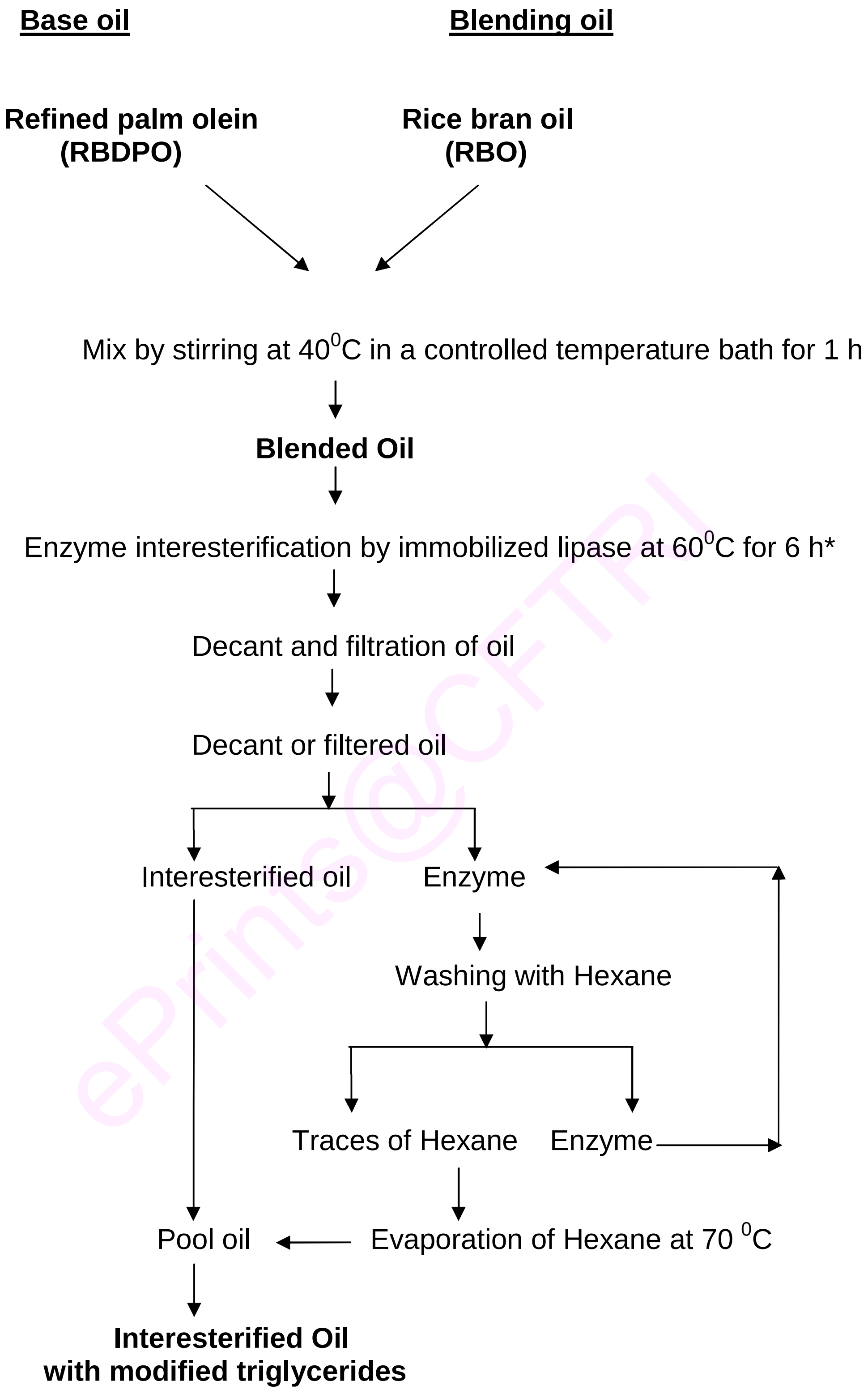
Preparation of oil blends

A reciprocating shaking water bath (model BS-31, Jeio Tech Co. Ltd., South Korea) was used for making different oil blends based on RBO, and RBDPO. Binary blends of RBO-RBDPO were made in the ratios of a) 20:80 (w/w), b) 30:70 (w/w), c) 40:60 (w/w), d) 50:50 (w/w), e) 60:40 (w/w), f) 70:30 (w/w) and g) 80:20 (w/w). The blending was carried out at the shaking speed of 150 rpm at 40 °C for 1 hr. Fatty acid composition of modified oils were determined by Gas Chromatography (AOCS: Ce 1-62, 1993) under nitrogen atmosphere.

Protocol for Enzymatic Interesterification of oil blends

The lipase-catalyzed interesterification (transesterification) reaction of RBO blended with RBDPO was carried out in a reciprocating shaking water bath (model BS-31, Jeio Tech Co. Ltd., South Korea) as described earlier

(Reena and Lokesh, 2007). The binary oil blends (BL) of RBO and RBDPO were taken in a round-bottomed flask in the ratio of i) 2:8, ii) 5:5 and iii) 8:2 (w/w) and placed on a constant temperature water bath at 60±1°C. Immobilized *Rhizomucor miehei* lipase (10% w/w based on oil) was added and incubation was continued at the shaking speed of 150 rpm for a period of 6 hr. The enzyme interesterified oil (EI) was separated from the enzyme by filtration. The products obtained were then tested for different physico-chemical, hydrodynamic, thermal, heat transfer. The modified oils were also monitored for apparent viscosity, FFA, PV, diene/triene value, TPM, kinematic viscosity, melting point, enthalpy, heat transfer coefficient, triacylglycerol molecular species and thermo-oxidative stability. The protocol used for the preparation of enzyme interesterified oils is given in Fig. 1.



(* Temperature and time condition for interesterification reaction was fixed for desired levels modifications)

Fig. 1 Protocol for the preparation of enzyme interesterified oils

Heating devise for measurement of kinematic viscosity

A custom designed air bath heater (cylindrical, 30 cm x15cm) shown in Fig. 2 was constructed for this research work. The air bath heater was fabricated with aluminum sheet layered with 3.0 cm of glass wool insulation and vertically fixed on a wooden table. The air bath heater's housing was made of 6.3 mm steel plate layered with 9.5 cm of silica fiber insulation and 30 mm of glass-wool insulation. A cylindrical hollow chamber with outer diameter 180 mm, inner diameter 110 mm and length 750 mm was made with the above mentioned steel plate and vertically fixed on a horizontal wooden table. The air was heated with a 1200 W hot gun (Milwaukee, model number-1220) with built-in blower and circulated through the bottom of the chamber. A 0.2 hp thermo-variac was attached to regulate and control the temperature inside the chamber.

A circular window of 75 mm diameter was made on the steel surface at the position where the bigger bulb of the viscometer could appear when inserted from the top of the chamber and a same size glass made of 3.17 mm Pyrex^{TM} was fixed there to view the meniscus markers on the viscometer clearly from the outside of the chamber. The chamber was well isolated from outside so that there would not be any heat loss from it and temperature remains stable. An aluminum duct of V-shape was formed to hang the viscometer inside the chamber from the top. The viscometer was attached to the lid of the heater with the V-shape aluminum duct allowing only its suction and fill ports to be exposed externally. Three T-type thermocouples (36-guage, Omega Engineering,

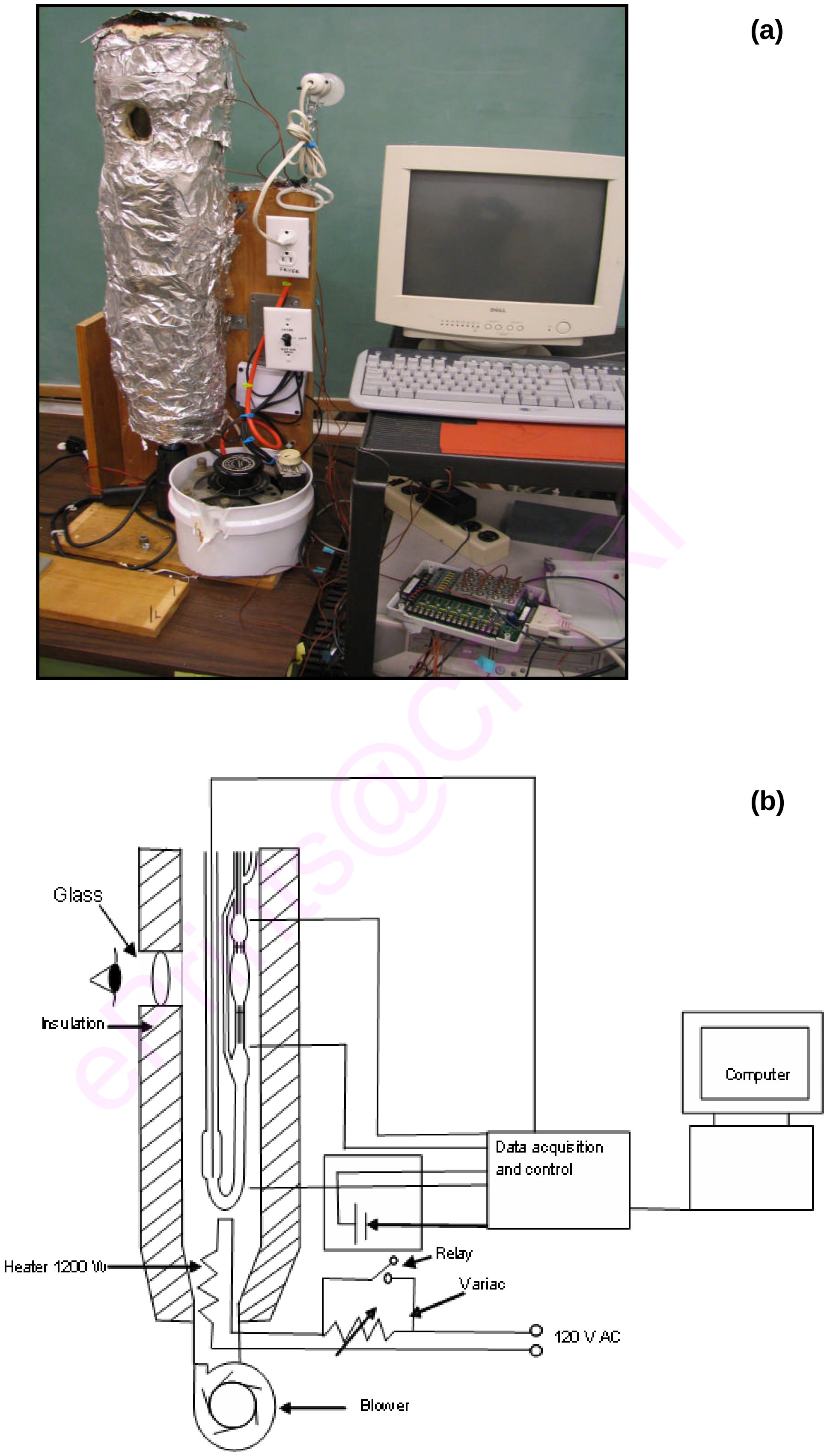


Fig. 2 (a) Set up of Air bath heater and (b) schematic presentation of Air bath heater

Stamford, USA) were calibrated using ice bath and boiling water reference and mounted in the chamber at lower, middle and higher positions. A solid-state control relay was fixed at 170°C, 180°C and 190°C, respectively to check the air temperature inside the chamber.

Data acquisition, temperature control and temperature measurement

Data acquisition and temperature control were performed using a closed loop control system with on / off control of the heating element. The 5 to 20 mV thermocouple signals were channeled to Iotech Data Shuttle High-Resolution DAS-8 eight channels analog-to-digital converter. Temperature of oil was recorded for every sec using T-type thermocouples immersed into the oil. The average temperature of the oil was recorded using two additional thermocouples (T-type) immersed into the same oil at different locations. The thermocouple signals (5-20V) were channeled through IOTECH Data Shuttle High-Resolution (DS-16-8-TC) eight channels analog-to-digital converter and timer counter interface, installed on a personal computer. This digital output was sent to a universal screw terminal accessory board connected to a solid state semiconductor relay (model: SSR ZC 20A, 75-125V AC, part no. AQR 20 A1-S-Z 4/6V DC, screw terminal type, Panasonic Electric Works, New Providence, USA). The relay turned the resistive heater on or off based on the thermocouple readings and system set-point. A multipurpose software (DASYLab-V 5.02.20) was used for data processing corresponding to air temperatures in the air heater bath or oil temperature of the equipment used for frying. DasyLab, multipurpose software package for data acquisition and control, processed the digital signals obtained from the Iotech Data Shuttle

DAS-8. Based on the values of the digital signals from the DAS-8 which corresponded to air temperatures in the heater, Iotech Data Shuttle prompted the digital input / output interface mounted on its mother board, to send out a specific digital signal (either 5 V to 9 V). This digital output was sent to the input / output universal screw terminal accessory board which allowed connection to a solid state relay. A solid state relay (Panasonic, model no- SSRZC20A, part no- AQR20A1-S-Z, 75-125 VAC, 4-6 VDC, 45 A) was used to control the heater. The relay turned the resistive heater on or off based on the thermocouple readings and system set-point.

Construction of cylindrical transducer

A cylindrical transducer made of 1100-alloy aluminum with 1 cm diameter and 4 cm length as shown in Fig. 3, was fabricated with a stainless aluminum guide wire attached at the one base of cylinder for support. Thermal conductivity, specific heat capacity and density of the transducer were 180 W/m ^{o}C, 962.9 J/kg ^{o}C and 2712.6 kg/m^3 respectively. A 36 gauge, T-type thermocouple was positioned in a small hole drilled to the center of the circular part of the transducer. To accomplish this task, a 0.8 mm hole was drilled in the center of the circular part of the transducer upto a depth equivalent to half the length of the cylinder. The thermocouple wire was sealed in the hole with 5 Minute®, a high thermal conductivity epoxy adhesive. The thermocouple was attached to Iotech Data Shuttle, eight-channel data analog board and data acquisition and control was processed by DasyLab software. Same relay with different switches was used to control the

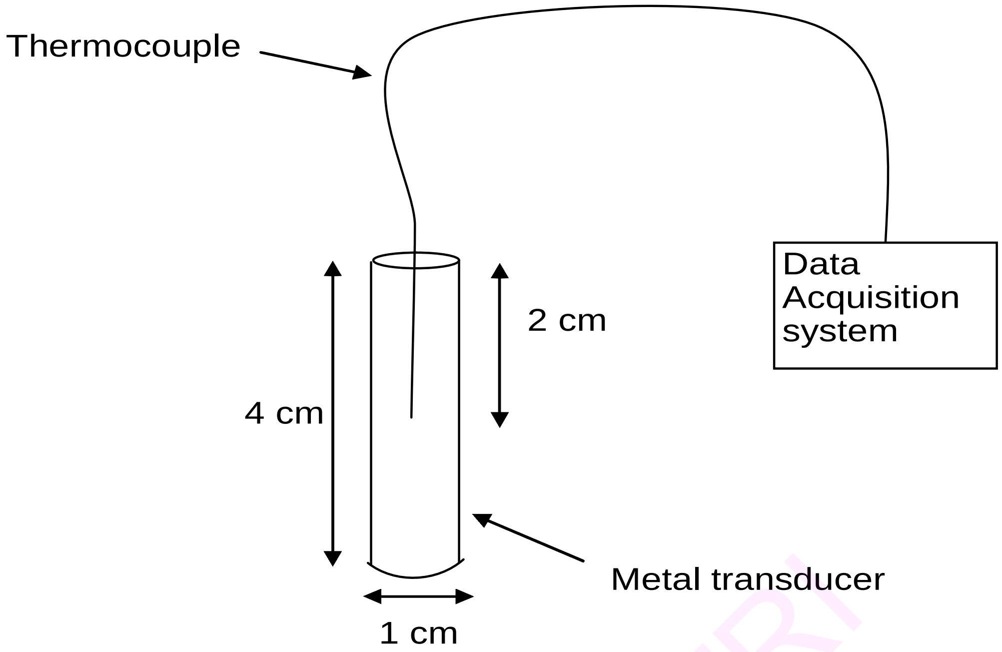


Fig. 3 Aluminum transducer attached with thermocouple and Data Acquisition System (DAS)

temperature of fryer. The relay turned the resistive heater on or off based on the thermocouple readings at system set point.

Kinematic viscosity

Kinematic viscosity of the different oils was measured with a calibrated Cannon^{TM} Ubbelhode capillary viscometer (size 75, Cannon Instruments company, State College, PA, USA) as per method reported by Debnath *et al*, (2010). A sketch of which is shown in Fig. 4. The T-type thermocouple was inserted directly to the oil to monitor its temperature during the experiment. The viscometer was calibrated first by using the procedure given in its manual.

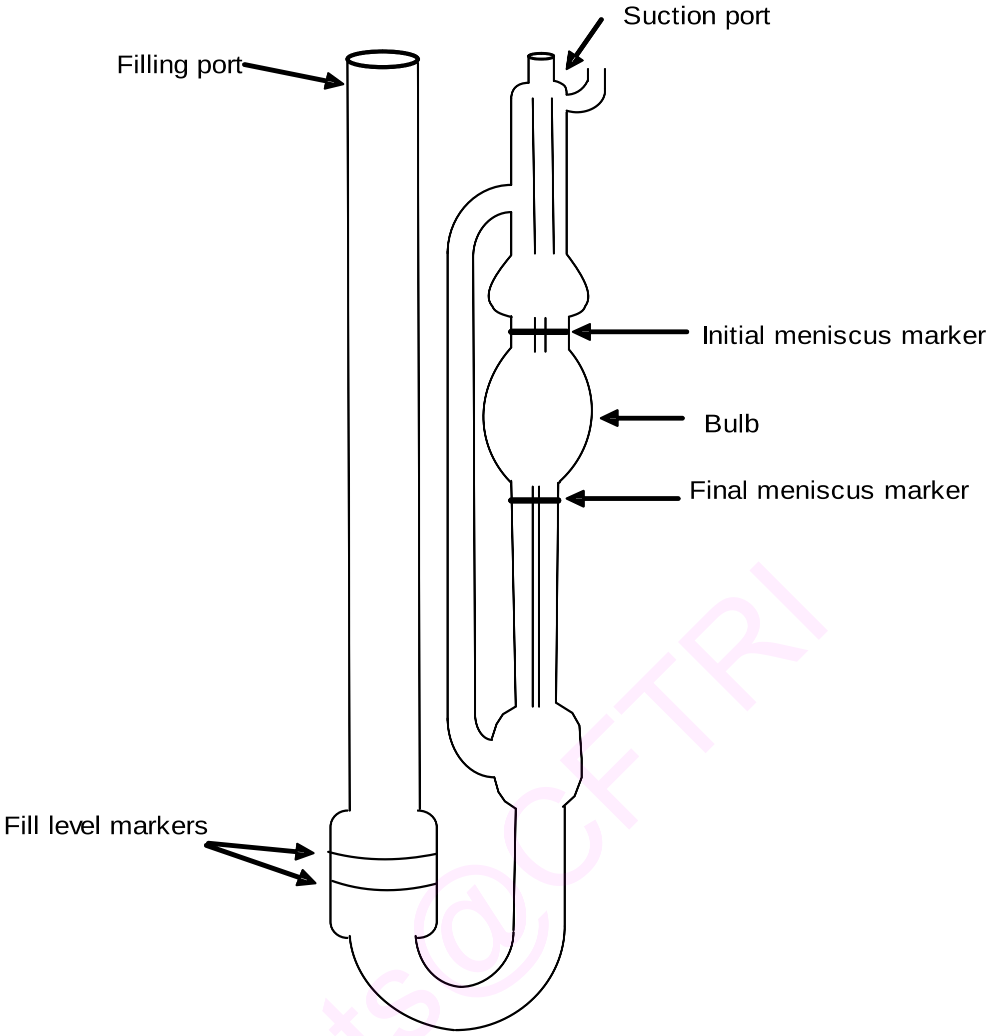


Fig. 4 Capillary viscometer used for measurement of kinematic viscosity

Heat transfer coefficient

An electric fryer (Presto, FryDaddy, 120V AC, 1200 watt, National Presto Industries, Inc., China) was used for heat transfer study (Fig. 5). Convective heat transfer coefficients were measured using a lumped capacity analysis method (Holman, 1997). A cylindrical metal transducer (type 1100 alloy aluminum, diameter 0.01m, length 0.04 m, density ρ = 2712.64 kgm^{-3}, specific heat, c_p = 962.9 Jkg^{-1} °C^{-1}) (Perry and Green, 1994) was used to measure heat transfer coefficient at 170 to 190±2°C. The heat transfer coefficient of oils were estimated during the experiment from time-temperature data obtained from the metal transducer. The dimension less temperature ratio $\ln\left(\frac{T_A - T_t}{T_A - T_i}\right)$ was plotted against the time (t) of different frying oil and their blends. The slope of the linear portion of the graph was considered and equated with $\left(\frac{hA}{\rho V c_p}\right)$ as per Eqn. (1) furnished below.

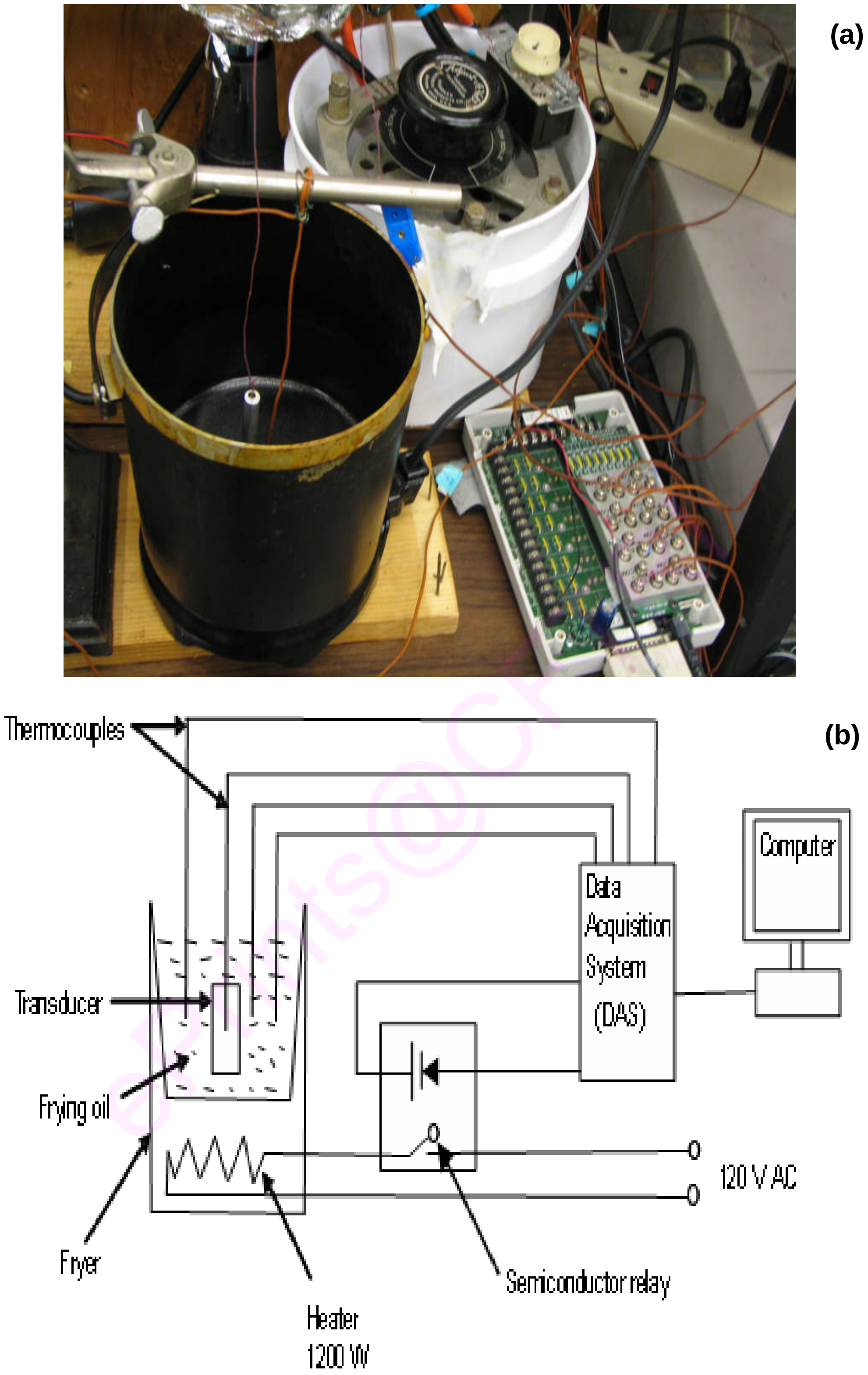


Fig. 5 (a) Set up of the fryer and (b) schematic Diagram of fry fryer set up

Heat transfer can be determined using Eqn. (3), since the area (A), density (ρ) and specific heat (c_p) of the metal transducer are known. The regression equation was fitted to the time-temperature data to the following Eqn. (1), using software, Microsoft Excel 2003. Heat transfer coefficient was calculated from the slope of the equn. (3).

$$\ln\left(\frac{T_A - T_t}{T_A - T_i}\right) = -\left(\frac{hA}{\rho V c_p}\right)t \quad \text{… Eqn. (1)}$$

$$Slope = M = \left(\frac{hA}{\rho V c_p}\right) \quad \text{… Eqn. (2)}$$

$$h = M\left(\frac{\rho V c_p}{hA}\right) \quad \text{… Eqn. (3)}$$

where, T_t - transducer temperature at any time, °C; T_A - oil temperature, °C; T_i - initial transducer temperature, °C; t - time, sec; A - area of transducer, m^2; ρ - density of transducer, kg/m^3 ; c_p - specific heat of transducer, J/kg °C, V- volume of transducer, m^3.

Activation energy

The influence of temperature on viscosity is explained (Fasina *et al.,* 2006) by an equation similar to Arrhenius type (Eqn. 4) given as follows:

$$\nu = A_0 \exp^{\left(-\frac{E_a}{RT}\right)} \quad \text{… Eqn. (4)}$$

where, ν - is the kinematic viscosity, m^2s^{-1}, A_0 - constant, E_a - activation energy, $Jmol^{-1}$, R - the universal gas constant, $Jmol^{-1}K^{-1}$, T- absolute temperature, K. The constant, E_a was calculated from the slope of the plot of ln ν (kinematic viscosity) against 1/T (1/absolute temperature) for selected oil blends.

Flow behaviour and apparent viscosity of oils

Apparent viscosities of the different oils used for frying were carried out using a controlled shear-stress viscometer (Model # RT 10, Haake Gmbh, Karlshruhe, Germany) consisting coaxial cylinder at a shear rate of 102 s^{-1} at 25±1°C and 50±1°C. Thermostatically controlled water bath was used for maintaining temperature of the oil during the experiment (Bhattacharya e*t al.,* 1999*)*. The shear-stress and shear-rate data were fitted to the power-law model (Eqn. 5) and flow behaviour index and consistency index were estimated by employing non-linear analysis of shear stress/shear-rate data, using the Microsoft Excel 2007 software.

$$\sigma = K \left(\dot{\gamma} \right)^{n} \qquad \text{…Eqn. (5)}$$

where, σ - shear stress (mPa), $\dot{\gamma}$ - shear-rate (s^{-1}), K - consistency index (mPa s^n) and n - flow behaviour index (dimensionless).

Hydrodynamic properties

Hydrodynamics involves the measurement of hydrodynamic properties, such as kinematic viscosity, density, surface tension. The kinematic viscosity of the native, blended and enzyme interesterified oils was measured with a Cannon^{TM} Ubbelohde calibrated capillary viscometer (size 75, Cannon

Instrument Company, State College, PA) as per method reported by Debnath *et al.* (2010a). A T-type thermocouple with digital temperature data logger (Hitech, Bangalore, India) connected to a personal computer was used to monitor temperature of the oil.

Specific gravity

Specific gravity is defined to as the ratio of the mass of a given volume of a substance to the mass of a different reference substance such as water. Specifc gravity was measured at 25°C according to the AOAC (1975) method.

Surface tension

The tensiometer (Tesiometer K100, Krüss GmbH, Humberg, Germany) was used for measuring surface tension between oil and air at 25°C following Du Noüy Ring method (Paddy and Russel, 1960).

Colour of oils

The colour of the oils was measured using Lovibond Tintometer (model F, Tintometer Ltd., Salisbury, U. K.). The analysis was carried out using 1 inch cell in the transmittance mode and expressed as Red (R) and Yellow (Y) units. Colour of the oil was measured before and after heating/frying operations.

Frying studies

Deep fat frying of potato slices

Potato fries, after cutting, were washed immediately for 1 min with distilled water to remove starch adhering onto the surface before frying. Frying was carried out using a laboratory model deep-fat fryer (Dormeyer, model DF3CH, Chicago, USA) filled with two liters of frying oil. For each experiment, ten potato slices were deep-fried usng 2.0 liters of oil (1:50 slice to oil ratio). The temperatures of potato at the surface (0.5 mm from surface) and geometric centre as well as frying oil were recorded by a thermocouple (T-type) using digital temperature indicator (Hitech, Bangalore, India) connected to a personal computer. Frying of the potato slices was carried out at a constant temperature of 180±1°C for 5 min. The samples were withdrawn after 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180, 240 and 300 s, and moisture as well as oil contents were determined. The samples were submerged in oil with the help of a wire mesh to ensure good contact between the potato slices and the oil. To hold the sample at the predetermined temperature after frying, the samples immediately after frying were transferred to preheated hot air oven maintained at the selected temperatures. The samples were withdrawn after 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180, 240 and 300 s for the determination of surface (oil fraction available on the surface) and structure oil (oil penetrated into the core of potato slice) content.

Surface, structure and total oil content

The surface oil content of immediately fried or fried sample held at various temperatures or times was estimated as per the procedure given by

Durán *et al.* (2007), by immersing the fried product into hexane for 20 s immediately followed by removal of product and evaporation of hexane. After the extraction of surface oil, structure oil content was estimated by drying the samples at 105°C for 24 h followed by soxhlet extraction with petroleum ether (AOAC, 1980). Oil content was expressed on dry weight basis as kg of oil/ kg dry solids. Total oil content was calculated by addition of surface oil and structure oil contents. All the experiments were done in triplicates and average values were presented.

Mass transfer coefficient

The equation for oil uptake was written, considering a first order mass transfer kinetics (Krokida *et al.*, 2001a & b; Debnath *et al.*, 2003) as

$$dO/dt = K_o(O_t - O_\infty) \qquad \text{....Eqn. (6)}$$

where K_o is the kinetic coefficient for oil transfer and is specific to geometry, initial composition and process conditions; O represents the oil content; subscripts t and ∞ represent the relevant concentration at t = t and t = ∞, respectively; O_∞ is the equilibrium oil content. Since O_∞ does not represent the physical driving potential for oil transfer, it was considered as pseudo coefficient, and has been referred to as equilibrium oil content in the text for the sake of convenience.

Integration of the Eqn. (6), with appropriate initial condition, resulted in the following equation (Krokida *et al.*, 2001a & b):

$$\ln\frac{(O_t - O_\infty)}{(O_o - O_\infty)} = \ln(O_r) = K_o t \qquad \text{.... Eqn. (7)}$$

where, O_r is the diffused oil ratio. The rate of change of oil content (dO/dt) was obtained from variation of oil content plot. (dO/dt) was plotted against average oil content (O) and the equilibrium oil (O_∞) content was inferred from slope of such plot. The experimental data (-ln O_r against t) was plotted as per Eqn. (6) and the value of K_O was obtained from the slope of such plot.

A correlation similar to Arrhenius equation (Eqn. 7) was proposed to relate the effect of holding temperature on mass transfer coefficients of oil (Durán et al., 2007). The coefficients of the equation were inferred from the intercept and slopes of the plot of –ln (K_o) and against 1/T. R is the gas constant (8.314 kJ/kg mol K).

$$K_o = 0.53 \cdot \exp\left(-\frac{7.28 \cdot 10^3}{RT}\right) \qquad \text{….Eqn. (8)}$$

This type of correlation indicated that the decrease in mass transfer coefficients of oil with an increase in holding temperature followed an exponential trend.

Measurement of oil partition coefficient

Oil partition coefficient (K_p) was calculated as follows:

$$K_p = \frac{\text{Oil content present on the surface}}{\text{Oil content present on the structure}} \qquad \text{….Eqn. (9)}$$

Preparation of Poori

Poori is an Indian traditional breakfast and snack food made of wheat flour. Frying of *poori* under controlled condition was carried out using a laboratory scale deep-fat fryer (Dormeyer, model DF3CH, Chicago, USA) using two liter of each oil. Dough was prepared by adding 600 ml of water to 1 kg of wheat flour, and mixing by a planetary mixer (Hobart, England). The dough was made into small balls (20 g each), which was hand pressed between two flat plates to form circular disc (dia: 70-80 mm, thickness: 2-3 mm) before frying. In each experiment, 6 *pooris* were fried for a duration of 3 min at a temperature of 173±1°C. The temperature of oil was measured by a thermocouple (T-type) connected to a personal computer via digital temperature indicator (Hitech, Bangalore, India). The *pooris* were submerged in oil during frying with the help of a wire mesh to ensure exposure of both the surfaces to the oil for effective contact. The frying was continued for 10 frying cycles.

The oil samples were withdrawn after frying, allowed to cool at 25±1°C, and immediately stored in the refrigerator for subsequent analysis.

Heating/simulated frying studies of oils

Heating of oil under controlled condition was carried out using a laboratory scale deep-fat fryer (Dormeyer, model DF3CH, Chicago, USA) filled with two liter of oil. The temperature of oil during heating was recorded at 173±1°C for 3 min by a thermocouple (T-type) using digital temperature indicator (Hitech, Bangalore, India) connected to a personal computer. Five batches of heating (each 3 min) were conducted in a day without any time lag and this exercise was repeated for 6 consecutive days. This was referred as heating/frying cycles in an intermittent mode. There was 24 h gap between the two cycles. After each cycle, the fryer was put off and heated oil was allowed to cool to ambient temperature (25±1°C) and the samples were drawn each day and stored in a brown bottle in refrigerator for subsequent analysis.

Intermittent frying of *poori*

The poori was frying under the same condition as mentioned above. In this case *poori* frying was carried out in a true manner.

ANALYSIS

Physicochemical properties of oils

The physicochemical properties of oils, viz., colour, free fatty acids (FFA), peroxide value (PV), total polar material (TPM), fatty acid composition, oryzanol content, radical scavenging activity (RSA) were determined. The methodologies for the determination of these parameters are given in the following sections.

Fatty acids composition of oils

The fatty acids composition of the native, blended and interesterified oils were determined as fatty acid methyl esters by using Gas Chromatograph (AOCS, Ce 1-62, 2002) (model GC-15A, Shimadzu Corporation, Kyoto, Japan) equipped with flame ionization detector (FID) and a data processor (model CR-4A). GC was fitted with a stainless steel column (3m length x 0.5 mm i.d.) packed with 15% diethylene glycol succinate (DEGS) supported on 60-80 mesh Chromosorb WAW (Shimadzu Corporation., Kyoto, Japan). The GC was operated at the conditions of column temperature 180°C, injector temperature 220°C, detector temperature 230°C, with a nitrogen flow of 40 ml/min; hydrogen flow 40 ml/min, air flow 300 ml/min. The fatty acids were identified by comparison to the retention times of standard fatty acid methyl esters. The content of saturates, monounsaturates and polyunsaturates were calculated from the fatty acids composition of the oils.

Triglyceride molecular species

The triacylglycerol molecular species of the native, blended and interesterified oils were identified according to the method of Che-Man *et al.* (1998a), using HPLC (Shimadzu, Kyoto) equipped with a LC-10AT pump, system controller CBM-10A, and RID-10A refractive index detector with the CLASS-LC10 software. The column used was (4.6x250 mm) packed with a particle size of 5 μm (Symmetry® RP-C18, Waters). The mobile phase and the flow rate used were a mixture of acetone-acetonitrile (63.5:36.5, v/v) and 1 ml/min, respectively. 20 μl of 20% (w/v) oil in chloroform was injected to HPLC system for the analysis. Triacylglycerol molecular species were identified on the basis of theoretical carbon number (TCN) and based on retention time of triacylglyceol standards, such as LLL, PPP and OOO (Bland *et al.*, 1991). It was observed from the fatty acid composition that the major portion of the RBO and RBDPO to be composed of palmitic (P), oleic (O) and linoleic (L) acids. Therefore, the elution sequence of triacylglycerols was predicted on the basis of TCN which was calculated by the formula as $TCN = [\{(CN)-(2n)\} - (0.7\ L) - (0.6\ O)]$, where CN is no. of carbon atoms in these three fatty acids of triacylglycerol and n the no. of double bonds (El-Hamdy and Perkins, 1981). To ascertain the triacylglycerol molecular species after its separation, individual fraction was collected from different runs of same composition for every peak, esterified to fatty acid methyl ester (FAME) and analysed by Gas Chromatograph. Each fatty acid was identified by correlating the retention value of standards.

Melting profile

The melting profiles of the native, blended and enzyme interesterified oils were determined using a differential scanning calorimeter (DSC) (Perkin Elmer, USA), consisting auto cool accessory connected to liquid nitrogen to cool the DSC cell at a specific cooling rate. The cell was also provided with purge gas (nitrogen) at the rate of 10 ml/ min to avoid thermal currents in the cell. The DSC was calibrated with indium, lead and tin as the reference standards provided by the supplier. A sample of 5-10 mg of triglycerides was placed in a hermetically sealed aluminum pan. An empty pan was used as reference. The data on heat flow and temperature were analyzed with the thermal software provided with the system. Analysis was done according to AOCS recommended DSC procedure (AOCS, CJ 1-94, 2000). The samples were initially subjected to tempering by heating rapidly (10°C/min) from room temperature to 80°C and holding at that temperature for10 min to destroy crystal memory. The samples were then cooled at the rate of 10°C/min to -40°C and held at this temperature for 3 min. Following this, the sample was heated again to 80°C at the rate of 10°C/min. Heating and cooling thermograms were recorded and used for the determination of temperature for onset, completion, melting and crystallization range and also for the enthalpy of melt and crystallization. The peak areas were measured at by using the software provided by the instrument supplier.

Thermo-oxidative stability

The thermal decomposition of the native, blended and enzyme interesterified oils were analyzed by Thermogravimetric analysis (TA) and Differential Thermogravimetric Analysis (DTA) method under air flow rate of 100 ml min^{-1} using a Thermogravimetric analyzer (Perkin Elmer, USA) at a constant heating rate of 10 $^{\circ}$C min^{-1} up to 600°C. About 8±0.5 mg of the oil was used for each experiment. The thermal stability of these oils were measured based on initial decomposition temperature.

Frying qualities

The frying qualities of oils were determined by their thermal stability during frying of formed products, such as potato slice, p*oori*. The thermal stability of the fresh oil, blended, interesterified oils was evaluated by the changes in oil colour, accumulation of degradation products after repeated frying of *poori* at 173±1°C for 3 min.

Free fatty acids

The free fatty acid (FFA) content of the oils were analysed by AOCS Official Method (AOCS O.M. No. Ca 5a-40, 2002). The neutralized alcohol was added to the oil sample and allowed to boil, and then titrated against sodium hydroxide using phenolphthalein indicator. The values were expressed as % oleic acid.

Peroxide value

The peroxide value (PV) of the oils were as determined by using standard procedures (AOCS O.M. No. Cd 8-53, 2002). The peroxides present in the oil oxidises KI to iodine and was determined quantitatively by titration with sodium thiosulphate and expressed as milliequivalents of oxygen per kilogram of oil. The oil sample was dissolved in acetic acid/chloroform mixture (3:2), followed by the addition of saturated KI and kept aside under the closed condition for a period of 1 min. The reaction was terminated with the addition of distilled water and was titrated against standard sodium thiosulfate using freshly prepared starch indicator till the blue colour disappeared. The values are expressed as milliequivalents of oxygen/ kg of oil.

Conjugated dienes and trienes of oils

Conjugated diene and triene (oxidation products) (AOCS O. M. No. Ti1a-64, 1977) of the native, blended, enzyme interesterified oils were analyzed by spectrophotometric measurement at the wavelengths of 233 and 266 nm, respectively. Assessments of these quality parameters for native, blended and interesterified oil samples were also monitored after deep fat frying of Poorias mentioned above.

Total polar material

In the present study, Fri-Check (Fri-Check bvba, Belgium) (Gertz, 2000) was used to measure the degradation of oils in terms of total polar material (TPM) before and after heating of oil and oil collected after frying of *poori*. Oil was filled up to the mark of the cylindrical sensor tube and inserted into the Fri-Check unit. The measurement starts with a beep sound and ends with display of total polar material. The sensor tube monitors the total material in terms of viscosity changes of degraded frying oils.

Proton ^{1}H NMR spectroscopy

Assessment of degradation of oils using proton NMR spectroscopic method is a sensitive method for quantification of protons formed during degradation of fats and oils during frying. The ^{1}H one-dimensional data were recorded on a Bruker 500 NMR spectrometer (Avance 500). A Bruker 5 mm proton probe was used. All experiments were conducted at 22°C. ^{1}H chemical shift was referenced to solvent $CDCl_3$ (7.25 ppm). The resonance frequencies are 500.11 MHz for ^{1}H. For ^{1}H one-dimensional NMR, the pulse length is 4 μs pulse (60 degree tip angle); the sweep width is 7 kHz; the data size is 32k; the number of transient is 32 with 2 dummy scans; the receiver gain is 15; the recycle delay is 1 second. The different fatty acid methyl esters were used before taking NMR of samples to standardize the different proton signals. NMR analyses of frying oils were carried out by taking samples in $CDCl_3$, with tetramethylsilane as an internal standard. The total protons present (absolute value) in the oils before and after frying were recorded.

Oryzanol content

Oryzanol content in native, heated and fried rice bran oil used for frying was determined by spectrophotometric (model UV-1601, Shimadzu Corporation, Kyoto) method (Gopala Krishna *et al.*, 2006) by dissolving 0.01 g of oil in 10 ml of hexane and reading the absorbance at 314 nm in a 1-cm cell. The oryzanol content was calculated as percent basis using the Eqn. 10.

$$Oryzanol \quad content \quad (\%) = [(A/M)(100/358.9)] \quad \text{… Eqn. (10)}$$

where, A is absorbance of the oil, M is weight of the oil in gram/100 ml, 358.9 is extinction coefficient of oryzanol.

Radical scavenging activity

The radical scavenging activity (RSA) of the oils was assayed by reduction of 2, 2 diphenyl 1-picrylhydrazyl (DPPH) radicals in toluene according to the procedure followed by Ramadan *et al*., (2003). A HPLC grade toluene solution of DPPH radicals at a concentration of (10^{-4} M) was freshly prepared and 50 mg of oil sample was added with 4 ml DPPH aliquot in toluene solution in a test tube and allowed to mix for about 20 s and kept aside for 60 min at ambient temperature. Afterwards, the absorption of DPPH radicals was recorded at 515 nm in a 1 cm quartz cell after 60 min of mixing against a blank of pure toluene with DPPH radicals using UV-visible spectrophotometer (model UV-1601, Shimadzu Corporation, Kyoto). The radical scavenging activity was calculated using the following Eqn. 11:

$$RSA \ (\%) = [(O.D. \ of \ control - O..D. \ of \ sample)/(O.D. \ of \ control)]*100$$

…Eqn. (11)

Moisture and oil content of product

The moisture content of the potato slice/*Poori*was determined by drying the sample in a hot air oven at 105°C for a period of 24 h (AOAC, 1980). The oil content was estimated by drying the samples at 105°C for 24 h followed by soxhlet extraction with petroleum ether (AOAC, 1980). Oil content was expressed on dry weight basis as % of total oil content. All experiments were carried out in triplicates and average values were presented.

Sensory odour profiles

For sensory odour profiling of fried oils, a group of 10 - 12 panelists were trained over three sessions for descriptive sensory analysis. The members of the panel were drawn from the scientific staff familiar with sensory analysis techniques working in lipid technology and related fields. The training included the development of a common lexicon of the sensory attributes in evaluation. Initially free choice profiling was carried out for all the individual oils. In order to assist the panelists in selection of descriptors, dominant odour notes of the oils used in this study and the reference compounds corresponding to the odour notes given in Table A were used. The panelists evaluated the oils and recorded the perceived attributes individually. Following this, open discussion was held to reach an agreement on appropriate descriptors, according to guidelines (Dravnieks *et al.*, 1985; Rajalakshmi and Narasimhan, 1996; Jowitt, 1974) to enable the panelists to characterize the odour of oils. The score cards developed for the quantitative descriptive analysis (QDA) had 15 cm scale with selected descriptors anchored at either end for low and high intensity (Stone *et al*., 1974). Sensory

Table A. Edible Oils and their Dominant Odours

Oil	Dominant odours
Groundnut oil (refined)	Nutty, sweet, fresh, oil-like
Sunflower oil (refined)	Seedy, sweet, fresh, oil-like
Coconut oil	Nutty, sweet, fresh, copra-like
Mustard oil	Sulphury, pungent, sour
Sesame oil	Nutty, earthy, oil-like
Rice bran oil (refined)	Branny, oil-like, husk-like
Refined palm olein	Husk-like, oil-like, beany

Sensory descriptors	Reference chemical compounds (Dravnieks, *et al.*, 1985)
Husk-like	Isopropyl quinoline
Branny	Isopropyl quinoline
Nutty	imethyl pyrazine or 2-ethyl pyrazine
Oil-like	2,4 trans-trans decadienal-1-heptanal
Earthy	Isopropyl quinoline
Sweet	Aldehyde or vanillin
Sulphur/sulfidic	Diethyl sulfide
Sour, acid, vinegar	Hexanonic acid
Pungent, aid	Tetrahydrothiophene
Beany	Isopropyl quinoline
Rancid	Butyric acid
Musty	Methyl iso-borneol
Hay-like	Isopropyl quinoline
Harsh	Acetic acid
Green	Hexanol

evaluation was done by sniffing method (BIS, 1971) using fresh citrate phosphate buffer (pH 4.0–4.5) as a carrier. Head space volatiles were allowed to accumulate by keeping the flask stoppered for 15 min. Oil samples dispersed in citrate phosphate buffer were evaluated against blank sample (without buffer) to identify and quantify the descriptors. Odour evaluation of the oils was carried out before and after 1st, 4th, 7th and 10th cycle of frying. Mean values of intensity scores were calculated for generating odour profiles. Odour profile was carried out for selected blended and interesterified oil samples of fresh and fried for 1st, 4th, 7th and 10th frying cycles. Overall quality of sensory odour profiling of native oils was evaluated based on based on Hedonic scale (9- point scale) to see the liking or preference.

Consumer acceptance study

Poori samples fried in selected blended and interesterified oils were evaluated for consumer acceptance using seven point ‘Hedonic scale’. The point scale ranged from ‘like very much’ (LVM) to ‘dislike very much’ (DVM) with ‘neither like nor dislike’ as the midpoint. Around 50 panelists evaluated the product.

Statistical analysis

Data were analyzed by multiple comparison test for comparison of means using Duncan Multiple Range Test (DMRT) (Duncan, 1955) for statistical significance ($p<0.05$).

Chapter-1

Evaluation of Edible Oils

Effect of Blending and Enzymatic Interesterification on

-Section 1A: Physico-chemical Properties

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-Section 1B: Thermal Properties

1.1 Introduction

Fats and oils are vital components in our daily diet, which provide energy, essential fatty acids, and serve as a carrier of fat soluble vitamins. In India, more than 80% of edible oils are produced from peanut, sunflower, safflower, rice bran, coconut and mustard. These vegetable oils are used for cooking and frying dishes, in which, heat transfer through cooking medium is an important criterion.

Each oil or fat is unique in nature with respect to the presence of its characteristic fatty acid composition and triglyceride molecules. The variety of fatty acids such as saturated, monounsaturated and polyunsaturated fatty acids in a triglyceride molecule play important role in the physico-chemical, thermal and functional properties of the fats and oils. The fatty acid composition of different edible oils available in the India used for culinary purposes indicates that there is a wide variation in their fatty acid composition. The exact combination of fatty acids in the triglyceride molecule of fats and oils recommended by the Nutritionists and Food Technologists can not always be fulfilled from a single native oil or fat. However, saturated fatty acids have higher oxidative stability. But at higher temperatures (>150°C) saturated fatty acids form carboxylic acids, aldehydes and ketones. Consumption of oil containing satutrated fatty acids is one of the major factors for increase in health risks which leads to cardiovascular diseases, diabetes, cancer and hyper tension. Monounsaturated fatty acids are relatively stable against oxidation. Although polyunsaturated fatty acids (PUFA) are beneficial to health, its oxidation may lead to oxidative stress. Further presence of high levels of PUFA in oils reduces its stability which undergoes degradation during frying (Brinkmann, 2000). Therefore, there is a need for modification of oils to balance the fatty acids to have desirable nutritional properties as well as improved physical, thermal, heat transfer and stability for culinary purposes. This can be achived by blending of two oils containing different fatty acids (Adolph, 1999). Blending of two edible oils is now allowed by the Government of India. The proportion of one vegetable oil in the blend has to be atleast 20% or more (BIS, 1995; Padmavathy *et al.*, 2001).

Oils containing triglycerides of higher amounts of saturated fatty acids will be in solid form at room temperature whereas oils containing triglycerides of lower-melting fatty acids (unsaturated) will exist in liquid form. The triglyceride molecular species in oils indicates its physical, thermal as well as nutritional properties. The triglyceride molecular species of the oils and fats are generally analysed by reverse phase HPLC (Ruiz-Sala, 1996).

The use of of blended oil for culinary uses are gaining importance as they provide nutritional benefits, improved thermal stability, economical alternatives or substitutes to expensive vegetable oils. According to Goburdhun *et al.* (1995) soybean oil blended with palm kernel olein provides a stable oil in terms of hydrolysis, oxidation of fats and rancidity development as compared to pure soybean oil. It is reported (Xu *et al.,* 2000) that blend of high oleic acid canola oil and palmolein showed higher oxidative stability, lower free fatty acid and polar compound formation as well as higher heat stability than palmolein alone. Su *et al.* (2004) showed that blending of linoleic acid rich soybean oil with high-oleate soybean oil had a significant effect on colour and oxidative stability during frying of bread cubes. Extensive research was done to describe the chemical changes in frying oils, but very few have attempted to relate the physical properties of oils to the thermal properties.

Lipases can modify oils and fats by catalyzing the transesterification or interesterification reaction at a specific reaction condition. This in turn changes the physical properties of triglyceride molecule present in blended oils (Casimir, 1997; Costa *et al*., 2010). Transesterification between two oils usually involves exchange of fatty acids between the native triglycerides resulting a change in the physical, thermal and chemical properties of the native oil. Change in fatty acids in a random manner with different levels of unsaturation can also influence the physical properties of the triglycerides in the oil. In comparison to physical blending, enzyme interesterification of oil improves the homogeneity of the oil, which, in turn, enhances the appearance and consumer acceptance (Sreerama *et al*., 2005). The different oils have different physical properties. The physical properties of specific oil determine its heat transfer properties during frying. The heat transfer coefficient is an important factor, which characterize heat flow during frying. The correlation among viscosity, oil temperature and heat transfer coefficient is one of the key factors in food processing industries. The physical properties of oils are the primary factors which control the quality of the fried food (Stern and Roth, 1959).

These observations underscore the need for developing oils with improved viscosity, heat transfer and stability. Recently, Reena and Lokesh (2007) have prepared combinations of blended and interesterified oils using coconut oil with rice bran oil or sesame oil with improved nutritional benefits. These oils are enriched in natural antioxidant molecules such as oryzanol or sesame lignans and hence expected to possess better thermal stability. These studies indicated that there may be differences in the viscosity, heat transfer and thermal and oxidative stability of blended and interesterified oil as compared to individual native oils. Such information is useful for developing oils suitable for frying dishes, which is a major utility for edible oils in India. The blended and interesterified oil, while having the desirable characteristics to be better suited for frying, should meet the sensory requirements as well. Research work on hydrodynamic, thermo-analytical, structural properties and thermo-oxidative stability of enzyme interesterified oils are very few in the literature.

Therefore, to fulfill the gap the present investigation has been undertaken for (a) screening the locally available native edible vegetable oils, their physico-chemical properties and (b) the quality assessments of the oils by frying of poori (a traditional Indian breakfast and snack food, made of wheat flour dough flattened to circular shape) and their sensory odour profiles.

1.2 Results

1.2.1 Physico-chemical studies for initial selection of oils

For initial selection of the oils, the physico-chemical properties of the different oils viz., coconut oil (CNO), ground nut oil (GNO), mustard oil (MO), rice bran oil, (RBO), refined palm olein (RBDPO), sesame oil (SESO) and sunflower oil (SFO) are given in Table 1.1. Each oil has unique chararacteristic colour. The lighter colour of oil is preferred by the consumer. In the present study the red colour value of oils varied from 0.0 red (R) for CNO to 5 red (R) Lovibond colour unit for MO during frying. It was found that the red colour value of GNO (2 R) and SFO (1.9 R) did not vary significantly with frying. The same for RBO, RBDPO and SESO increased with frying from 2 to 4 (R), 1 to 1.4 (R) and 2.2 to 2.7 (R), respectively. It was observed also that the yellow colour value varied from 0.4 yellow (Y) for CNO to 19.7 yellow (Y) units Lovibond colour units for MO. The yellow colour value for RBO (19 Y) and SESO (18.5 Y) did not vary significantly with frying. The yellow colour value for GNO, RBDPO and SFO varied from 3.8 to 4.5 (Y), 7 to 10 (Y) 3 to 4.5 (Y) with increasing frying cycles.

The viscosity of individual oils also differed significantly from 80.8 m Pas for CNO to 128.3 mPas for RBDPO at 25±1°C. The FFA, PV and TPM of individual oils varied from 0.12 to 0.35%, 1.47 to 4.35% and 0.0 to 7.2%, respectively.

Table 1.1 Physicochemical properties of different native oils

Oils	Colour (Lovibond colour unit)		Apparent viscosity (mPa s)		FFA (%)	PV ($meqO_2$/kg)	TPM (%)
	Red	Yellow	25±1 °C	50±1 °C			
CNO	0.0	0.4±0.01	80.8±0.02	57.4±0.01	0.16±0.2	1.97±0.1	0.0
GNO	2.0±0.01	3.8±0.02	99.3±0.03	63.6±0.01	0.14±0.1	1.89±0.4	2.3±0.3
MO	5.0±0.01	19.7±0.01	109.7±0.02	67.8±0.03	0.34±0.3	2.53±0.3	7.2±0.5
RBO	2±0.02	19.0±0.01	106.0±0.01	59.6±0.02	0.15±0.2	1.63±0.2	3.5±0.6
RBDPO	1.0±0.02	7.0±0.01	128.3±0.02	61.6±0.01	0.12±0.1	1.47±0.2	2.4±0.2
SESO	2.2±0.01	18.5±0.01	97.1±0.01	60.5±0.02	0.35±0.2	3.11±0.3	2.3±0.4
SFO	1.9±0.01	3.0±0.01	95.2±0.03	58.8±0.01	0.27±0.1	4.35±0.5	2.3±0.3

where, CNO - coconut oil, GNO - ground nut oil, MO - mustard oil, RBO - rice bran oil, RBDPO – refined palm olein, SESO - sesame oil, SFO - sunflower oil, FFA- free fatty acids, PV- Peroxide value, TPM-total polar material

1.2.2 Frying studies for initial selection of oils

During repeated frying of oils, the colour values in MO and RBO increased (Fig. 1.1). No significant changes in free fatty acid (FFA) values were observed upto 10 cycles of frying (Fig. 1.2a). Peroxide values (PV) increased in MO and GNO in repeated cycles of frying (Fig. 1.2b). Total polar materials showed an increase after 7 cycles of frying for all the oils except CNO (Fig. 1.2c).

Earlier studies from the laboratory have also indicated that blends of oils containing RBO has better cholesterol lowering properties when fed to rats (Reena and Lokesh, 2007). RBO contains endogenous antioxidants, such as oryzanol while RBDPO contains tocotrienols. Together these antioxidants may provide better stability to oils when RBO and RBDPO are blended. Hence, binary oil blends of RBO and RBDPO were prepared in different proportions and evaluated for properties having a bearing on frying characteristics of oils.

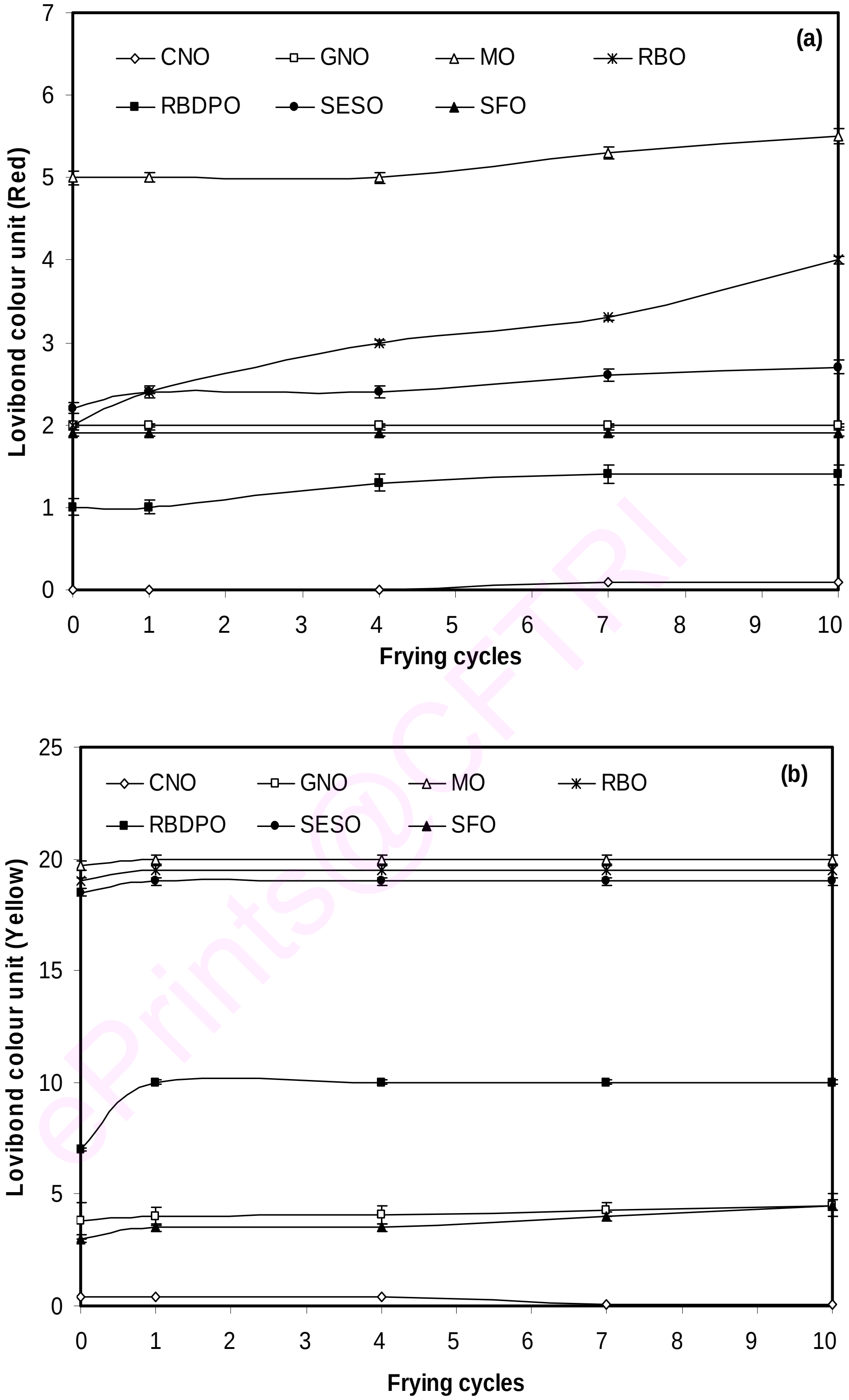


Fig. 1.1 Lovibond (a) red and (b) yellow colour units of different oils at different frying cycles

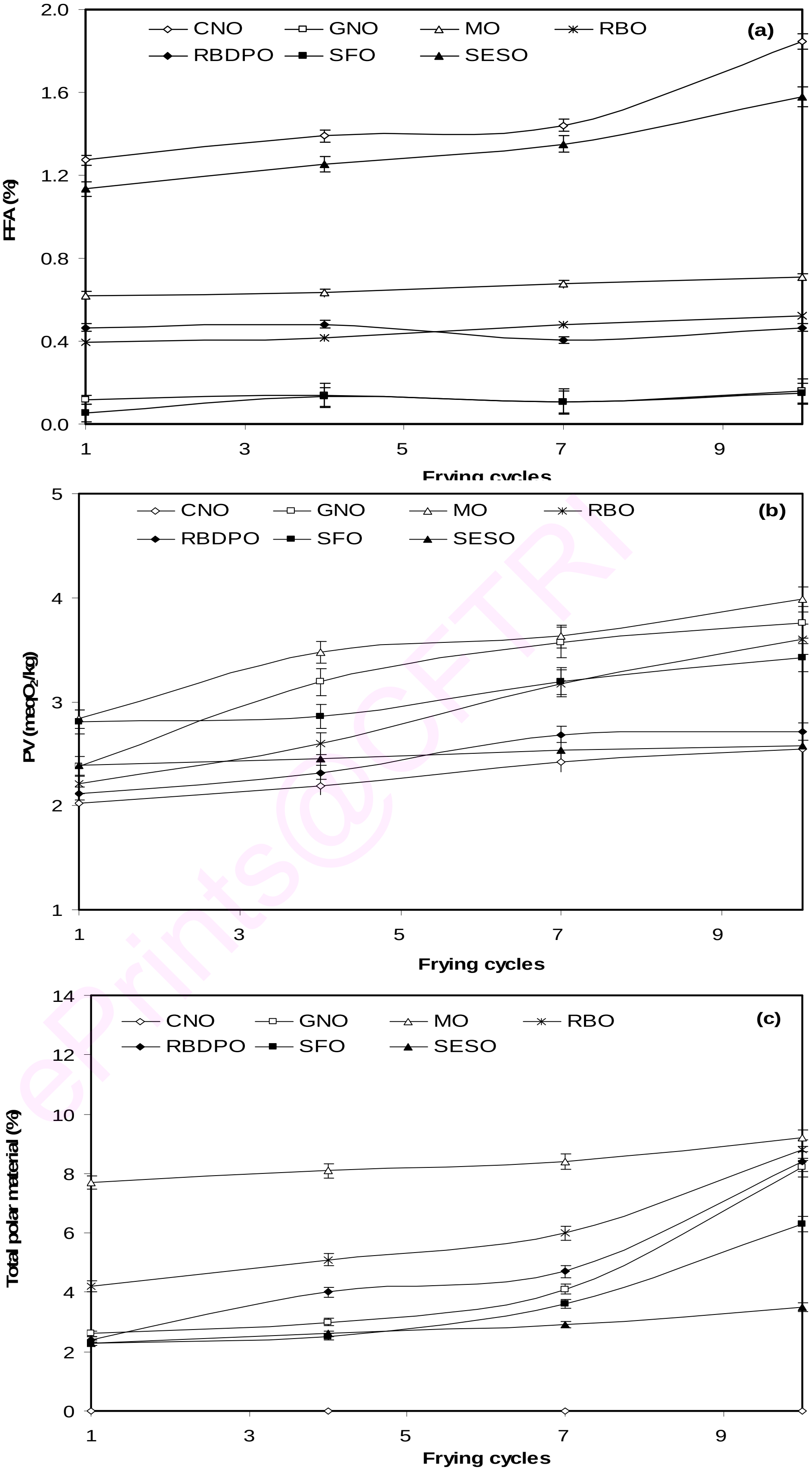


Fig. 1.2 Changes in (a) FFA, (b) PV and (c) TPM of oils during frying at 173±1°C

1.2.3 Sensory evaluation

Each oil has its own characteristic odour such as copra-like, nutty, beany and earthy aroma etc. which varies with consumers' preference. In the present study sensory odour profile of RBO and RBDPO for frying of *poori* by the panelists indicated acceptance for *poori* fried in RBO and RBDPO (Table 1A). The sensory profilogram of *poori* fried in CNO (Table 1Aa), GNO (Table 1Ab), SFO (Table 1Ac) and SESO (Table 1Ad) indicated that fried *poori* samples had more of typical copra-like, nutty, beany and earthy aroma, respectively.

Product fried in MO (Table 1Ae) was least preferred because of pungent and strong sulphury odour. Sensory evaluation of *pooris* fried in RBO (Table 1Af) and RBDPO (Table 1Ag) was preferred by the panelists. Similar observation was reported by Che Man and Wan-Hussin (1998) while frying of potato chips using RBDPO and refined bleached deodorized coconut oil (RBDCO). They observed that RBDPO was better than RBDCO as preferred by sensory panelists. A significant difference ($p<0.05$) in overall sensory quality of the product was observed. Rice bran oil had higher intensity of branny, beany, oil-like attributes. These odour notes gradually reduced with the increasing frying trials (Table 1Af). Ravi *et al.* (2005) reported also similar observations for rice bran oil. Refined palm olein had more of nutty note (Table 1Ag).

However, there was no significant difference between fresh and fried oils (RBDPO, P1, P4, P7, P10) observed. Similar trend was seen for green, hay-like and sweet notes. Typical cooking oil notes were perceived in both

Table 1A Sensory odour profile of different edible oils

Table 1Aa. Sensory odour profile of Coconut oil (CNO)

Oil	Coconut-like	Sweet	Fresh	Copra-like	Nutty	Heated oil	OQ
CNO	8.0	6.1	8.0	4.6	5.2	2.1	5.1
CNO 1	7.5	6.0	7.5	4.4	5.0	1.9	4.7
CNO 4	7.2	5.6	7.0	4.0	4.8	1.7	4.5
CNO 7	7.0	5.2	6.5	3.5	4.5	1.4	4.4
CNO 10	6.7	5.0	6.0	3.0	4.0	1.2	4.4

Table 1Ab. Sensory odour profile of Ground nut oil (GNO)

Oil	Nutty	Sweet	Earthy	Green	Branny	Husk like	Oil like	Heated oil	OQ
GNO	9.5	9.0	5.0	7.0	2.0	2.0	8.0	3.0	7.3
GNO 1	8.0	8.0	4.6	6.0	2.0	2.0	7.0	4.0	7.2
GNO 4	7.5	7.0	4.5	4.5	1.5	1.5	6.5	4.0	7.2
GNO 7	7.3	7.0	4.0	4.0	1.5	1.5	6.0	5.0	7.0
GNO 10	7.1	7.0	4.0	4.0	1.5	1.5	6.0	5.0	7.0

Table 1Ac. Sensory odour profile of Sunflower oil (SFO)

Oil	Oil like	Sweet	Seedy	Green	Beany	Heated oil	Rancid	OQ
SFO	8.0	8.0	9.0	8.0	5.3	3.0	7.5	7.5
SFO 1	7.5	6.5	8.2	7.0	3.5	5.0	7.4	7.4
SFO 4	6.5	4.5	8.0	6.0	4.0	6.0	7.2	7.2
SGNO 7	6.0	4.0	7.5	6.5	4.5	7.0	7.1	7.1
SFO 10	5.5	3.5	7.0	7.0	5.0	7.5	7.0	7.0

Table 1Ad. Sensory odour profile of Sesame oil (SESO)

Oil	Seedy	Sweet	Oil-like	Nutty	Husk - like	Beany	Heated oil	Earthy	Branny	OQ
SESO	8.0	6.1	9.0	8.0	4.0	5.0	2.1	6.2	5.2	6.4
SESO 1	7.5	5.5	8.6	7.5	3.7	4.5	2.0	6.0	5.0	6.3
SESO 4	6.5	5.0	8.0	6.6	3.1	4.3	2.0	5.7	4.5	6.2
SESO 7	6.0	4.6	7.5	6.0	2.7	4.2	1.7	5.4	4.2	6.1
SESO 10	5.5	4.0	7.1	5.5	2.4	4.0	1.5	5.0	4.5	5.9

CNO - Coconut oil, GNO - Ground nut oil, SFO - Sunflower oil, SESO - Sesame oil, 1 – 1st frying; 4 – 4th frying; 7 – 7th frying, 10 – 10th frying, OQ- overall quality based on Hedonic scale (9 point scale)

Table 1Ae. Sensory odour profile of Mustard oil (MO)

Oil	Sulphury	Pungency	Harsh	Vinegar like	Heated oil	OQ
MO	8.0	9.0	4.5	2.1	5.0	6.3
MO 1	7.5	8.5	4.3	2.5	5.8	6.2
MO 4	6.5	8.0	4.0	3.0	6.6	6.2
MO 7	6.0	7.5	3.5	3.2	7.3	6.1
MO 10	5.5	7.0	3.2	3.5	8.0	6.0

Table 1Af. Sensory odour profile of Rice bran oil (RBO)

Oil	Branny	Beany	Oil-like	Husk -like	Earthy	Harsh	Heated oil	Rancid	Musty	OQ
RBO	6.6	5.5	7.5	3.5	4.0	4.5	2.0	-	5.2	8.6
RBO 1	5.8	4.1	7.2	3.2	4.1	4.6	3.2	-	5.0	8.5
RBO 4	5.6	4.4	7.4	3.3	3.9	4.2	4.1	-	4.5	8.4
RBO 7	5.2	4.3	7.2	3.8	4.2	4.4	5.2	3.5	4.2	8.3
RBO 10	5.1	4.1	7.3	3.7	4.1	4.7	5.1	3.8	4.5	8.1

Table 1Ag. Sensory odour profile of Refined palm olein (RBDPO)

Oil	Nutty	Green	Hey -like	Sweet	Cooking oil	Heated oil	Harsh	Rancid	Musty	OQ
RBDPO	6.2	3.5	4.2	2.9	8.5	2.5	3.1	-	2.1	8.8
RBDPO 1	5.9	3.4	4.2	2.8	8.2	3.2	3.2	2.5	2.2	8.6
RBDPO 4	5.7	3.2	4.1	2.5	7.9	3.6	3.9	2.9	2.6	8.5
RBDPO 7	5.6	3.1	4.1	2.1	7.9	4.1	4.1	3.1	2.8	8.4
RBDPO 10	5.5	3.1	4.0	2.0	7.8	4.2	4.4	3.2	3.0	8.2

MO - Mustard oil, RBO - Rice bran oil, RBDPO - Refined palm olein, 1 – 1st frying; 4 – 4th frying; 7 – 7th frying, 10 – 10th frying, OQ- overall quality based on Hedonic scale (9 point scale) rice bran oil and refined palm olein. Heated oil aroma increased marginally with frying trial.

Based on the above results, the rice bran oil (RBO) and refined palm olein (RBDPO) were chosen for the further studies. These oils are chosen also for these studies owing to presence of high amounts of endogenous antioxidants. These oils are making entry into mainstream of cooking oils in India.

1.3 Discussion

The intial screening of the oils required for the subsequent blending and interesterification is carried out. The physico-chemical properties such as colour, apparent viscosity, free fatty acids, peroxide value and total polar matetial of the locally available seven major edible vegetable oils were stuied. The oils selected for intial screening are: coconut oil (CNO), ground nut oil (GNO), mustard oil (MO), rice bran oil, (RBO), refined palm olein (RBDPO), sesame oil (SESO) and sunflower oil (SFO). The Lovibond colour units and the apparent viscosity of these oils at different temperatures are significantly different. After examining the physico-chemical properties of thes oils, continuous frying studies of *poori* (an Indial traditional fried snack) was carried out. Frying was continued upto 10 frying cycles. The physico-chemical properties such as colour, FFA, PV, viscosity and total polar material (TPM) of these oils were monitored upto 10 frying cycles. This provided an information about the colour changes and increase in FFA, PV, viscosity and TPM for all the oils. Among the seven different oils, the sensory panelists preferred the RBO as well as RBDPO for frying.

The physical properties of oil influence its heat transfer properties during frying. The convective heat transfer coefficient is a useful parameter in characterizing heat flow across a fluid/solid interface. The relationship between oil temperature, viscosity and convective heat transfer coefficient play important role in the preparation of processed foods. This also needs to be considered during modeling of the frying process. Extensive research data has been reported in literature to describe the chemical changes in oils upon frying, but very few attempted to relate these chemical changes to the thermal properties of the oil.

Santos *et al.* (2005), studied rheological behavior of un-used and used cooking oils and observed that the oils show Newtonian behavior above 10 s^{-1} shear rates. Further, the viscosity during heating and cooling remained unchanged up to the temperature of 80°C but increased after frying at 190°C. According to Fasina *et al.* (2006) viscosity of different vegetable oils over a temperature range of 5 to 95°C decreased exponentially with temperature and highly correlated with mono and poly unsaturated fatty acids of oils.

In recent times, there is a demand for blended oils for culinary uses because they provide improved thermal stability, nutritional benefits, substitutes to pure vegetable oils and ability to tailor the desired properties. It is reported that enzyme modification of palm oil blended with canola or soybean oil showed improvement in the handling properties at low temperature (Kurashige *et al.,* 1993). Solid fats of tallow and butterfat interesterified with high oleic sunflower oil and soybean oil showed improved physical and thermal characteristics (Foglia, *et al*., 1993). Kinematic viscosity of the interesterified castor oil blended with coconut stearin, palm stearin and sal fat using *Mucor miehei* was studied by Ghosh *et al.* (1999). They observed that there was a significant decrease in viscosity with temperature in interesterified castor oil and its blends.

However, during frying several undesirable physical and chemical changes occur in oils/fats (Robertson and Morrison, 1977; Fritsch, 1981; Cuesta *et al.*, 1993; Tyagi and Vasishtha, 1996) such as hydrolysis, oxidation, thermal decomposition and thermal polymerization (Blumenthal, 1991; Houhoula *et al.,* 2003) with the production of lipid dimers, polymers, *trans*-isomers and cyclic substances (Melton *et al*., 1994) which affects the quality of the left over frying oils (Sulthana and Sen, 1990; Marquez-Ruiz *et al.*, 1992). In addition, these products may also have harmful effects on human health (Billek, 1985). Oxygen concentration is a critical parameter causing oil deterioration during frying, but at elevated temperatures, oxygen supply is limited. A steam blanket (Gertz, *et al.,* 2000) is created on the oil surface by water vapour (Saguy and Dana, 2003) evaporated from the fried food, which acts as physical barrier minimizing contact between oxygen and oil. Blumenthal and Stier (1991) showed that quality of fried food deteriorates with increasing concentration of degradation products in the oil which act as surfactants. They showed that rate of heat transfer from oil to food changes as the oil quality deteriorates during frying.

Enzyme interesterification changes also the thermal behavior of the oils. Dian *et al.* (2006) studied changes in DSC melting properties of palm oil, sunflower oil and palm kernel oil and their blends in various ratios using blending and chemical interesterification. They observed the modification in melting properties in the blends and triacylglycerol composition and improved miscibility of the oil blends. Jin *et al.* (2008) found physical properties such as solid fat content and melting characteristics of ternary blends of palm kernel oil, tallow and palm olein and found that as the palm olein content increased in the blend, the solid fat content decreased, while the solid fat content increased with increasing tallow content. Hayati *et al.* (2000) studied melting characteristics and solid fat content of anhydrous milk fat, soft palm oil stearin, hard palm oil stearin and their interesterified blends. They found that this modification resulted in a better miscibility and a decrease in melting enthalpy and solid fat content of interesterified blends.

Thermal behaviour of oils and fats can be employed to explain their physical and chemical properties. Triglyceride molecules of each oil control its thermal profiles of oil (Md Ali, 1994). Oils and fats show multifaceted thermal behaviour (Tan and Che Man, 2000). Oils and fats show melting/crystallization profiles instead of exhibiting specific melting and crystallization temperatures. The melting and crystallization behaviours of edible oils and fats are important properties for providing information related to functionality of several processed food products.

Senanayake and Shahidi (2007) studied oxidative stability of enzyme modified and unmodified oils during storage using proton NMR and showed that significant changes occurred in aliphatic to olefinic and aliphatic to diallylmethylene proton ratios during oxidation.

Buzas *et al.* (1979) studied the oxidative stability and oxidation state of edible oils using thermogravimetry. They observed that thermal decomposition under dynamic conditions allows the detection of thermal and oxidative degradation of oils. Ushikusa *et al.* (1994) investigated thermal stability of different synthetic antioxidants using programmed thermogravimetry and found that antioxidants showed characteristic weight loss patterns which were not influenced by the gas used (air or nitrogen) in determination. Thermal stability and kinetic parameters of a few commercial edible oils were investigated by thermogravimetry and the kinetic parameters were observed to be dependent on composition of fatty acids and by presence of antioxidants (Santos *et al.,* 2002). These studies indicated that the thermogravimetric stability of oil is influenced by structure of triacylglycerol molecules.

In the subsequent sections the physico-chemical properties of the blended and enzyme interesterified oil based on RBO and RBDPO and the influence of blending and enzymatic interesterification on thermal properties and thermo-oxidative stability of oils are explained.

Section 1A

Physico-chemical Properties

1.4 Preamble

In this section, the results of the physico-chemical properties such as colour, flow behaviour, fatty acid composition, FFA, PV, diene triene, proton NMR, TPM, fatty acid composition, triacylglycerol molecular species for RBO and RBDPO, their blends and that of interesterified oils are presented in this section.

1.5 Results

1.5.1 Effect of blending and interesterification of oils on flow behaviour

The values of apparent viscosity of the native, blended and enzyme interesterified frying oils are shown in the Table 1.2, which followed a shear thinning behaviour (Fig. 1.3).

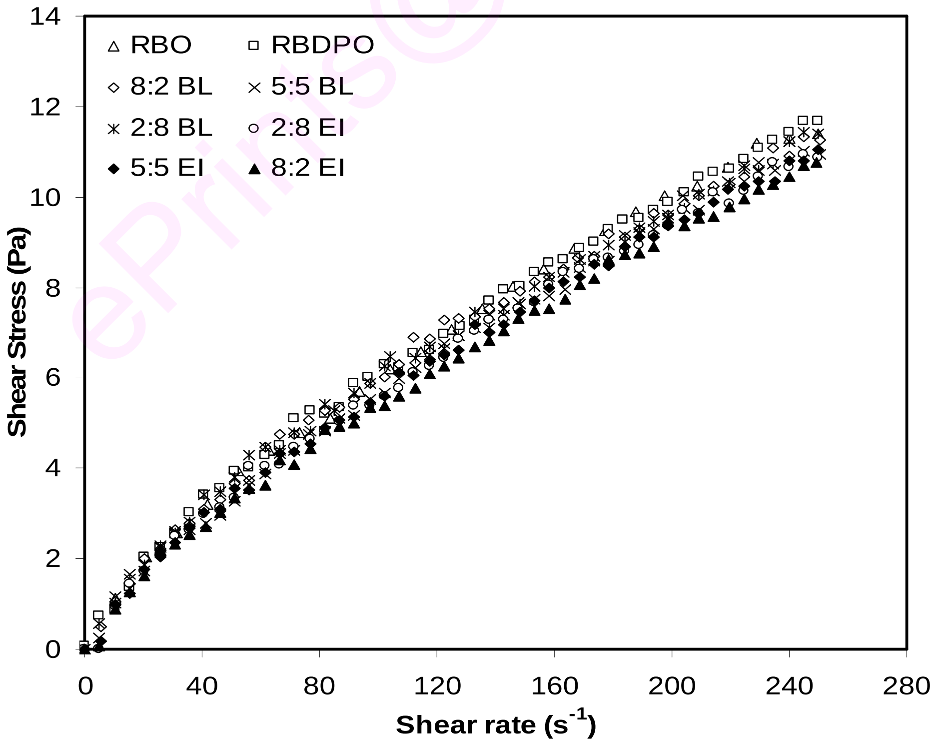


Fig. 1.3 Rheogram of selected native, blended and enzyme interesterified oils
RBO- rice bran oil, RBDPO- refined palm olein, BL- blended oil, EI- enzyme interesterified oil, 8:2, 5:5, 2:8 - ratio of blend of RBO and RBDPO

RBDPO showed highest apparent viscosity (61.5 mPas). However, apparent viscosity of RBDPO was found to decrease from 61.5 to 55.45 mPas (9.9% decrease) when blended with RBO in different proportions. The interesterified oils with the same combination were found to have apparent viscosity, which decreased further from 55.45 to 52.8 mPas (4.8% decrease as compared to blended oil). The change in viscosity could be due to the different levels of saturated, mono and polyunsaturated fatty acids contents present in oils and oil blends as observed in the Table 1.3. This can be corroborated from the Fig. 1.4. This results indicated that viscosity is better correlated with the change in unsaturated fatty acids (R^2 for SFA: 0.52, MUFA: 0.80, PUFA: 0.81). The effect of change in different levels of fatty acids present in different vegetable oils such as rapseed, sunflower olive corn, soybean and their selected blends was also observed by Santos *et al* (2005).

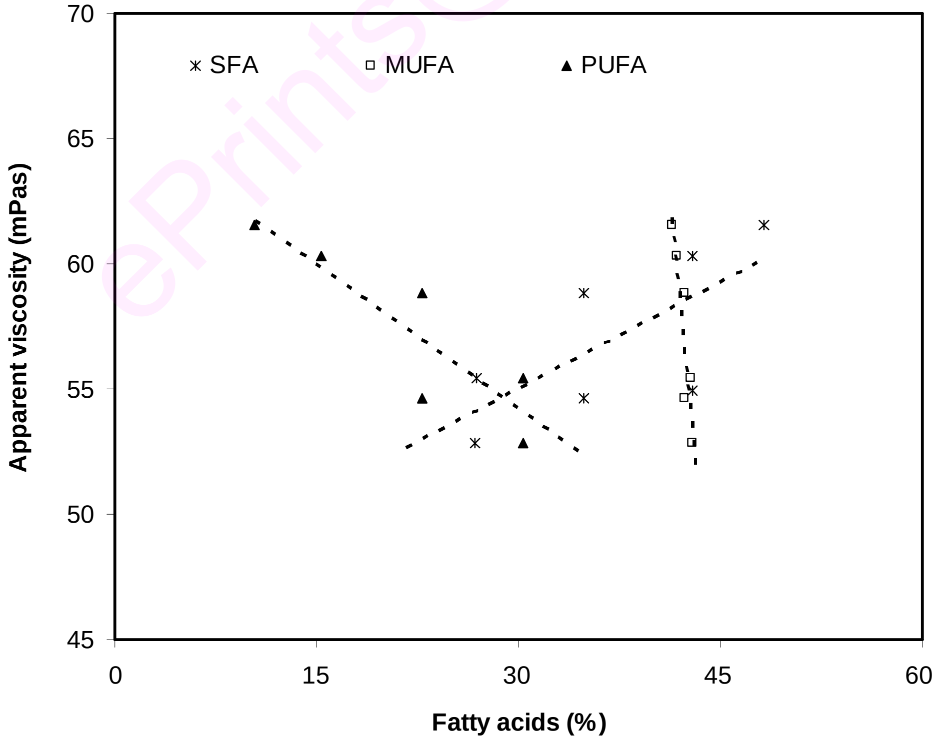


Fig. 1.4 Relation between viscosity with different fatty acids concentration
SFA- saturated fatty acids, MUFA- monounsaturated fatty acids, PUFA-polyunsaturated fatty acids

However, the exchange of fatty acids within and between triglyceride molecular species after enzyme mediated interesterification resulted in further modification in viscosity of oils (Reena *et al.,* 2009). The enzyme-mediated interesterification lowers the viscosity in native and blended oils of castor oil with sal fat, palm stearin and coconut stearin as reported by Ghosh *et al.* (1999). Our results are in agreement with their findings.

Table 1.2 Rheological status of native, blended and interesterified oils

Oils	Apparent viscosity (mPa s) at 50±1 °C	Flow behaviour index (n) (-)	Consistency index (K) (mPa s^n)
Native oils			
RBO	59.6±0.02	0.73	0.21
RBDPO	61.5±0.01	0.69	0.24
Blended oils			
8:2 BL	55.4±0.01	0.80	0.14
5:5 BL	58.8±0.02	0.75	0.18
2:8 BL	60.3±0.01	0.73	0.20
Interesterified oils			
8:2 EI	52.8±0.02	0.91	0.08
5:5 EI	54.6±0.01	0.76	0.17
2:8 EI	54.9±0.01	0.74	0.18

where, RBO-rice bran oil, RBDPO- refined palm olein, BL-blended oil, EI-enzyme interesterified oil, 8:2, 5:5, 2:8 - different ratio of blends of RBO and RBDPO

The apparent viscosity of oils, however, was found to increase after frying (Fig. 1.5a) and was found to be directly related to the formation of polar maerials due to exposure to oxygen and temperature during prolonged frying at higher temperature (Warner, 2002; Kochhar and Gertz, 2004). The formation of polar material increased with frying time correlating with the change in oil viscosity (r=0.97) (Fig. 1.5b). The change of viscosity may be due to formation of degraded products of oxidationas well as leaching of compound from the fried product during frying.

1.5.2 Fatty acid composition of oils

Fatty acids of the native, blended and interesterified oil were monitored by GC. The analysis indicated that out of total fatty acids 21.8% and 47.7% are saturated fatty acids and 35.5% and 10.8% are poly unsaturated fatty acids in RBO and RBDPO, respectively. These two oils are blended in equal amounts by which PUFA content in RBDPO increased by 2-fold. There was no significant change in fatty acid compositions of blended oils (saturated-35.6%, polyunsaturated-21.5%) and enzyme interesterified (saturated-35.7%, polyunsaturated-21.3%) oils (Table 1.3).

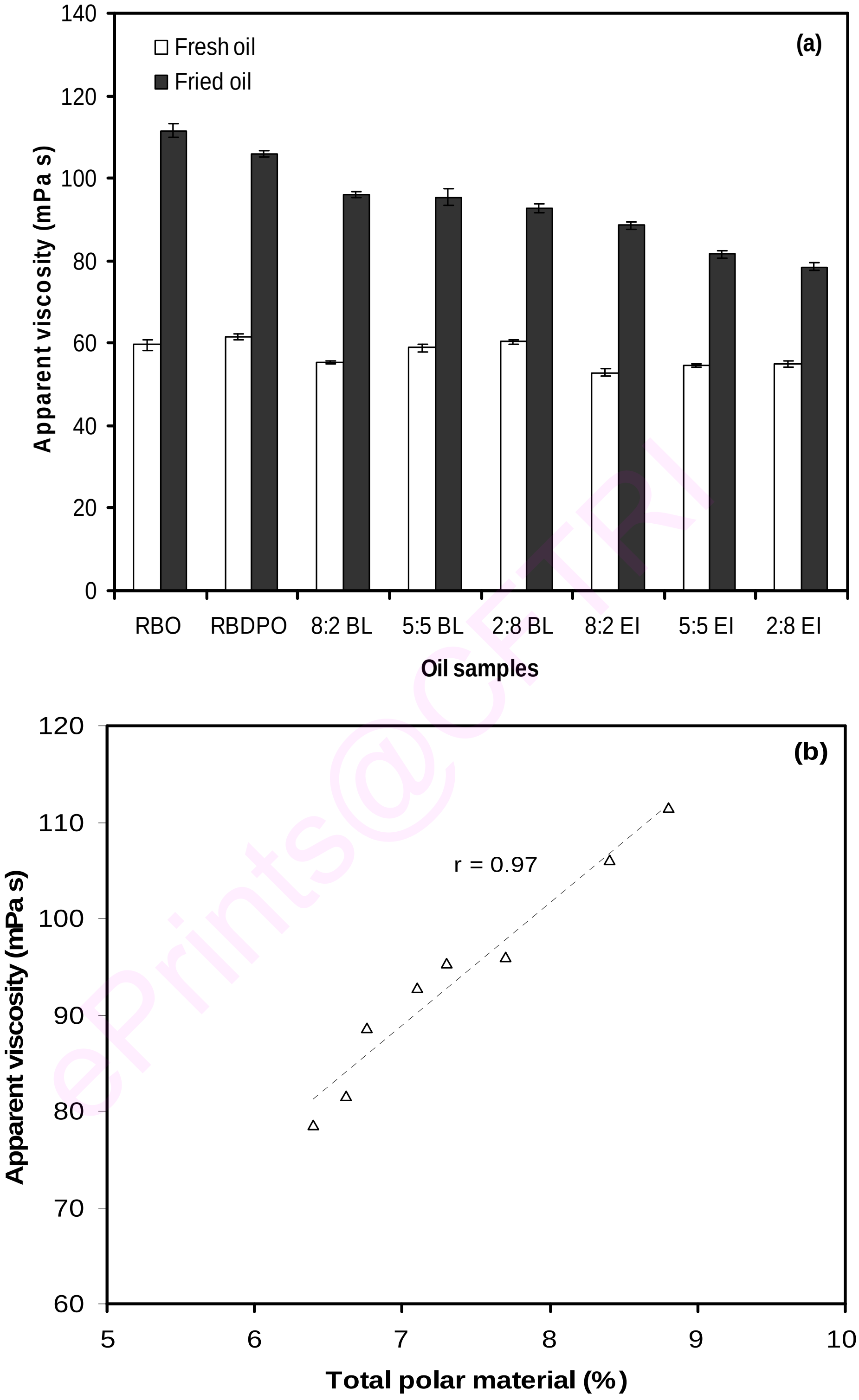


Fig. 1.5 (a) Changes in apparent viscosity of fresh and fried oils and (b) Correlation of total polar material with change of apparent viscosity
RBO-rice bran oil, RBDPO-refined palm olein, BL-blended oil, EI-enzyme interesterified oil

1.5.3 Changes in colour

The change of colour of the native, blended and interesterified oils before and after used for frying upto 10 cycles is shown in Fig. 1.6. The colour in all the oils were found to increase in colour after repeated frying cycles as compared to fresh samples. This may be partially due to formation of degraded compounds by oxidation and may also be due to the leaching of compound (s) from the fried product (Augustin *et al.*, 1987). A significant reduction ($p<0.05$) in colour formation was observed during frying in interesterified oils as compared to blended oils (Fig. 1.6).

1.5.4 Changes in FFA, PV, conjugated diene and triene formation

Free Fatty acid content is the most common indicator of oil quality as it leads to development of off-flavor in oils and fried products. FFA increases during frying mainly due to hydrolysis, thermal oxidation and polymerization. The degradation of different oils during frying was observed to increase FFA, PV, diene and triene conjugates of oils over repeated frying period (Table 1.4). FFA content and PV values were found to increase marginally in blended and interesterified oils as in Table 1.4. Similar observation was reported by Seriburi and Akoh (1998) when Lard and high-oleic sunflower oil were interesterified with SP435 lipase at 55°C for 24 h to produce plastic fats.

Table 1.3 Major fatty acids and distribution of SFA, MUFA and PUFA of native, blended and interesterified oils

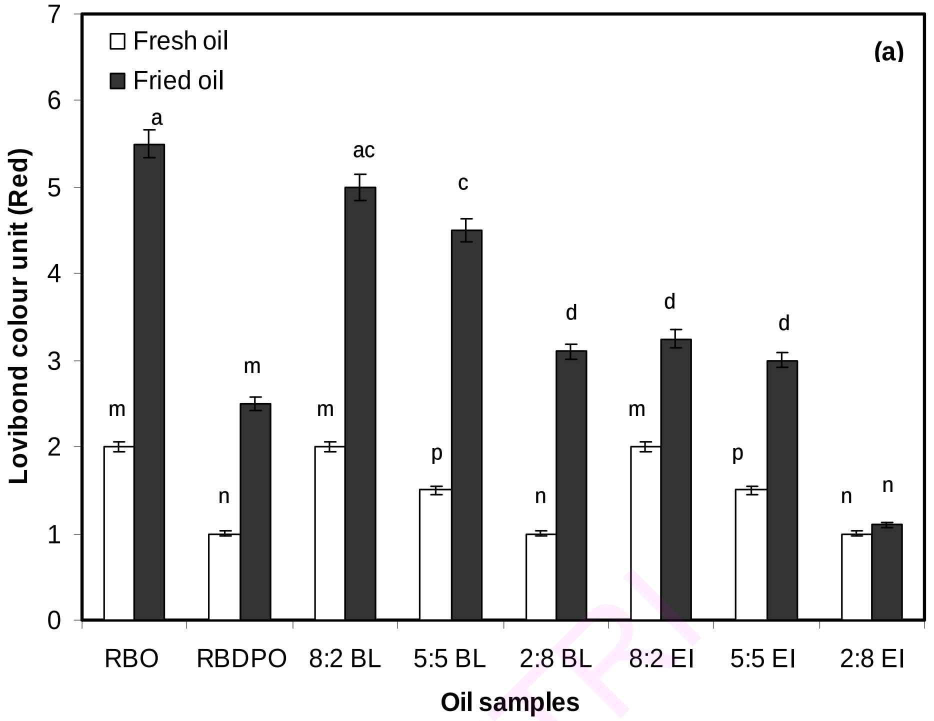
(a) Fatty acids composition (% Area)

	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Native oils							
RBO	nd	0.3±0.1	20.1±0.1	1.3±0.2	43.3±0.1	33.4±0.2	1.6±0.1
RBDPO	0.5±0.1	1.0±0.2	43.0±0.2	3.7±0.2	41.4±0.1	10.2±0.1	0.2±0.2
Blended oils (RBO+RBDPO)							
8:2 BL	0.1±0.1	0.4±0.2	24.7±0.2	1.8±0.2	42.9±0.1	28.8±0.1	1.3±0.2
5:5 BL	0.2±0.1	0.6±0.2	31.6±0.1	2.5±0.2	42.4±0.1	21.8±0.2	0.9±0.1
2:8 BL	0.4±0.2	0.9±0.2	38.4±0.2	3.2±0.1	41.8±0.1	14.8±0.2	0.5±0.1
Interesterified oils (RBO+RBDPO)							
8:2 EI	0.1±0.2	0.4±0.1	24.6±0.1	1.7±0.2	42.9±0.1	28.9±0.2	1.4±0.2
5:5 EI	0.2±0.2	0.5±0.1	31.7±0.2	2.5±0.1	42.3±0.2	21.9±0.1	0.9±0.1
2:8 EI	0.4±0.1	0.9±0.2	38.5±0.1	3.1±0.2	41.9±0.2	14.7±0.1	0.5±0.1

(b) Distribution of different classes of fatty acids in native, blended and interesterified oils

	SFA	MUFA	PUFA
Native oils			
RBO	21.7±0.1	43.3±0.1	35.0±0.2
RBDPO	48.2±0.2	41.4±0.2	10.4±0.1
Blended oils			
8:2 BL	27.0±0.2	42.9±0.2	30.1±0.1
5:5 BL	34.9±0.1	42.4±0.2	22.7±0.2
2:8 BL	42.9±0.2	41.8±0.1	15.3±0.1
Interesterified oils			
8:2 EI	26.8±0.2	42.9±0.2	30.3±0.1
5:5 EI	34.9±0.2	42.3±0.1	22.8±0.2
2:8 EI	42.9±0.1	41.9±0.2	15.2±0.1

Mean ± SD of three samples, RBO- rice bran oil, RBDPO- refined palm olein, BL- blended oil, EI-enzyme interesterified oil, SFA-saturated fatty acids, MUFA-monounsaturated fatty acids, PUFA-polyunsaturated fatty acids, 8:2, 5:5, 2:8 – ratio of RBO in the RBO-RBDPO blend, nd - not detected



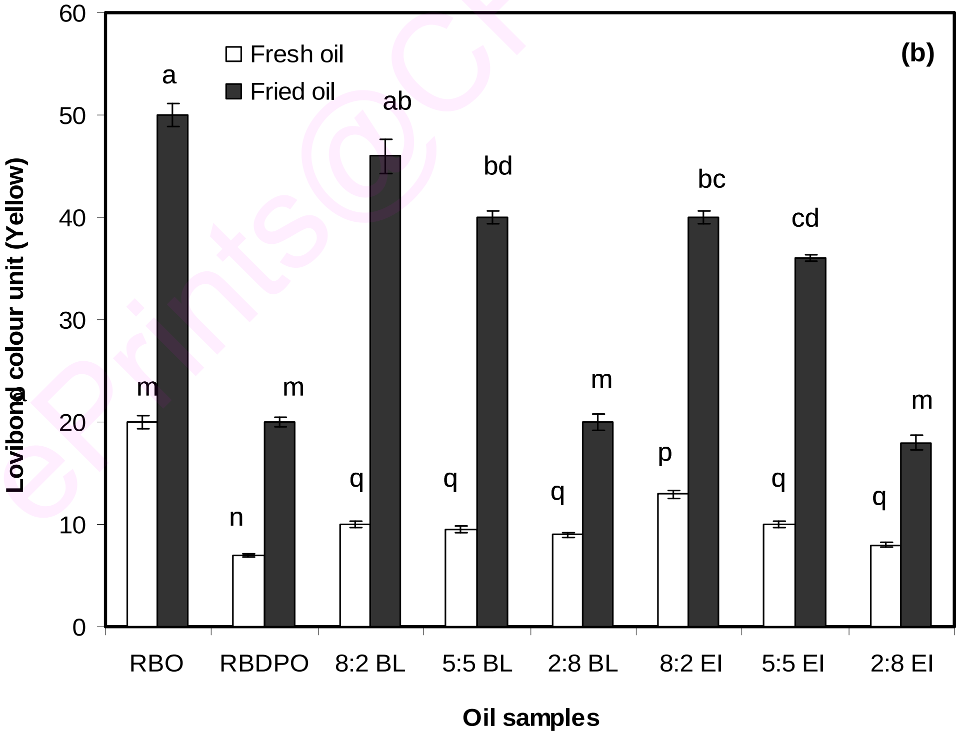


Fig. 1.6 Changes in Lovibond (a) red and (b) yellow colour units in fresh and fried samples of different native, blended and interesterified fried oils

RBO-rice bran oil, RBDPO-refined palm olein, BL-blended oil, EI-enzyme interesterified oil, 8:2, 5:5, 2:8 - ratios of blends of RBO and RBDPO, Different superscripts in the bar diagram indicate significantly different values ($p < 0.05$, Duncan's Multiple Range Test)

Initially oil, when abused forms hydroperoxides, a primary product and a good indicator of lipid oxidation. However, at high temperatures it forms secondary oxidation products. The changes in conjugated dienene (linoleic hydroperoxides) and triene (ethylenic diketones) compounds indicate the rate of oxidation (Gray, 1978). A significant ($p<0.05$) difference in increase of conjugated diene and triene values were observed in the blended oil as compared to interesterified oils after repeated frying of *poori* (Table 1.4). Similar observation was reported by Warner (2002) in fried oil.

The interesterified oils showed a comparatively lesser degree of oil degradation than corresponding blends and native oils during frying as monitored in terms of diene and triene formation.

1.5.5 Changes in total polar material (TPM)

The polar material is an indicator for the deterioration of oils used for frying. The TPM consists of polymeric, cyclic non-volatile substances including soluble constituents leached from the fried products. In Europe, the level of polar material was fixed to 24 % as the threshold point for discarding frying oil (Firestone, 1993; Sanibal and Mancini-Filho, 2004). In our studies a significant difference ($p < 0.05$) in TPM (1.7-8.8%) was observed for all the oil systems during repeated frying (Debnath *et al.,* 2010b).

Oil blends produced marginally higher amounts of TPM than corresponding interesterified oil blends when used for frying under similar conditions (Fig. 1.7).

Table 1.4 Chemical properties of native, blended and interesterified oils

Oil status	Oils	FFA (%)	PV (meqO_2/kg)	Diene (%)	Triene (%)
Fresh oil	Native oils				
	RBO	0.15±0.1	1.63±0.2	0.91±0.1	0.36±0.1
	RBDPO	0.12±0.1	1.47±0.2	0.16±0.1	0.07±0.2
	Blended oils				
	8:2 BL	0.18±0.2	1.71±0.1	0.80±0.2	0.26±0.2
	5:5 BL	0.17±0.1	1.61±0.1	0.57±0.2	0.19±0.1
	2:8 BL	0.15±0.2	1.54±0.2	0.44±0.1	0.15±0.1
	Interesterified oils				
	8:2 EI	0.43±0.2	4.49±0.3	0.91±0.2	0.21±0.2
	5:5 EI	0.39±0.2	4.34±0.2	0.84±0.1	0.18±0.3
	2:8 EI	0.37±0.3	4.13±0.1	0.78±0.1	0.06±0.1
Fried oil	Native oils				
	RBO	0.61±0.3	3.60±0.1	2.39±0.1	0.70±0.1
	RBDPO	0.54±0.2	2.71±0.2	1.78±0.1	0.59±0.2
	Blended oils				
	8:2 BL	0.57±0.1	4.98±0.3	2.09±0.1	0.65±0.2
	5:5 BL	0.55±0.2	4.60±0.2	1.93±0.2	0.57±0.2
	2:8 BL	0.53±0.1	4.14±0.1	1.72±0.1	0.49±0.1
	Interesterified oils				
	8:2 EI	0.68±0.1	5.31±0.2	1.85±0.2	0.50±0.1
	5:5 EI	0.66±0.3	5.19±0.2	1.68±0.1	0.45±0.1
	2:8 EI	0.63±0.2	4.98±0.1	1.58±0.2	0.34±0.1

Mean ± SD values of triplicate data were presented in the table, BL-blended oil, EI-enzyme interesterified oil, 8:2, 5:5, 2:8- ratio of RBO and RBDPO blend

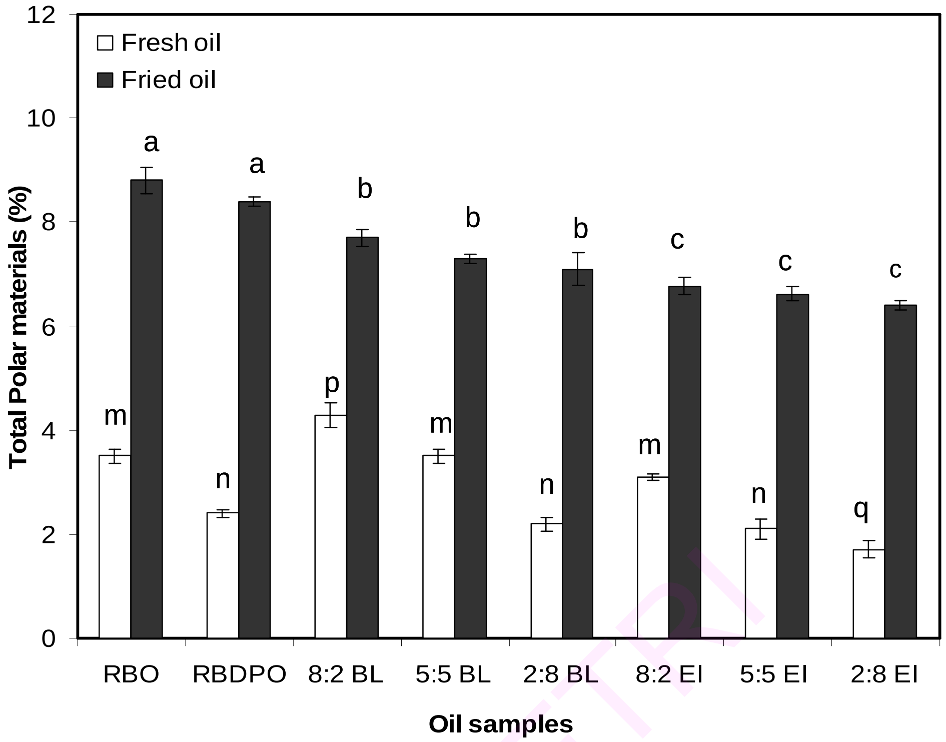


Fig. 1.7 Changes in total polar materials in native, blended and interesterified oils
RBO-rice bran oil, RBDPO-refined palm olein, BL-blended oil, EI-enzyme interesterified oil, 8:2, 5:5, 2:8 - ratio of blends of RBO and RBDPO, Different superscripts in the bar diagram indicate significantly different values ($p < 0.05$, Duncan's Multiple Range Test)

1.5.6 Changes in Allylmethylene, Olefinic and Diallylmethylene protons

The changes in oil during the degradation process caused by frying were monitored by NMR spectroscopic method. The assignment of protons for triglycerides and fatty acid esters is presented in the Table 1.5.

The NMR spectral analysis of the native oils (RBO and RBDPO), their binary blends and interesterified oils were recorded before and after frying of *poori* and the significant proton shifts in oils due to frying conditions are presented in the Table 1.6.

Table 1.5 The ^{1}H NMR spectrums of pure triglycerides and fatty acid esters (assignment of proton signals)

Signal	Structure	^{1}H NMR, Chemical shift (δ, ppm)
a	*CH3-*	0.87
g	*-CH=CH-*	5.35
k	*=CH-CH2-HC=*	2.8
i	*CH2CHCH2*	5.3
h	*CH2CHCH2*	4.28, 4.13
e'	*CH3-CH2-CH=*	0.96
f	*-CH2-CH=*	2.05
b	*-CH2-COO*	2.30
c	*-CH2-CH2COO*	1.61
d	*-CH2-*	1.32 – 1.25

Table 1.6 Total allylmethylene, olefinic proton and diallylmethylene proton of native, blended and enzyme interesterified oils before and after frying

Oil samples	*-CH2-CH=* *Allylmethylene* (δ = 2.05 ppm)		*-CH=CH-* *Olefinic* (δ = 5.35ppm)		*=CH-CH2-HC=* *Diallylmethylene* (δ = 2.8 ppm)	
	Before frying	After frying	Before frying	After frying	Before frying	After frying
RBO	9.72	8.30	6.76	5.69	1.99	1.55
RBDPO	6.56	5.99	3.84	3.49	0.67	0.52
8:2 BL	7.95	7.23	5.27	4.47	1.37	0.93
5:5 BL	8.33	7.06	5.38	4.48	1.33	1.07
2:8 BL	9.32	6.25	6.44	3.54	1.68	0.64
8:2EI	10.51	9.29	7.20	5.33	1.91	1.52
5:5EI	9.10	7.57	5.98	4.80	1.40	1.28
2:8EI	8.26	6.69	5.04	3.98	1.01	0.85

where, RBO-rice bran oil, RBDPO-refined palm olein, BL-blended oil, EI-enzyme interesterified oil, δ-chemical shift, 8:2, 5:5, 2:8 – ratio of the blends of RBO and RBDPO

The NMR spectra of RBO and RBDPO are presented in the (Fig. 1.8 (a) and Fig. 1.8 (b)). It was observed that, during repeated frying, the intensity of allylmethylene protons (signal at 2.05 ppm) was found to decrease from 9.32 to 6.25 in blended oil (2:8 BL) and from 8.26 to 6.69 when frying was carried out in corresponding interesterified oils. The change in diallylmethylene protons (signal at 2.8 ppm) was found to decrease from 1.68 to 0.64 in blended oil (2:8 BL) and from 1.01 to 0.85 in case of corresponding interesterified oils. The change in olefinic proton (signal at 5.35 ppm) was found to decrease from 6.44 to 3.54 in blended oil (2:8 BL) and from 5.04 to 3.98 in case of interesterified oils (Table 1.6). Similar changes were observed in other combination of blended (8:2 BL) and interesterified (8:2 EI) oils. Minimum changes in the blend of 5:5 BL blend and 5:5 EI interesterified oil were observed. Similar decreasing trends of unsaturated related protons and diallyl protons were observed with olive and sunflower oils during heating at frying temperature by Valdes and Garcia (2006). Although the specific structure of compounds for these key changes was not determined, the chemical shift in the protons indicated significant polymerization in oils during prolonged frying (Khatoon *et al*., 1998).

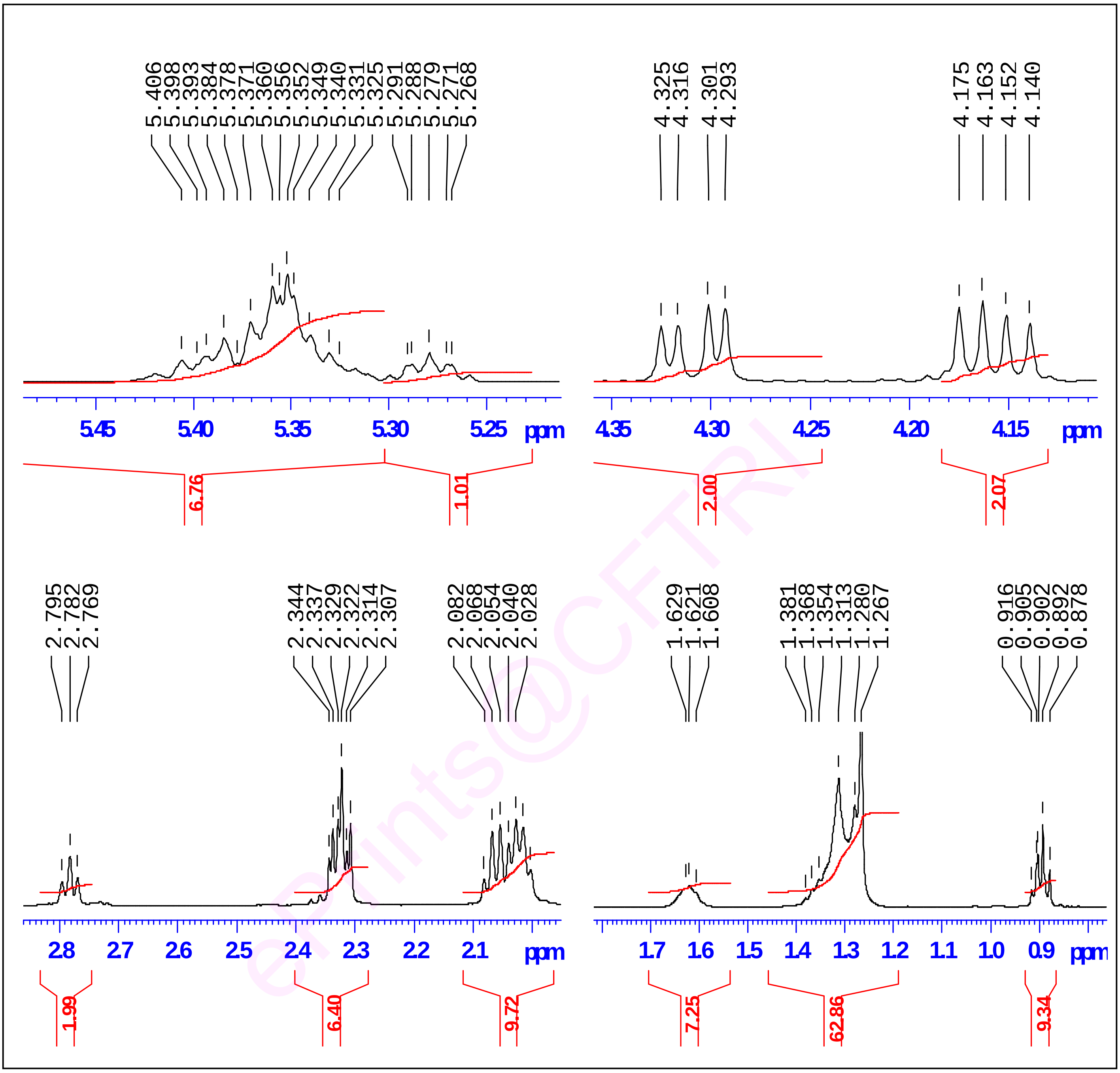


Fig. 1.8 (a) NMR spectra (^{1}H one-dimensional) for native RBO

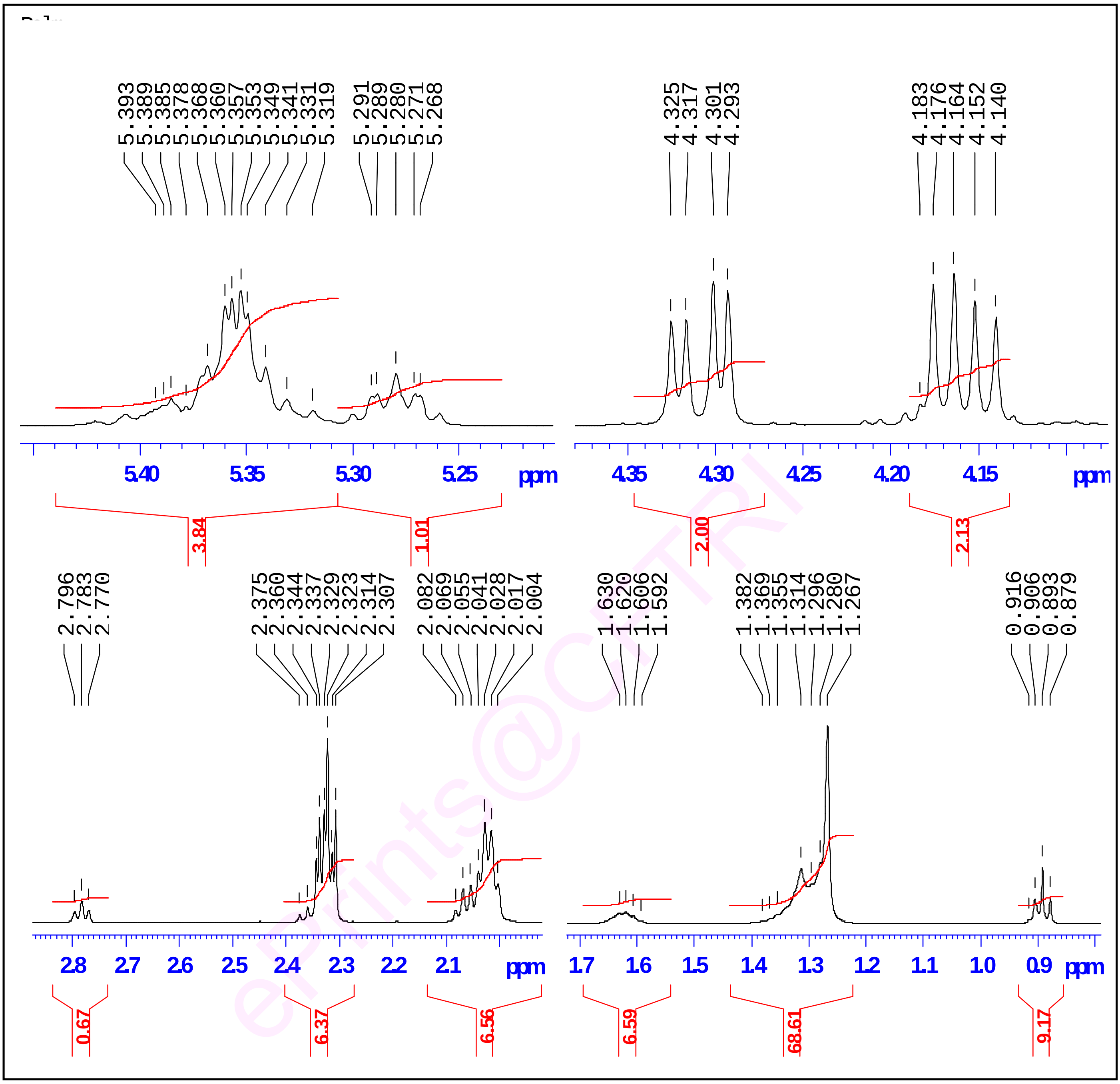


Fig. 1.8 (b) NMR spectra (^{1}H one-dimensional) for native RBDPO

1.5.7 Triacylglycerol molecular species by HPLC

The stereospecific structure of triacylglycerols changes their physical, biochemical and thermal properties of oils (Small, 1991; Bracco, 1994), This may influence the hydrodynamic and heat transfer properties as well as thermal and oxidative stabilities of oils. The triacylglycerol molecular species of the native and blended and interesterified oils are presented in the Table 1.7(a). The triacylglycerol molecular species of the native and selected blended and interesterified oils are presented in the Table 1.7(b). Twelve triacylglycerol molecules were identified in RBDPO which were similar to those reported by other researchers (Siew *et al.,* 2000).

About 54% of the triacylglycerols in RBDPO contained di- and trisaturated fatty acids. Of these, 49.9% was contributed by disaturated (SSU) and 4% by trisaturated (SSS) triacylglycerols. These components have melting point above 25±1°C, which resulted in the semisolid physical state of RBDPO at ambient temperature (25±1°C).

Similarly, twelve triacylglycerol species were identified in RBO and these triacylglycerols were similar to those reported in the literature (Marini *et al.,* 2003). The RBO contained mainly triunsaturated (UUU, 42.2%) and monosaturated (SUU, 44.8 %) triacylglycerols (Table 1.8a). Blended oil (BL) containing equal proportions of RBO and RBDPO was found to have an increase in unsaturated triacylglycerols (by 4.9 times) and decrease in trisaturated triacylglycerols (by 3.3 times) as compared to native RBDPO. Variation in blending of RBO with RBDPO at the proportion of 2:8 to 8:2 resulted in decrease of high-melting disaturated triacylglycerols from 32.7 to 19.3 % (Table 1.8a). Blending of RBO with RBDPO at a proportion of 5:5

resulted in a decrease in high-melting disaturated triacylglycerols by 52.9%. This resulted in a decrease in the individual triacylglycerols with higher melting point, such as, POP, which decreased by 56.2% in the blended oil (5:5 ratio) in comparison to RBDPO (Table 1.7a). Interesterification of RBO-RBDPO blend resulted in an increase in monosaturated triacylglycerols by 10.7% (Table 1.8b) and triunsaturated triacylglycerols by 7.6% and a decrease in high-melting trisaturated triacylglycerols by 50% (Fig. 1.9).

Table 1.7 Triacylglycerol molecular species

(a) RBO and RBDPO based blended oils

Triglyceride molecular species (Tg)	RBO	(Area %)[a]			
		RBDPO	8:2 BL	5:5 BL	2:8 BL
LLL	4.9±0.1	nd	1.3±0.3	0.6±0.2	0.3±0.2
LLO	11.7± 0.3	0.5±0.2	8.8±0.2	6.0±0.1	3.1±0.3
MMM	nd	1.8± 0.1	nd	nd	nd
PLL/MPL	9.5±0.2	2.2± 0.1	5.5± 0.1	4.5±0.2	2.6±0.1
MMP	nd	0.4±0.3	nd	nd	nd
LOO	14.9± 0.3	1.1±0.2	15.2±0.1	12.9±0.1	7.8±0.3
PLO	20.0± 0.2	11.4±0.2	21.1±0.2	21.2±0.2	17.4±0.1
PLP	5.3±0.2	10.5±0.1	5.7±0.1	6.1±0.3	7.6±0.1
OOO	10.7±0.1	3.8±0.1	9.3±0.1	7.2± 0.2	7.2± 0.2
POO	14.1±0.2	27± 0.2	17.8± 0.2	22.1±0.3	25.7±0.2
POP	5.8±0.1	33.1±0.1	12.0±0.1	14.5± 0.1	22.1±0.1
PPP	1.6±0.1	1.8± 0.2	0.5± 0.2	1.2±0.2	2.3±0.1
OOS	1.2±0.2	nd	1.1± 0.2	0.8± 0.1	0.9± 0.2
POS	0.2±0.2	6.3±0.1	1.7±0.1	2.9±0.2	3.0±0.1

(b) Native, selected blended and enzyme interesterified oils

Triglyceride molecular species (Tg)	(Area %)[a]			
	RBO	RBDPO	5:5 BL	5:5 EI
LLL	4.9±0.1	nd	0.6±0.2	3.1±0.1
LLO	11.7± 0.3	0.5±0.2	6.0±0.1	9.1±0.2
MMM	nd	1.8± 0.1	nd	nd
PLL/MPL	9.5±0.2	2.2± 0.1	4.5±0.1	3.7±0.1
MMP	nd	0.4±0.3	nd	nd
LOO	14.9± 0.3	1.1±0.2	12.9±0.1	9.5±0.2
PLO	20.0± 0.2	11.4±0.2	21.2±0.2	17.2±0.1
PLP	5.3±0.2	10.5±0.1	6.1±0.1	7.8±0.1
OOO	10.7±0.1	3.8±0.1	7.2± 0.2	7.2± 0.1
POO	14.1±0.2	27± 0.2	22.1±0.1	21.4±0.2
POP	5.8±0.1	33.1±0.1	14.5± 0.1	16.2±0.1
PPP	1.6±0.1	1.8± 0.2	1.2±0.2	0.6±0.2
OOS	1.2±0.2	nd	0.8± 0.2	1.1± 0.1
POS	0.2±0.2	6.3±0.1	2.9±0.1	3.1±0.1

[a]Mean ± SD of three samples

RBO- rice bran oil, RBDPO- refined palm olein, BL- blended oil, EI-enzyme interesterified oil, M - myristic, P - palmitic, S - stearic, O - oleic, L – linoleic, 5:5 - ratio of blend of RBO and RBDPO nd, not detected

Table 1.8 Proportion of trisaturated (SSS), monounsaturated (SSU), diunsaturated (SUU) and triunsaturated (UUU) triacylglycerol molecular species of native, selected blended and interesterified oils

(a) Native and blended oils

Triacylglycerol molecular species (Tg)	RBO	(Area %)[a]			
		RBDPO	8: 2 BL	5:5 BL	2:8 BL
UUU	42.2±0.2	5.4±0.1	34.7±0.3	26.7±0.2	18.5±0.1
SUU	44.8±0.1	40.6±0.1	45.5±0.1	48.6±0.1	46.6±0.1
SSU	11.3±0.1	49.9±0.2	19.3±0.1	23.5±0.2	32.7±0.3
SSS	1.6±0.2	4.0±0.2	0.5±0.2	1.2±0.2	2.2±0.2

(b) Native, selected blended and interesterified oils

Triacylglycerol molecular species (Tg)	(Area %)[a]			
	RBO	RBDPO	5:5 BL	5:5 EI
UUU	42.2±0.2	5.4±0.1	26.7±0.2	28.9±0.1
SUU	44.8±0.1	40.6±0.1	48.6±0.1	43.4±0.2
SSU	11.3±0.1	49.9±0.2	23.5±0.2	27.1±0.2
SSS	1.6±0.2	4.0±0.2	1.2±0.2	0.6±0.1

[a] Mean ± SD of three samples

RBO- rice bran oil, RBDPO- refined palm olein, BL- blended oil, EI-enzyme interesterified oil, U-unsaturated fatty acids, S-saturated fatty acids, 5:5 - ratio of blend of RBO and RBDPO

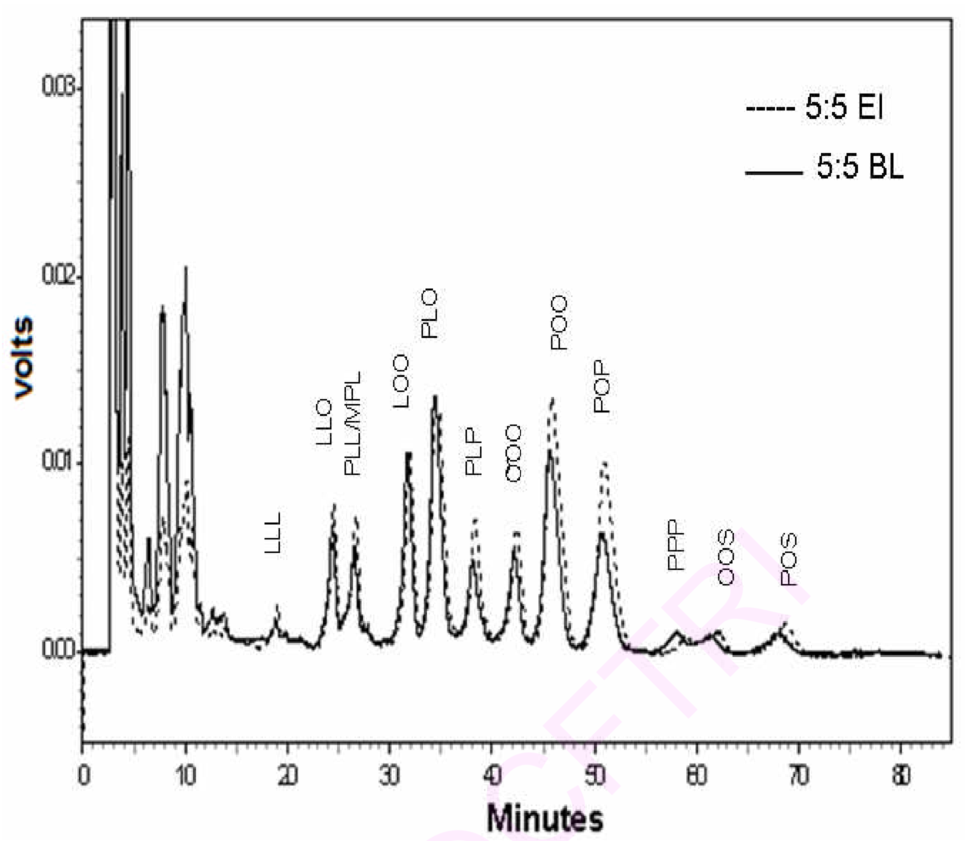


Fig. 1.9 Triacylglycerol molecular species of blended and interesterified oils of RBO with RBDPO
M-myristic, P-palmitic, S-stearic, O-oleic, L-linoleic, BL- blended oil, EI-enzyme interesterified oil, 5:5 - ratio of the blend of RBO and RBDPO

Section 1B

Thermal Properties

1.6 Preamble

In this section, the results of the thermal properties covering the heat transfer coefficients, melting properties, crystallization behaviour, enthalpy measured in DSC, thermal stabililty of the rice bran oil, refined bleached, deodorized palm olein and their blend are presented. The results are compared with that of intereterified oil.

1.7 Results

1.7.1 Melting profiles of native, blended and interesterified oil blends

The DSC endogram of RBDPO showed three endothermic peaks (Fig. 1.10) over different temperature range, 5.98 to 12.38 °C, 13.09 to 16.99 °C and 31.79 to 33.49 °C, which indicated that RBDPO contains heterogeneous

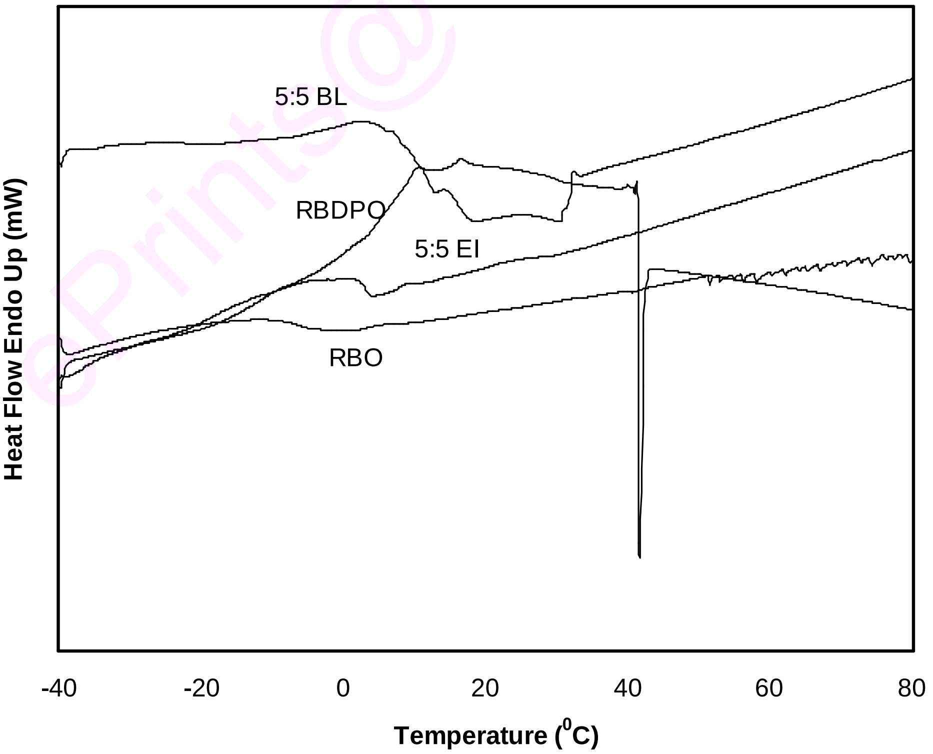


Fig. 1.10 DSC thermograms of RBO, RBDPO, 5:5 BL and 5:5 EI
RBO – rice bran oil, RBDPO- refined palm olein, BL- blended oil, EI- enzyme interesterified oil, 5:5 - ratio of blend of RBO and RBDPO

triacylglycerols with diverse unsaturation. The melting profile of RBO showed a single melting peak at very low temperatures (-24.5 to -5.6°C), which may be due to higher levels of unsaturated triacylglycerols present in RBO. Blending as well as enzymatic interesterification of RBO with RBDPO resulted in the emergence of two endothermic peaks in different temperature ranges.

Variation in blending of RBO with RBDPO in the ratio of 2:8 to 8:2 resulted in shifting in low-melting peak from -12.75 to 10 °C, whereas the high melting peak was found to shift from 16.75 to 22.31 °C (Table 1.9a).

In the blended oil (5:5 ratio), the low-melting peak was observed in the range of –11.9 to 9.6°C. The high-melting peak of the blended oil (5:5 ratio) containing the RBO and RBDPO was observed at 14.94 to 17.6 °C. It can be seen from the Table 1.9b that the same blend (5:5 ratio) shifted the peak melting point of the triacylglycerols towards a lower temperature range from 10.36 °C (RBDPO) to 2.97 °C (BL). It further decreased to – 4.76 °C in the interesterified oil (EI). Similar observation was made by Reshma *et al.* (2008) while preparation of zero trans shortening with bioactive phytochemicals by lipase catalyzed interesterification of blends based on palm stearin and rice bran oil.

The shifting of the melting point towards lower temperatures in the EI can be attributed to the decrease in the disaturated triacylglycerols (45.7%) and trisaturated triacylglycerols (85%) (Table 1.9b). The variation in the melting point towards lower temperatures in the blended oil containing RBDPO and RBO may be due to the decrease in the disaturated triacylglycerols (52.9%) and trisaturated triacylglycerols (70%) (Table 1.9b). However, the enthalpy (0.7 J/g) for the high melting peak of the interesterified oil (5:5 EI) was decreased by 44.3% in comparison to the enthalpy (0.39 J/g) blended oil (5:5 BL) (Table 1.9b). This may be due to the decrease (50%) in trisaturated triacylglycerols in the interesterified oil as compared to its blended oil counterpart.

Table 1.9 Peak temperatures and enthalpies of native, blended and interesterified oil

(a) Native and blended oils

Oils	Peak 1				Peak 2				Peak 3			
	Onset (^{0}C)	Peak (^{0}C)	End set (^{0}C)	ΔH (J/g)	Onset (^{0}C)	Peak (^{0}C)	End set (^{0}C)	Δ H (J/g)	Onset (^{0}C)	Peak (^{0}C)	End set (^{0}C)	ΔH (J/g)
RBO	-24.5	-12.3	-5.6	6.48	-	-	-	-	-	-	-	-
RBDPO	5.98	10.36	12.38	6.39	13.09	14.73	16.99	1.05	31.79	32.3	33.49	0.93
2: 8 BL	-12.75	4.14	9.23	37.75	16.75	21.83	28.66	1.6566	-	-	-	-
5:5 BL	-11.9	2.97	9.60	34.4	14.94	16.56	17.60	0.70	-	-	-	-
8:2 BL	-0.90	17.16	10.0	41.47	18.99	20.64	22.31	0.24	-	-	-	-

(b) Native, blended and interesterified oils

Oils	Peak 1				Peak 2				Peak 3			
	Onset (^{0}C)	Peak (^{0}C)	End set (^{0}C)	ΔH (J/g)	Onset (^{0}C)	Peak (^{0}C)	End set (^{0}C)	Δ H (J/g)	Onset (^{0}C)	Peak (^{0}C)	End set (^{0}C)	ΔH (J/g)
RBO	-24.5	-12.3	-5.6	6.48	-	-	-	-	-	-	-	-
RBDPO	5.98	10.36	12.38	6.39	13.09	14.73	16.99	1.05	31.79	32.3	33.49	0.93
5:5 BL	-11.9	2.97	9.60	34.4	14.94	16.56	17.60	0.70	-	-	-	-
5:5 EI	-21.8	-4.76	3.81	26.6	7.14	9.02	12.04	0.39	-	-	-	-

RBO- rice bran oil, RBDPO- refined palm olein, BL- blended oil, EI-enzyme interesterified oil, 2:8, 5:5, 8:2-ratio of RBO and RBDPO, U-unsaturated fatty acids, S-saturated fatty acids, nd – not detected, ΔH- enthalpy

1.7.2 Crystallization behaviour of native, blended and interesterified oil

Cooling curves of the selected oils show the crystallization temperature of triacylglycerol species. The blended oils of RBO with RBDPO and their interesterified counterparts resulted in one and two exothermic peaks in their crystallization profile, respectively (Table 1.10).

Table 1.10 Crystallization behaviour of native, blended and interesterified oils

Oils	Peak 1				Peak 2			
	Onset (°C)	Peak (°C)	End set (°C)	ΔH (J/g)	Onset (°C)	Peak (°C)	End set (°C)	Δ H (J/g)
RBO	nd	nd	nd	nd	nd	nd	nd	nd
RBDPO	11.01	8.78	-5.62	-35.49	-18.19	-19.90	-30.09	-3.18
5:5 BL	6.10	-0.12	-8.32	-4.96	nd	nd	nd	nd
5:5 EI	9.68	7.94	5.50	-1.64	3.56	1.38	-4.83	-3.69

RBO- rice bran oil, RBDPO- refined palm olein, BL- blended oil, EI-enzyme interesterified oil, 5:5-ratio of RBO and RBDPO, U-unsaturated fatty acids, S-saturated fatty acids, nd – not detected, ΔH- enthalpy

The onset temperature for the peaks crystallizing in the high temperature range for blended as well as interesterified oil containing on RBO and RBDPO shifted to a lower temperature in comparison to the corresponding temperature observed in case of RBDPO. The peak crystallizing temperature of the blend was found to be in the range of 6.1 to -8.32 °C and for interesterified oil, it was in the range of 9.68 to 5.5°C. The peak crystallizing temperature for the interesterified oil shifted to a lower temperature range of 3.56 to – 4.83 °C, which indicates homogeneity of triacylglycerols in the interesterified oil blend (Fig. 1.11).

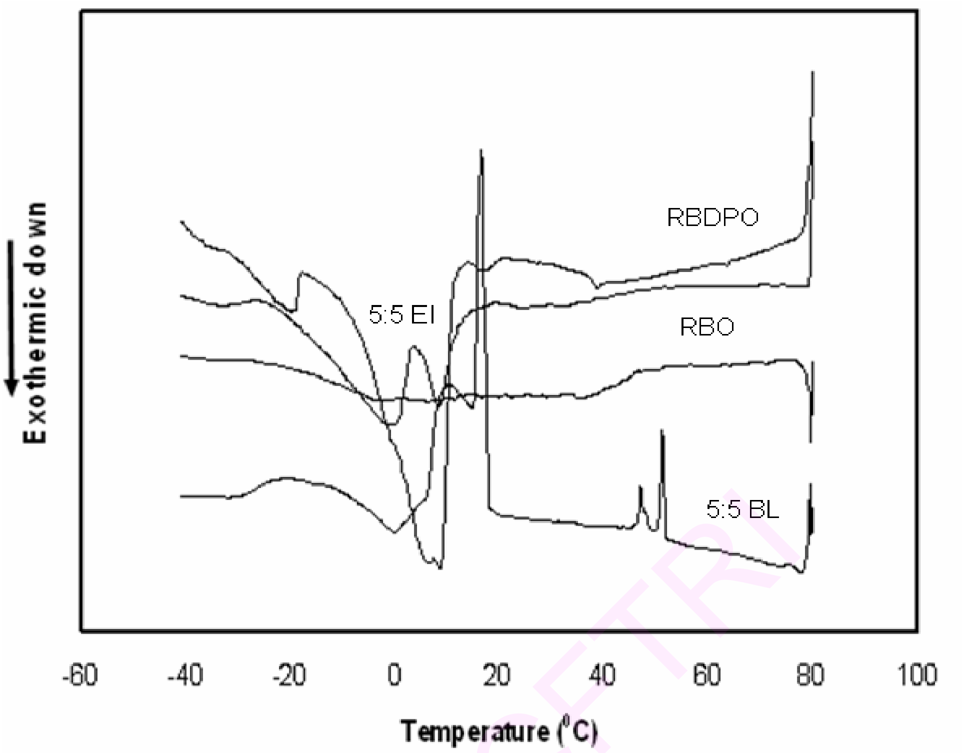


Fig. 1.11 Crystallization exotherms of RBO, RBDPO, BL and EI
RBO- rice bran oil, RBDPO- refined palm olein, BL- blended oil, EI-enzyme interesterified oil, 5:5-ratio of blend of RBO and RBDPO

1.7.3 Thermo-oxidative stability of oils

The thermal stability of the native, blended and interesterified oils was analysed by thermogravimeter in presence of oxygen. In this method, the temperature at which the beginning of the decomposition starts indicates the thermal stability of the specific oil. The results showed that after interesterification, temperature of the first thermal decomposition increased from 190.0 °C (RBDPO) to 230.9 °C (EI) with corresponding mass loss of 34.7 and 41.4%, respectively (Table 1.11).

Three stages of decomposition was observed in case of RBO, RBO-RBDPO blend and interesterified oil blend while RBDPO showed four stages as can be seen from Fig. 1.12. RBO showed (Fig. 1.12a) higher degree of stability as compared to RBDPO (Fig. 1.12b). This may be due to presence of natural antioxidants (Santos *et al.,* 2002) in RBO, such as oryzanol. The interesterified oil (EI) showed (Fig. 1.12d) comparatively lesser degree of degradation than corresponding native and blended oils (BL) (Fig. 1.12c). This may be due to combined effect of two antioxidants such as oryzanol in RBO and tocotrienols in RBDPO and changed triacylglycerol composition after blending and interesterification of these two oil blends.

Table 1.11 Thermal decomposition data of native, blended and interesterified oils obtained from TG/DTG curves

Oils	First step		Second step		Third step		Fourth step	
	T* (°C)	Dm* (%)	T* (°C)	Dm* (%)	T* (°C)	Dm* (%)	T* (°C)	Dm* (%)
RBO	212.7-327.2	28.7	327.2-369.1	50.2	327.2-415.2	66.7	nd	nd
RBDPO	190.0-342.1	34.7	342.1-365.5	43.7	365.5-388.4	57.2	388.4-417.3	65.9
5:5 BL	220.8-372.5	55.5	372.1-427.3	75.5	427.3-457.6	83.3	nd	nd
5:5 EI	230.9-380.3	41.4	380.3-426.3	63.6	426.3-456.5	89.0	nd	nd

T* - Temperature of thermal degradation, Dm*- Mass loss,
RBO- rice bran oil, RBDPO- refined palm olein, BL- blended oil, EI-enzyme interesterified oil, 5:5-ratio of blend of RBO and RBDPO, nd – not detected,

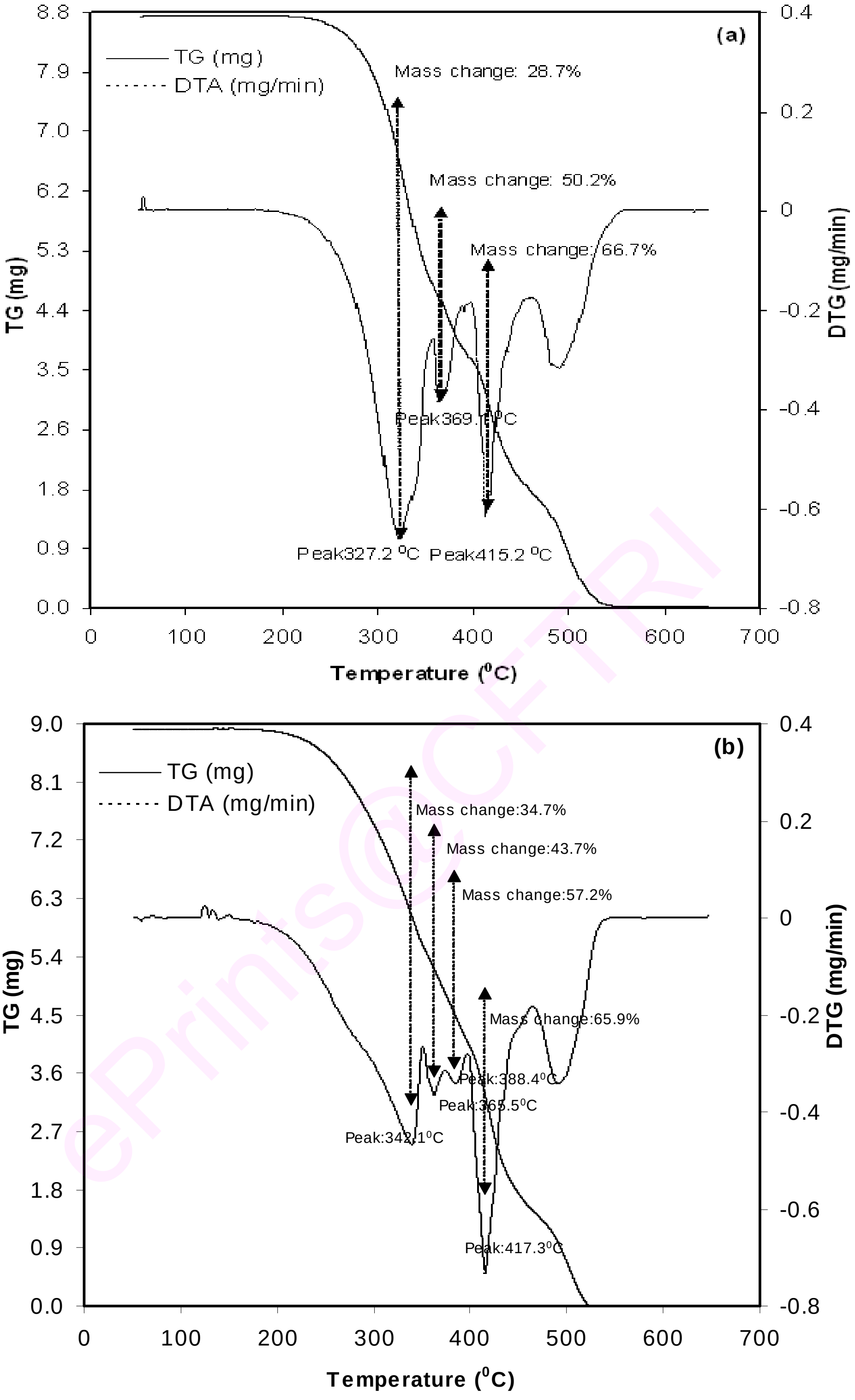


Fig. 1.12 TG and DTG curves under oxygen atmosphere (a) RBO and (b) RBDPO
RBO- rice bran oil and RBDPO- refined palm olein

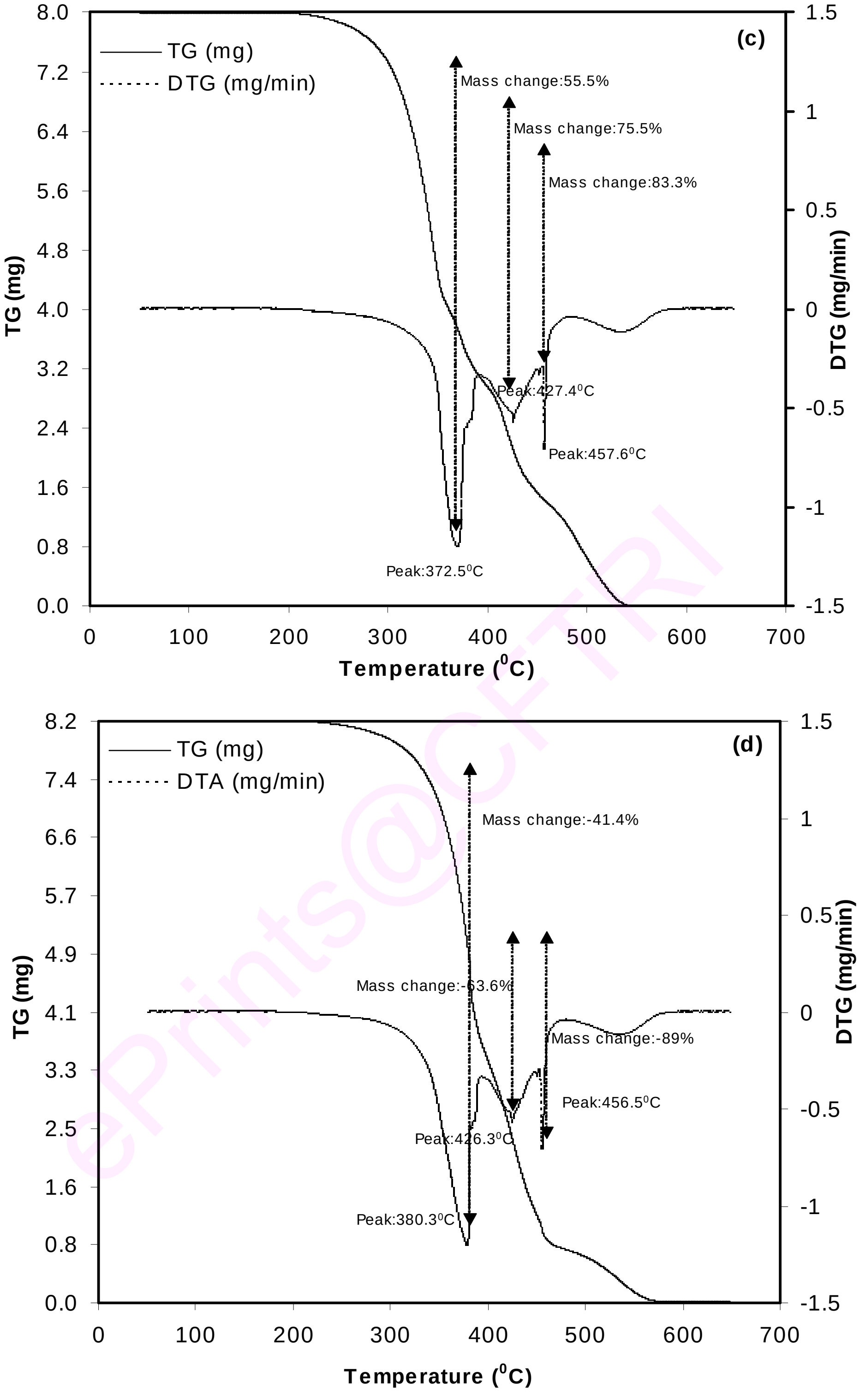
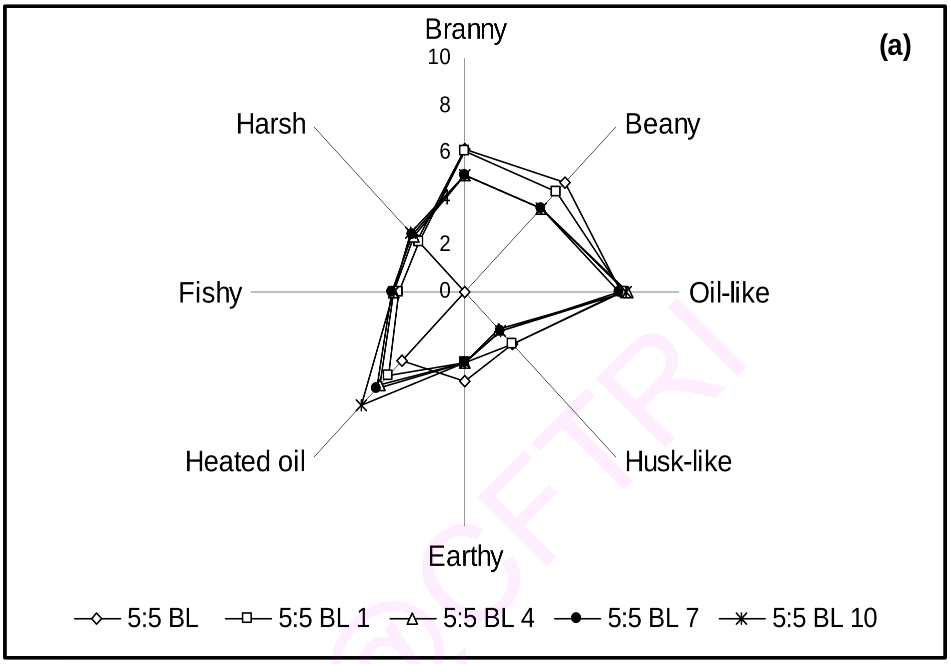


Fig. 1.12 TG and DTG curves under oxygen atmosphere (c) 5:5 BL and (d) 5:5 EI
BL- blended oil, EI-enzyme interesterified oil, 5:5- ratio of oil blend of RBO and RBDPO

1.7.4 Sensory odour profiles of blended (5:5 BL) and interesterified (5:5 EI) oils

Sensory odour profile of blended and interesterified oils are shown in the Fig. 1.13.



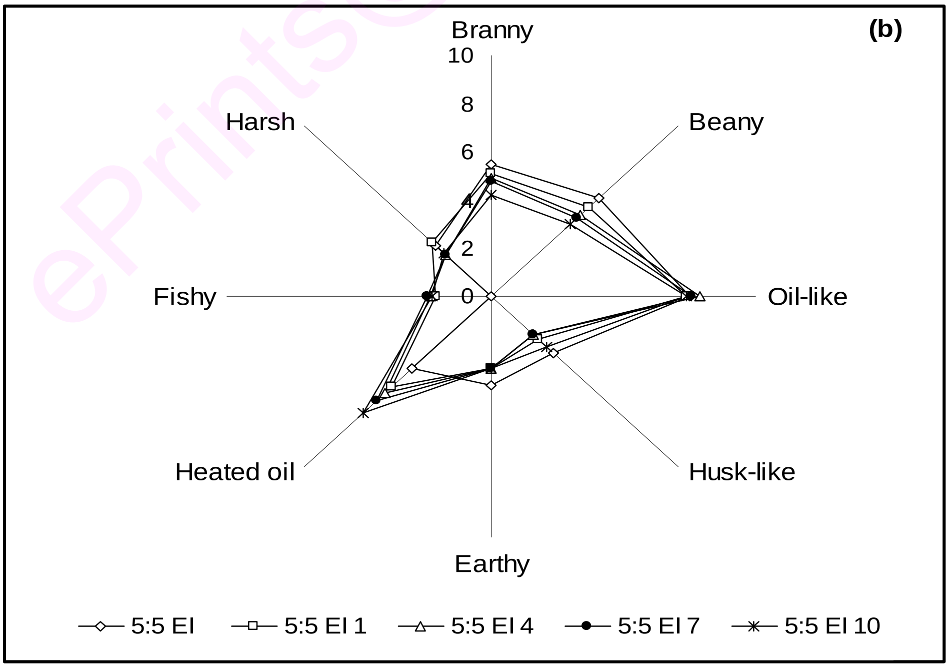


Fig. 1.13 Sensory odour profile of (a) blended and (b) enzyme interesterified oils BL 1, BL 4, BL 7, BL 10 – blended oil and EI 1, EI 4, EI 7, EI 10 - enzyme Interesterified oil after 1st, 4th, 7th and 10th frying cycles, respectively, 5:5 – ratio of blend of RBO and RBDPO

Beany and branny notes in *5:5 BL* and *5:5 EI* showed a decreasing trend with the increasing frying cycles. Heated oil note, perceived by the panelists showed with increasing frying cycles. Other attributes sch as oil-like, husk-like, earthy did not show significant ($p>0.05$) changes with increasing number of frying cycles. Both blended oil and interesterified oil samples exhibited similar sensory notes for the unheated and frying oil samples. Therefore, interesterification of oils did not change the typical sensory odour profiles of oils as indicated in this the study.

1.7.5 Consumer acceptance study

The sensory scores of the *poori* fried using blended (5:5 BL) and interesterified oils (5:5 EI) are shown in Fig. 1.14. The *poories* fried using blended and interesterified oil blend have shown desirable and good consumer acceptance score. The blended oil (5:5 BL 1) obtained after first frying cycle was rated as 'Like very much' by 14% of the respondents. 70% and 16% of the population rated as 'Like moderately' and 'Like slightly' for the 5:5 BL 1 sample. However, 8% of panelists rated sample 5:5 EI 1 as 'Like very much', 85% as 'Like moderately' and 7% as 'Like slightly'. Samples 5:5 BL 10 and 5:5 EI 10 are rated 'Like moderately and 'Like slightly' by the respondents. However, all the ratings have fallen on 'Like' category and the *poori* samples are acceptable. Both the fried products (*poori*) in the 1st frying cycle using blended (5:5 BL 1) and interesterified oils (5:5 EI 1) have shown higher acceptability (Debnath *et al.,* 2010b).

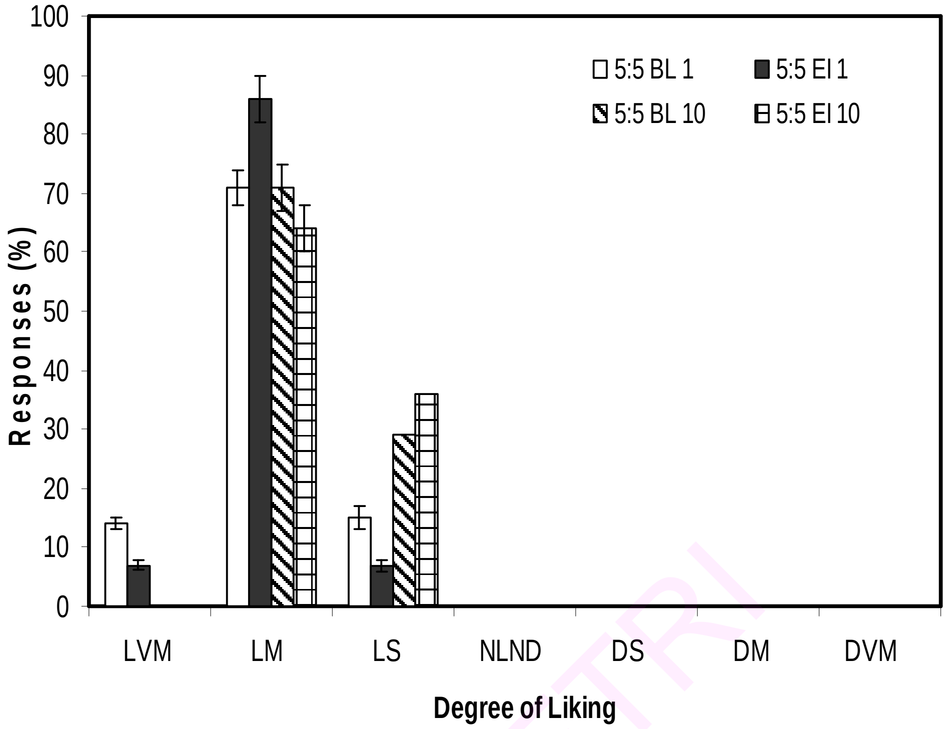


Fig. 1.14 Consumer acceptance study of *poori* fried in blended and interesterified oils
BL 1, BL 10 – blended oil after 1st and 10th frying cycle, EI 1, EI 10 – enzyme interesterified oil after 1st and 10th frying cycles, 5:5 - ratio of blend of RBO and RBDPO
LVM – Like Very Much, LM – Like Moderately, LS – Like Slightly, DS - Dislike Slightly, DM – Dislike Moderately, DVM – Dislike Very Much, NLND – Neither Like Nor Dislike

1.8 Discussion

Blending of edible vegetable oils gives more flexibility in getting an oil with desired physical, thermal properties needed for making specific food products (Chen *et al*., 2007). Enzyme interesterification is preferred for modifying the oils over the physical blending of oils as the physical mix of oils may not produce oils with desired properties.

In the present investigation we prepared physical blends based of RBO and RBDPO at the different ratio of 2:8, 5: 5 and 8:2. These blended oils were subjected to lipase (1, 3-specific lipase RM IM) catalyzed interesterification reaction to rearrange the fatty acids in the triglyceride molecule. This modification may change its physical, thermal, hydrodynamic as well as nutritional properties (Rousseau *et al.,* 1998). Several products have been developed during last decades, such as low fat butter products, fat substitutes and zero calorie fats using such modified oils. The overall fatty acid composition and their arrangement/location in the triglyceride moiety can influence the physical properties of the fat (Rodrigues and Gioielli, 2003). A single triglyceride molecule can show multiple physical properties due to presence of different crystal forms known as polymorphism (Lopez, 2005). The polymorphism influences melting points, solid fat content, plasticity and brittleness of fats.

Triglycerides of RBDPO are primarily rich in saturated fatty acids. About 54% of the triacylglycerols in RBDPO contained di- and trisaturated fatty acids. Of these, 49.9% was contributed by disaturated and 4% by trisaturated triacylglycerols. This resulted in semisolid physical state of RBDPO at ambient temperature (25±1°C). After blending with RBO and subsequent interesterification, the level of trisaturated triglycerides decreased which lowered the melting point of interesterified oils. These results are in agreement with those reported by Ribeiro *et al.* (2009) who showed that interesterified blends of soybean and fully hydrogenated soybean oil showed reduction in solid fat content and melting properties as compared to its physical blends.

The thermograms for RBO and RBDPO indicated a wide range of melting temperatures, which is influenced by the number of carbon atoms present in the triaclyglycerol molecular species and the number of unsaturations in the oil. Similar observation was reported by Costa *et al*. (2010) when dealing with solid-liquid equilibrium of tristearin with refined rice bran and palm Oils. RBDPO showed three endothermic peaks indicating the heterogeneous nature of triacylglycerols. The melting profile of RBO showed a single melting peak at very low temperatures due to higher levels of unsaturated triacylglycerols present in it.

Blending as well as enzymatic interesterification of RBO with RBDPO resulted in the emergence of two endothermic peaks in different temperature ranges. The blending of these oils shifted the peak melting point of the triacylglycerols towards a lower temperature range from 10.36 °C (RBDPO) to 2.97 °C (5:5 Blend). It further decreased to – 4.76 °C after enzymatic interesterification (5:5 interesterified soil) due to decrease in the disaturated triacylglycerols and trisaturated triacylglycerols.

The cooling curves of blended oils of RBO with RBDPO and their interesterified counterparts resulted in one and two exothermic peaks in their crystallization profile, respectively. The peak crystallizing temperature for the interesterified oil shifted to a lower temperature range of 3.56 to – 4.83 °C, indicating more homogeneity of triacylglycerols in the interesterified oil.

The flow behavior of the native blended and interesterified oils indicated that these oils follows marginally shear thinning behavior. This study also indicated that the apparent viscosity of oils decreases after blending followed by interesterification. Our results are in agreement with the findings of Ghosh *et al.* (1999) who observed that interesterified blends of castor oil with sal fat, palm stearin and coconut stearin showed a reduction in viscosity.

The interesterified oil blends was found to have higher thermo-oxidative stability than native and blended oils as revealed by chemical, thermogravimetric and differential thermogravimetric analysis. These studies therefore indicated that the blended and interesterified oils provide an alternate system for frying oils with improved thermophysical properties. This work may be helpful in many physical processes such as frying, cooking and baking.

Sensory evaluation of the product (*poori*) fried using in these modified oils revealed no significant ($p>0.05$) differences in sensory attributes and its acceptance by its panelists. The *poori* fried in blended oil and interesterified oil showed desirable (fresh oil note) sensory odour profiles and were found to have good consumer acceptance score (Debnath *et al.,* 2010b).

Chapter-2

Hydrodynamic and Heat Transfer Properties of Enzyme Interesterified Oils

2.1 Introduction

Individual oils have unique physical properties. The physical properties of specific oil determine its heat transfer properties during frying. The heat transfer coefficient is an important factor, which characterize heat flow in the oil during frying. The correlation between viscosity, oil temperature and heat transfer coefficient is one of the key factors for quality of food prodcts. The physical properties therefore are the primary factors which control the quality of the fried food (Stern and Roth, 1959).

The use of blended oil for culinary uses are gaining attention as they provide nutritional benefits, improved thermal stability, cheaper alternatives or substitutes to expensive vegetable oils and suitable for tailoring the desired properties. Various research was done to describe the chemical changes in frying oils, but very few have attempted to relate the physical changes to the thermal properties of the different oils and oil blends. Miller *et al.* (1994) showed that viscosity and convective heat transfer coefficients vary between 2.5 to 3.95 $m^{-2}s^{-1}$ and 250.7 to 276.2 $Wm^{-2}°C^{-1}$ for canola, palm, corn and soybean oil over a temperature range of 170 to 190°C. They also observed that frying time affects the oil viscosity significantly. The influence of oil temperature on convective heat transfer coefficient and heat flux was reported by Hubbard and Farkas (2000). They found that an increase in oil temperature causes an increase in maximum convective heat transfer coefficient, heat flux, and rate of moisture removal.

The objectives of this part of investigation is to determine (a) the hydrodynamic (kinematic viscosity) and heat transfer properties (heat transfer coefficient) of blended and enzyme interesterified oils containing RBO and

RBDPO and (b) optimization of enzyme based process for preparation of interesterified oil with desirable hydrodynamic (kinematic viscosity) and heat transfer properties. This will enable us to develop suitable oil combinations for frying purposes.

2.2 Results

2.2.1 Typical Time-Temperature profile of aluminum transducer immersed in RBDPO heated at different temperatures

It is known that the heating rate of oil increased with increasing frying oil temperatures (Fig. 2.1). The plot between dimensionless temperature ratio (in logarithmic form) i.e., $\ln\left(\frac{T_A - T_t}{T_A - T_i}\right)$ against time of frying oil over the temperature of 170°C to 190°C were presented in (Fig. 2.2). The slope of the linear portion of the plot i.e., $\left(\frac{hA}{\rho V c_p}\right)$ was considered while linear regression analyses for the evaluation of convective heat transfer coefficients.

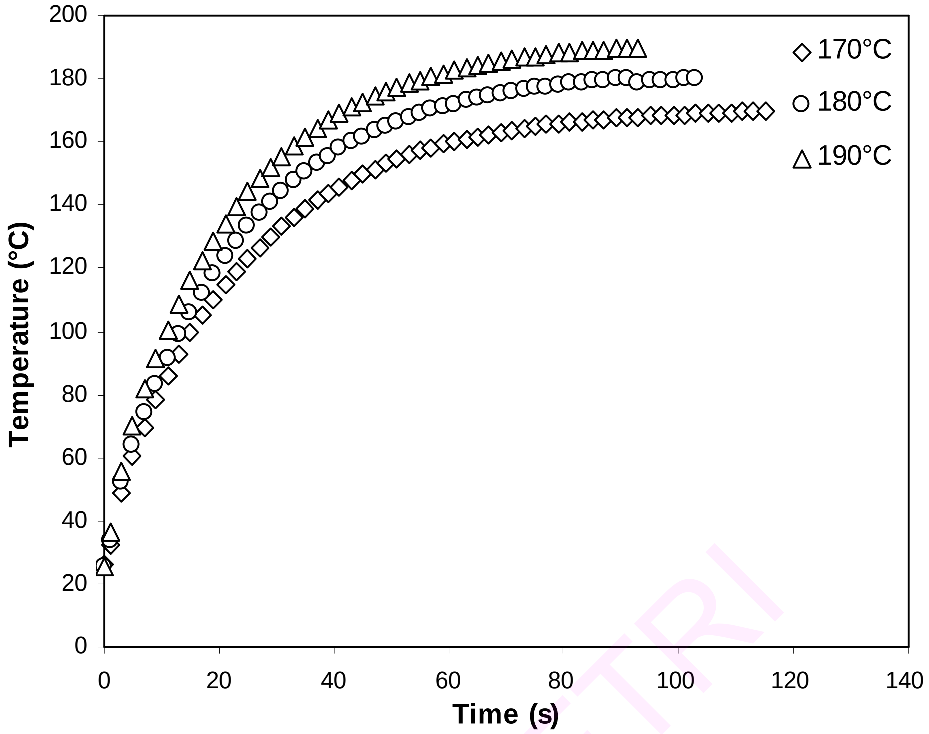


Fig. 2.1 Typical Time-Temperature profile of aluminum transducer immersed in RBDPO heated at different temperatures

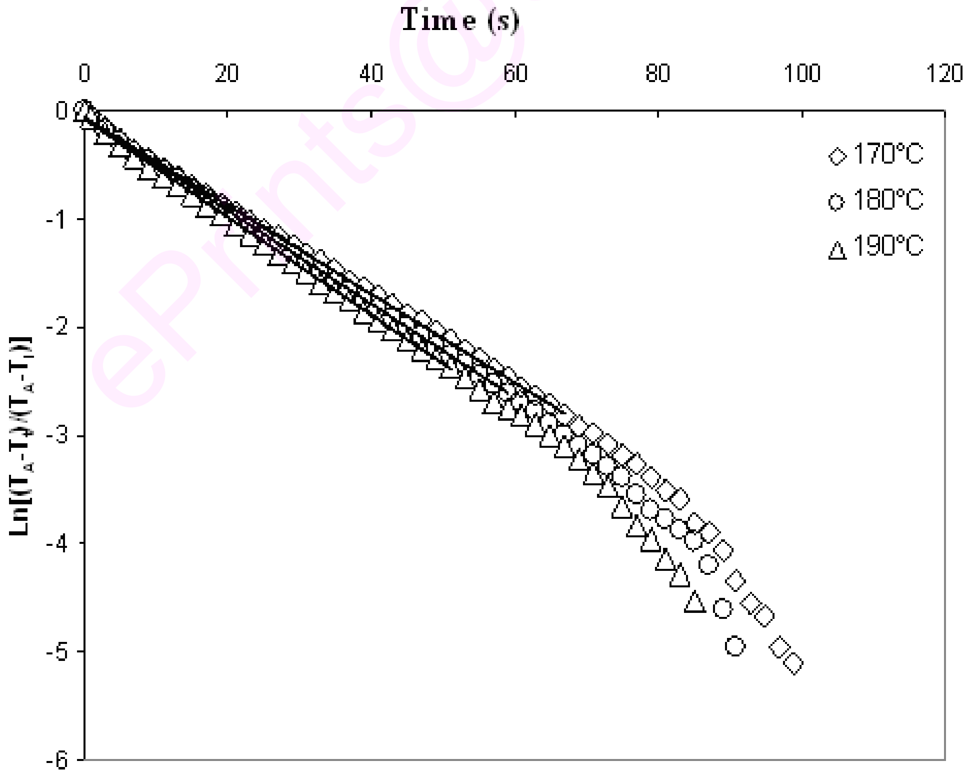


Fig. 2.2 Typical plots of dimensionless temperature ratio vs time and their slopes of linear portions used for calculation of heat transfer coefficients of RBDPO

2.2.2 Effect of blending on viscosity and heat transfer characteristics of oils

RBDPO has the higher kinematic viscosity in the temperature range 170 to 190^0C (Table 2.1) as compared to RBO. Over an elevated range of temperature (170°C to 190°C), kinematic viscosity of RBDPO as well as selected blends of RBO-RBDPO was found to decrease from 3.617×10^{-6} to 2.955×10^{-6} $m^2 s^{-1}$ (18.3% decrease) when RBO was changed from 20-80% (w/w) in the blend. These values are presented in the Fig. 2.3a. These values are similar to viscosity values reported by other researchers (Miller *et al.,* 1994) for canola, corn, palm and soybean oil. The change of viscosity of blends of oils can be attributed to the levels of saturated, mono and polyunsaturated fatty acids contents in different oils (Santos *et al.*, 2005). Results showed a good correlation between kinematic viscosity ($r > 0.997$) of frying oils/ oil blends with temperature. The interaction between oil type (control /blend) and oil temperature was found to have a significant ($p<0.05$) effect on viscosity of oil.

It is seen from the Table 2.1 that RBO has the higher value for the convective heat transfer coefficient as compared to RBDPO. The convective heat transfer coefficients of the selected blends were found to increase from 221.0 to 265.8 W m^{-2} $°C^{-1}$ (20.27% increase) in case of RBO-RBDPO blend with the increasing levels of RBO from 20-80% (w/w) in the blend. The values of heat transfer coefficients for these blends are presented in Fig. 2.3b. These values are similar to the range of heat transfer coefficient values reported in the literature (Miller *et al.,* 1994) for canola, corn, palm and soybean oil. Results showed a good correlation between heat transfer coefficient ($r > 0.993$) of native and blended oils with temperature over an elevated range.

Therefore, blending and temperature had a significant ($p<0.05$) effect on heat transfer coefficient of the oils.

Table 2.1 Kinematic viscosity and heat transfer coefficient of unmodified oils used for frying

Oils	Kinematic viscosity ($\times10^{-6}$ m^2s^{-1})			Heat transfer coefficient (Wm^{-2} $°C^{-1}$)		
	170 °C	180 °C	190 °C	170 °C	180 °C	190 °C
RBO	3.392	3.191	2.946	233.3	243.2	247.5
RBDPO	3.617	3.418	3.100	221.0	237.2	244.4

where, RBO – Rice bran oil, RBDPO – Refined palm oilein

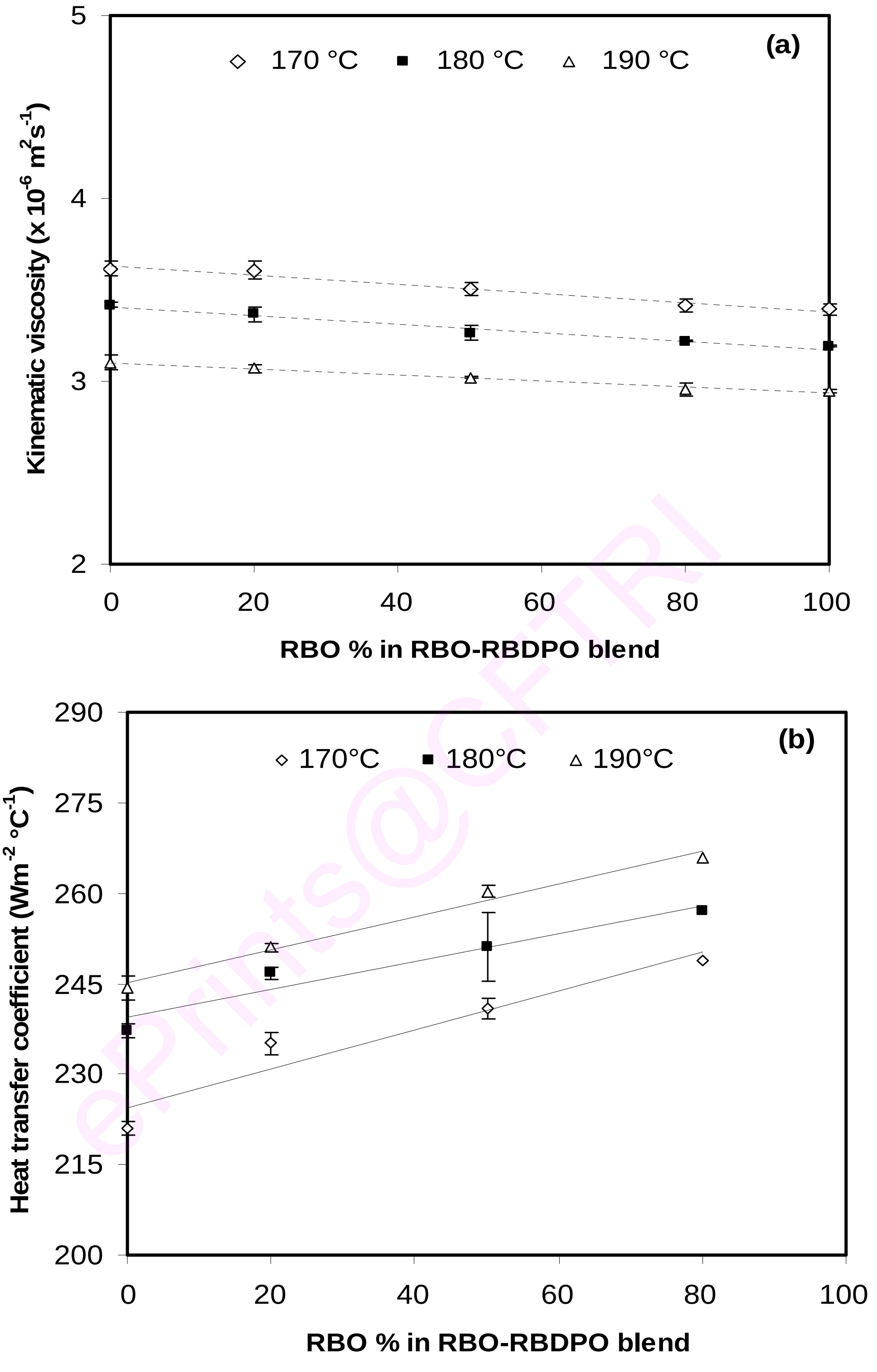


Fig. 2.3 (a) Viscosity and (b) heat transfer coefficient of RBO-RBDPO blends at elevated temperatures

2.2.3 Effect of blending on activation energy

The variation of kinematic viscosity with absolute temperature was explained with the Arrhenius type equation (Eqn. 4 in Materials and Methods). The activation energy of RBO-RBDPO blend was found to decrease from 13.7 kJ/mol to 12.0 kJ/mol with increasing level of RBO from 20-80% (w/w), in the blend (Fig. 2.4). The results illustrated that the oil blends having lower viscosities resulted in lower magnitude of activation energies (Ewell, 1938). These values are similar to activation energy values as reported for corn oil by other researchers (Da Silva and Singh*,* 1995) for corn oil.

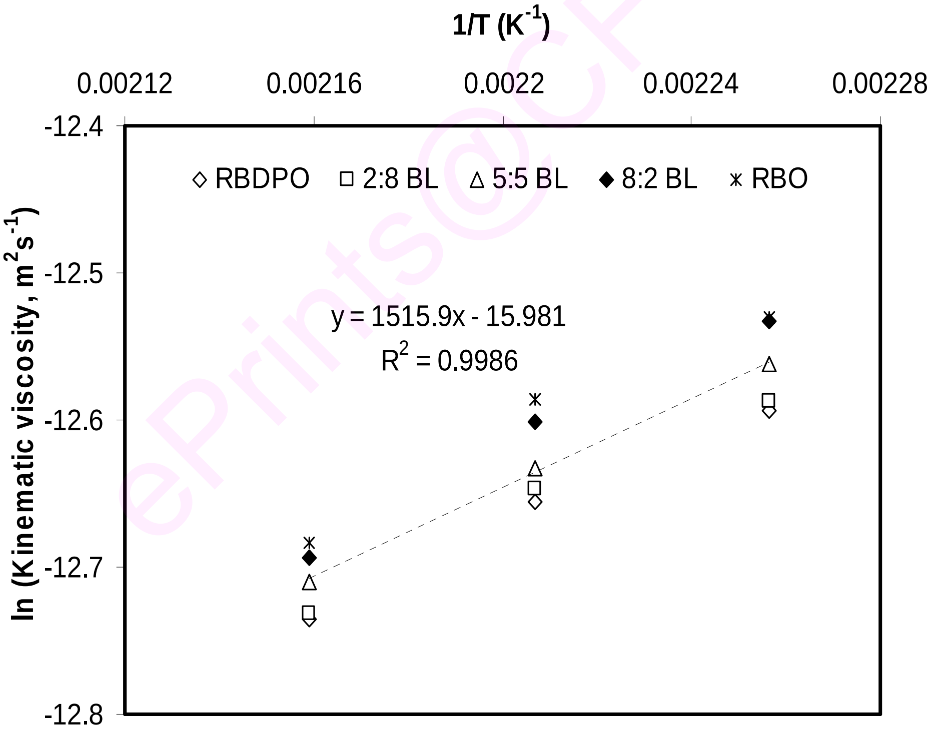


Fig. 2.4 Arrhenius type plot of change in kinematic viscosity of RBO-RBDPO blend with absolute temperature
RBO-rice bran oil, RBDPO-refined palm oil, BL-blended oil, 8:2, 5:5 and 2:8- ratios of blends of RBO and RBDPO

2.2.4 Effect of interesterification on hydrodynamic property (kinematic viscosity) of oils

The kinematic viscosity of the oil blends varied from (3.518×10^{-6} to 2.89×10^{-6} $m^2 s^{-1}$) as shown in Fig. 2.5. The kinematic viscosities of binary oil blends were found to decrease from 3.518×10^{-6} to 3.316×10^{-6} $m^2 s^{-1}$ at a frying temperature of 173±1 °C with an increase of RBO in RBO-RBDPO blend from 20 to 80 % (w/w). The convective heat transfer coefficient was observed to decrease further from 3.316×10^{-6} to 2.89×10^{-6} $m^2 s^{-1}$ in interesterified oil blends at a frying temperature of 173±1 °C. These values are presented in the Fig. 2.5. These values are similar to the kinematic viscosity values reported by Debnath *et al.* (2010a).

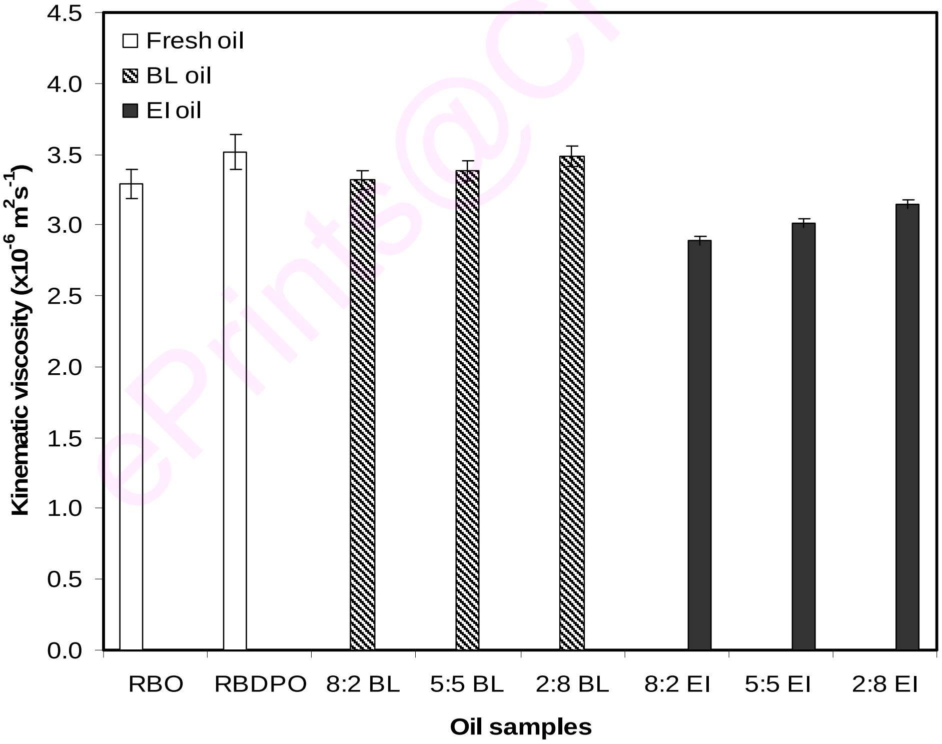


Fig. 2.5 Changes in hydrodynamic property (kinematic viscosity) in native, blended and interesterified oil blends based on RBO and RBDPO at 173°C
RBO-rice bran oil, RBDPO-refined palm olein, BL-blended oil, EI-enzyme interesterified oil , 8:2, 5:5, 2:8 - ratios of blends of RBO and RBDPO

This change of viscosity can be attributed to the levels of saturated, mono and polyunsaturated fatty acids contents in different oils due to blending and enzyme interesterification (Fig. 1.5 in Section-1A). Therefore, the interaction between oil type (control/ blended/ interesterified oil) and oil temperature was found to have a significant ($p<0.05$) effect on viscosity of oil.

2.2.5 Effect of interesterification on heat transfer coefficient

The convective heat transfer coefficient of the oil blends varied from (229.1 to 258.0 $Wm^{-2}\,^{\circ}C^{-1}$) as shown in Fig. 2.6. The convective heat transfer coefficients of binary oil blends were found to increase from 229.1 to 253.0 $W\,m^{-2}\,^{\circ}C^{-1}$ at a frying temperature of 173±1 °C with an increase of RBO in RBO-RBDPO blend from 20 to 80 % (w/w). The convective heat transfer coefficient was observed to increase further from 253.0 to 258.7 $W\,m^{-2}\,^{\circ}C^{-1}$ in interesterified oil blends at a frying temperature of 173±1 °C. These values are similar to the kinematic viscosity values reported by Debnath *et al.* (2010b). It may be noted that, with increasing temperature of the oil, the intermolecular interactions among the aclylglycerides increase which, in turn, decrease the oil viscosity and consequently increase the heat transfer coefficient (Toro-Vazquez *et al.,* 1993*).* Interesterification of the oil blends containing RBO and RBDPO has affected the solid fat content and thermal profiles of oils (Reena *et al.*, 2009). The blending as well as enzyme interesterification changes the triglyceride profile (discussed in Section-1A) which could be responsible for the variation of kinematic viscosity and in turn heat transfer coefficient. Results showed a good correlation between heat transfer coefficient ($r > 0.97$) of native and blended oils used for frying at different temperatures. Thus the enzymatic interesterification of oil blends and

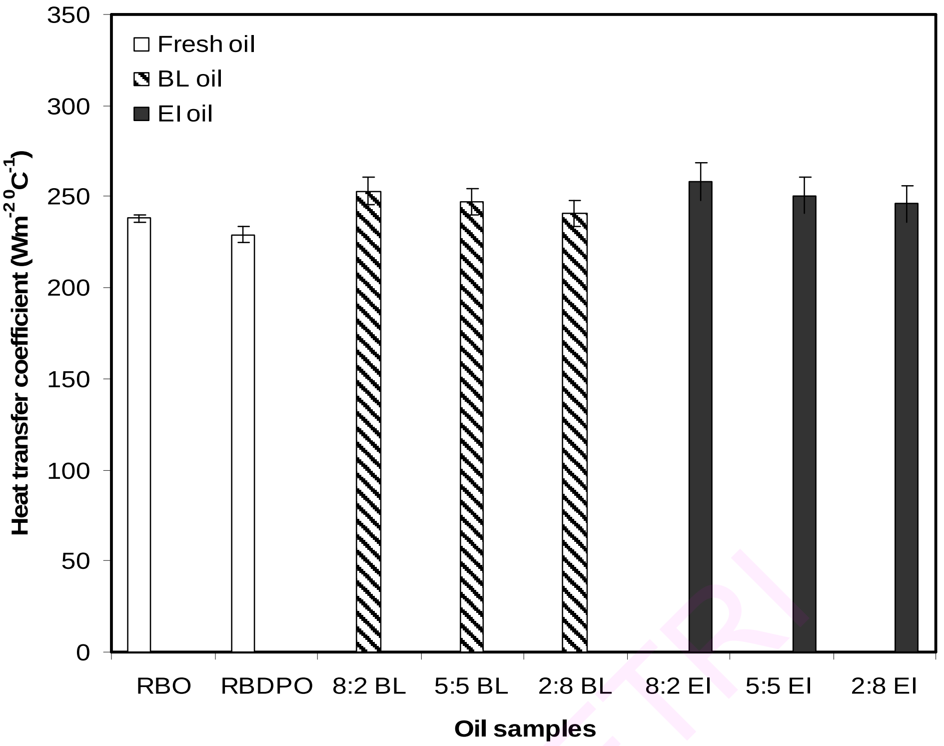


Fig. 2.6 Changes in heat transfer coefficient in native, blended and interesterified oil blends based on RBO and RBDPO at 173˚C
RBO-rice bran oil, RBDPO-refined palm olein, BL-blended oil, EI- enzyme interesterified oil , 8:2, 5:5, 2:8 - ratios of blends of RBO and RBDPO

temperature were found to have a significant ($p<0.05$) effect on heat transfer coefficient.

2.2.6 *Preliminary optimization of interesterification process based on single parameter*

(a) Effect of RBO level (%) in RBO-RBDPO blend

The effect of RBO level in RBO-RBDPO blend on interesterification reaction for achieving low hydrodynamic properties and highheat transfer coefficient at the constant reaction temperature (45^0C), enzyme concentration (7%) and reaction time (7h) was investigated. The results indicated that a minimum kinematic viscosity (2.93×10^{-6} m^{2}s^{-1}) and maximum heat transfer coefficient (254.8 Wm^{-2} $^{\circ}$C $^{-1}$) can be achieved in the oil blend containing 80% of RBO and 20% of RBDPO (Fig. 2.7).

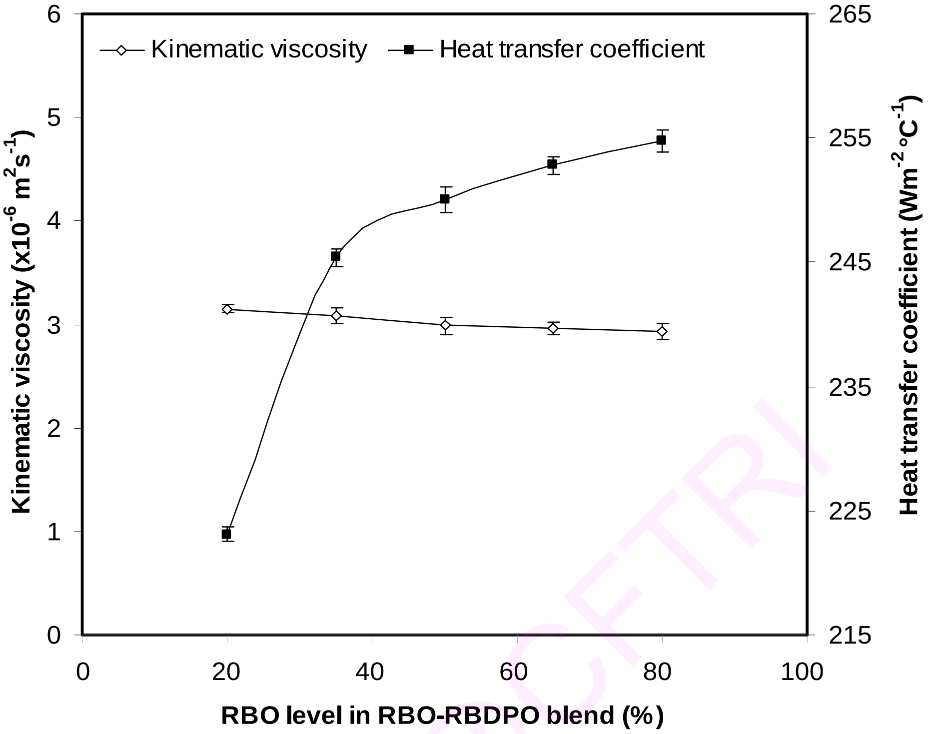


Fig. 2.7 Effect of RBO level in RBO-RBDPO blend on kinematic viscosity and heat transfer coefficient of interesterified oil

(b) Effect of reaction temperature

The effect of reaction temperature on interesterification reaction for achieving low hydrodynamic properties and high heat transfer coefficient was studied at the constant RBO level in the RBO-RBDPO blend (50%), enzyme concentration (7%) and reaction time (7h). The result indicated that a minimum kinematic viscosity (3.01x10^{-6} m^{2}s^{-1}) and maximum heat transfer coefficient (252.03 Wm^{-2} $^{\circ}$C^{-1}) can be achieved at a given reaction temperature of 60 $^{\circ}$C (Fig. 2.8).

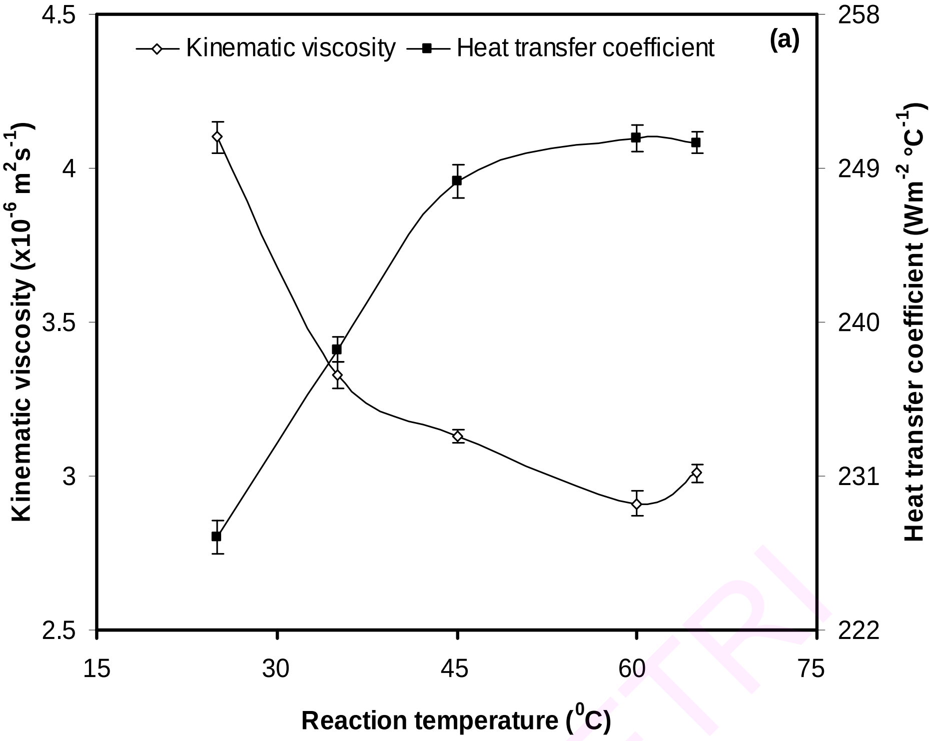


Fig. 2.8 Effect of reaction temperature on kinematic viscosity and heat transfer coefficient of interesterified oil

(c) Effect of enzyme concentration

The influence of enzyme concentration on interesterification reaction for achieving low hydrodynamic properties and high heat transfer coefficient was studied at the constant RBO level in the RBO-RBDPO blend (50%), reaction temperature (45°C) and reaction time (7h). The result indicated that a minimum kinematic viscosity (2.99 x10^{-6} m^{2}s^{-1}) and maximum heat transfer coefficient (250.04 Wm^{-2} $^{\circ}$C^{-1}) can be achieved at a given enzyme concentration of 7% (Fig. 2.9).

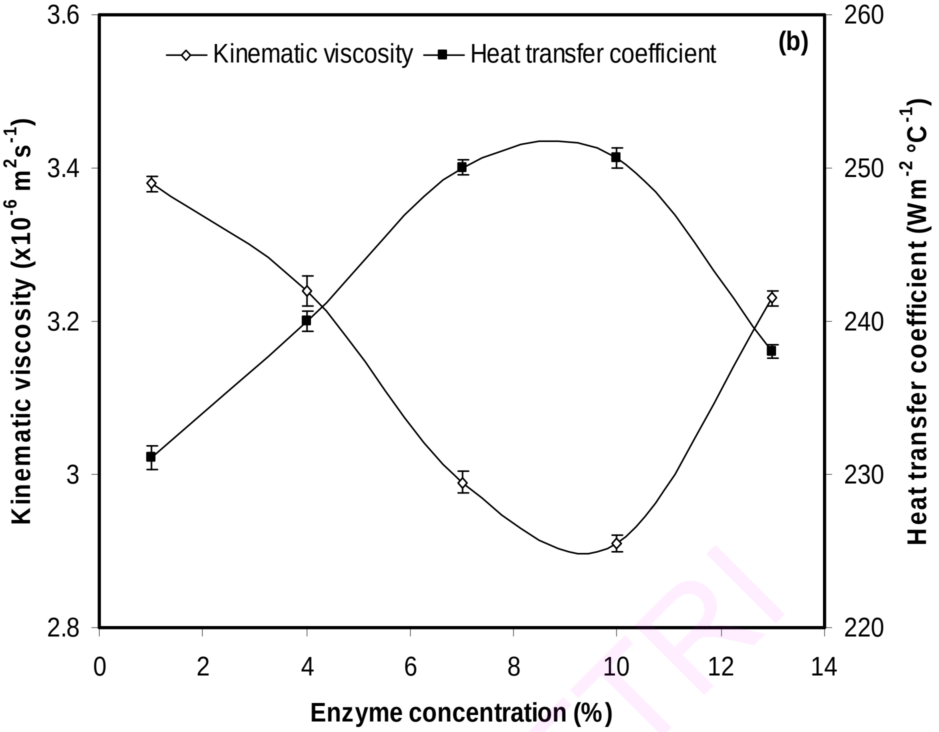


Fig. 2.9 Effect of enzyme concentration on kinematic viscosity and heat transfer coefficient of interesterified oil

(d) Effect of reaction time

The effect of reaction time on enzyme interesterification reaction for achieving low hydrodynamic properties and highheat transfer coefficient was observed at a constant RBO level in the RBO-RBDPO blend (50%), reaction temperature (45 ˚C) and enzyme concentration (7%). The result indicated that a minimum kinematic viscosity (2.99 x 10^{-6} m^2s^{-1}) and maximum heat transfer coefficient (250.04 Wm^{-2} ˚C^{-1}) which can be achieved at a given reaction time of 7h (Fig. 2.10).

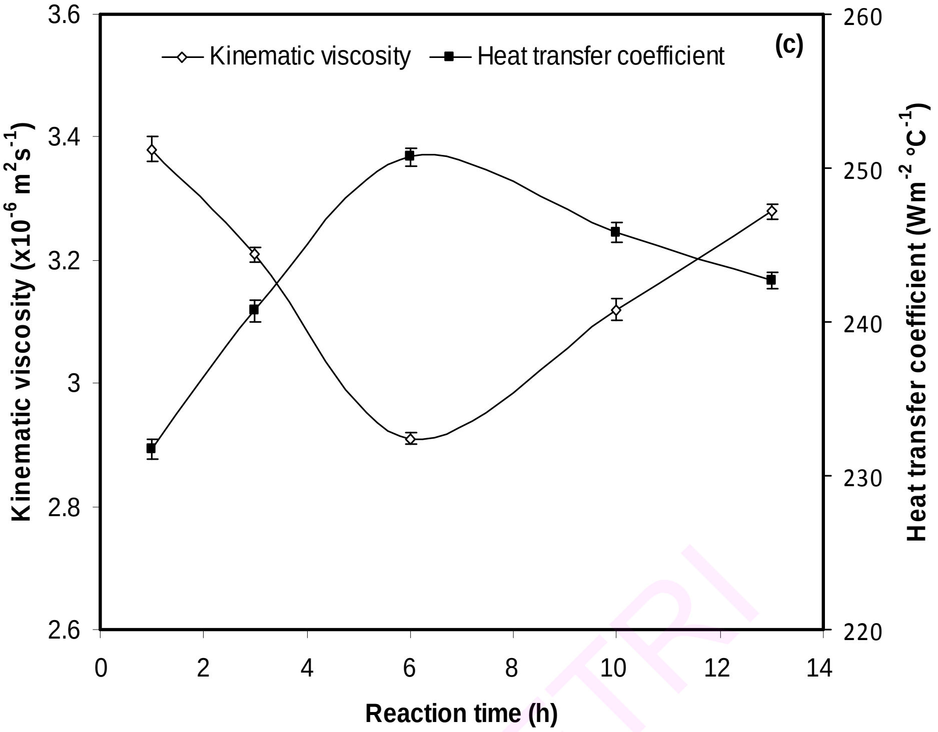


Fig. 2.10 Effect of reaction time on kinematic viscosity and heat transfer coefficient of interesterified oil

2.3 Discussion

In this study, the methodology for determination of kinematic viscosity and convective heat transfer coefficients of frying oils and their selected blends and interesterified counterparts were carried out over a range of elevated temperatures (170, 180 and 190°C). Blended and interesterified oils exhibited desired low levels of viscosity and higher levels of heat transfer properties than naturally occurring oils and therefore, are physico-thermally better suited for frying. The change in viscosity with the varied ratio of the oil blend may be due to change in proportion of unsaturated fatty acids in the oil containg pi-bonds as reported by Santos *et al.* (2005). Our results also supported this observation.

The blended as well as interesterified oils were found to show lesser time to reach the frying temperature and consequently higher heat transfer coefficient (229.1 to 253 Wm^{-2} $°C^{-1}$) (when varied the ratio from 2:8 to 8:2) in comparison to their corresponding native oils (Debnath *et al.*, 2010b). The blended oil of the same ratio again showed higher level of heat transfer (253 to 258 Wm^{-2} $°C^{-1}$). Hence use of blended and enzyme interesterified oils for frying can be a cost effective proposition for frying process. A good correlation between kinematic viscosity ($r > 0.997$) or heat transfer coefficient ($r > 0.993$) was observed with these oils and their selected binary blends with selected temperatures.

Apart from lower level of viscosity and higher level heat transfer, the blended as well as interesterified oil provide higher level of oxidative and thermal stability. It was reported that the blended oil containing palm kernel olein and soybean oil provides a stable oil with respect to hydrolysis, oxidation and rancidity in comparison to soybean oil (Goburdhun *et al.,* 1995).

The desired level of kinematic viscosity and heat transfer coefficient were found to change with individual treatment of different parameters, such as proportion of individual oil in the oil blend, reaction temperature, enzyme concentration, reaction time on these properties of oils. Hence there is a need to optimize the enzyme interesterification reactions following suitable statistical models. The next chapter addressed the optimization of enzymatic interesterification reaction condition following response surface methodology.

Chapter-3

Optimization of Enzymatic Interesterification Process by Response Surface Methodology

3.1 Introduction

Optimization of the different parameters in a batch or continuous reactor for enzyme interesterification enables one to obtain the maximum product yield. The response surface methodology is a statistical design that enables one to determine optimal conditions for enzyme-assisted reactions by carrying out minimum number of experiments. Several authors have worked on modification of lipids by interesterification. Zhou *et al.* (2001) investigated the production of structured triacylglycerols containing short-chain fatty acids in a batch reactor with a solvent-free system using immobilized *Rhizomucor miehei* lipase following response surface methodology. They observed optimal reaction conditions to be: reaction time (17 h), enzyme load (14 wt. %), ater content (10 wt.%) and reaction temperature (65°C) for 55 mol% incorporation of caproic acid in structured triacylglycerols. Huang and Akoh (1996) investigated optimized synthesis of structured lipids using immobilized SP435 lipase by transesterification of ethyl caprylate, soybean oil and sunflower oil in hexane using response surface methodology. They found that optimum conditions for synthesis of structured lipids were incubation time (26.4 h), molar ratio of ethyl caprylate to total triglycerides (8), percentage of soybean oil as source of long chain triglycerides (75%) and long chain triglyceride concentration (0.58M). Rao *et al.*, (2002) studied the optimization of the reaction for modifying coconut oil TAG by using lipase-catalyzed acidolysis in hexane to incorporate n-3 or n-6 PUFA using immobilized lipase, Lipozyme IM60, from *Rhizomucor miehei* by response surface methodology. They found that maximal incorporation of n-3 PUFA occurred at a 1:4 molar ratio of TAG/FFA when incubation was carried out for 34 h at 54°C and for n-6

FA was optimum conditions were shown to be at a 1:3 molar ratio of TAG/FFA when incubated for 48.5 h at 39°C which yielded structured lipids with 13.65 and 45.5% of n-3 and n-6 fatty acids, respectively. Rapeseed oil and capric acid were interesterified (Xu *et al*., 1999a) in solvent-free media catalyzed by Lipozyme IM from *Rhizomucor miehei* to produce specific-structured lipids. Ming *et al*. (1998) investigated the enzymatic transesterification of palm stearin – palm karnel olein (40:60) in a solvent free system to improve the physical and melting characteristics of solid fats. They found that enzymatic transesterification was able to produce fat mixture with substantially lower melting points by repositioning the fatty acids of triglycerides from higher melting range to form lower or middle melting components. Senanayake and Shahidi (2002) investigated the modification of fatty acid composition of borage oil in hexane, by incorporation of docosahexaenoic acid. They achieved the optimum incorporation of DHA, up to 34.1% at enzyme concentration of 165 units, after 25 h of reaction at 50 °C. Cheong *et al.* (2007) investigated the production of diacylglycerol (DAG)-enriched palm olein by partial hydrolysis using Lipozyme RM IM lipase. They achieved the optimal reaction conditions at 50 wt% water content, 10 wt% enzyme load, 65°C of reaction temperature and 12 h of reaction time. Liu *et al*. (2007) optimized conditions for enzymatic synthesis of acylglycerols from glycerol and n-3 polyunsaturated fatty acids concentrates, prepared from tuna oil. They achieved the optimum condition of esterification at glycerol 2.5 g, hexane 5 ml, the initial water content 0.60%, temperature 40 °C, and molecular sieves 1 g. Sharma *et al*. (2009) studied the production of structured lipid containing 1:1 ratio of n-3 and n-6 fatty acids by incorporating α-linolenic acid from linseed oil into groundnut oil using lipase. They achieved optimal conditions at enzyme concentration 3.75% (w/w), temperature 37.5 °C, incubation time 30.81 h and ratio of free fatty acid concentrate from linseed oil to groundnut oil 1.16 (w/w). The effects and relationship amongst reaction temperature, reaction time, enzyme load and substrate mole ratio were studied by Koh *et al*. (2010) for the production of healthy functional cooking oil for long-term dietary treatment. They observed that fatty acids used for the production greatly influenced the physical and chemical properties of the product.

Very little information on effects of enzyme transesterification on physical and thermal changes such as kinematic viscosity and heat transfer coefficients of oils at frying temperatures are available. Therefore, the objective of this research work was to optimize the synthesis of enzyme interesterified oil containing rice bran oil (RBO) and refined palm olein (RBDPO) to obtain oil with optimum hydrodynamic (kinematic viscosity) and heat transfer (heat transfer coefficient) properties. The effect of four variables namely, RBO level in the RBO-RBDPO blend, reaction temperature, enzyme concentration and reaction time were investigated using response surface methodology.

3.2 Interesterification and optimization

3.2.1 Interesterification of oil blends

The lipase-catalyzed interesterification (transesterification) reaction of RBO blended with RBDPO was carried out in a reciprocating shaking water bath (model BS-31, Jeio Tech Co. Ltd., South Korea). The binary oil blends of

RBO and RBDPO were taken in a round-bottomed flask in the different mass (w/w %) ratio of (20-80%) and 1, 3-specific lipase (Lipozyme RM IM, from *Rhizomucor miehei*) was added at different concentrations (1-13% w/w) at constant temperature (over the range 25-65°C). Incubation was continued at the shaking speed of 150 rpm for different time periods (1-13 h). The enzyme was separated from mixture by filtration. The products obtained were then tested for kinematic viscosity and heat transfer coefficient, fatty acid composition and triacylglycerols.

3.2.2 Experimental design

A four variable, five levels Central Composite Rotatable Design (CCRD) face centered cube design (Cochran and Cox, 1957) was used to study the surface response model for determining the optimum combination of independent variables. In the present study 31 design points were chosen. Experiments were performed in duplicates. The coded independent variables (X_i, i = 1, 2, 3 and 4) investigated were RBO level in RBO-RBDPO blend (20-80% RBO-RBDPO), reaction temperature (25 - 65ºC), enzyme concentration (1-13%, w/w) and reaction time (1-13 h) (Table 3.1). Independent variables and their range were selected based on the prior experiment trials conducted. In this present study seven replicates (treatment 25 - 31) were included to estimate the experimental error.mThe polynomial equation fitted was expressed as surface plots in order to envisage the relationship between the response and experimental levels of each variable.

3.2.3 Statistical analysis and optimization

A second-order regression equations/models were used to fit the responses. The second order polynomial equation (Eqn. 3.1) was used to fit the experimental data given in Table 3.1. Responses or dependent variables were studied for viscosity and heat transfer coefficient measurement.

$$Y = a_0 + \sum_{i=1}^{k} a_i X_i + \sum_{i=1}^{k} a_{ij} X_i^2 + \sum_{i_i<j}^{k} \sum_{j}^{k} a_{ij} X_i X_j + e \qquad \text{......... Eqn. (3.1)}$$

where, Y is the predicted response for kinematic viscosity and heat transfer coefficient of oils and a_0 is a constant, i and j are linear and quadratic coefficients, k is the number of factor studied and optimized in the experiment, a is a regression coefficient of the model and X_i and X_j are independent variables in coded values, e is the random error. Optimization of polynomial equation fitted was accomplished by a non-linear mathematical optimization method. A canonical analysis was employed to obtain the canonical equation and to investigate the nature of the surface (Wu and Hamada, 2000) using the software package Statistica (1999) (StatSoft v5.5, Statsoft Inc., Tulsa, USA). Response surfaces of the present design were obtained by using the fitted model and keeping two independent variable constant and varying the other two variables. The experiments were confirmed by using mixtures of variables at different levels.

3.2.4 Validation of the model

Optimization of reaction conditions in terms of RBO level in RBO-RBDPO blend, reaction temperature, enzyme concentration and reaction time

Table 3.1 Experimental design: CCR design with independent and dependent variables

Runs	Independent variables								Dependent variables[a]	
	Coded levels				Actual levels				Kinematic viscosity ($x10^{-6}$ m^2s^{-1})	Heat transfer coefficient (Wm^{-2} $^{\circ}C^{-1}$)
	X_1	X_2	X_3	X_4	x_1	x_2	x_3	x_4		
1	-1	-1	-1	-1	35	35	4	4	3.61±0.001	221.61±0.017
2	-1	-1	-1	+1	35	35	4	10	3.60±0.001	222.33±0.027
3	-1	-1	+1	-1	35	35	10	4	3.59±0.001	223.42±0.057
4	-1	-1	+1	+1	35	35	10	10	3.58±0.001	226.87±0.006
5	-1	+1	-1	-1	35	55	4	4	3.57±0.001	226.90±0.035
6	-1	+1	-1	1	35	55	4	10	3.56±0.001	227.07±0.008
7	-1	+1	+1	-1	35	55	10	4	3.55±0.001	227.27±0.001
8	-1	+1	+1	+1	35	55	10	10	3.54±0.001	228.36±0.041
9	+1	-1	-1	-1	65	35	4	4	3.52±0.007	229.07±0.008
10	+1	-1	-1	+1	65	35	4	10	3.51±0.015	229.32±0.000
11	+1	-1	+1	-1	65	35	10	4	3.39±0.004	231.01±0.000
12	+1	-1	+1	+1	65	35	10	10	3.27±0.003	236.75±0.037
13	+1	+1	-1	-1	65	55	4	4	3.47±0.001	230.43±0.071
14	+1	+1	-1	+1	65	55	4	10	3.27±0.003	236.42±0.028
15	+1	+1	+1	-1	65	55	10	4	2.96±0.001	259.41±0.035
16	+1	+1	+1	+1	65	55	10	10	2.71±0.002	263.71±0.035
17	-2	0	0	0	20	45	7	7	3.15±0.014	223.09±0.014
18	+2	0	0	0	80	45	7	7	2.93±0.002	254.80±0.023
19	0	-2	0	0	50	25	7	7	4.10±0.003	227.42±0.049
20	0	+2	0	0	50	65	7	7	3.01±0.001	252.03±0.004
21	0	0	-2	0	50	45	1	7	3.38±0.004	231.07±0.014
22	0	0	+2	0	50	45	13	7	3.23±0.000	238.03±0.004
23	0	0	0	-2	50	45	7	1	3.38±0.004	231.76±0.049
24	0	0	0	+2	50	45	7	13	3.28±0.001	242.69±0.068
25	0	0	0	0	50	45	7	7	3.11±0.000	248.18±0.082
26	0	0	0	0	50	45	7	7	3.13±0.003	248.24±0.044
27	0	0	0	0	50	45	7	7	3.10±0.000	248.38±0.021
28	0	0	0	0	50	45	7	7	3.05±0.001	249.41±0.042
29	0	0	0	0	50	45	7	7	3.02±0.001	249.91±0.020
30	0	0	0	0	50	45	7	7	2.99±0.001	250.04±0.002
31	0	0	0	0	50	45	7	7	3.10±0.001	249.62±0.025

where,

[a] Mean ± SD

X_1/x_1= RBO level in RBO-RBDPO blend (%), X_2/x_2= Reaction temperature (°C)

$X_3//x_3$= Enzyme concentration (%), X_4/x_4 = Reaction Time (h)

RBO-Rice bran oil, RBDPO-Refined palm olein were calculated using the predictive equation obtained from RSM. The production of enzyme interesterified oil was carried out at the optimum conditions and compared with the predicted value. The enzyme interesterified oil obtained after optimization reaction following RSM is abbreviated to EIO. The corresponding blended oil is abbreviated as BLO.

3.3 Results

3.3.1 Response surface analysis

3.3.1.1 Effect of process parameters and conditions

The effect of RBO levels in RBO-RBDPO blend, temperature, enzyme concentration and reaction time on the kinematic viscosity and heat transfer coefficient is presented in Table 3.2 as the coefficients of second order polynomials.

Table 3.2 Estimated regression coefficients for selected parameters and responses

	Kinematic viscosity (x10^{-6} m^{2}s^{-1})			Heat transfer coefficient (Wm^{-2} °C^{-1})		
	Estimated coefficients	Standard error	t-value	Estimated coefficients	Standard error	t-value
Constant	5.3532	1.1944	4.6313***	155.9679	38.4542	3.8999**
X_1/x_1 (L)	0.0327	0.0191	1.3875	0.5242	0.6162	0.6854
X_1/x_1 (Q)	0.0000	0.0001	0.3745	-0.0151	0.0041	-3.6956**
X_2/x_2 (L)	-0.1024	0.0314	-3.2554**	1.7689	1.0124	1.7473
X_2/x_2 (Q)	0.0014	0.0003	4.8879***	-0.032	0.0092	-3.4833**
X_3/x_3 (L)	0.0229	0.0896	0.2556	-0.2116	2.884	-0.0404
X_3/x_3 (Q)	0.0085	0.0032	2.697	-0.4992	0.102	-4.892***
X_4/x_4 (L)	-0.0655	0.0896	-0.7313	5.3077	2.884	1.8404
X_4/x_4 (Q)	0.0092	0.0032	2.9161**	-0.4249	0.102	-4.1638***
1L by 2L	-0.0005	0.0003	-1.8355	0.0202	0.0082	2.4663*
1L by 3L	-0.0019	0.0008	-2.2289*	0.08	0.0273	2.9336**
1L by 4L	-0.0008	0.0008	-0.885	0.0151	0.0273	0.5523
2L by 3L	-0.0015	0.0013	-1.1472	0.0879	0.0409	2.1487*
2L by 4L	-0.0007	0.0013	-0.5244	0.0029	0.0409	0.0708
3L by 4L	-0.0011	0.0042	-0.2622	0.0517	0.1364	0.3792

where, L = linear effect, Q = quadratic effect, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
X_1/x_1= RBO level in RBO-RBDPO blend (%), X_2/x_2= Reaction Temp. (°C), $X_3//x_3$= Enzyme Conc. (%), X_4/x_4 = Reaction Time (h), RBO-Rice Rice bran oil, RBDPO-Refined palm olein

Multiple regression coefficients obtained by interaction between the variables using least square method for predicting the second-order polynomial model for optimization of kinematic viscosity and heat transfer coefficients were presented in the Table 3.2. Estimating the regression coefficients with t- test indicated that temperature had significant effect on kinematic viscosity of interesterified oils. RBO level in RBO-RBDPO blend and enzyme concentration had a positive effect on kinematic viscosity. For example, at the RBO level (50%) in RBO-RBDPO blend, increasing the enzyme concentration from 1 to 13% and running the reaction at a temperature of 45°C led to an decrease in kinematic viscosity from 3.28 to 3.23 x 10^{-6} m^2s^{-1}. However, at a constant level of enzyme concentration (7%), increasing the RBO level in the RBO-RBDPO blend from 20 to 80% resulted in a decrease of kinematic viscosity from 3.29 to 2.93 x 10^{-6} m^2s^{-1} (Table 3.1). Different researchers (Xu *et al.,* 1998; Zhou *et al.,* 2001) also found that the higher concentration of the substrate is the most important factor responsible for increasing the yield of products. Our results are in agreement with these results.

The product of the two variables (RBO level in RBO-RBDPO vs. enzyme concentration) or (temperature vs. enzyme concentration) had a significant positive influence on increasing heat transfer coefficient at higher enzyme level and longer reaction time. For example, at a fixed level of temperature (55°C), increasing the enzyme concentration from 4 to 10% at RBO level of 65% in the RBO-RBDPO blend led to increase in heat transfer coefficient from 236.42 to 263.71 Wm^{-2} $°C^{-1}$. Similarly, by increasing the RBO levels in the RBO-RBDPO blend from 50 to 80% at fixed enzyme concentration of 7% led to increase in heat transfer coefficient from 248.24 to 254.8 Wm^{-2} °C^{-1} (Table 3.1).

The ANOVA of responses parameters is given in Table 3.3. The ANOVA indicates that the model is highly appropriate for the prediction. The coefficient of determination (R^2) is 0.88 for kinematic viscosity and 0.94 for heat transfer coefficient indicate that the model adequately represented the relationship among the selected parameters. The relationship between the responses and process parameters were examined by three dimensional surface plots (Figs. 3.2a & b and Figs. 3.3a to 3.3d) with two variables fixed at optimum level and changing other two variables within the experimental range. In the surface plots, only the significant effects of the interactions are furnished below based on the F-values from ANOVA table (Table 3.3).

Table 3.3 ANOVA results for selected parameters and responses

	Kinematic viscosity (x10^{-6} m^{2}s^{-1})	Heat transfer coefficient (Wm^{-2} °C^{-1})
X_1/x1 (L)	0.6536	1486.416
X_1/x_1 (Q)	0.0203	392.401
X_2/x_2 (L)	0.4602	673.047
X^2/x_2 (Q)	0.4556	292.638
X_3//x_3 (L)	0.3802***	419.521
X_3//x_3 (Q)	0.1693***	577.200
X_4/x_4 (L)	0.028	79.097
X_4/x_4 (Q)	0.2198***	423.155
1L by 2L	0.0784**	146.712
1L by 3L	0.1156***	207.576
1L by 4L	0.0182	7.357*
2L by 3L	0.0306	111.355
2L by 4L	0.0064	0.120
3L by 4L	0.0016	3.468
Pure error	0.0163	4.009
R^2	0.88	0.940

where,

L = linear effect, Q = quadratic effect, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

X_1/x_1= RBO level in RBO-RBDPO blend (%), X_2/x_2= Reaction Temp. (°C)

X_3//x_3= Enzyme Conc. (%), X_4/x_4 = Reaction Time (h), RBO-Rice Rice bran oil, RBDPO-Refined palm olein

3.3.2 Response surface for kinematic viscosity

3.3.2.1 Effect of interaction of reaction temperature and RBO level in RBO-RBDPO blend

Fig. 3.2a showed the response surface plots as a function of reaction temperature and RBO level in RBO-RBDPO blend and their mutual effects on the synthesis of interesterified oils with low kinematic viscosity. As shown in Fig. 3.2a, the interaction of RBO level in RBO-RBDPO blend and reaction temperature has a significant positive effect on kinematic viscosity. Thus it is observed that an increase in temperature from 25°C to 65°C at a constant level of RBO in RBO-RBDPO blend of 50% of each oil, the kinematic viscosity decreased from 4.10 to 3.01 x 10^{-6} $m^{-2}s^{-1}$. The kinematic viscosity was found to decrease with an increase in reaction temperature up to 55°C. Further increase in temperature slow change was observed in kinematic viscosity. This may be due to inactivation of enzyme at this point. However, with increase in RBO level in RBO-RBDPO blend from 20 to 80%, the kinematic viscosity was found to decrease from 3.19 to 2.93 x 10^{-6} $m^{-2}s^{-1}$.

3.3.2.2 Effect of interaction of enzyme concentration and RBO level in RBO-RBDPO blend

Fig. 3.2b showed the response surface plots as function of enzyme concentration and RBO level in RBO-RBDPO blend and their mutual effects on the synthesis of interesterified oils with low kinematic viscosity. The interaction of enzyme concentration and RBO level in RBO-RBDPO blend has a significant effect in decreasing the kinematic viscosity (Fig. 3.2b). The increase in enzyme concentration from 1 to 13% with moderate level of RBO in RBO-RBDPO blend (50%) leads to decrease in kinematic viscosity from

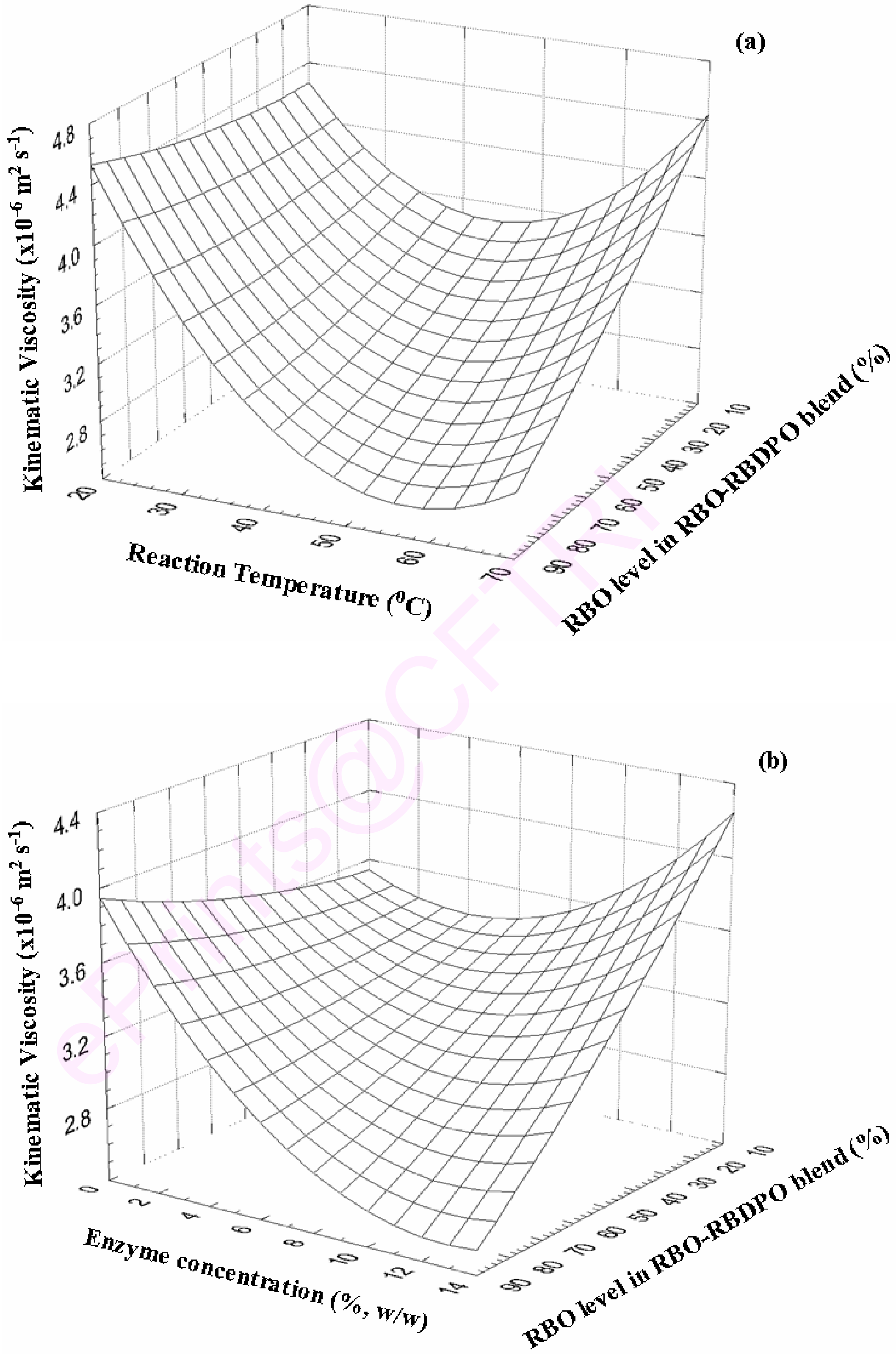


Fig. 3.1 Response surface for kinematic viscosity as a function of
(a) reaction temperature and RBO Level in RBO-RBDPO blend and
(b) enzyme concentration and RBO level in RBO-RBDPO blend

3.38 x 10^{-6} to 3.23 x 10^{-6} $m^{-2}s^{-1}$. If the RBO level in the RBO-RBDPO blend increased from 35 to 65% at a specific enzyme concentration of 10%, the kinematic viscosity was found to decrease from 3.59 x 10^{-6} to 2.96x10^{-6} $m^{-2}s^{-1}$. The kinematic viscosity was found to decrease with an increase in RBO level in the RBO-RBDPO blend up to 65%. Further increase in RBO level in RBO-RBDPO blend resulted an increase in kinematic viscosity. Higher incorporation of RBO level beyond 65% may have an inhibition effect.

3.3.3 Response surface for heat transfer coefficient

3.3.3.1 Effect of interaction of reaction temperature and RBO level in RBO-RBDPO blend

Fig. 3.2a showed the response surface plots as a function of reaction temperature and RBO level in RBO-RBDPO blend and their mutual effects on the synthesis of interesterified oils with high heat transfer coefficient. As shown in Fig. 3.2a the interaction of reaction temperature and RBO level in RBO-RBDPO blend has a significant effect on kinematic viscosity. It was found that an increase in temperature along with an increase in RBO level in RBO-RBDPO blend leads to decrease in kinematic viscosity. For example, with an increase in temperature from 25°C to 65°C at a constant RBO level of 50% in RBO-RBDPO blend, the heat transfer coefficient was found to increase from 227.42 to 252.03 Wm^{-2} $°C^{-1}$. The heat transfer coefficient was found to increase also with an increase in reaction temperature up to 55°C. Further increase in temperature slow change in heat transfer coefficient was observed. However, with an increase in RBO level in RBO-RBDPO blend from 20 to 80%, the heat transfer coefficient was found to increase from 223.09 to 254.8 Wm^{-2} $°C^{-1}$. Koh *et al*. (2010) also showed that reaction temperature and substrate mole ratio strongly affect the synthesis of medium and long-chain triacylglycerols using the enzyme, Lipozyme RM IM.

3.3.3.2 Effect of RBO level in RBO-RBDPO blend and enzyme concentration

Fig. 3.2b showed the response surface plots as a function of RBO level in RBO-RBDPO blend and enzyme concentration and their mutual effects on the synthesis of interesterified oils with high heat transfer coefficient. The interaction of RBO level in RBO-RBDPO blend and enzyme concentration has a significant effect in increasing heat transfer coefficient as shown in Fig. 3.2b. It can be observed that an increase in enzyme concentration along with an increase in RBO level in RBO-RBDPO blend leads to an increase in heat transfer coefficient. The increase in enzyme concentration from 4 to 10% with an increase in RBO level in RBO-RBDPO blend from 35 to 65% at a reaction temperature of 55°C leads to an increase in heat transfer coefficient from 226.9 to 259.41 Wm^{-2} $°C^{-1}$. The heat transfer coefficient was found to increase with an increase in RBO level in RBO-RBDPO blend up to 65%. Further increase in RBO level in RBO-RBDPO blend has no effect on increase in heat transfer coefficient.

3.3.3.3 Effect of the interaction of reaction temperature and enzyme concentration

Fig. 3.2c showed the response surface plots as a function of reaction temperature and enzyme concentration and their mutual effects on the synthesis of interesterified oils with high heat transfer coefficient. The interaction of reaction temperature and enzyme concentration has a significant effect in increasing heat transfer coefficient as shown in Fig. 3.2c. It was observed that an increase in reaction temperature along with

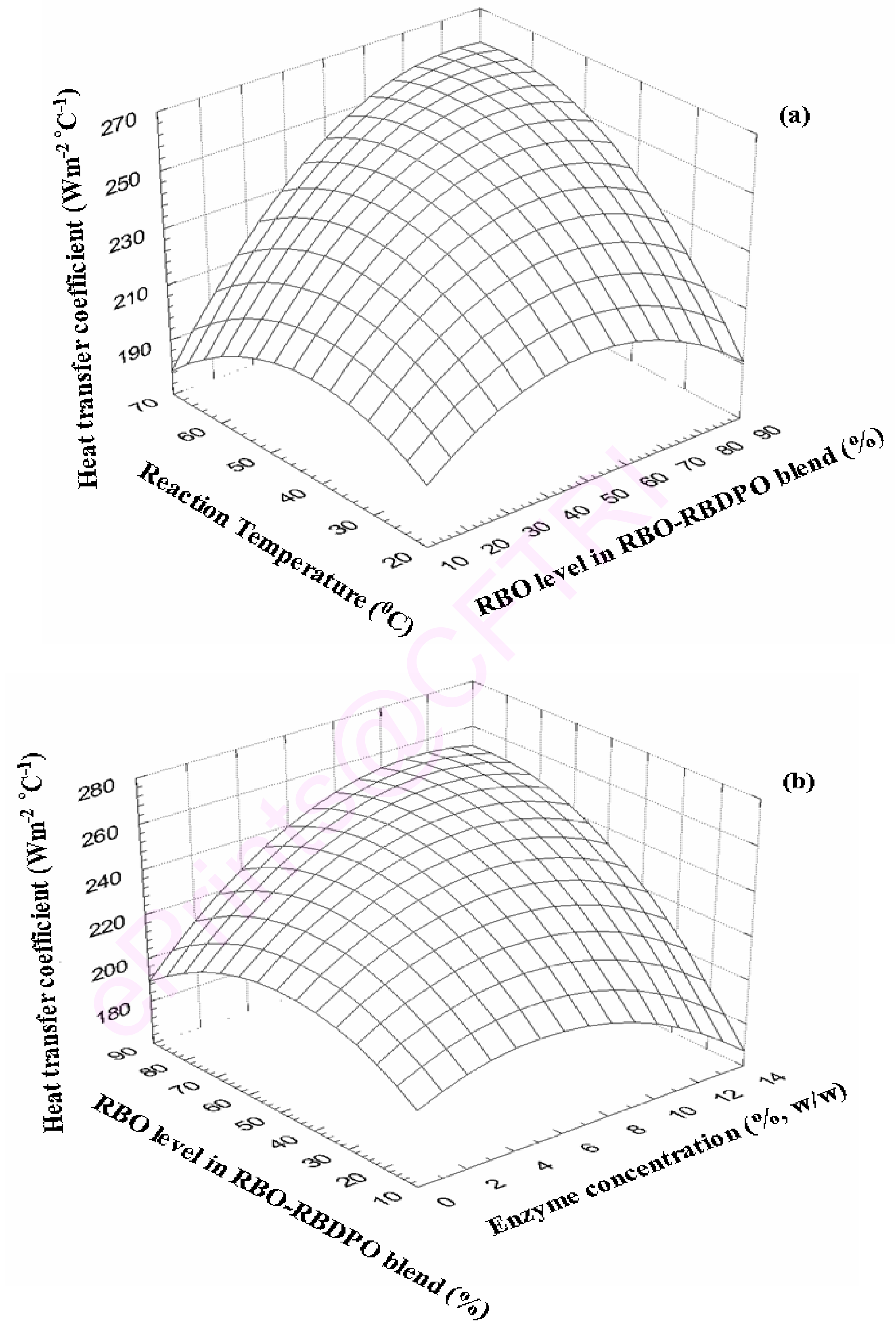


Fig. 3.2 Response surface for heat transfer coefficient as a function of
(a) reaction temperature and RBO level in RBO-RBDPO blend,
(b) RBO level in RBO-RBDPO blend and enzyme concentration

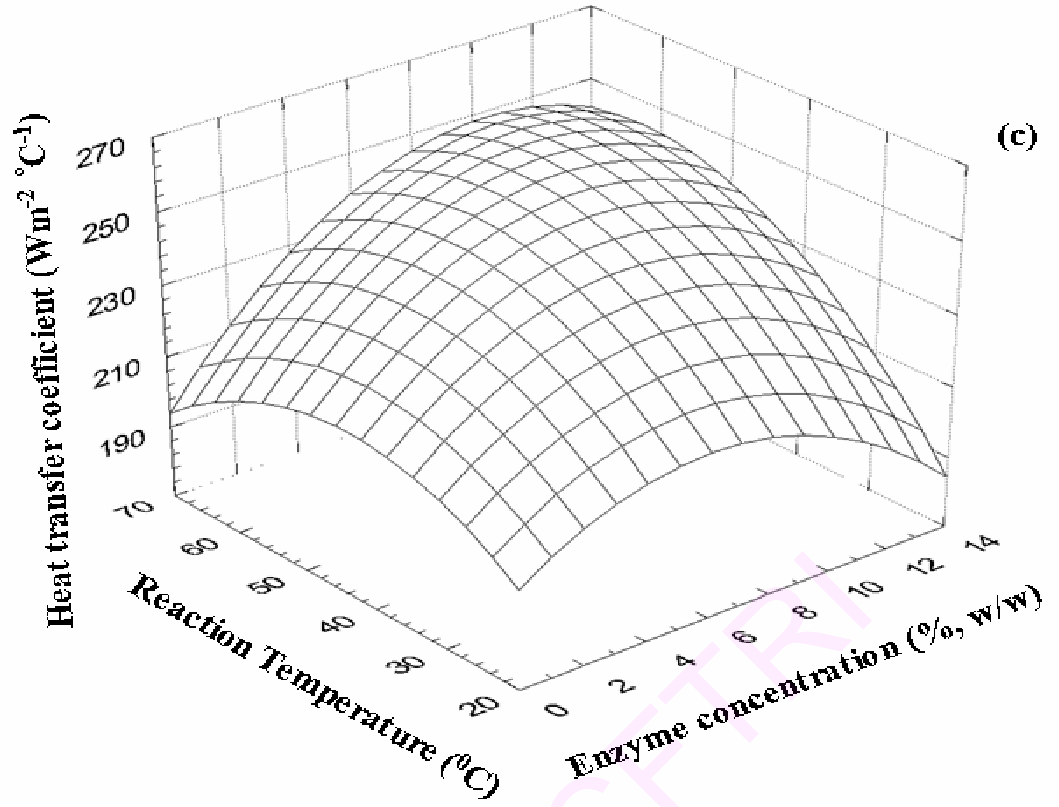
increase in enzyme concentration leads to increase in heat transfer coefficient. Thus, the increase in enzyme concentration from 4 to 10% with increase in reaction temperature from 35 to 55°C led to increase in heat transfer coefficient from 229.32 to 263.71 Wm^{-2} $°C^{-1}$ at a constant reaction time of 10 h. The heat transfer coefficient of interesterified oil was found to increase with an increase in temperature up to 55^0C.

3.3.3.4 Effect of the interaction of reaction time and RBO level in RBO-RBDPO blend

Fig. 3.2d showed the response surface plots as a function of reaction time and RBO level in RBO-RBDPO blend and their mutual effects on the synthesis of interesterified oils with high heat transfer coefficient. The interaction of reaction time and RBO level in the RBO-RBDPO blend has a significant effect in increasing heat transfer coefficient as shown in Fig. 3.2d. Increase in RBO level in RBO-RBDPO blend (35 to 65%) with increasing reaction time (4 to 10 h), increases the heat transfer coefficient from 226.9 to 236.42 Wm^{-2} $°C^{-1}$ at a fixed enzyme concentration of 4% and temperature of 55°C. Other researcher (Huang and Akoh,. 1996) also found that the molar ratio and reaction time are important factors for enzymatic interesterification and their interaction play significant role on response variables. They observed this during optimization of enzymatic synthesis of structured lipids by transesterification of ethyl caprylate and soybean oil and sunflower oil in hexane using RSM. Our results are in agreement with these results.

3.3.4 Kinematic viscosity and heat transfer coefficient

The ideal conditions for this reaction would be to use the least amount



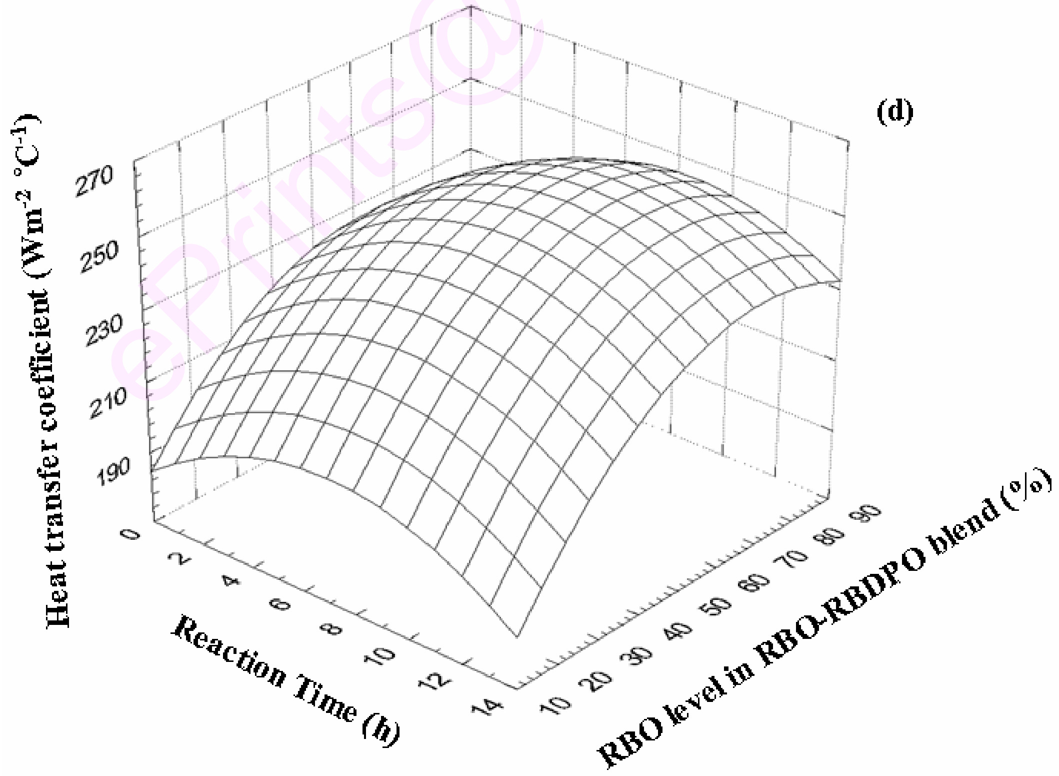


Fig. 3.2 Response surface for heat transfer coefficient as a function of (c) reaction temperature and enzyme concentration and (d) reaction time and RBO level in RBO-RBDPO blend

of enzyme to achieve lower kinematic viscosity and higher heat transfer coefficient at the low temperature with the lower level of RBO in the RBO-RBDPO blend. Based on the results of the experimental conditions were adapted to get minimum value of kinematic viscosity and maximum value of heat transfer coefficient of the enzyme interesterified oil (EIO) after simultaneous optimization (Ravi and Susheelamma, 2005). As indicated in Table 3.1, the minimum value of kinematic viscosity and the maximum value of heat transfer coefficient were observed when reaction conditions were set at temperature 55°C, enzyme concentration 10%, RBO level in RBO-RBDPO blend is 65% and reaction time 10 h. Under these conditions, a minimum kinematic viscosity of $2.71x10^{-6}$ $m^{-2}s^{-1}$ and a maximum heat transfer coefficient of 263.7 Wm^{-2} $°C^{-1}$ were achieved in interesterified oil. These values were close to the predicted values from the model used (predicted minimum kinematic viscosity of $2.63x10^{-6}$ $m^{-2}s^{-1}$ and maximum heat transfer coefficient of 262.0 Wm^{-2} $°C^{-1}$). Thus, it is possible to optimize the conditions for getting interesterified oil through RSM with desired amount of kinematic viscosity and heat transfer coefficient using lipase-catalyzed interesterification reaction.

3.3.5 Validation of the model

Predicted response variables were verified by performing the experiments at the optimized levels (Table 3.3). The model fitting is validated based on high R^2 (coefficient of determination) values. No significant difference ($p>0.05$) was observed between the predicted and observed values indicating the goodness of the fit (Montgomery, 1984) (Table 3.4).

Table 3.4 Simultaneous optimization: predicted and experimental process variables

Independent variables		Dependent variables			
		Kinematic viscosity ($x10^{-6}$ m^{-2} s^{-1})		Heat transfer coefficient (Wm^{-2} K^{-1})	
		Predicted	Experimental	Predicted	Experimental
Level of RBO in RBO-RBDPO oil blend (%, w/w)	62.0	2.63	2.71	262.0	263.7
Reaction temperature (°C)	65.0				
Enzyme concentration (%,w/w)	10.0				
Reaction time (h)	6.4				

RBO- rice bran oil, RBDPO- refined palm olein

3.4 Discussion

The response surface methodology was adopted for standardizing the conditions to develop interesterified oil having minimum kinematic viscosity and maximum heat transfer coefficient. The oil combinations used contain rice bran oil and refined palm olein for lipase-catalyzed transesterification. This approach provided an insight into interaction among various parameters based on which the optimum combination of variables (within the specific range) was identified for obtaining interesterified oil with desired properties using a relatively small number of experiments. By solving the regression equation the optimum conditions for interesterification were determined. This exercise was undertaken to get an oil with desired levels of kinematic viscosity and heat transfer coefficient of oil, important for controlling heat transfer during frying process. Four variables, such as, RBO level in RBO-RBDPO blend, reaction temperature, enzyme concentration and reaction time were selected and optimization using RSM coupled with CCRD. Simultaneous optimization results predicted the optimum reaction conditions for enzyme interesterified oil. With decreasing RBO level in RBO-RBDPO the kinematic viscosity was found to increase. This could be due to the increase in the proportion of saturated fatty acids in interesterified oil. Earlier investigators (Xu *et al.,* 1998; Zhou *et al.,* 2001) also found that the higher concentration of the substrate is the most important factor responsible for increasing the yield of products. The interaction of reaction time and substrate i.e., RBO level in the RBO-RBDPO blend has a significant effect in increasing heat transfer coefficient. Other researcher (Huang and Akoh, 1996) also found that the interaction between substrate, i.e. molar ratio and reaction time play significant role on response variables during enzymatic interesterification. The enzyme acts as a catalyst in the interesterification process and beyond certain temperature the enzyme stops functioning. Similar phenomena were observed by Koh *et al*. (2010) who also reported the low operational stability of the enzyme at high temperatures due to change in enzyme conformation.

This investigation could help food industries to develop suitable oils for frying operations. Optimization studies also helped in developing of enzyme interesterified oil with improved hydrodynamic and heat transfer properties. The present study showed that it is possible to adopt statistical techniques such as RSM to synthesize interesterified oil with improved hydrodynamic and thermal properties at desired levels without losing its frying capability. Since oils are ultimately used in frying or cooking, the mass transfer characteristics of the native and modified oils are tested in the frying process. Results are reported in the subsequent chapters.

Chapter-4

Mass Transfer Study of a Frying Process

4.1 Introduction

Frying is one of the unit operations in food processing that involves simultaneous heat and mass transfer. Deep-fat frying is commonly used for preparing fried foods. In this process heat is transferred between a fluid medium (oil) to the solid (food material). In general, convective heat transfer coefficient is used for measurement of heat transfer. Mass transfer is related to the oil uptake/oil inflow by/to the product and the moisture outflow from the product. The mass transfer characteristics are measured by the term mass transfer coefficient. Therefore, it is important to understand the frying mechanism, and how the oil gets absorbed and distributed in the food material during frying. Deep-fat fried savory snacks generally contain 35-40% oil. However, due to increasing consumer awareness on nutrition related to obesity and cardiovascular diseases (Krokida *et al.,* 2001a), much research has been aimed towards the development of fried food products having reduced fat content (Dueik *et al.,* 2010). Deep fat frying generally involves three types of mass transfer such as (a) migration of water from the core of the food to surface, which is removed during frying, (b) absorption of frying oil into the food and (c) leaching of liquefied food components from the food (Blumenthal, 1991). In order to produce low fat snack products, it is necessary to understand mechanism of frying process. Different studies have shown that most of the oil during frying is retained on the surface of fried products sch as fried potatoes (Pedreschi *et al.,* 1999; Bouchon *et al.,* 2001; Bouchon *et al.,* 2003). During cooling the oil penetrates into structure of the product (Aguilera *et al.,* 2000; Bouchon *et al*., 2003; Ufheil and Escher, 1996). There are mainly two oil fractions that are observed in fried potato cylinders - surface oil (oil content available on the surface) and structural oil (oil content penetrated in the core). The total oil content in the product is defined as the sum of surface and structure oil contents (Bouchon *et al*., 2003). Durán *et al.* (2007) studied oil partition in pre-treated potato slices during frying at 120-180°C and cooling. They observed that potato chips absorbed about 65% of total oil content and 35% oil remained at the surface. Ufheil and Escher (1996) studied the dynamics of oil penetration during the deep fat frying of potato slices and suggested that oil uptake corresponded mainly to a surface phenomenon. Moreira *et al.* (1997) found that during frying of tortilla chips, 64% of the total oil content was absorbed by the chips and 36% of the total oil remained at the surface. After frying, oil present on the surface migrates into core structure by the vacuum created due to the condensation of vapor inside the product (Gamble *et al.,* 1987; Moreira *et al.,* 1997).

It is hypothesized that, if the cooling of the product is delayed and the product is held at elevated temperature, it will delay the onset of vacuum (or oil migration into the structure) and subsequently the oil can be removed from the surface by adopting suitable technique for oil adsorption and thereby will result in significant reduction of oil content in the final product. However, the very little work is done on kinetics of oil partitioning between surface and structure during frying and cooling.

In this chapter the kinetics of oil partitioning and distribution of oil between surface and structure of fried potato slices during holding at elevated temperatures is investigated. The suitable alternative method for removal of surface oil and hence reduction of the total oil content of the product is also evaluated. The details of the materials required and methods for studying the effect of oil absorption (a) onto surface and (b) into structure (core), oil partition coefficient, oil mass transfer coefficient, sensory evaluation of the fried potato slice during frying using RBDPO are presented in the 'Materials and Methods' section.

4.2 Results

4.2.1 Effect of frying on moisture and oil content

The changes in the moisture and oil content during deep fat frying of potato slices at 180°C is presented in Fig. 4.1, which indicated that the moisture content of the potato slices was reduced from 5.33 to 1.22 kg/kg and oil content was increased up to 0.44 kg/kg. We have earlier shown that the mass transfer coefficient for oil and moisture are related to each other and were found to have linear relation between them Debnath *et al.* (2003).

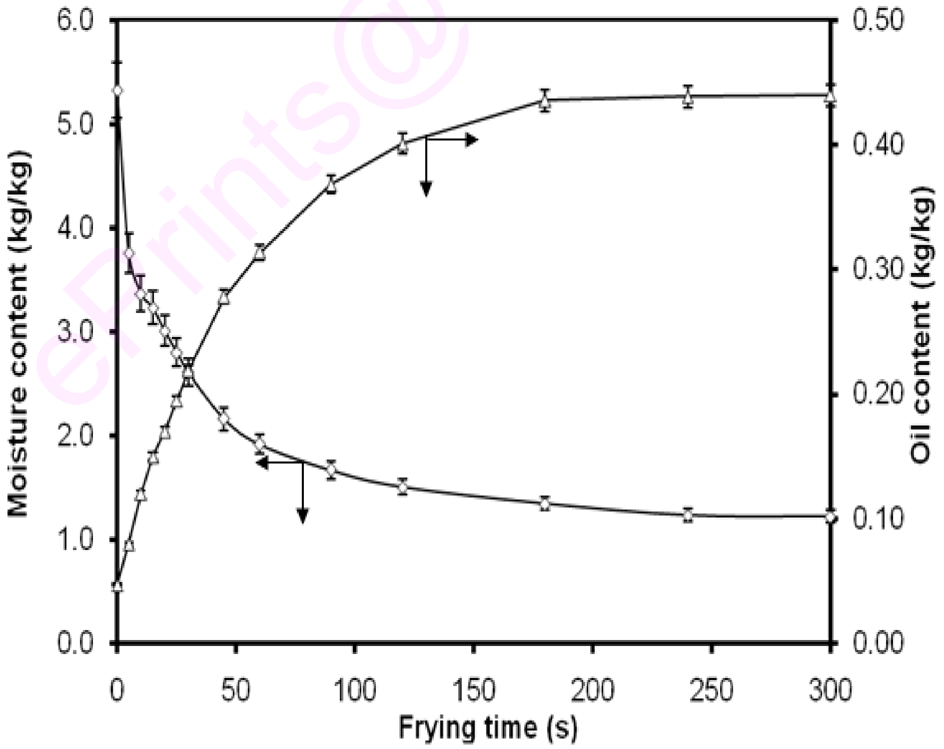


Fig. 4.1 Changes in moisture and oil content during frying at 180°C using RBDPO

Gamble *et al.* (1987) and Kozempel *et al.* (1991) reported that decrease in moisture and increase in oil content during frying are the two simultaneously occurring operations.

4.2.2 Effect of frying on oil absorption (a) onto surface and (b) into structure (core)

Due to formation of crust during deep-fat frying, the oil absorbed remained on the surface of the product and penetrates into the structure during cooling or holding. Results given in Fig. 4.2 (a & b) indicates the changes in oil content on the surface and into the surface of potato slices, which were deep fat fried at 180°C and held at ambient temperature (control) as well as at 100, 120, 140, 160 and 180°C. Immediately after the completion of frying the sample showed that out of the total oil content in it (0.440 kg/kg), 92% of oil was available on the surface (0.406 kg/kg) and 8% of oil penetrated (0.034 kg/kg) inside the product. These results indicate that the most of oil absorbed during frying remained on the surface due to crust formation. Similar observations were also reported by the other researchers (Farkas *et al.*, 1992; Aguilera and Gloria-Hernandez, 2000; Bouchon *et al.,* 2003).

4.2.3 Effect of frying and holding temperatures of the fried potato on oil partition coefficient

The partition coefficient of oil immediately after the frying was defined as the ratio of surface oil to structure oil and it was found to be 11.94 for the potato fried in this study (Fig. 4.2c). The holding of fried sample at ambient temperature resulted in decrease in surface oil content from initial value of 0.406 kg/kg to equilibrium oil content of 0.152 kg/kg. Consequently, structure oil content increased from initial value of 0.034 kg/kg to 0.288 kg/kg. The results indicated that holding of fried sample at ambient condition resulted in migration of oil from surface to the structure. The partition coefficient of oil at the equilibrium condition (after 300 s of holding) was found to be 0.530 kg/kg (Fig. 4.2c). The increase in the temperature during holding from 100 to 180°C was found to result in the availability of higher amount of oil on the surface and lower amount in the structure. For instance, the holding temperature at 180°C resulted in higher surface equilibrium oil content (0.332 kg/kg) and lower structure equilibrium oil content (0.108 kg/kg), which indicated that around 75% of total oil content of fried product remained on the surface and only 25% migrated into the structure. The partition coefficient of oil at the equilibrium condition at 180°C was found to be 3.06. The oil holding at different temperature indicates that the partition coefficient of oil in the product immediately after the frying was very high (e.g. 11.94) and holding at ambient condition decreased significantly to 0.530. Further, increase in holding temperatures resulted in increase in oil partition coefficient. However, for all the conditions, the oil partition coefficient was found to be less than the oil partition coefficient obtained immediately after the frying (Fig. 4.2c). Higher oil partition coefficient indicates the availability of higher amounts of oil on the surface (or lower amount into structure) of the fried product.

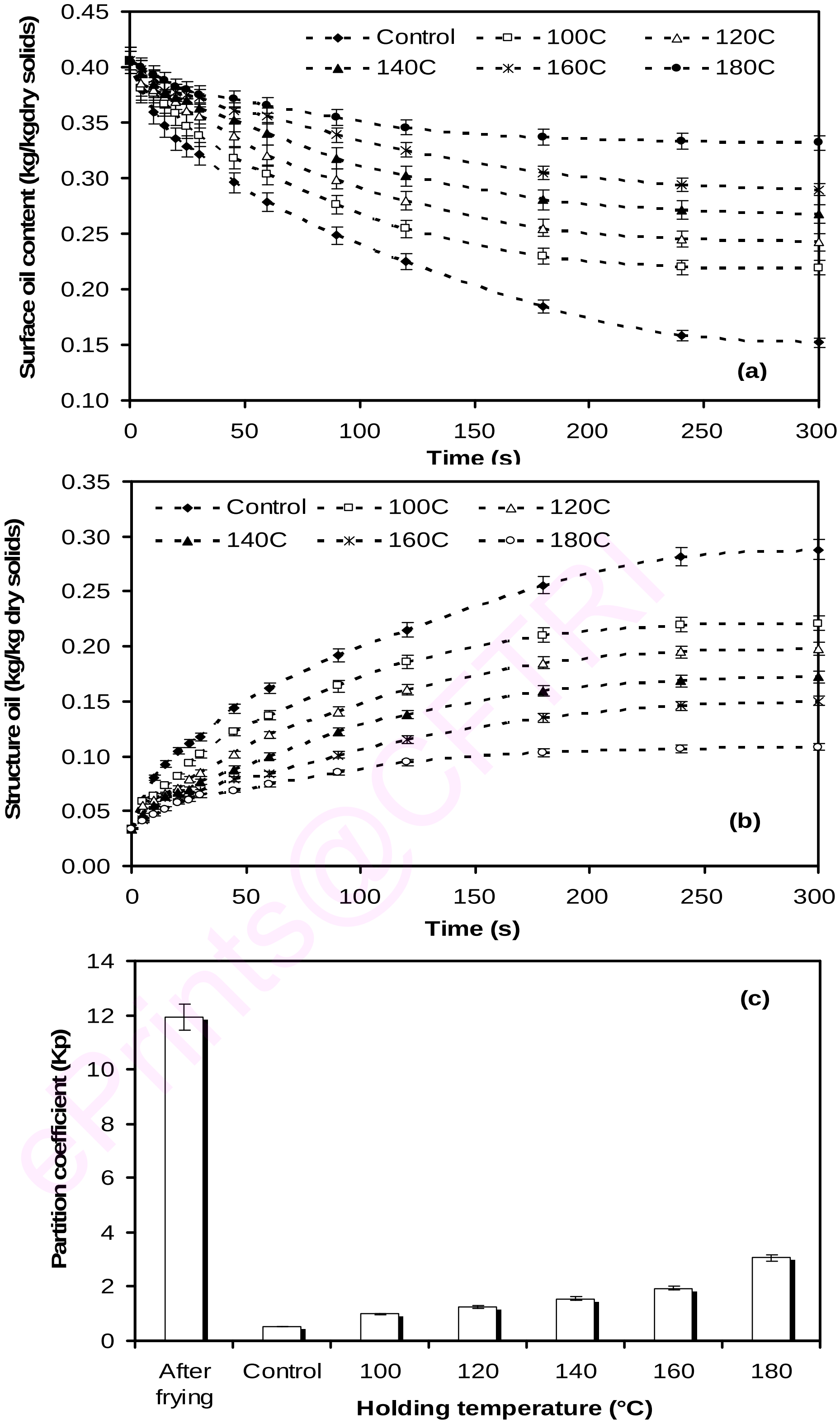


Fig. 4.2 Pattern of oil absorption (a) onto surface, (b) into structure (core) after frying and holding the fried products at different temperatures and (c) plot of partition coefficient (Kp) at the equilibrium condition (after 300 s of holding) with holding temperature

4.2.4 Effect of frying and holding temperatures on mass transfer coefficient: Arrhenius Equation

Mass transfer coefficient of oil was calculated by -ln (O_r) with time (Fig. 4.3) as per Eqn. (2.6) and the relevant values are reported in Table 4.1. The results indicated the decrease in mass transfer coefficient of oil with an increase in holding temperature of deep fat fried product.

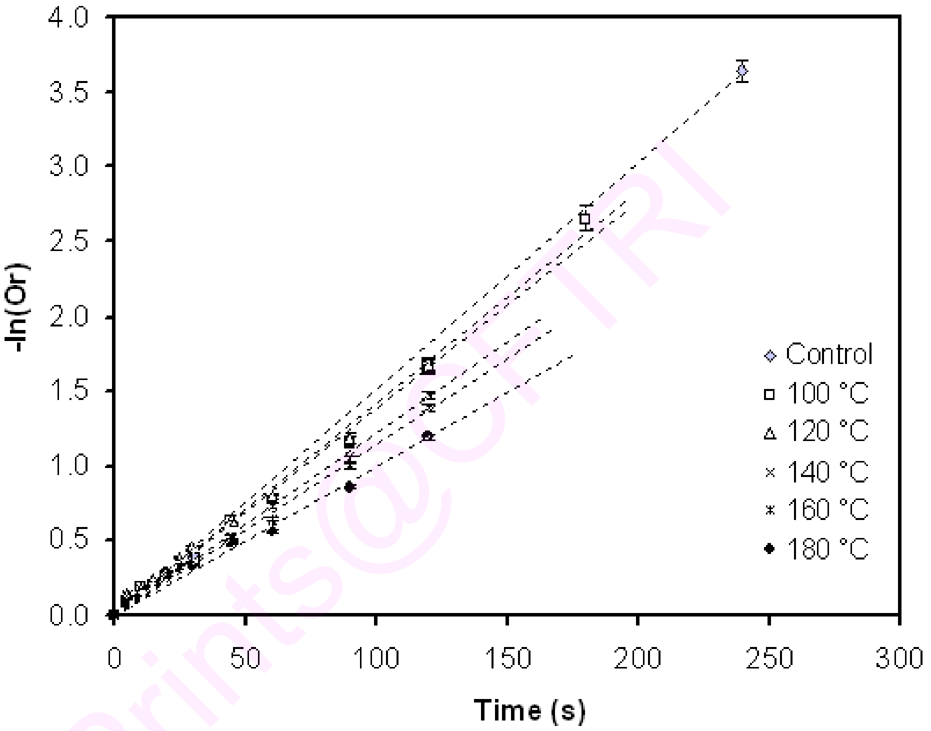


Fig. 4.3 Plots of –ln (O_r) against holding time during absorption of oil into the structure

A correlation similar to Arrhenius equation (Eqn. 4.1) is proposed to relate the effect of holding temperature on mass transfer coefficients of oil (Durán *et al.*, 2007). The coefficients of the equation were inferred from the intercept and slopes of the plot of –ln (K_o) and against 1/T (as shown in Fig. 4.4). R (8.314 kJ/kg mol K) is the gas constant.

$$K_o = 0.53 \cdot \exp\left(-\frac{7.28 \cdot 10^3}{RT}\right) \qquad \text{....Eqn. (4.1)}$$

This type of correlation (r=0.96) indicated that the decrease in mass transfer coefficients of oil with holding temperature followed an exponential trend.

Table 4.1 Kinetic parameters of potato slices after frying and holding at different temperatures

Temperature (°C)	K_o (s^{-1})	R^2	Equilibrium oil content (kg/kg)	
			Surface	Structure
Control	0.015	0.99	0.152±0.002	0.288±0.002
100	0.014	0.98	0.219±0.001	0.221±0.002
120	0.013	0.99	0.242±0.002	0.198±0.001
140	0.012	0.99	0.268±0.001	0.172±0.003
160	0.011	0.99	0.290±0.001	0.150±0.001
180	0.009	0.98	0.332±0.003	0.108±0.002

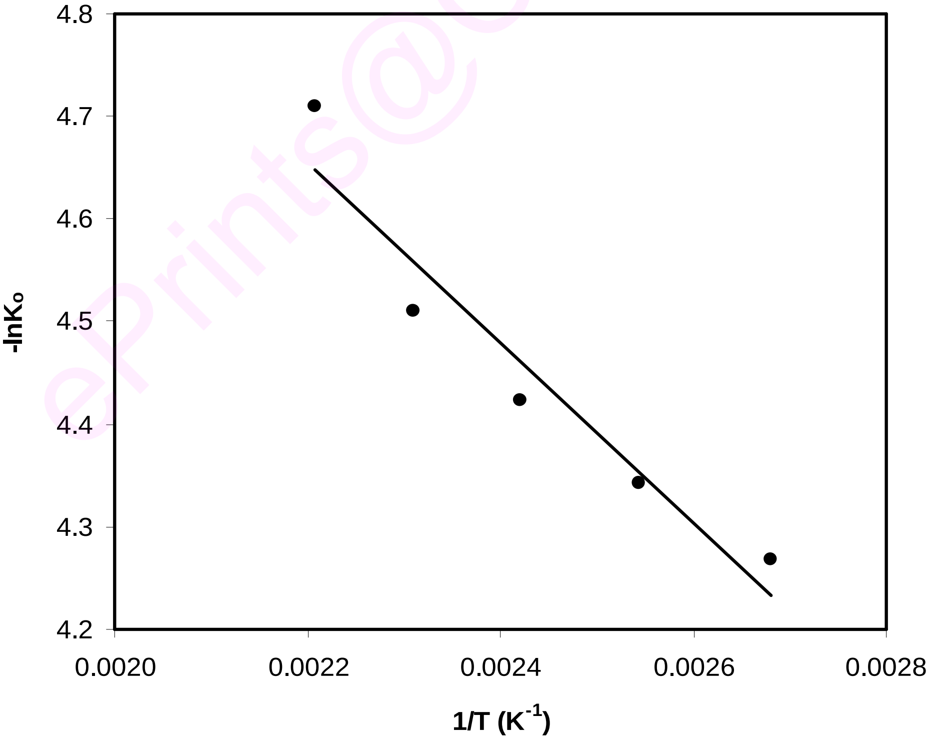


Fig. 4.4 Plot of -ln (K_o) with 1/T; The relationship -ln (K_o) = -875.63 (1/T) + 6.58 (R^2 = 0.92) was found to fit the presented data

In order to further investigate the reasons for the reduced mass transfer of oil from surface to the structure of fried product during holding at ambient and at higher temperatures, the temperature variations at the surface and in the structure (core) during holding were plotted with time (Fig. 4.5). The temperature during holding at ambient temperature indicated that both the surface as well as the core temperatures stabilise to around 38°C. Whereas holding at elevated temperature resulted in higher surface and core temperatures. For instance, holding at 180°C resulted in ~179.5°C and ~115°C surface and core temperature respectively. At this temperature, the moisture present in the product will still be

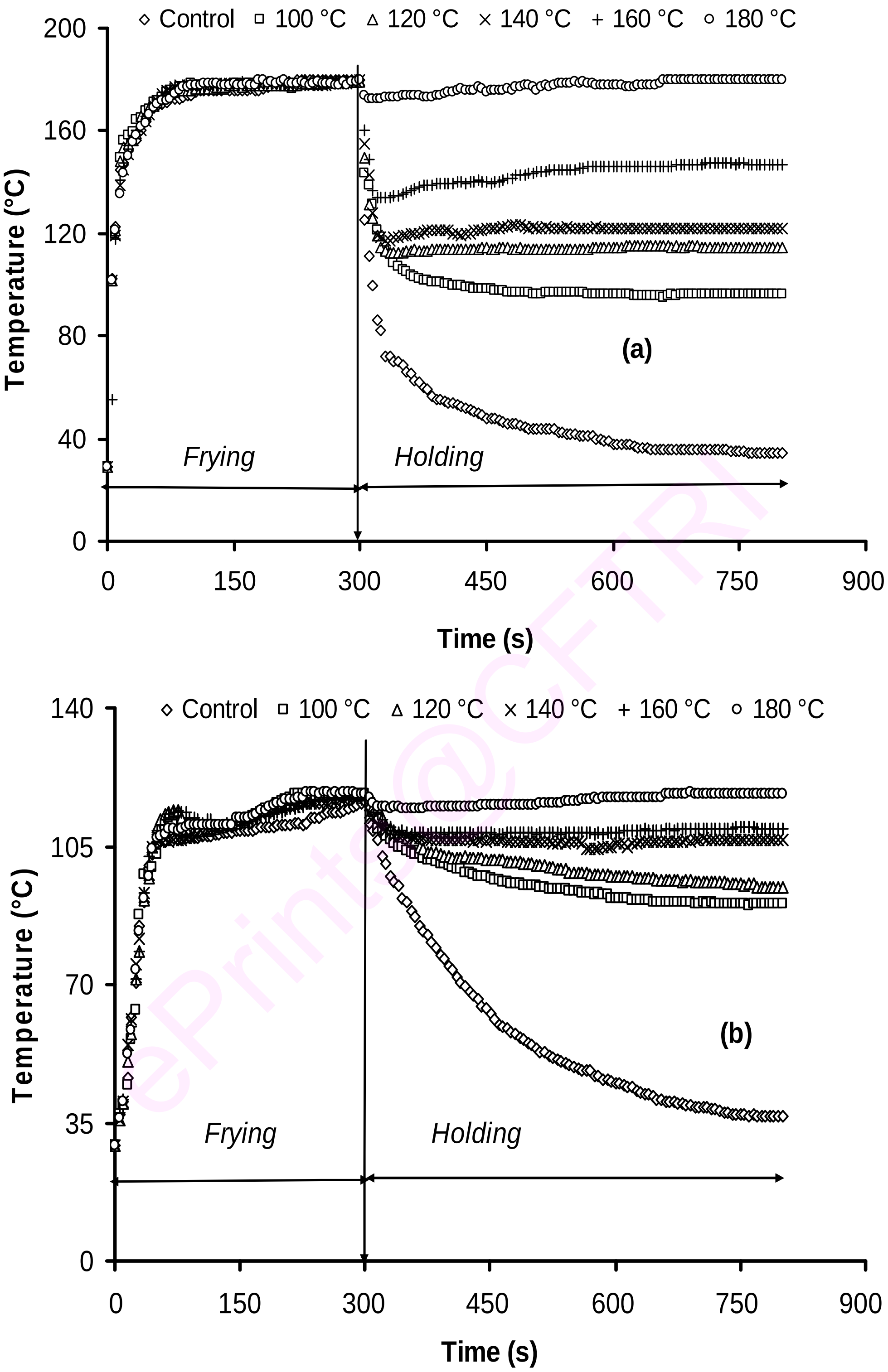


Fig. 4.5 (a) Surface temperature and (b) core temperature during frying of potato slices and holding product at control temperature (25°C)

in the form of vapor and there will not be any condensation of vapor, which will not trigger the onset of vacuum. Consequently, the pressure inside the core and the atmosphere will not be significantly different. Perhaps, this insignificant difference in pressure would not cause migration of oil in fried product from surface to the structure. These observations are in agreement with our earlier proposed hypothesis. In order to remove the oil available on the surface due to holding the product at elevated temperature, a high absorbent paper was used. The results indicated that this technique could reduce the total oil content from 0.44 to 0.33 kg/kg dry solids.

4.2.5 Sensory evaluation

The sensory scores of the fried potato slices with (experimental) and without the surface oil removal (control) are shown in Fig. 4.6. All the attributes namely appearance, colour, crispness, taste and overall acceptability were compared with those of control sample using t-test for comparison of two means. The results indicated that there was no significant differences ($p>0.05$) between the experimental and control samples in terms of appearance, taste, crispness and overall acceptability, However, the difference in colour was found to be significant at $p<0.05$.

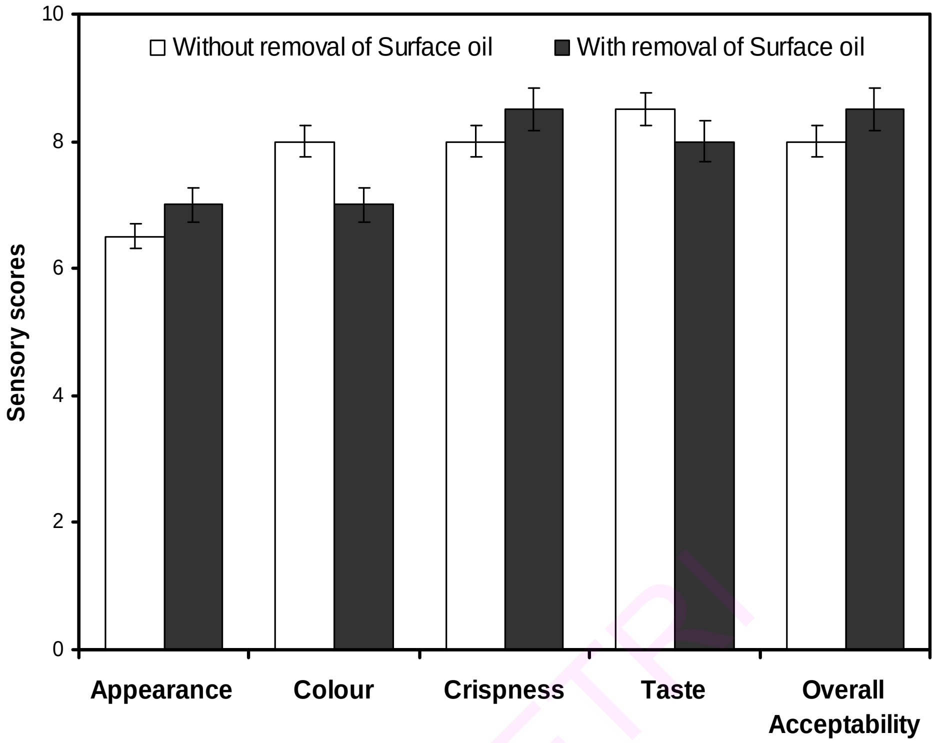


Fig. 4.6 Sensory scores of fried potato sample with and without removal of surface oil (control).

4.3 Discussion

During deep-fat frying most of the oil remained on the surface but migrates into the core during holding after frying. During frying of potato cylinders, generally two major oil fractions such as surface oil and structural oil (Bouchon *et al*., 2003). The total oil content can be obtained from these oils. Durán *et al.* (2007) showed that during frying of pre-treated potato slices at 120-180°C and cooling potato chips absorbed about 65% of total oil content and 35% oil remained at the surface. The surface oil can be prevented from penetrating into the core by following suitable technique. Holding of product immediately after deep-fat frying at elevated temperature resulted in significant mass transfer from surface to core, thereby making oil available on the surface. This surface oil can be removed by blotting with absorbent paper (Debnath *et al*., 2009). The sensory analysis indicated that the removal of surface oil did not result in any significant changes in the product overall quality. The present work dealt with the kinetics of partitioning of oil on the surface as well as in the structure during holding of fried potato slices at elevated temperatures (100, 120, 140, 160 and 180°C) and compared with the sample held at 25 °C. After frying, the oil present on the surface migrated into structure due to condensation of vapor inside the product resulting in creation of vacuum. However, the oil present in the surface does not migrate into structure, if the fried product was held at elevated temperatures. This excess oil can be removed by absorbent paper. Thus, oil partly remains on surface and partly migrates. Oil partition coefficient, defined as the ratio of oil present on the surface to the structure, during holding of samples at 180°C was found to be 3.06, whereas the oil partition coefficient of sample held at ambient temperature was 0.53, which indicates the availability of oil on the surface of fried product held at elevated temperature. The mass transfer coefficient of oil corroborated with the above findings. The holding of deep-fat fried product at 180°C followed by the removal of oil from surface using high absorbent paper reduced the oil content of the final product singnificantly.

The present work thus widens the scope for further development of the suitable method for obtaining low fat fried product for health conscious individuals without sacrificing the quality of the product. Much research has been aiming towards the development of fried food products having reduced fat content using vacuum frying techniques (Dueik *et al.,* 2010). This is a preliminary study to understand the movement of oil in the product. The frying performances of the oils developed through blending and enzyme interesterification was evaluated. The effect of heat and mass transfer properties of native, blended and interesterified oils is presented in the next chapter.

Chapter-5

Frying Quality of Enzyme Interesterifed Oils

5.1 Introduction

Frying is a traditional heat processing method of food preparation used in different parts of the world. Deep fat frying involves simultaneous heat and mass transfer processes. The quality of the products from deep-fat frying depends not only on the frying conditions but also on the type of oils and foods used during the process. Oils play a dual role in the preparation of fried foods, they serve as a heat transfer medium between the food and the fryer, and they also contribute to the food texture and flavour characteristics. There are no specific criteria to determine the relative fry-life (duration over which oil can be safely used for frying) of specific oil. However, it is a common practice to discard frying oil when the percentage of free fatty acid (FFA) reaches 1.0% (Smith *et al*., 1986) or the percentage of total polar materials (TPM) goes above 27% (Billek *et al*., 1978). TPM can be regarded more profoundly as an indicator for oil deterioration instead of colour or FFA of the oil (Fritsch, 1981; Yoon *et al*., 1985). Ideally, a frying oil must be low in free fatty acids and polar material content, with a high breakdown resistance during continuous use.

In addition to the chemical alterations that occur during frying, changes in the oil's physical and thermal properties such as surface tension (Tseng *et al*., 1996), contact angle (Rossi *et al.,* 2009), viscosity, density, and convective heat transfer coefficient can affect food quality. Gutierrez *et al*. (1988) showed that the increase in viscosity and density of frying oil was the consequence of a polymerization reaction. Deep fat frying is normally carried out at high temperatures (between 160 and 180 °C) and in the presence of air and moisture. These frying oils undergo physical and chemical deterioration, which affects their frying performance and the storage stability of the fried products. The prolonged use of frying oil causes adverse effect on flavor, stability, color and texture of fried products and such products may be harmful to human health (Ramadan *et al*., 2006). Degraded products formed during frying are of both volatile and non-volatile nature. Most of the volatile fractions are lost during the frying process, whereas, the non-volatile fractions produced primarily by thermal oxidation and polymerization of unsaturated fatty acids (Chang *et al*., 1978; Fritsch, 1981) are retained in the frying oil and the fried product. These compounds are accumulated in the frying oil and cause further degradation. These products are absorbed by the fried food and enter the diet and affect the health. Highly oxidized oils may also produce polyaromatic hydrocarbons, which have carcinogenic effect (FAO/WHO, 1988).

Due to these changes, the quality of the oil may be degraded to an extent above which it is not possible to prepare high-quality fried products and such oil should be discarded (Stevenson *et al*., 1984; Sebedio *et al*., 1990; Ramadan *et al*., 2003). Therefore, nutritional quality and shelf life of the fried products depend upon the quality of the oil used for frying (Rani and Chauhan, 1995). Miller *et al*. (1994) found that frying time and temperature significantly affected viscosity of oils. A good correlation was observed between viscosity and the convective heat transfer coefficient for corn oil. Tseng *et al*. (1996) noticed that the convective heat transfer coefficient changed more rapidly after 30 h of frying which correlated well with the viscosity of the frying oil.

These observations underscore the need for developing oils with improved viscosity, heat transfer and stability. Blending and interesterification are efficient means to modify the physical and chemical properties of oils and their blends (Soares *et al*., 2009). Chotimarkorn and Silalai (2008) observed that mixing of rice bran oil to soybean oil retarded the oxidative as well as hydrolytic rancidity in the fried dough during storage. The frying strength of the enzymatically synthesized palm-based medium- and long-chain triacylglycerols (MLCT) oil with the aid of different antioxidants under deep-frying conditions was assessed by Koh *et al*. (2008). They found that palm-based MLCT oil in the presence of synthetic or natural antioxidants showed significantly better thermal resistance and oxidative strength than its control oil.The addition of sesame oil and rice bran oil increases the frying stability of canola oil during frying of of potato pieces (Farhoosh and Kenari, 2009). Recently, Reena and Lokesh (2007) have prepared combinations of blended and interesterified oils using coconut oil with rice bran oil or sesame oil with improved nutritional benefits. These oils are rich in natural antioxidant molecules such as oryzanol or sesame lignans and hence expected to possess better thermal stability.

These various studies indicated that there may be differences in the viscosity, heat transfer and thermal and oxidative stability of blended and interesterified oil as compared to individual native oils. Such information is useful for developing oils suitable for deep fat frying, which is a major utility for edible oils in India.

Several researchers worked on deep fat frying process under simulated frying conditions (Yanishlieva *et al*., 1997; Gertz and Kochhar, 2002; Smith, *et al*., 2007) to understand the quality of the oil itself. In the present chapter, simulated frying condition is followed by merely heating of the oil at frying condition without adding the food material into it. Then the deep fat frying was carried out following the real system and examined the frying performances/qualities of the oil in both the systems.

Poori is an Indian traditional breakfast and snack food. It is prepared by deep-fat frying of 3-4 inch diameter circular sheet of wheat flour dough prepared by kneading. Rice bran oil and refined palm olein are used as frying oil in many Indian households as these are found to be stable to heating and provide health promoting compounds such as oryzanol, β-carotene etc. which also act as good antioxidants.

The present work is carried out to evaluate the effect of frying cycle under simulated and real conditions on the chemical (free fatty acid, peroxide value, total polar compound and radical scavenging activity) and physical (colour, viscosity) and thermal (heat transfer coefficient) properties of blended and enzyme interesterified oil containing rice bran oil and refined palm olein. The effect of intermittent frying cycles on sensorial attributes of *poori* prepared using these oils was also determined. In this chapter, the enzyme interesterified oil obtained after optimization of interesterification reaction following response surface methodology is undertaken alongwith corresponding blended oil BLO (RBO:RBDPO::62:38).

5.2 Results

5.2.1 Effect of frying cycles on chemical properties of oil

5.2.1.1 Fatty acid composition

Fatty acid profile influences the nutritional value of the oil. Generally, fatty acids with double bonds are labile for oxidation during heating, owing to presence of π (pi) bonds, which consequently produces degradable products. Arroyo *et al*. (1995) and Jorge *et al*. (1997) indicated that heat treatment of fat induces modifications of fatty acids with two or three double bonds. Similar observations are made by Garrido-Polonio *et al*. (1994) and Sharma *et al.* (2006).

(a) *Effect of heating*

Rice bran oil has a balanced fatty acid composition in terms of saturates, monounsaturates and polyunsaturates in the proportion of 23:44:33. A minor changes in the total unsaturated fatty acids level of the RBO was observed when subjected to heating for 1 to 6 frying cycles. Marginal changes were observed in the total unsaturated fatty acids level (51.7 to 49.9%) of the RBDPO (**Table 5.1a**) subjected to heating at similar condition. Only moderate changes observed in unsaturated fatty acids (68.1 to 65.7%) in case of blended oil (BLO) and minor changes were found (68.2 to 66.2%) in case of enzyme interesterified oil (EIO) subjected to heating for 1 to 6 frying cycles (**Table 5.1b**).

Table 5.1 (a) Changes in fatty acid (relative % area) composition of RBO and RBDPO during heating

Oil	Fatty acids	Frying cycles						
		0	1	2	3	4	5	6
RBO	C12:0	nd	nd	nd	nd	nd	nd	nd
	C14:0	0.2±0.1	0.3±0.1	0.3±0.2	0.3±0.1	0.3±0.2	0.3±0.2	0.3±0.1
	C16:0	20.1±0.2	20.3±0.1	20.4±0.2	20.4±0.2	20.5±0.2	20.5±0.1	20.6±0.1
	C18:0	2.4±0.2	2.5±0.1	2.5±0.1	2.6±0.1	2.6±0.1	2.7±0.2	2.7±0.2
	C18:1	43.9±0.2	43.7±0.1	43.6±0.2	43.5±0.2	43.4±0.1	43.3±0.2	43.3±0.1
	C18:2	31.6±0.1	31.5±0.2	31.4±0.2	31.3±0.1	31.3±0.1	31.3±0.1	31.3±0.2
	C18:3	1.7±0.2	1.7±0.1	1.7±0.2	1.6±0.1	1.6±0.2	1.5±0.2	1.5±0.2
	SFA	22.8±0.1	23.1	23.2±0.2	23.4±0.1	23.4±0.2	23.6±0.1	23.6±0.2
	MUFA	43.9±0.2	43.7±0.3	43.6±0.1	43.5	43.4±0.2	43.3±0.1	43.3±0.1
	PUFA	33.3±0.2	33.2±0.1	33.1±0.2	33.0±0.1	32.9±0.3	32.8±0.1	32.8±0.2
RBDPO	C12:0	0.5±0.2	0.5±0.1	0.5±0.1	0.5±0.2	0.5±0.1	0.5±0.1	0.5±0.2
	C14:0	1.0±0.1	1.0±0.1	1.0±0.2	1.0±0.1	1.0±0.2	1.0±0.2	1.0±0.1
	C16:0	43.0±0.2	43.2±0.1	43.3±0.2	43.4±0.2	43.6±0.1	43.9±0.1	44.1±0.2
	C18:0	3.7±0.2	3.8±0.2	3.9±0.1	4.0±0.2	4.2±0.2	4.3±0.1	4.5±0.1
	C18:1	41.3±0.1	41.3±0.2	41.2±0.2	41.2±0.1	41.0±0.2	40.9±0.1	40.6±0.2
	C18:2	10.2±0.2	10.1±0.1	10.0±0.1	9.9±0.2	9.6±0.1	9.3±0.2	9.2±0.1
	C18:3	0.2±0.1	0.1±0.2	0.1±0.2	0.1±0.1	0.1±0.2	0.1±0.2	0.1±0.1
	SFA	48.2±0.1	48.5±0.2	48.7±0.3	48.9±0.1	49.3±0.1	49.7±0.2	50.1±0.3
	MUFA	41.3±0.2	41.3±0.1	41.2±0.2	41.2±0.2	41.0±0.3	40.9±0.1	40.6±0.4
	PUFA	10.4±0.1	10.2±0.2	10.1±0.3	10.0±0.1	9.7±0.2	9.4±0.2	9.3±0.3

SFA- saturated, MUFA-Monounsaturated, PUFA-Polyunsaturated fatty acids

Table 5.1 (b) Changes in fatty acid (relative % area) composition of BLO and EIO during heating

Oil	Fatty acids	Frying cycles						
		0	1	2	3	4	5	6
BLO	C12:0	0.2±0.2	0.2±0.1	0.2±0.1	0.2±0.2	0.2±0.1	0.2±0.1	0.2±0.1
	C14:0	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.2	0.6±0.2	0.6±0.1	0.6±0.1
	C16:0	28.9±0.2	29±0.2	29.2±0.1	29.4±0.1	29.6±0.2	29.8±0.2	30.0±0.2
	C18:0	2.3±0.1	2.6±0.1	2.8±0.1	3.0±0.2	3.2±0.1	3.3±0.2	3.5±0.2
	C18:1	42.5±0.2	42.3±0.2	42.1±0.2	41.9±0.2	41.7±0.2	41.6±0.2	41.4±0.2
	C18:2	24.5±0.2	24.4±0.1	24.2±0.1	24±0.2	23.7±0.1	23.5±0.2	23.3±0.2
	C18:3	1.1±0.1	1.0±0.2	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.2	1.0±0.1
	SFA	31.9±0.3	32.3±0.1	32.7±0.2	33.1±0.1	33.6±0.1	33.9±0.2	34.3±0.2
	MUFA	42.5±0.1	42.3±0.2	42.1±0.1	41.9±0.3	41.7±0.1	41.6±0.2	41.4±0.3
	PUFA	25.6±0.2	25.4±0.1	25.2±0.2	25±0.1	24.7±0.1	24.5±0.2	24.3±0.2
EIO	C12:0	0.2±0.1	0.2±0.2	0.2±0.1	0.2±0.2	0.2±0.1	0.2±0.2	0.2±0.1
	C14:0	0.6±0.1	0.6±0.2	0.6±0.1	0.6±0.2	0.6±0.1	0.6±0.1	0.7±0.2
	C16:0	28.8±0.2	28.9±0.2	29.1±0.1	29.3±0.2	29.4±0.2	29.6±0.1	29.8±0.1
	C18:0	2.2±0.1	2.4±0.1	2.6±0.2	2.7±0.1	2.9±0.2	3.0±0.1	3.1±0.1
	C18:1	42.6±0.2	42.5±0.1	42.3±0.2	42.2±0.1	42.0±0.2	41.9±0.1	41.7±0.1
	C18:2	24.6±0.1	24.5±0.1	24.3±0.2	24.1±0.1	24.0±0.1	23.8±0.2	23.6±0.1
	C18:3	1.0±0.2	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.2	0.9±0.2	0.9±0.1
	SFA	31.8±0.2	32.1±0.3	32.5±0.2	32.8±0.1	33.1±0.2	33.4±0.1	33.8±0.1
	MUFA	42.6±0.2	42.5±0.1	42.3±0.1	42.2±0.2	42.0±0.3	41.9±0.2	41.7±0.2
	PUFA	25.6±0.1	25.4±0.1	25.2±0.2	25.0±0.1	24.9±0.3	24.7±0.1	24.5±0.1

SFA- saturated, MUFA-Monounsaturated, PUFA-Polyunsaturated fatty acids

(b) Effect of frying

During frying of poori using RBO for 1 to 6 frying cycles, minor changes was observed in the total unsaturated fatty acids level (76.5 to 75.0 %) of RBO. But in case of frying of poori for 1 to 6 frying cycles using RBDPO marginal changes was found in the total unsaturated fatty acids level (51.3 to 48.5%) of the RBDPO (**Table 5.2a**). Similarly, when (BLO) is exposed to 1 to 6 frying cycles, moderate changes in total unsaturated fatty acids level (68.1 to 65.7%) observed in the blended oil. In case of enzyme interesterified oil (EIO) changes was obsereved at a minor level (68.2 to 66.2%) under the similar frying condition (**Table 5.2b**). In general, polyunsaturated fatty acids are decreased and total saturated fatty acid were increased during frying. Similar observation was reported by Che Man *et al*. (2003) while frying of banana chips for five days using RBDPO.

However, incase of of frying the rate of degradation for all the oils are observed more. This could be due to the effect vaporization of moisture from the product to oil during frying.

Table 5.2 (a) Changes in fatty acid (relative % area) composition of RBO and RBDPO during frying

Oil	Fatty acids	Frying cycles						
		0	1	2	3	4	5	6
RBO	C12:0	nd	nd	nd	nd	nd	nd	nd
	C14:0	0.2±0.1	0.3±0.2	0.3±0.2	0.3±0.1	0.3±0.1	0.3±0.2	0.3±0.2
	C16:0	20.1±0.2	20.3±0.2	20.4±0.1	20.5±0.2	20.7±0.1	20.7±0.1	20.7±0.2
	C18:0	2.4±0.2	2.7±0.1	2.7±0.2	2.7±0.1	2.9±0.1	2.9±0.1	2.9±0.2
	C18:1	43.9±0.2	43.9±0.1	43.9±0.2	43.9±0.2	43.9±0.2	43.9±0.1	43.9±0.1
	C18:2	31.6±0.1	31.3±0.2	31.2±0.2	31.1±0.2	31.0±0.1	30.9±0.1	30.8±0.1
	C18:3	1.7±0.2	1.7±0.1	1.6±0.2	1.6±0.1	1.5±0.2	1.4±0.1	1.4±0.2
	SFA	22.8±0.1	23.3±0.2	23.5±0.3	23.7±0.2	23.8±0.1	23.9±0.2	23.9±0.3
	MUFA	43.9±0.2	43.4±0.1	43.3±0.2	43.2±0.3	42.9±0.1	42.8±0.2	42.8±0.2
	PUFA	33.3±0.2	33.0±0.1	32.8±0.2	32.7±0.2	32.5±0.1	32.3±0.3	32.2±0.1
RBDPO	C12:0	0.5±0.2	0.5±0.2	0.5±0.2	0.5±0.1	0.5±0.1	0.5±0.2	0.5±0.1
	C14:0	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.2	1.0±0.1	1.1±0.2	1.1±0.2
	C16:0	43.0±0.2	43.3±0.1	43.7±0.2	43.9±0.1	44.7±0.2	44.9±0.1	45.2±0.1
	C18:0	3.7±0.2	3.9±0.1	4.1±0.2	4.2±0.1	4.3±0.2	4.4±0.1	4.7±0.2
	C18:1	41.3±0.1	41.2±0.1	40.9±0.1	40.7±0.1	40.5±0.2	40.4	40.0±0.1
	C18:2	10.2±0.2	10.0±0.2	9.7±0.1	9.6±0.1	8.9±0.1	8.6±0.2	8.4±0.2
	C18:3	0.2±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.2	0.1±0.2
	SFA	48.2±0.1	48.7±0.2	49.3±0.4	49.6±0.3	50.5±0.1	50.9±0.2	51.5±0.4
	MUFA	41.3±0.2	41.2±0.4	40.9±0.2	40.7±0.3	40.5±0.1	40.4±0.2	40.0±0.3
	PUFA	10.4±0.4	10.1±0.3	9.8±0.2	9.7±0.1	9.0±0.1	8.7±0.2	8.5±0.4

SFA- Saturated, MUFA-Monounsaturated, PUFA-Polyunsaturated fatty acids

Table 5.2 (b) Changes in fatty acid (relative % area) composition of BLO and EIO during frying

Oil	Fatty acids	Frying cycles						
		0	1	2	3	4	5	6
BLO	C12:0	0.2±0.2	0.2±0.2	0.2±0.1	0.2	0.2±0.1	0.2±0.2	0.2±0.2
	C14:0	0.5±0.1	0.5±0.1	0.6±0.2	0.6±0.1	0.6±0.1	0.6±0.1	0.6±0.1
	C16:0	28.9±0.2	29.2±0.1	29.4±0.1	29.8±0.1	30.1±0.2	30.3±0.1	30.5±0.2
	C18:0	2.3±0.1	2.7±0.1	3.0±0.2	3.2±0.1	3.4±0.2	3.6±0.2	3.8±0.2
	C18:1	42.5±0.2	42.2±0.2	41.8±0.2	41.5±0.1	41.3±0.2	41±0.2	40.9±0.2
	C18:2	24.5±0.2	24.2±0.1	24.0±0.1	23.7	23.4±0.1	23.3±0.2	23.0±0.1
	C18:3	1.1±0.1	1.0±0.1	1.0±0.2	1.0±0.2	1.0±0.1	1.0±0.1	1.0±0.1
	SFA	31.9±0.3	32.6±0.2	33.2±0.2	33.8±0.1	34.3±0.2	34.7±0.1	35.1±0.1
	MUFA	42.5±0.1	42.2	41.8±0.2	41.5±0.1	41.3±0.1	41±0.3	40.9±0.2
	PUFA	25.6±0.2	25.2±0.2	25±0.1	24.7±0.1	24.4±0.2	24.3±0.1	24.2±0.2
EIO	C12:0	0.2±0.1	0.2±0.2	0.2±0.1	0.2±0.1	0.2±0.2	0.2±0.1	0.2±0.1
	C14:0	0.6±0.1	0.6±0.1	0.6±0.2	0.7±0.1	0.7±0.2	0.7±0.2	0.7±0.1
	C16:0	28.8±0.2	29.1±0.2	29.3±0.1	29.5±0.2	29.7±0.1	29.9±0.1	30.1±0.2
	C18:0	2.2±0.1	2.4±0.2	2.5±0.1	2.6±0.1	2.8±0.2	3.0±0.2	3.2±0.1
	C18:1	42.6±0.2	42.3±0.1	42.2±0.1	42.0±0.2	41.9±0.1	41.7±0.2	41.5±0.1
	C18:2	24.6±0.1	24.5±0.2	24.3±0.2	24.1±0.1	23.8±0.2	23.6±0.1	23.4±0.2
	C18:3	1.0±0.2	0.9±0.2	0.9±0.1	0.9±0.2	0.9±0.2	0.9±0.1	0.9±0.1
	SFA	31.8±0.2	32.3±0.3	32.6±0.2	33.0±0.1	33.4±0.1	33.8±0.2	34.2±0.1
	MUFA	42.6±0.2	42.3±0.2	42.2±0.1	42.0±0.2	41.9±0.3	41.7±0.2	41.5±0.2
	PUFA	25.6±0.1	25.4±0.2	25.2±0.1	25.0±0.1	24.7±0.2	24.5±0.2	24.3±0.3

SFA- Sturated, MUFA- Monounsaturated and PUFA- Polyunsaturated fatty acids

5.2.1.2 Free fatty acid content

Free fatty acids (FFA) content is considered to be an indicator of oil quality as it leads to development of off-flavor in oils and fried products. FFA increases during frying mainly due to hydrolysis, thermal oxidation and polymerization.

(a) *Effect of heating*

FFA was found to increase with an increase in frying cycles in case of simulated frying (heating). The percentages of FFA of fresh RBO, RBDPO, BLO and EIO were 0.15, 0.12, 0.17 and 0.22%, respectively. FFA of RBO, RBDPO, BLO and EIO was found to increase from 0.15 to 0.64, 0.12 to 0.44, 0.17 to 0.36 and 0.22 to 0.30% after subjected to heating for 1 to 6 consecutive cycles, respectively (Fig. 5.1a).

(b) Effect of frying

In case of frying of poori, FFA was found to increase with an increase in frying cycles. FFA of RBO, RBDPO, BLO and EIO was found to increase after frying of poori for 1 to 6 consecutive cycles from 0.15 to 0.69 , 0.12 to 0.48, 0.17 to 0.44 and 0.22 to 0.41%, respectively (Fig. 5.1b).

The change in FFA after frying was found to be more in all the oils in comparison to simulated frying (heating only).

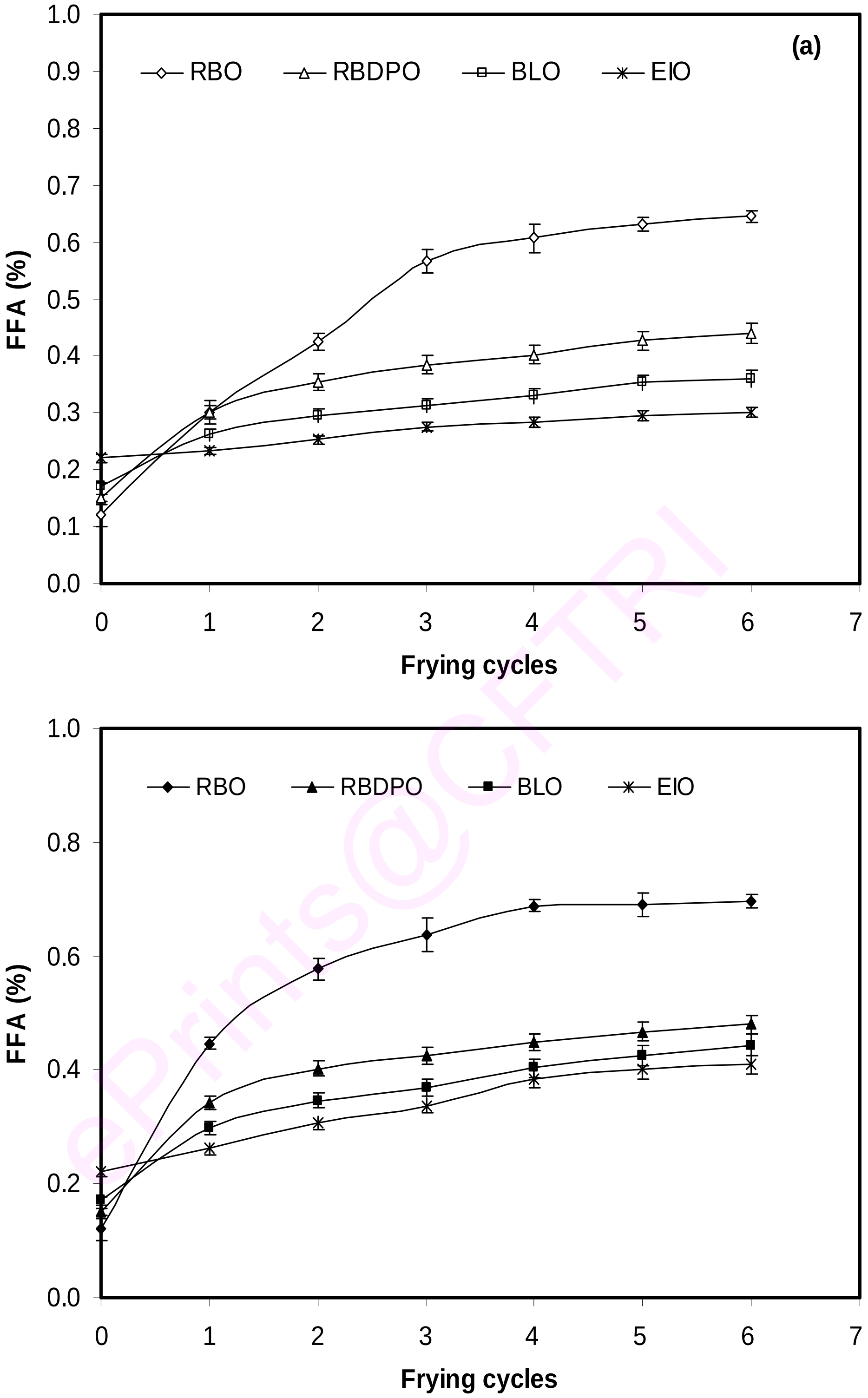


Fig. 5.1 Changes in free fatty acid content during (a) heating and (b) frying of poori using RBO, RBDPO, BLO and EIO
RBO-rice bran oil, RBDPO-refined palm olein, BLO-blended oil and EIO-enzyme interesterified oil

5.2.1.3 Peroxide value

Peroxides are the foremost initial reaction products of lipid oxidation, which are responsible for primary oxidation. Oil, initially forms hydroperoxide compounds, which are good indicators of lipid oxidation under normal conditions. However, at high temperatures, they break and form secondary oxidation products during cooling.

(a) *Effect of heating*

The peroxide value of fresh RBO, RBDPO, BLO and EIO was 1.63, 1.47, 1.71, 3.71 meqO_2/kg. After heating for 1 to 6 cycles, the peroxide value of RBO, RBDPO, BLO and EIO was found to increase from 1.63 to 7.7, 1.47 to 3.6, 1.71 to 4.79 and 3.71 to 4.28 meqO_2/kg in case of heating upto 6 cycles, respectively (Fig. 5.2a).

(b) Effect of frying

After frying for 1 to 6 cycles, the peroxide value of RBO, RBDPO, BLO and EIO was increased from 1.63 to 11.5, 1.47 to 4.05, 1.71 to 6.9 and 3.71 to 5.9 meqO_2/kg, respectively. The peroxide value was found to increase from 1.47 meqO_2/kg to 7.7 and to 11.7 meqO_2/kg in case of heating and frying, respectively (Fig. 5.2b).

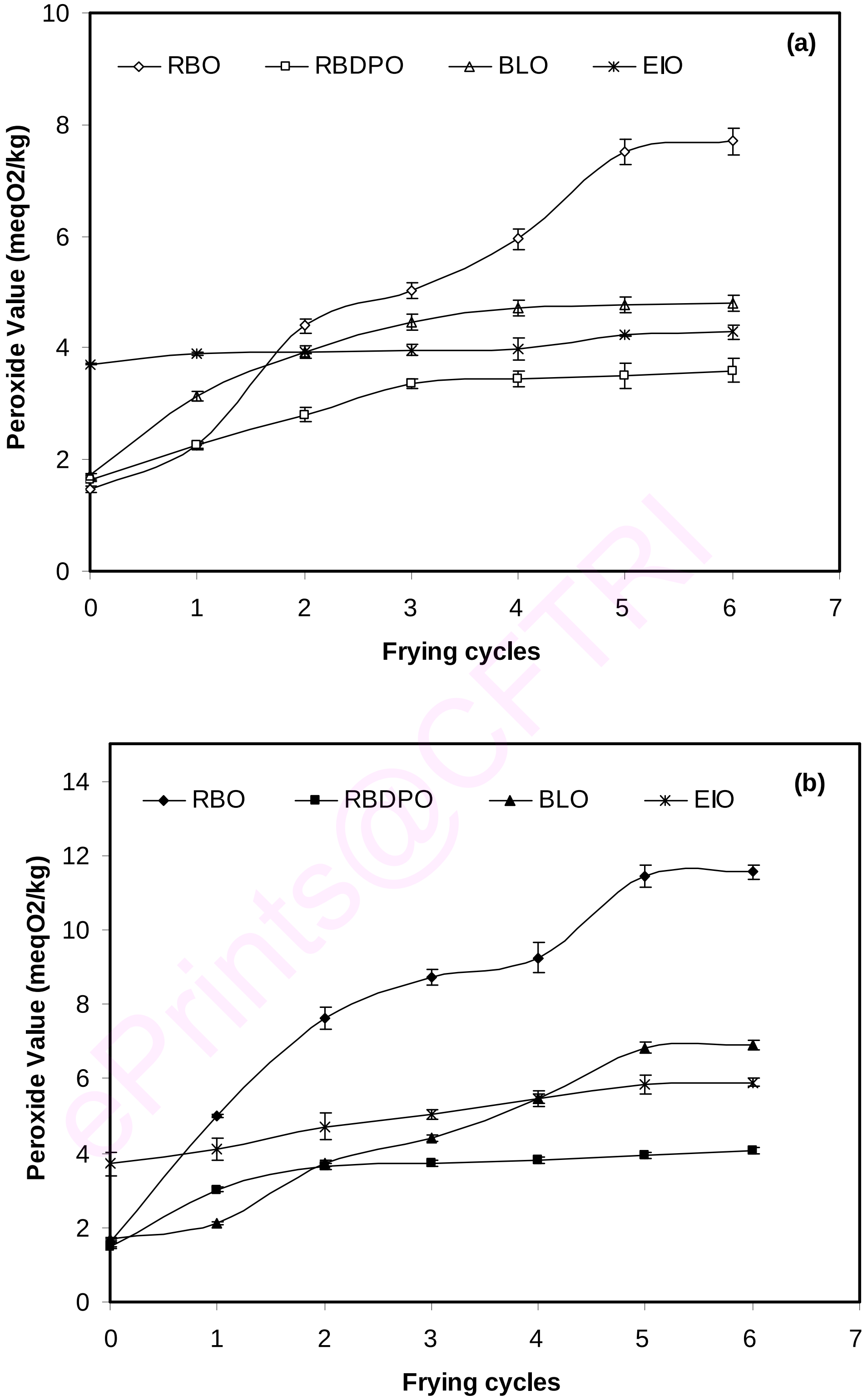


Fig. 5.2 Changes in peroxide value during (a) heating and (b) frying using RBO, RBDPO, BLO and EIO RBO-rice bran oil, RBDPO-refined palm olein, BLO- blended oil and EIO-enzyme interesterified oils

The above oils showed a comparatively higher degree of degradation during frying as compared to oil subjected to heating only. Peroxide value is useful as an indicator of oxidation at the initial stages. It is related to the formation and breakdown of oxidation products. Peroxides are unstable and decompose at frying temperature. Peroxides may even increase after the sample is taken from the fryer.

5.2.1.4 Radical scavenging activity (RSA)

Antioxidant is a substance which is capable of preventing or delaying the oxidation reaction during processing such as heating or frying of foods. RBO contains natural antioxidants such as oryzanol, tocopherol and tocotrienol, which were able to quench free radicals to some extent during heating and frying experiments.

(a) *Effect of heating*

Fig. 5.3a showed that after 6 cycles of simulated frying (heating), the ability of RBO, RBDPO, BLO and EIO to quench DPPH radicals changed from 56.2 to 54.92, 41.46 to 17.33, 53.4 to 46.1 and 54.2 to 50.92%, respectively.

(b) Effect of frying

When the above oils are subjected to frying for 6 cycles, the ability of quenching DPPH free radicals is changed in the order of 56.2 to 53.2, 41.46 to 12.52, 53.4 to 43 and 54.2 to 48.3%, respectively. Therefore, the radical scavenging activity is found to increase from 41.46% (in RBDPO) to 54.2% in enzyme interesterified oil (EIO) (Fig. 5.3b). In case of RBO scavenging abilkity is more. This may be due to presence of micronutrients such as oryzanol.

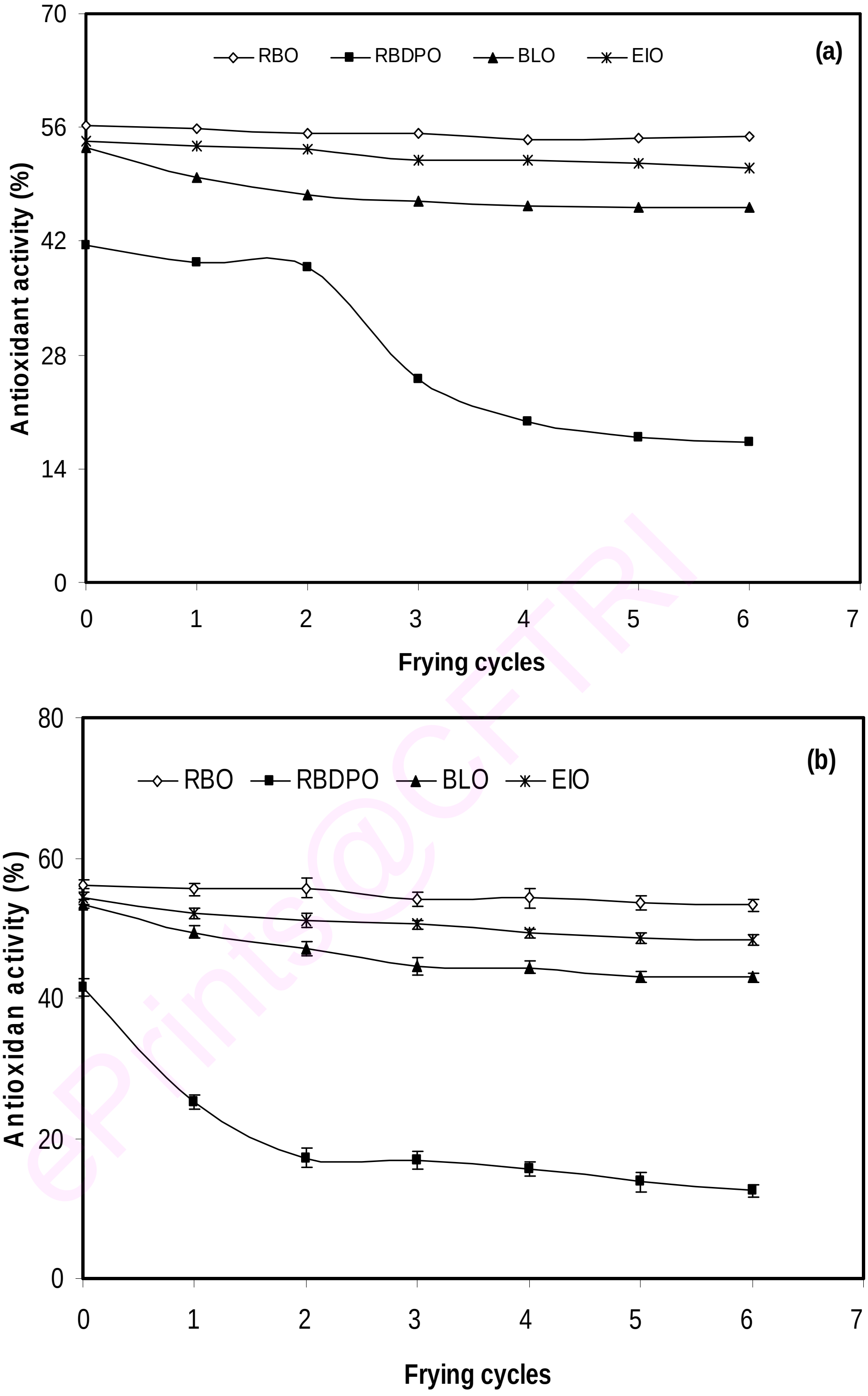


Fig. 5.3 Changes in radical scavenging effect during (a) heating and (b) frying of poori using RBO, RBDPO, BLO and EIO
RBO-rice bran oil, RBDPO-refined palm olein, BLO-blended oil and
EIO-enzyme interesterified oil

The loss of antioxidant activity in frying experiments was significantly higher than in the heating experiments. The loss of antioxidant activity in RBDPO was higher than RBO and its enzyme interesterified counterpart. The loss of antioxidant activity from fried oil can be attributed to the volatilization of antioxidant through evaporation and/or decomposition of the antioxidant.

5.2.1.5 Specific gravity

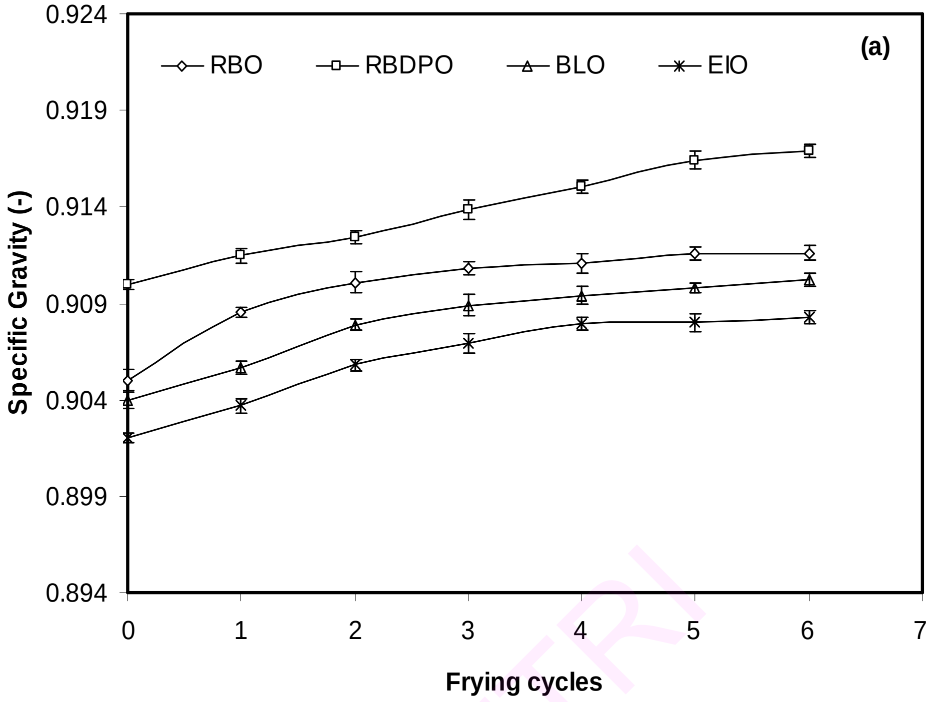
The specific gravity of the oil changes during heating or frying. The specific gravity increases with oil deterioration.

(a) Effect of heating

The specific gravity of RBO, RBDPO, BLO and EIO are found to increase from 0.905 to 0.912, 0.910 to 0.917, 0.904 to 0.910 and 0.902 to 0.908, respectively. and with increasing frying cycle due to oil degradation. It was observed that the values (Fig. 5.4a) were not significantly ($p>0.05$) different.

(b) Effect of frying

In case of frying, the specific gravity of RBO, RBDPO, BLO and EIO are found to change from 0.905 to 0.913, 0.910 to 0.918, 0.904 to 0.912 and 0.902 to 0.911, respectively, with increasing frying cycle. The observed values (Fig. 5.4b) were not significantly ($p>0.05$) different.



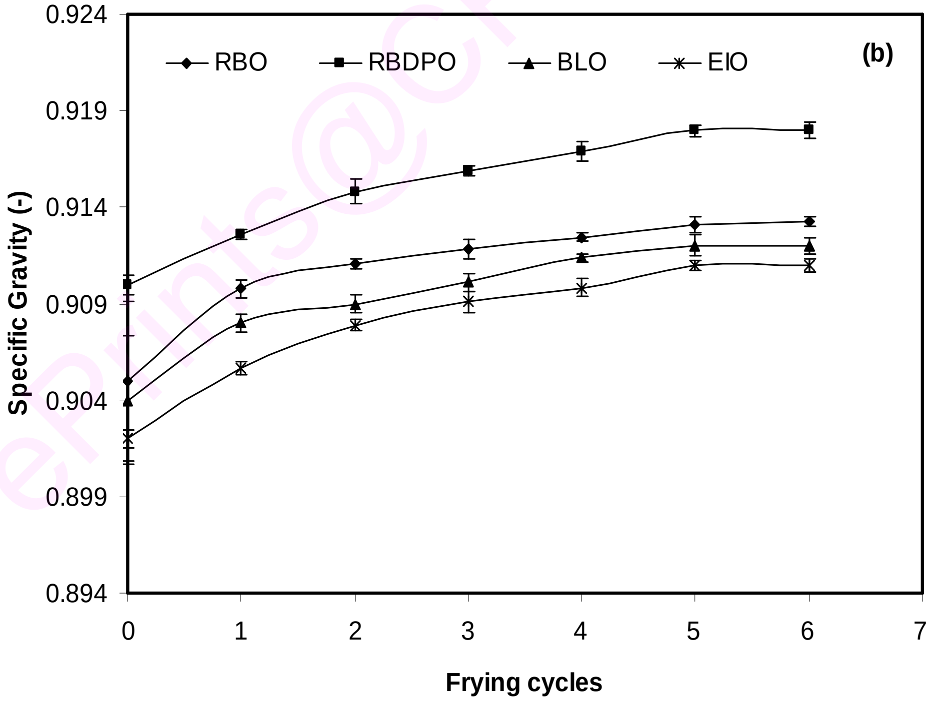


Fig. 5.4 Changes in specific gravity during (a) heating and (b) frying of poori using RBO, RBDPO, BLO and EIO

RBO-rice bran oil, RBDPO-refined palm olein, BLO-blended oil and EIO-enzyme interesterified oil

5.2.1.6 Surface tension

The surface tension of oil also changes during heating or frying. The surface tension oil decreases with increasing oil degradation.

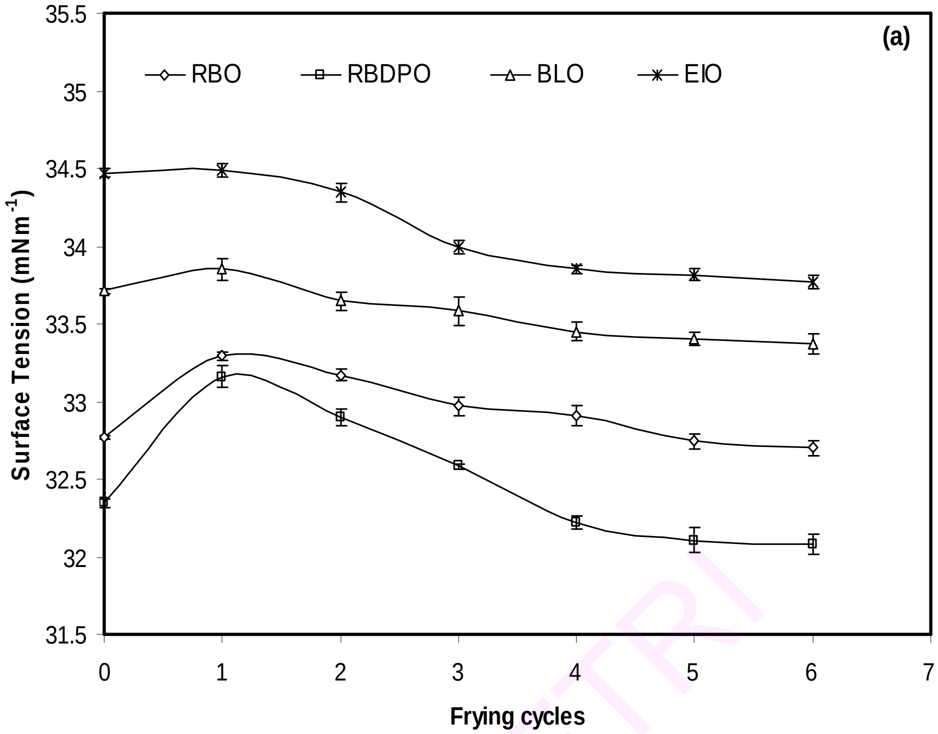
(a) *Effect of heating*

It was seen that the surface tension of the RBO, RBDPO, BLO and EIO decreased from 32.77 to 32.7, 32.3 to 32.08, 33.71 to 33.36 and 34.7to 33.77 mNm^{-1} with increase in frying cycle (Fig. 5.5a) during simulated frying (heating).

(b) *Effect of frying*

From the Fig. 5.5b is observed that the surface tension of the RBO, RBDPO, BLO and EIO decreased with increase in frying cycles, from 32.77 to 32.46, 32.34 to 32.0, 33.71 to 32.9 and 34.47 to 33.5 mNm^{-1} , respectively,.

Therefore, the surface tension of these oils was found inversely proportional to the frying cycle. A good correlation (r= 0.88) between the surface tension and viscosity of these oils was observed. Similar observations were made by Tseng *et al.* (1996). They observed the soybean oil degradation at frying temperature during preparation of tortilla chips. They showed that surface tension decreases significantly with oil degradation. The decrease of surface tension in the heated or fried oil could be due to the formation of mono and diglycerides due to hydrolysis of triglycerides during prolonged frying.



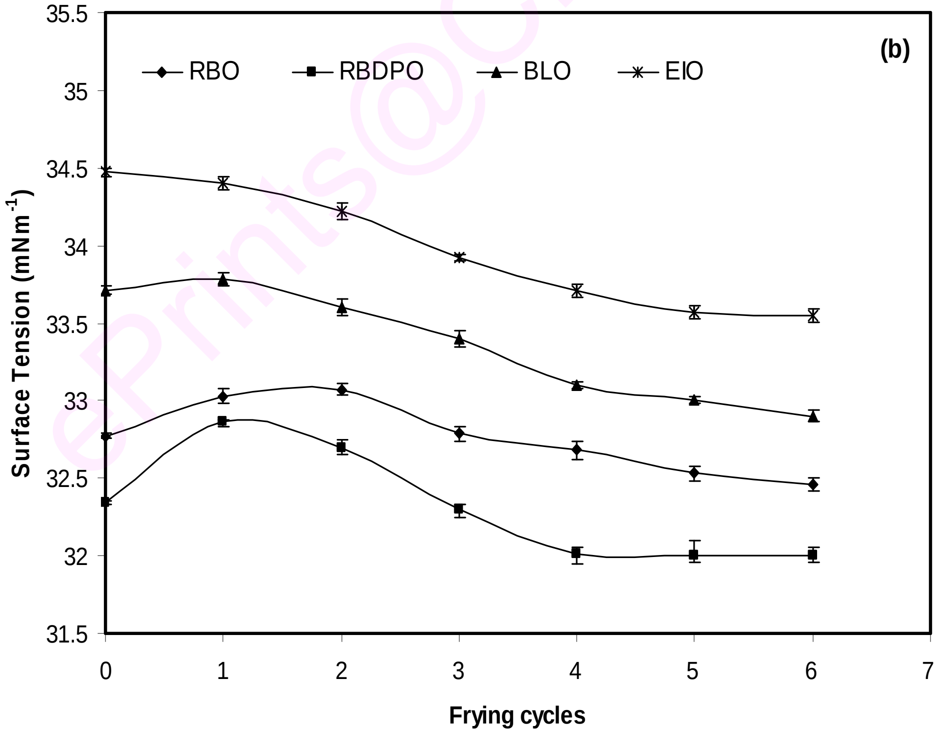


Fig. 5.5 Changes in surface tension of oils during (a) heating and (b) frying of poori RBO-rice bran oil, RBDPO-refined palm olein, BLO- blended oil and EIO- enzyme interesterified oil

5.2.1.7 Oryzanol content of RBO

Oryzaol is a minonstituent present in rice bran oil. This component provides the stability of this oil. This component has nutritional and health benefits also as hypochlesterolemic agent.

(a) Effect of heating

The oryzanol content in the native, heated rice bran oil was shown in Fig. 5.6a. Oryzanol content was found to change from 1.429 % to 1.427 % in case of heating , for 6 consecutive frying cycles which was not significant ($p>0.05$).

(b) Effect of frying

The oryzanol content in the native, fried rice bran oil was shown in Fig. 5.6b. Oryzanol content was found to change from 1.429 % to 1.426 % in case of frying for 6 consecutive frying cycles. There was no significant ($p>0.05$) changes observed in oryzanol content in rice bran oil during heating as well as frying of *poori*. This observation indicated that the rice bran oil is stable oil useful for frying (Gopalakrishna *et al.*, 2001).

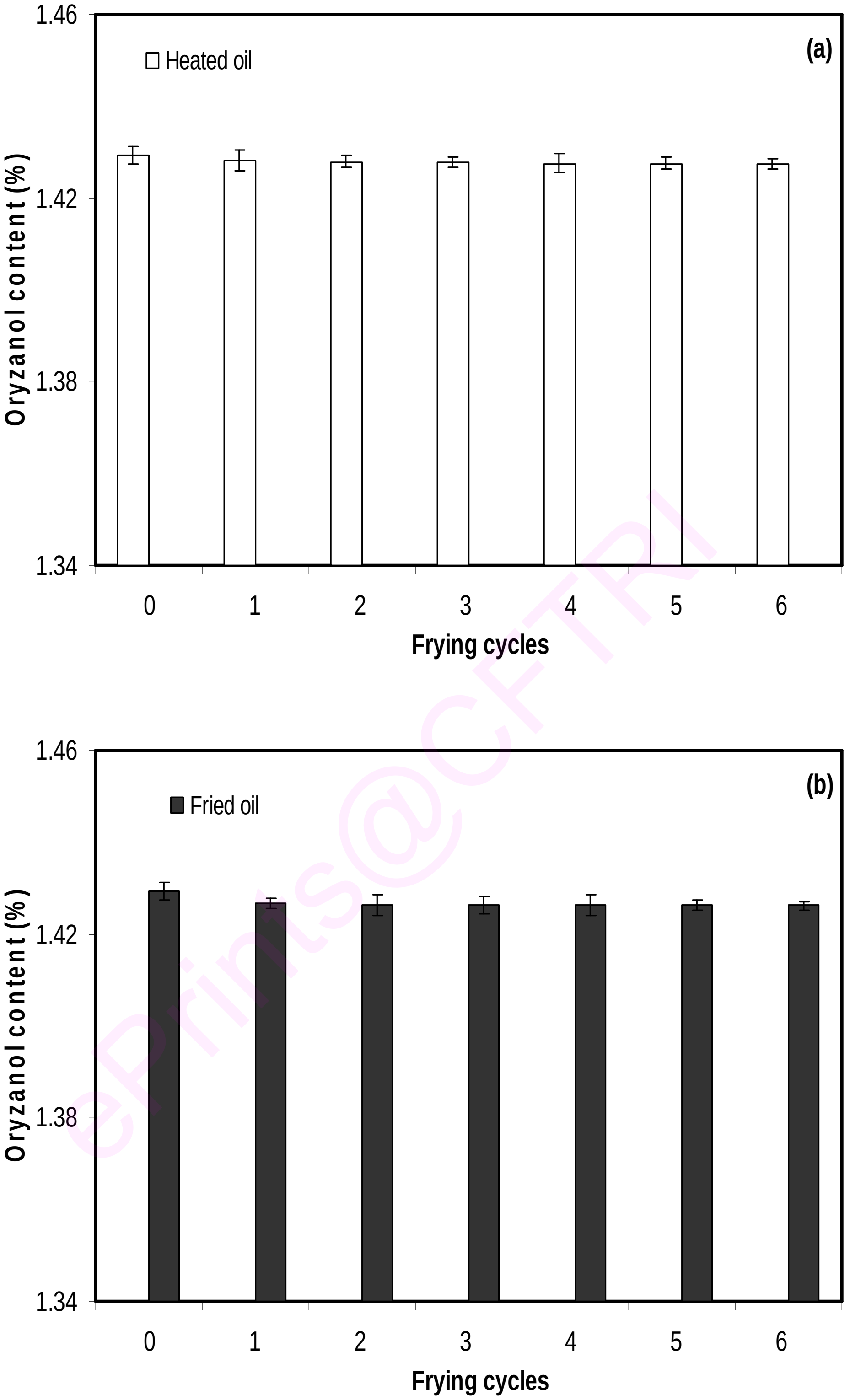


Fig. 5.6 Changes in oryzanol content of RBO during (a) heating and (b) frying of *poori*

5.2.1.8 Total polar material and oil discarding point after frying

Total polar material (TPM) is one of the most valid and objective criteria for the deterioration of oils and fats during deep-fat frying. During frying, as peroxides/hydroperoxides breaks down, short chain acids, aldehydeds, ketones, alcohols and non-volatile end products are formed in the frying oil. These cause molecules in oils to become polar (Xu *et al*., 1999b). The polar material is an indicator for the extent of deterioration of oils used for frying.

The total polar material consists of polymeric, cyclic nonvolatile substances (resulting from oxidation and hydrolysis of oil) including soluble constituents leached from the fried products. The level of polar material was fixed to 25-27% as the threshold point for discarding frying oil (Mellema, 2003; Sanibal and Filho, 2004; Fauziah *et al*., 2000). European countries have set a maximum level of total polar material of 24% (Stier, 2001). As the viscosity of the oil increases during frying, the polar materials also increased with the consecutive frying for 6 frying cycles.

(a) *Effect of heating*

The total polar material of native RBO, RBDPO, blended oil (BLO) and interesterified oil (EIO) are 3.5, 2.4 and 3.8 and 3.6%, respectively. The TPM of RBO, RBDPO, BLO and EIO was found to increase from 3.5 to 7.3, 2.4 to 2.9, 3.8 to 6.8 and 3.6 to 6.0 % in case of heating, respectively for 1 to 6 consecutive frying cycles (Fig. 5.7a).

(b) Effect of frying

From the Fig 5.7b it is observed that the TPM of RBO, RBDPO, BLO and EIO increased from 3.5 to 10.2, 2.4 to 7.1, 3.8 to 8.1 and 3.6 to 7.7 % after frying poori for 6 frying cycles, respectively (Fig. 5.7b).

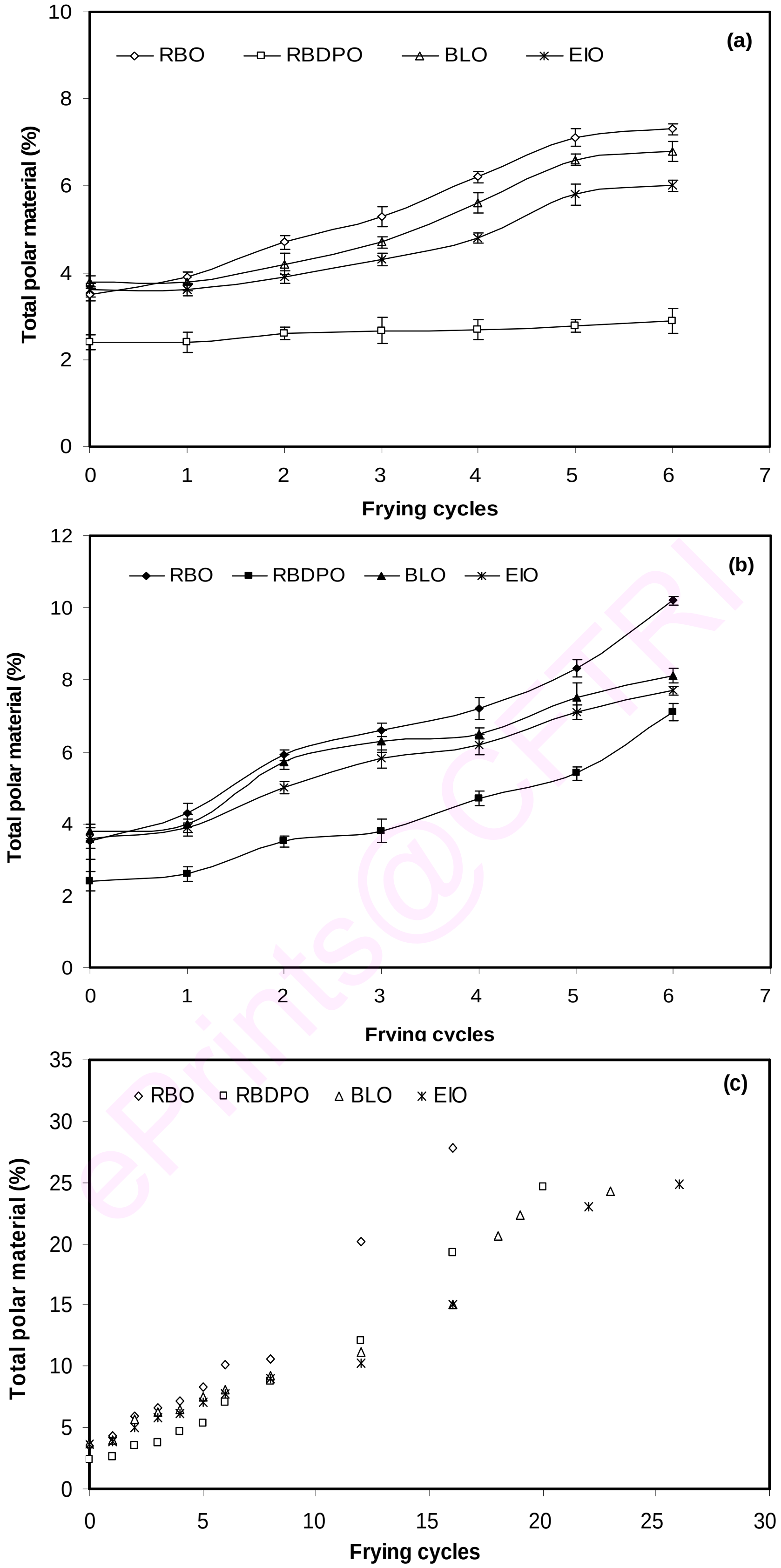


Fig. 5.7 Changes in total polar material during (a) heating and (b) frying of *poori* using RBO, RBDPO, BLO and EIO and (c) oil discarding point
RBO-rice bran oil, RBDPO-refined palm olein, BLO- blended oil and EIO-enzyme interesterified oil

The total polar material was found to increase linearly with the number of frying cycles during heating as well as frying. Simar observation was reported by Bansal *et al.* (2010) while the stability of RBDPO was studied under repeated frying and controlled heating conditions.

(c) Oil discarding point

Since oil causes health hazards after reaching its total polar material to 24% as discussed above (Stier, 2001), it needs to be discarded. From the Fig. 5.10c, it was observed that during frying, the TPM reached to a discarding point of 24% for RBO, RBDPO, BLO and EIO after 15, 21, 23 and 25 frying cycles, respectively. This indicated that interesterified oil is more resistant than the corresponding blended oil and native oils. This indicated the suitability of blended as well as interesterified oils for domestic or industrial frying.

5.2.2 Effect of frying cycles on physical properties of oil

5.2.2.1 Colour

During heating or frying at higher temperature the colour of the frying media changes.

(a) Effect of heating

The change in the colour of the oils subjected to 6 cycles of heating experiments is shown in Table 5.3a. It is observed that the red colour of the oil increased from the control value of 2.0 (RBO) to 7.0 (RBDPO) Lovibond colour units. Similarly the yellow colur varied from 6 (RBDO) to 20 (RBO) Lovibond colour units.

(b) Effect of frying

The oils subjected to 6 cycles of frying experiments and the result is shown in Table 5.3b. It is found hat the red colour of the oil increased from the control value of 2.0 (RBO) to 4.0 (RBO) Lovibond colour units. Similarly the yellow colur varied from 6 (RBDO) to 20.5 (RBO) Lovibond colour units.

The colour of the oil was found to increase after frying due to presence of unsaturated carbonyl compounds, formation of degraded compounds by oxidation, non-polar compounds of food stuff leached in frying oil (Augustin *et al.*, 1987; Gutierrez *et al.,* 1988). The colour of oil samples was found to change drastically after frying in comparison to heating. Burton (1989) pointed out that the alpha and beta unsaturated carbonyl compounds derived from sugars are first formed intermediates that react with substances containing alpha amino groups to give carbonyl-nitrogen compounds which conjugate to form brown products.

Table 5.3 Effect of frying cycles on colour of RBO, RBDPO, BLO and EIO

(a) Heating effects

Oil	Fatty acids	Fresh	Frying cycles					
		0	1	2	3	4	5	6
RBO	Red	2.0±0.1	3.0±0.2	3.0±0.1	3.0±0.1	3.0±0.2	3.3±0.2	3.0±0.1
	Yellow	15.0±0.1	15.0±0.2	16.5±0.1	19.5±0.2	19.5±0.1	19.5±0.1	20.0±0.2
RBDPO	Red	1.0±0.2	1.5±0.2	1.5±0.1	2.0±0.2	2.0±0.2	1.0±0.1	1.0±0.1
	Yellow	6.0±0.1	7.0±0.1	7.0±0.2	8.0±0.1	8.0±0.2	7.0±0.1	7.0±0.1
BLO	Red	2.0±0.2	2.0±0.1	2.0±0.2	2.1±0.1	2.1±0.2	2.2±0.1	2.2±0.1
	Yellow	15.0±0.1	15.0±0.1	16.0±0.2	18.0±0.1	19.0±0.1	19.0±0.2	19.0±0.1
EIO	Red	1.8±0.2	1.8±0.1	1.8±0.1	1.9±0.1	2.0±0.2	2.0±0.2	2.0±0.1
	Yellow	16±0.2	16±0.3	16.5±0.2	16.5±0.1	17.0±0.2	18.0±0.1	18.0±0.1

(b) Frying effects

Oil	Fatty acids	Fresh	Frying cycles					
		0	1	2	3	4	5	6
RBO	Red	2.0±0.1	3.0±0.2	3.5±0.1	4.0±0.2	4.5±0.1	4.5±0.2	4.0±0.1
	Yellow	15.0±0.1	20.0±0.2	20.0±0.1	20.0±0.2	20.0±0.1	20.0±0.1	20.5±0.2
RBDPO	Red	1.0±0.2	1.0±0.2	0.7±0.1	0.6±0.2	0.6±0.2	0.6±0.1	0.6±0.1
	Yellow	6.0±0.1	7.0±0.1	6.0±0.2	5.0±0.1	4.0±0.2	4.0±0.1	4.0±0.1
BLO	Red	2.0±0.2	2.2±0.1	2.2±0.2	2.2±0.1	2.2±0.2	2.2±0.1	2.2±0.1
	Yellow	15.0±0.1	16.0±0.1	20.0±0.2	20.0±0.1	20.0±0.1	20.0±0.2	20.0±0.1
EIO	Red	1.8±0.2	1.8±0.1	1.9±0.1	1.9±0.1	2.0±0.2	2.1±0.2	2.1±0.1
	Yellow	16.0±0.2	16.1±0.3	16.1±0.2	16.3±0.1	17.1±0.2	18.4±0.1	19.0±0.1

RBO-rice bran oil, RBDPO-refined palm olein, BLO- blended oil and EIO-enzyme interesterified oil

5.2.2.2 Moisture and oil uptake

The moisture as well as oil uptake in the intermittent use of frying oil up to 6 cycles during deep-fat frying of *poori* are shown in Fig. 5.8. It was found that there was no significant (p>0.05) difference in the moisture content of *poori* fried in oils subjected to increased number of frying cycles. Similar trends for moisture content observed for the *poori* fried using all the oils in the present sudy. The oil uptake in the product was found to increase significantly from 28.46 to 37.75 % with an increase in number of frying cycles from 1 to 6. The higher oil uptake may be due to the increase in degradation products and viscosity of the frying medium due to thermal polymerization and/or oxidative deterioration during cooling, which

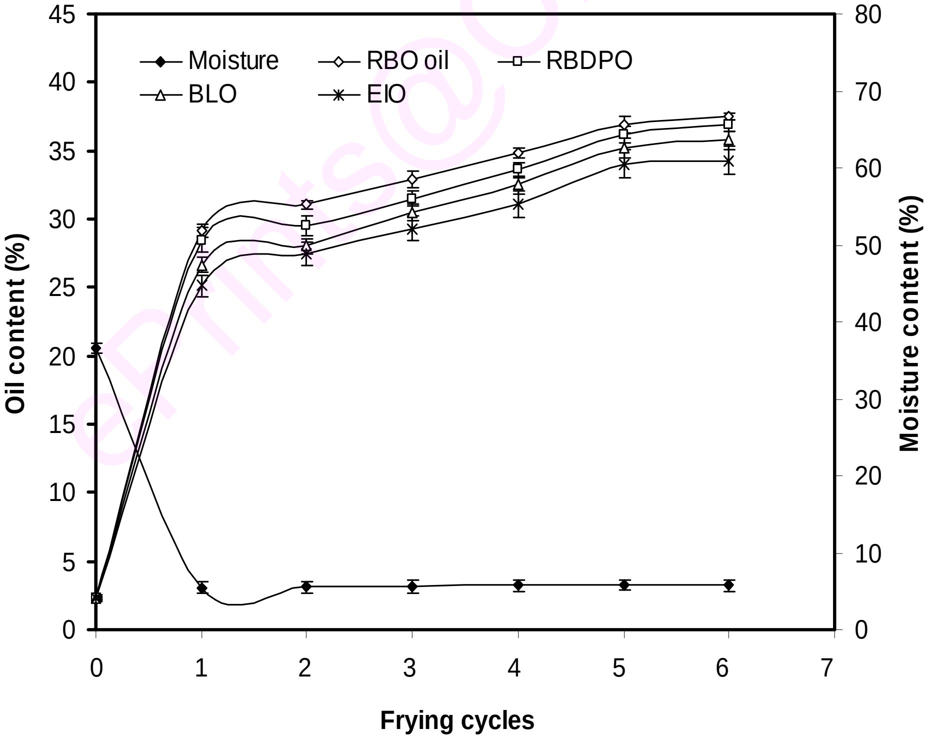


Fig. 5.8 Effect of frying cycles on moisture content and oil uptake during frying of *poori* using RBO, RBDPO, BLO and EIO
RBO-rice bran oil, RBDPO-refined palm olein,
BLO-blended oil and EIO-enzyme interesterified oil

leads to the higher oil absorption by the fried product (Blumenthal, 1991). The increase in viscosity of the oil with an increase in frying cycles results in formation of thick boundary layer of oil between the food being fried and the oil, which consequently results in higher oil uptake (Bouchon, *et al.*, 2003, Moyano and Pedreschi, 2006).

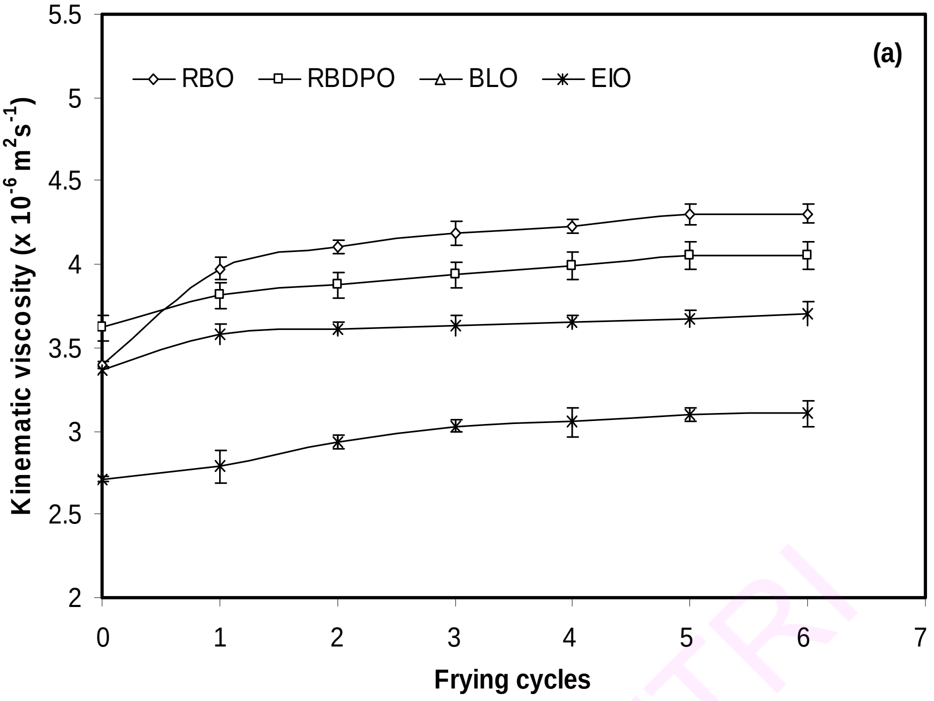
5.2.3 Kinematic viscosity

(a) Effect of heating

The kinematic viscosity of fresh RBO, RBDPO, blended oil (BLO) and the interesterified oil (EIO) are 3.39 x 10^{-6} m^2s^{-1}, 3.617 x 10^{-6} m^2s^{-1}, 3.36 x 10^{-6} m^2s^{-1} and 2.71 x 10^{-6} m^2s^{-1}, respectively. During heating for six frying cycles, the kinematic viscosity of these oils was found to increase from 3.39 x 10^{-6} to 3.938 x 10^{-6} m^2s^{-1}, 3.617 x 10^{-6} to 4.304x x 10^{-6} m^2s^{-1}, 3.36 x 10^{-6} to 3.704 x 10^{-6} m^2s^{-1} and 2.71 x 10^{-6} to 3.104 x 10^{-6} m^2s^{-1}, respectively for heating (Fig. 5.9a).

(b) Effect of frying

The kinematic viscosity of RBO, RBDPO, BLO and EIO was found to increase from 3.392 x 10^{-6} to 4.304 x 10^{-6} m^2s^{-1}, 3.617 x 10^{-6} to 4.67 x 10^{-6} m^2s^{-1}, 3.36 x 10^{-6} to 4.20 x 10^{-6} m^2s^{-1} and 2.71 x 10^{-6} to 3.4 x 10^{-6} m^2s^{-1}, repectively, for frying experiments for 1 to 6 consecutive cycles of frying (Fig. 5.9b). This increase in viscosity of oil was probably due to formation of higher molecular weight compounds involving carbon to carbon and carbon to oxygen to carbon bonds between oxidised fatty acids (Lalas *et al*., 2006). Teah (1988) and Berger (1984) indicated that polymeric materials are mainly responsible for the increase in viscosity. It was also found that because of



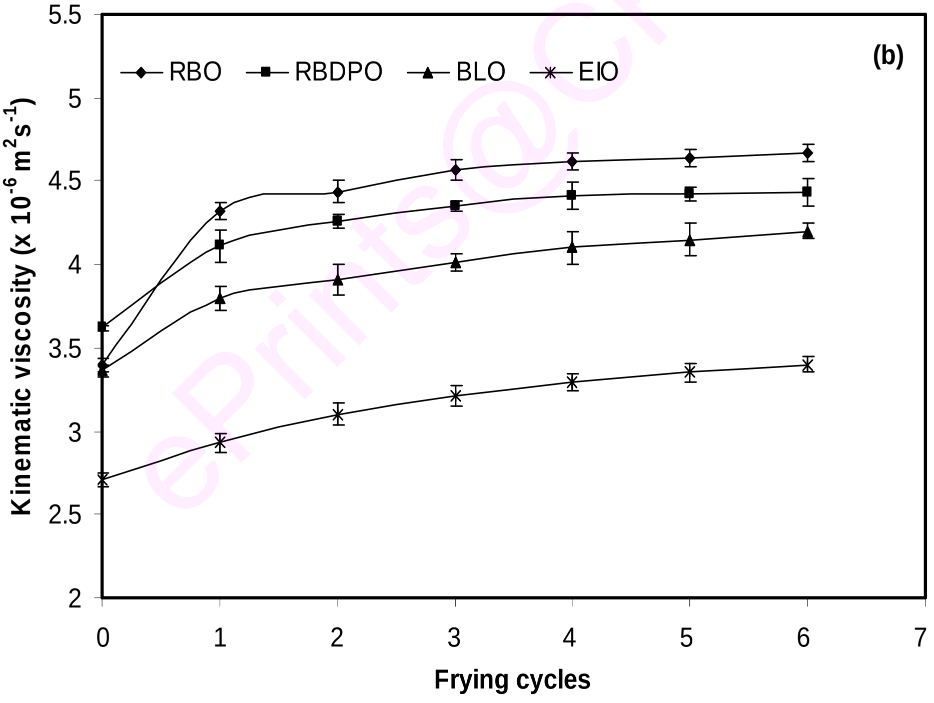


Fig. 5.9 Effect of frying cycles on kinematic viscosity of RBO, RBDPO, BLO and EIO during (a) heating and (b) frying
RBO-rice bran oil, RBDPO-refined palm olein, BLO-blended oil and EIO-enzyme interesterified oil

high water content leaching from fried products, the polymerization of oil increased during the frying process. It is directly related to the formation of polymeric compounds due to exposure of oxygen at high temperature during frying (Kochhar and Gertz, 2004).

5.2.4 Effect of frying cycle on heat transfer coefficient

(a) Effect of heating

The convective heat transfer coefficient of fresh RBO, RBDPO, blended (BLO) and enzyme interesterified oil (EIO) were 238.3 $Wm^{-2}\,°C^{-1}$, 221 $Wm^{-2}\,°C^{-1}$, 252 $Wm^{-2}\,°C^{-1}$ and 263.4 $Wm^{-2}\,°C^{-1}$, respectively. During heating for 1 to 6 cycles of frying at 173±3°C the heat transfer coefficient of the oils were found to decrease from 238.3 to 235.6 $Wm^{-2}°C^{-1}$, 221 to 215.5 $Wm^{-2}°C^{-1}$, 258 to 253 $Wm^{-2}°C^{-1}$ and 263.4 $Wm^{-2}°C^{-1}$ to 258.0 $Wm^{-2}°C^{-1}$, respectively (Fig. 5.10a).

(b) Effect of frying

In case of frying for 1 to 6 cycles at 173±3°C the heat transfer coefficient of these oils were found to decrease from 238.3 to 233 $Wm^{-2}°C^{-1}$, 221 to 212.2 $Wm^{-2}\,°C^{-1}$, 258 to 251 $Wm^{-2}\,°C^{-1}$ and 263.4 $Wm^{-2}\,°C^{-1}$ to 257.0 $Wm^{-2}°C^{-1}$, respectively (Fig. 5.10b).

The values of heat transfer coefficients were of the same order as that of reported by Miller *et al* (1994) and Debnath *et al.* (2010a). Heat transfer coefficient was found to be decreased marginally by the viscosity of the oil

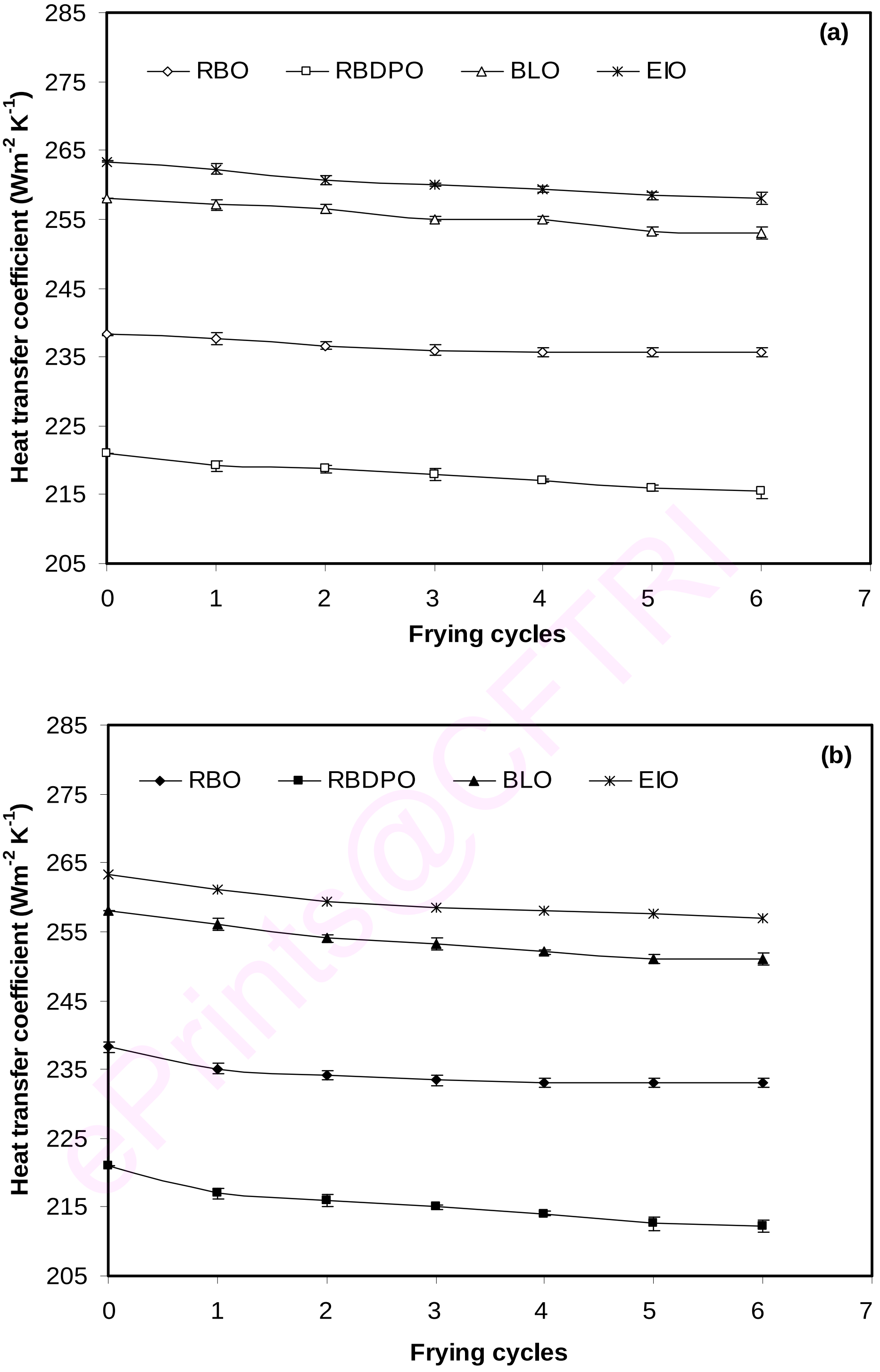


Fig. 5.10 Effect of frying cycles on heat transfer coefficient of (a) heated and (b) fried oil samples of RBO, RBDPO, BLO and EIO
RBO-rice bran oil, RBDPO-refined palm olein, BLO-blended oil and EIO-enzyme interesterified oil

and the correlation coefficient between viscosity and heat transfer coefficient of RBO during heating and frying were - 0.85 and - 0.98, respectively. Tseng *et al.* (1996) reported similar correlation between the heat transfer coefficient and viscosity in case of tortilla chips fried in soybean oil.

5.2.5 Effect of frying cycles on sensorial properties of poori

The scores for the *poori* fried at 173 ^{o}C for 3 min using RBO, RBDPO, blended oil (BLO) and the interesterified oil (EIO) have shown in Table 5.4 for sensory attributes, like appearance, colour, taste, crispness and overall acceptability. The sensory data showed no significant differences ($p>0.05$) in terms of appearance, colour, taste, crispness and overall acceptability for *poori* fried using RBO, RBDPO, BLO and EIO for 6 cycles.

Table 5.4 Sensory scores of the *poori* during prolonged frying (intermittent) with RBO, RBDPO, BLO and EIO

Sensory Attributes	Oil samples	Frying cycles						
		0	1	2	3	4	5	6
Appearance	RBO	8.0±0.2	8.0±0.4	7.6±0.5	7.6±0.5	7.5±0.6	7.5±0.5	7.4±0.6
	RBDPO	8.0±0.1	8.0±0.2	7.6±0.2	7.6±0.4	7.5±0.1	7.5±0.2	7.4±0.2
	BLO	8.0±0.2	8.0±0.1	7.6±0.1	7.6±0.1	7.4±0.1	7.4±0.2	7.4±0.2
	EIO	8.0±0.1	8.0±0.2	7.6±0.1	7.6±0.3	7.5±0.2	7.4±0.1	7.4±0.1
Colour	RBO	8.5±0.2	8.5±0.5	8.5±0.4	8.3±0.4	8.2±0.5	7.4±0.5	7.4±0.5
	RBDPO	8.5±0.1	8.4±0.3	8.4±0.3	8.2±0.2	8.2±0.5	7.4±0.3	7.4±0.2
	BLO	8.5±0.1	8.4±0.2	8.4±0.2	8.2±0.2	8.1±0.3	7.4±0.4	7.3±0.3
	EIO	8.5±0.1	8.4±0.2	8.4±0.1	8.2±0.1	8.1±0.2	7.3±0.3	7.3±0.3
Crispness	RBO	8.5±0.2	8.5±0.3	8.5±0.4	8.2±0.6	8.1±0.7	7.5±0.7	7.5±0.7
	RBDPO	8.5±0.2	8.5±0.1	8.4±0.3	8.2±0.4	8.1±0.5	7.5±0.7	7.4±0.6
	BLO	8.5±0.2	8.5±0.1	8.5±0.1	8.2±0.2	8.1±0.1	7.5±0.2	7.4±0.1
	EIO	8.5±0.1	8.5±0.2	8.4±0.2	8.1±0.3	8.0±0.3	7.4±0.5	7.4±0.5
Taste	RBO	8.5±0.2	8.5±0.2	8.5±0.5	8.1±0.4	7.8±0.7	7.7±0.7	7.7±0.7
	RBDPO	8.5±0.1	8.5±0.2	8.4±0.4	8.0±0.4	7.7±0.6	7.6±0.7	7.5±0.7
	BLO	8.5±0.2	8.5±0.2	8.4±0.1	8.1±0.3	7.6±0.4	7.5±0.5	7.5±0.4
	EIO	8.5±0.1	8.5±0.1	8.4±0.5	8.1±0.3	7.6±0.6	7.5±0.7	7.5±0.7
Overall Acceptability	RBO	8.4±0.1	8.4±0.1	8.2±0.4	8.1±0.7	7.5±0.6	7.5±0.6	7.5±0.6
	RBDPO	8.4±0.2	8.4±0.2	8.1±0.3	8.1±0.6	7.5±0.5	7.5±0.5	7.4±0.6
	BLO	8.4±0.2	8.4±0.3	8.1±0.2	8.0±0.3	7.4±0.4	7.4±0.5	7.4±0.3
	EIO	8.4±0.1	8.4±0.2	8.1±0.3	8.0±0.7	7.4±0.6	7.4±0.5	7.4±0.5

RBO-Rice bran oil, RBDPO- Refined palm olein, BLO-blended oil, EIO- Enzyme interesterified oil

5.3 Discussion

In this study, the frying performances of enzyme interesterified oil selected from optimization studies was evaluated. Effect of frying cycles during intermittent frying of *poori* on physical chemical and heat transfer quality of enzyme interesterified oil alongwith its native and blended counterparts are examined. Fatty acid profile influences the nutritional value of the oil. Generally, fatty acids with double bonds are labile for oxidation during heating, owing to presence of π (pi) bonds, which consequently produces degradable products. Arroyo *et al*. (1995) and Jorge *et al*. (1997) indicated that heat treatment of fat induces modifications of fatty acids with two or three double bonds. Similar observations are made by Garrido-Polonio *et al*. (1994) and Sharma *et al.* (2006). Free fatty acid, peroxide values were found to increase with an increase in frying cycles, whereas, radical scavenging activity of RBO and enzyme interesterified oil was found to decrease marginally and highly in RBDPO. Additional loss of antioxidant activity in frying experiment occurs through steam distillation resulting from the introduction of water present in food, absorption and interaction of antioxidant with food (Augustin and Berry, 1983).

There was significant ($p<0.05$) increase observed in total polar materials with prolonged frying. The discarding point of fried oil differs from oil to oil, such as the cut-point of palm olein is 25% (Sánchez-Muniz and Bastida , 2003) whereas the same for sunflower oil is 20% (Farhoosh and Tavassoli-Kafrani, 2011) fried oil. In the present case, it is clear that that during frying, the TPM reached to a discarding point of total polar material to 24% for RBO, RBDPO, BLO and EIO after 15, 21, 23 and 25 frying cycles, respectively, which clearly indicated that enzyme intesterified oil are more heat resisitant and stable.

No significant (p>0.50) change was found in oryzanol content in RBO with an increase in frying cycles upto 6. With regard to physical properties, the colour of the oil was found to increase with an increase in frying cycles. There was no significant increase in viscosity during heating or frying cycles in interesterified oil. There was no significant (p>0.05) change in sensory attributes of the *poori* fried using these oils. Therefore, enzyme interesterified oil may be considered to be a stable oil and can be used as a suitable frying medium.

General Discussion and Summary

Biotechnological approaches for modification of fats and oils is a relatively new field. Significant progress has been made in genetic engineering area for changing the composition of oilseeds and oils. Genetic modification of cotton seed oil to alter the fatty acid profile to provide high-oleic and high stearic cotton seed provided highly stable frying oil and oil for hard stock in margarine production (Liu *et al*., 2000). Genetic modification of Canola-quality Indian mustard is exploited for an alternate to canola of higher oxidative stability (Wijesundera *et al*., 2008) than canola.

The modification of fats and oils by enzymatic interesterification processes is also being exploited for value addition to oils and fats. Severeal commercial applications of microbial Lipases, plant lipases, lipases isolated from animal tissues are now exploited for biotransformations of fats and oils. In enzymatic interesterification, the reaction can be directed based on enzyme specificity towards substrates and hence can be exploited for the preparation of specific modified oils and fats or structured lipids. In chemical interesterification reactions such specificity is not seen (Mukherjee, 1990).

Oils and fats are vital constituents in our daily diet, which provide energy, essential fatty acids, and serve as a carrier of fat soluble vitamins. In India, about 80% of the edible oils used are from peanut, sunflower, safflower, rice bran, coconut and mustard. These vegetable oils are used for cooking and frying in which heat transfer through cooking medium is an important criterion. Natural oils and fats are the mixtures of different types of triacylglycerols containing different proportions of fatty acid moieties. Each oil is has characteristic fatty acid composition and triglyceride molecules. The different proportion of saturated, monounsaturated and polyunsaturated fatty acids in a triglyceride molecule play important role in physico-chemical, hydrodynamic, thermal and functional properties of the lipids. There is a wide variation in their fatty acid composition of different edible oils available in India used for cooking or frying purposes. The desired combination of fatty acids in the triglyceride molecule of fats and oils recommended by the Nutritionists and Food Technologists can not always be met from a single native oil or fat. Moreover, consumption of oil containing higher amount satutrated fatty acids could increase health hazards. However, saturated fatty acids have higher oxidative stability. But at frying/cooking temperatures these fatty acids form carboxylic acids, aldehydes and ketones. Monounsaturated fatty acids have moderate oxidative stability, whereas polyunsaturated fatty acids undergo severe degradation during frying aithough it is good for health (Brinkmann, 2000).

Therefore, efforts have been made to modify the selected oils by following suitable technique to have a stable oil with good frying stability and acceptability without removing the natural component from them.

The physical properties of oil influence its heat transfer properties during frying. The convective heat transfer coefficient is a useful parameter in characterizing heat flow across a fluid/solid interface. The relationship between oil temperature, viscosity and convective heat transfer coefficient play important role in the preparation of processed foods. This also helps in modeling of optimal processing conditions for frying. In recent times, there is a demand for blended oils for culinary uses because they provide improved thermal stability, nutritional benefits and provide to tailor made desirable properties. It is reported that solid fats of tallow and butterfat interesterified with high oleic sunflower oil and soybean oil showed improved physical and thermal characteristics (Foglia *et al*., 1993).

In the present study, enzyme interesterified oils were prepared from the blend of rice bran oil (RBO) and refined palm olein (RBDPO). Earlier studies have indicated that blends of oils containing RBO has better cholesterol lowering properties when fed to rats (Reena and Lokesh, 2007). RBO contains endogenous antioxidants, such as oryzanol while RBDPO contains beta-carotene, tocotrienols, tocopherols. Together these antioxidants may provide better stability to oils when RBO and RBDPO are blended.

The modification of oils was carried out by physical blending of two oils (RBO and RBDPO) as in proportion of 20 to 80% of each oil (BIS, 1995; Padmavathy *et al*., 2001). After preparing binary oil blends in different proportions with RBO and RBDPO, these oils are evaluated for properties having a bearing on frying characteristics of oils. The sensory evaluation of poori and odour profile of fried oils, after frying *poori* when evaluated in blended and interesterified oils. The consumer acceptance of fried *poori* prepared using these oils were also evaluated.

The change in the physical, hydrodynamic and thermal properties of rice bran oil and refined palm olein as affected by levels of saturated, unsaturated fatty acids when these oils were blended in different ratios were studied.

Response Surface Methodology coupled with CCRD was adapted for optimizing the synthesis of interesterified oil having minimum viscosity and maximum heat transfer coefficient. The interesterified oil obtained after optimization was evaluated for its physico-chemical, thermal properties and frying performances in terms of sensory attributes. Several researchers have used Response Surface Methodology to optimize reaction conditions for enzymatic interesterification reactions for different usages such as synthesis of structured lipids using ethyl caprylate, soybean oil and sunflower oil (Huang and Akoh, 1996; Xu *et al.,* 2000), production of structured triacylglycerols containing short-chain fatty acids in a a solvent-free system (Zhou *et al.,* 2001), synthesis of structured lipids based on coconut oil (Rao *et al.*, 2002).

Interesterification reaction involves hydrolysis and esterification reaction of fatty acids to produce new triglyceride species (Reyes and Hill, 1994). Synthesis of interesterified oil with immobilized 1, 3-specific lipase RM IM have shown that reaction parameters such as level of individual oil in the oil blend, reaction temperature, enzyme concentration and reaction time influence the change in proportion of TAG molecular species in the tricglyceride moiety. In the present study we noticed that RBO level in RBO-RBDPO blend and enzyme concentration separately had a positive effect on kinematic viscosity. Different researchers (Xu *et al.,* 1998; Zhou *et al.,* 2001) also found that the higher ratio of the substrate is the most important factor responsible for increasing the yield of products. The product of these two variables (RBO level in RBO-RBDPO and enzyme concentration) and (temperature and enzyme concentration) had a significant influence on increasing heat transfer coefficient at high enzyme level and long reaction time. The interaction of RBO level in RBO-RBDPO blend and reaction temperature as well as interaction of enzyme concentration and RBO level in RBO-RBDPO blend has a significant effect on kinematic viscosity. The interaction of RBO level in RBO-RBDPO blend and enzyme concentration as well as interaction of reaction temperature and enzyme concentration has a significant effect in increasing heat transfer coefficient. The interaction of reaction time and RBO level in the RBO-RBDPO blend has a significant effect on heat transfer coefficient. Earlier researcher (Huang and Akoh, 1996) have also found that the molar ratio and reaction time are important factors for enzymatic interesterification and their interaction play significant role on response variables.

During enzyme interesterification of RBO and RBDPO blend, it was observed that the melting profile of the blend as well as interesterified oil were modified. The native RBDPO showed three melting endotherms which represents polymorphic crystal forms, whereas there are two melting endograms observed in the interesterified oil. It is reported that triglycerides blends having asymmetrical distribution of fatty acids exhibits a lower melting point (Rousseau *et al.,* 1996). It is also reported that effectiveness of the interplanar packing of hydrocarbon chains is reduced when triglycerides of different chain length are blended (Grati *et al.,* 1989). The lowering of melting point in interesterified oil as compared to refined palm olein may be due to the above reason. This indicated that, melting profile may be changed due to change in stereochemical distribution of fatty acids in triglyceride moiety. The distinctive physical properties of the fats and oils depend on the types as well as the distribution of fatty acids on the triacylglycerol molecule.

The edible vegetable oils are generally used for culinary purposes, such as for cooking and frying. To tailor make the oils suitable for frying purposes one need to understand the mechanism of frying process. The mechanism of partitioning of oil into the different parts of the food materials need to be understood. Therefore, frying study was carried out by taking a potato slice as a model food materials to understand the mass transfer phenomena (oil uptake and moisture outflow).

During frying most of the oil in potato slices were retained onto the surface. Oil moves in fast initially into the product because of the large void spaces created in the crust but as the crust gets filled up, movement of oil slows down and ultimately stops. Holding the product at elevated temperature (100-180°C) restricted the oil migration from the surface into the structure due to delayed onset of vacuum (or oil migration into the structure) for its subsequent removal by adopting suitable technique, such as wipe up the outer surface with a absorbent paper by maintaining the fried food at frying temperature.

Frying causes several undesirable physical and chemical changes such as hydrolysis, oxidation as well as thermal decomposition and thermal polymerization in oils/fats (Blumenthal, 1991; Houhoula *et al.,* 2003). Therefore, the frying performances/frying quality as well as sensory attributes of the oils developed through modification was studied. The suitability of the blended and interesterified oils were used as a frying media. The frying quality of these modified oils were compared with that of control oils (RBO, RBDPO). Different physical, chemical, thermal, hydrodynamic properties and sensory parameters of the modified oils before and after frying of poori were evaluated. Enzyme interesterified oils are stable than its native and blended oils as supported with the chemical, hydrodynamic (density, surface tension), proton NMR and thermogravimetric analysis. Thermal properties (heat transfer coefficient) decreases with intermittent frying as the viscosity increases with time of frying. Oil uptake increases with intermittent frying time because oil with higher viscosity increases contact between food and oil (thick boundary layer) resulting in slow release from food into the oil surrounding it.

Fried oil causes health hazards after reaching its total polar material to 24% (Stier, 2001), it needs to be discarded. Poori was fried in the intermittent mode till the fried oil reaches to its discarding point. It was observed that during frying of poori, the TPM reached to a discarding point of 24% for RBO, RBDPO, BLO and EIO after 15, 21, 23 and 25 frying cycles, respectively. Therefor it can be concluded that the interesterified oil is more resistant than the corresponding blended oil and native oils. This also confirmed suitability of blended as well as interesterified oil suitable for frying.

These studies indicated that the enzyme interesterified oil containing RBO and RBDPO with improved physical, hydrodynamic and thermal properties, thermo-oxidative stabilities for preparing fried product opens up the scope for increased use of rice bran oil and refined palm olein for cooking purposes in India. The presence of endogeneous antioxidants such as oryzanol, tocotrienol in rice bran oil and β-carotene in refined palm olein further helps in getting a stable oil which has also nutritional benefits.

SUMMARY

- Interesterified oils were prepared from the blend of rice bran oil and refined palm olein by using immobilized 1, 3- specific lipase from *Rhizomucor miehei* as a catalyst for transesterification reactions.
- Enzyme-mediated interesterification modulated the physical and thermal properties of oil.
- The rheological and thermal properties of oils influenced by different factors such as amount of individual oil in the blend, reaction temperature, enzyme concentration and reaction time.
- The enzymatic interesterification minimized hydrodynamic (kinematic viscosity), maximize thermal (heat transfer coefficient) properties and improved themo-oxidative stability of oils.
- Response surface methodology was used to optimize reaction variables and predict conditions to minimize hydrodynamic and maximize thermal properties of oils.
- Enzyme interesterified oil showed lower melting temperature than that of refined palm olein and oil blends containing rice bran oil and refined palm olein.
- Interesterified oils showed higher heat transfer coefficient as indicated by lesser time to reach the frying temperature and higher heat transfer coefficient. Blended and enzyme interesterified oil blends can provide a better oil medium for frying suitable for food industries which utilise frying operations.
- The change in thermal properties in oil blends may be due to change in molecular rotation of fatty acids in the triglyceride moiety, whereas

changes observed in interesterified oil blends could additionally be due to change in molecular species/conformational changes of triacyglycerol species in oils.

- During frying of potato slices most of the oil retained onto the surface. Oil moves inside the core of the product initially because of the large void spaces created in the crust but as the crust gets filled up, movement of oil slows down and ultimately stops. Holding the product at an elevated temperature range of (100-180°C) restricted oil migration from surface into core of structure due to delayed onset of vacuum, which can be subsequently removed by using absorbants.
- Heating in open pan fryer caused deterioration in control oil and blended oil faster than that observed in enzyme interesterified oils.
- Enzyme interesterified oils are stable than oils as observed with the chemical, hydrodynamic (kinematic viscosity, density, surface tension), proton NMR and thermogravimetric analysis.
- Thermal properties of oils (heat transfer coefficient) decreases with intermittent frying as the viscosity increases with time of frying.
- Oil uptake in *poori* increased with intermittent frying time as a result of increased viscosity which, in turn, increase contact between food and oil (thick boundary layer) resulting in slow release of oil from food into its surroundings.
- Thus biotechnologically modified oils are better suited for frying operations

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Research Publications

1. Sukumar Debnath, N. K. Rastogi, A. G. Gopala Krishna and B. R. Lokesh (2009), Oil partitioning between surface and structure of deep-fat fried potato slices: A kinetic study, *LWT-Food Science and Technology*, 42, 1054-1058

2. Sukumar Debnath, Sriram Kumar Vidyarthi and R. Paul Singh (2010), Impact of blending of frying oils on viscosity and heat transfer coefficient at elevated temperatures, *Journal of Food Process Engineering,* 33, 144-161.

3. Sukumar Debnath, Maya Prakash and B. R. Lokesh (2010), Lipase mediated interesterification of oils for improving rheological, heat transfer properties and stability during frying, *Food and Bioprocess Technology: An International Journal (Accepted for Publication, DOI: 10.1007/s11947-010-0485-3*)