Encapsulation of Edible Lipids for Functional Food Applications: Process Development and Characterization

By

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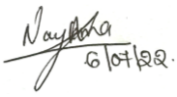

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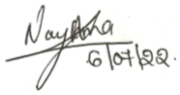

Nayana N.

Dedicated to

My family and beloved ones who taught me about dreams

and how to catch them….

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ABBREVIATIONS

SFA	SATURATED FATTY ACIDS
MUFA	MONOUNSATURATED FATTY ACIDS
PUFA	POLYUNSATURATED FATTY ACIDS
WHO	WORLD HEALTH ORGANISATION
LDL	LOW DENSITY LIPOPROTEIN
EPA	EICOSAPENTAENOIC ACID
BHA	BUTYLATED HYDROXYANISOLE
BHT	BUTYLATED HYDROXYTOLUENE
PG	PROPYL GALLATE
TBHQ	TERTIARY BUTYL HYDROQUINONE
LD_{50}	LETHAL DOSE
ADI	ACCEPTABLE DAILY INTAKE
FSSAI	FOOD SAFETY AND STANDARD AUTHORITY OF INDIA
USFDA	UNITED STATES FOOD AND DRUG ADMINISTRATION
CODEX	CODEX ALIMENTARIUS COMMISSION
SFO	SUNFLOWER OIL
CO	COCONUT OIL
FSO	FLAX SEED OIL
RBO	RICE BRAN OIL
RPO	RED PALM OIL
OL	OLIVE OIL
SFO+	BLENDED SUNFLOWER OIL
CO+	BLENDED COCONUT OIL
FSO+	BLENDED FLAX SEED OIL
RBO+	BLENDED RICE BRAN OIL
OL+	BLENDED OLIVE OIL
FFA	FREE FATTY ACID
PV	PEROXIDE VALUE
PAV	PARA ANISIDINE VALUE
PSO	POMEGRANATE SEED OIL
BPSO	BLANCHED POMEGRANATE SEED OIL
TPC	TOTAL PHENOLIC CONTENT

TCC	TOTAL CATECHIN CONTENT
TIFAC	TECHNOLOGY INFORMATION FORECASTING ASSESSMENT COUNCIL
EE	ENCAPSULATION EFFICIENCY
WPC	WHEY PROTEIN CONCENTRATE
SC	SODIUM CASEINATE
O/W	OIL IN WATER EMULSION
W/O	WATER IN OIL EMULSION
MD	MALTODEXTRIN
DHA	DOCOSAHEXAENOIC ACID
TAG	TRIACYLGLYCERIDES
EFA	ESSENTIAL FATTY ACIDS
CVD	CARDIOVASCULAR DISEASES
TMOP	TECHNOLOGY MISSION ON OIL SEEDS AND PULSES
SD	STANDARD DEVIATION
RDA	RECOMMENDED DAILY ALLOWANCE
AOAC	ASSOCIATION OF ANALYTICAL COMMUNITIES
SD	SPRAY DRYING
GA	GUM ARABIC
FAME	FATTY ACID METHYL ESTERS
a_w	WATER ACTIVITY
CI	CREAMING INDEX
TO	TOTAL OIL
SO/EO	SURFACE OIL
TPA	TEXTURE PROFILE ANALYSIS
TSS	TOTAL SOLUBLE SOLIDS
SGF	SIMULATED GASTRIC FLUID
SIF	SIMULATED INTESTINAL FLUID
FTIR	FOURIER TRANSFORM INFRARED SPECTROSCOPY
BD	BULK DENSITY
TD	TAPPED DENSITY
PDI	POLY DISPERSITY INDEX
SEM	SCANNING ELECTRON MICROSCOPY

SYMBOLS

β	Beta
μ	Micro
-	Negative
+	Positive
=	Equal to
~	Approximate
h	Hour
min	Minute
cm	Centimetre
μm	Micrometre
g	Gram
mg	Milligram
μg	Microgram
%	Percentage
°C	Degree Celsius
mL	Millilitre
μl	Microlitre
M	Molar
mM	Millimolar
μM	Micromolar
N	Normality
nm	Nanometre
mV	Milli Voltage
ω	Omega
meq/kg	milliequivalent/Kg
ppm	Parts per million
V	Volts
w/w	Weight / weight
s	Second
N	Newton
ε	Porosity

PREFACE

Food is consumed not only to satisfy hunger, but also to supplement necessary nutrients for humans, at the same time to prevent nutrition-related diseases and to improve physical and mental well-being. The household and industrial market indicates a steady increase in the demand for healthy oils, with added health benefits e.g., optimum fatty acid profile and bioactive compounds, though the amount of fat that is consumed daily is a topic of controversy. Deficiency due to lack of balanced fatty acids in the diet, especially polyunsaturated fatty acids (PUFA), is one of the significant global nutritional problem. According to the Food and Nutrition Board's Committee on diet and health, the recommended ratio of SFA (saturated fatty acid)/MUFA (monounsaturated fatty acid)/PUFA (polyunsaturated fatty acid) in edible oils is 1:2:1 and that of omega-3/ omega-6 fatty acid is 0.05. The blending of edible oils is considered as the least expensive approach that results in desired fatty acid composition. However, PUFA is susceptible to oxidative degradation due to its high degree of unsaturation. Various encapsulation techniques have been proposed for improving oxidative stability of PUFA, by converting oils in to powder or by encapsulating the oil using a carrier matrix. The oxidative stability is one of the key factors in determining the use of vegetable oil in foods and industrial application.

Considering the microencapsulation approach, edible oils can be converted in to powder using appropriate techniques to improve storage stability and application, besides facilitating easy handling and transport. Therefore, converting oil to powder can be an innovative and consumer-friendly approach to improve the nutritional and oxidative stability of oils and to widen their applications in the culinary/health/functional food and nutraceutical sectors. Various encapsulation techniques have been employed for converting oils into powder form by utilizing a wide range of carrier or wall materials. The commonly used microencapsulation techniques include emulsification, spray drying, and freeze drying. Among the above, spray

drying is considered as an industry-friendly drying-cum-encapsulation technique. Choosing an ideal wall material is the key to achieve optimal encapsulation efficiency. The general traits expected of a wall material include, but are not limited to, bland flavour, high solubility, emulsification ability, film-forming, and drying properties. Diverse wall materials have been used for encapsulating oils, including gums (gum arabic, xanthan), proteins (whey protein, soy protein), polysaccharides, and modified starches. **'Oil to powder technologies for food' is one of the areas identified under the 'Technology vision document 2035' prepared by Technology Information, Forecasting and Assessment Council (Technology vision 2035, TIFAC, 2015). The document emphasises the need for targeted research in this area for technology development**. With this back ground, we focussed on developing oil blends with optimum fatty acid profile enriched with bio actives, and development of powdered oils/fats (vegetable oils, butter, ghee, and red palm olein etc.), by microencapsulation using different combinations of wall materials. The resulting encapsulated products can be used for regular culinary purpose, as well as, as an ingredient in various food products, for providing nutritional benefits and functionality.

Chapter 1 gives a general introduction and review of literature about blending of oils, various encapsulation techniques, spray drying, and application of encapsulates in nutraceutical and functional food sectors.

Blending of oils in appropriate proportions could result in a product with improved fatty acid composition, enriched nutritional content and improved shelf stability. **Chapter 2** deals with the optimisation of blending of β-carotene rich RPO with different edible vegetable oils. The ratio has been finalised according to the government regulations and GC-MS/MS profiling to obtain the optimum fatty acid composition. The thermal, oxidative and storage stability of the blended oils in comparison with the base oils has been characterized and β-carotene content was assessed. It was found that RPO blended with other oils have improved shelf and thermal

stability. Since the blended oils exhibited greater stability to oxidation, blending can be opted as a method for reducing the oxidation levels of oils, and at the same time enhances the nutritional quality.

Physical states of oils sometimes make them inconvenient to handle. If oils/fats can be converted in to powder form, it may improve convenience for usage during food preparations, and improve the shelf stability. Oil in the form of microencapsulated powders have been used to a limited extend, mainly in food and non-food applications. The conversion of oil into powder form will also facilitate easy incorporation in nutritional products, functional foods and nutraceutical products. **Chapter 3** focus to deliver an encapsulated oil blend in powder form to deliver PUFA and oil soluble vitamins with balanced fatty acid composition. The selected blended oils had improved fatty acid composition, and enriched beta carotene content. The peroxide value (PV) and free fatty acids (FFA) values of the component oils, during the shelf life studies, were less than the reported rancidity values, which indicated that quality of the oil was within the prescribed limits. The carotene content of the blend was found to be 270 ppm. The GC-MS/MS profiling of the oil blend confirmed its improved fatty acid composition. Further, we carried out optimization of the encapsulation process of RPO/FSO blends. The optimized conditions presented a core-to-wall ratio of 1:2, oil payload of 34 % and inlet temperature of 180 °C. The developed encapsulate was utilized as a partial fat substitute and carotenoid fortificant in bakery products, which revealed that the developed oil powder can act as a butter replacer, without affecting the sensorial properties of the product. The application studies met the RDA of β- carotene. The possibility of replacing synthetic fats with encapsulate has been hence established.

Increasing the oil pay load from 50 % to 70 %, with fat as the core component has been less addressed in the previous chapter. Inorder to tackle this, an attempt to improve the oil pay

load through the development of emulsion has been tried. Trials has been carried out to optimize the wall material composition and homogenisation conditions to fabricate a kinetically stable emulsion. Conversion of fat encapsulted higher oil payload emulsions into powder is a novel attempt, both in the aspect of fat encapsulation as well as in terms of utilizing spray drying method for converting higher oil pay load emulsions into powder. Hence, we attempted the preparation of encapsulated powder with better yield and encapsulation efficiency by optimising the process conditions of spray drying. **Chapter 4** deals with the optimization of process conditions for fabricating fat encapsulates (butter/ghee) with higher oil pay load for food and health care applications. In order to develop a fat substitute in the form of a powder, it is important to fabricate a stable emulsion without affecting the inherent properties of the fat. Hence, this chapter is divided in to two subchapters **4.a** and **4.b**, where in 4.a. focused on developing a stable emulsion with higher oil payload and 4.b. dealt with conversion of the stable emulsion to fat encapsulated powder. The optimization was carried out in terms of oil payload, wall material composition and homogenization conditions. The oil load was increased from 50 % to 70 % (dry basis) at a total soluble solids (TSS) content of 30 %. The optimized emulsions were kinetically stable even after 24 h of storage at ambient conditions (29 to 35 °C). Thermal analysis (DT-TGA) and FTIR-ATR analysis of the emulsions revealed that, core component (oil) was well protected when whey protein was used as the wall material. These thermally and kinetically stable emulsions were further taken for encapsulation studies.

Chapter 4.b. deal with the development of encapsulated lipids (butter/ghee), for product development and application level studies. The stable emulsions fabricated using 50 %, 60 % and 70 % of ghee, from the previous chapter was converted in to powder using spray drying. The process conditions for spray drying were optimized with an aim to obtain better encapsulation efficiency and yield. The fat encapsulates were characterized in terms of

powder properties (flow-ability, moisture content, water activity, solubility etc.), and morphology (SEM, fluorescent microscopy). In-vitro release and core release studies were conducted under simulated environments. Besides this, stability (oxidative and thermal) and food application studies were also carried out. Encapsulation efficacy evaluation confirmed better incorporation of lipids in the system. The in-vitro release analysis of oil under simulated oral and gastric conditions ensured very low release kinetics in mouth and sustained release in gastro-intestinal fluids at different pH values. The morphological characterisation of emulsion using SEM and fluorescent microscopy confirmed better encapsulation of oil. Encapsulates were further analysed to understand it's suitability to get utilized in various food applications. When the oil load was increased from 50 % to 70 %, at optimized conditions, the emulsion stability did not varied, and the powder form of this showed better stability to oxidation and heat. The simulated treatments confirmed the optimal release behaviour, thus encapsulates could be used to deliver thermally stable bioactive compounds.

Thus the present study advocate blending of edible oils rich in nutritionally important compounds and phytochemicals to modify fatty acid profile which can be fine-tuned to deliver the optimum fatty acid composition required for the maintenance of health. Blending also improves the oxidative, shelf and thermal stability of oils as compared to the same properties of the individual oils. Blends in the form of encapsulated powder could be used for delivering lipids with improved nutritional and bioactive properties. This powdered oil could be used as conventional fat replacer which could be exploited further. Edible lipids in the form of microencapsulates as demonstrated in the study using ghee, offers a very promising platform in the convenient food sector. The higher oil payload and the encapsulation efficiently achieved in the present study is a positive drive in this direction.

Chapter 1

Introduction and Review of literature

1. Introduction

Consumer's expectations and demands for processed foods has changed considerably over the years as the foods that are reported to directly contributes to health and preventive management of diseases are in high demand (Molleta and Rowland, 2002). Food is consumed not only to satisfy hunger but also supplement necessary nutrients for humans, at the same time prevent nutrition-related diseases and improve physical and mental well-being. The consumption pattern of the modern consumers and their concern about health, contributes to the high demand for foods that contain natural bioactive or functional ingredients that increase the nutritional value and health benefits of food and functional foods play an important role (Roberfroid, 2007). Foods containing plant extracts with antioxidant properties, polyunsaturated fatty acids, probiotics, vitamins, and minerals are the main targets of this consumption trend. However, many of these components are unstable under normal conditions limiting their application. Thus, it is necessary to use techniques that can guarantee the stability of these functional constituents and allow their application in diverse food matrices. Various sectors of the food industry have a demand for the enrichment of foods with functional compounds. The major challenge in the use of enriched foods is to obtain stable products and maintain their functional properties during processing, storage, until consumption. The development of novel functional food products open up an increasingly challenging opportunity and emerging technologies opens up new avenues for developing functional foods.

Dietary lipids are the building blocks of membranes. They contributes a major role in growth and maintenance of good health as carriers of fat soluble vitamins and antioxidants and also as mediators of pro- and anti-inflammations (Sharifi-Rad et al., 2020; Ganesan et al., 2014). In foods, lipids are mainly found in the form of triacylglycerides (TAG), (Fennema, 1996) and

are composed of triacylglycerol units with one glycerol molecule and three fatty acids (Figure-1).

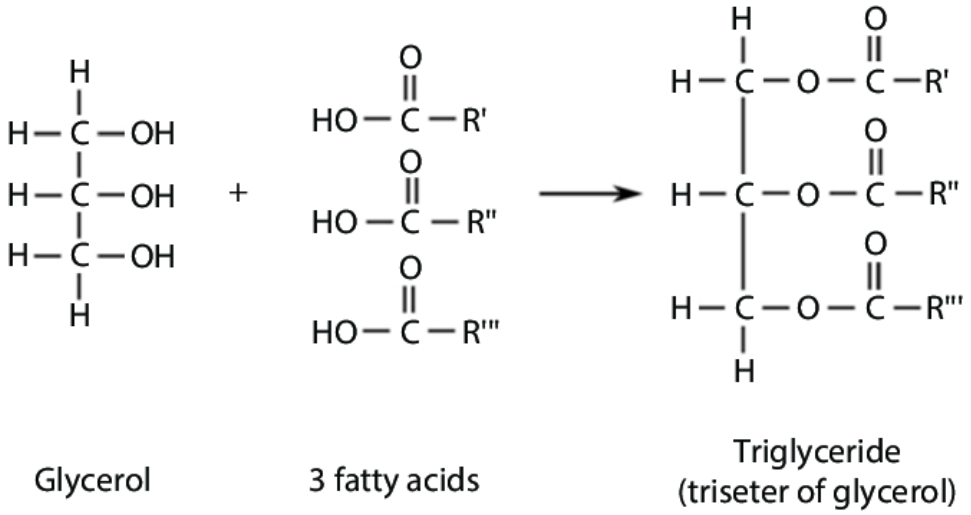


Figure: 1.1 Triglyceride formation

The term “Fat” is generally used for solid lipids, whereas “Oil” is used for liquid lipids. Based on the structure of fatty acids attached to the glyceride backbone, the cooking oil can be considered as that contain saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The term ‘saturated’ indicates that the maximum possible number of hydrogen atoms are bonded to each carbon in the molecule. The molecule is very stable (usually solid at room temperature) and hard to break up that allows it to store in circulatory systems. Saturated fats are thus usually considered unhealthy fats. Unsaturated fatty acids have one or more carbon-carbon double bonds. They are divided into polyunsaturated fatty acids and monounsaturated fatty acids. Fatty acids containing more than one carbon-carbon double bond is known as polyunsaturated fatty acids are found in relatively minor amounts. Fatty acids containing one double bond is known as monounsaturated fatty acids. Dietary fats play a major role in providing desirable physical, nutritional, organoleptic properties to the food. Vegetable oils used for culinary purpose and

processed foods are the main source of fat in the diet. Cooking oils can be categorized into three groups, viz. that containing SFA, MUFA and PUFA. Examples of some of the major sources of fatty acids are listed below in Table- 1.

Table – 1.1 Common fatty acids and its food sources

Common name	Type of fatty acid	Systematic name	Food Sources
Lauric acid (C12:0)	**SFA**	n-dodecanoic acid	Coconut oil, palm kernel oil, nutmeg oil
Myristic acid (C14:0)		n-tetradecanoic acid	Palm kernel oil, Coconut Oil
Palmitic acid (C16:0)		n-hexadecanoic acid	Palm oil, Olive oil,
Stearic acid (C18:0)		n-octadecanoic acid	Palm oil , Cocoa butter, animal lipids
Behenic acid (C22:0)		n-docosanoic acid	Moringa olefera seed oil, Peanut oil, peanut butter, wheat germ oil
Palmitoleic acid (C16:1)	**MUFA**	cis-9-hexadecenoic acid	Macadamia nuts
Oleic acid (C18:1)		cis-9-octadecenoic acid	Olive oil, avocado oil, sunflower oil, canola, mustard, rice bran, and groundnut oils
Erucic acid (C22:1)		cis-13-docosenoic acid	Rapeseed oil, Mustard Oil
Linoleic acid (C18:2)	**PUFA**	cis-9-, cis-12-octadecadienoic acid	Sunflower, safflower, soybean, corn, and canola oils as well as nuts and olive oil
Linolenic acid (C18:3)		cis-9-, cis-12-, cis-15-octadecatrienoic acid	Flaxseed oil, rapeseed oil, soybean oil , Perilla seed oil
Arachidonic acid (C20:4)		5,8,11,14-eicosatetraenoic acid	Dietary animal sources

In the present scenario, health aspect of fats and oils has gained more attention as the consumption of fats and oil are reported to be linked with lifestyle diseases e.g., circulatory or nervous system associated (Dhayani et al., 2018). WHO (2017), reported 17.7 million deaths globally due to cardiovascular diseases which is projected to reach 23.3 million in future which has generated more attention to the consumption pattern of fats and oil. According to various clinical and animal studies, it is proved that saturated fat increases the blood and plasma cholesterol level (Sura et al., 2020). Consumption of a diet that is rich in PUFA and MUFA decreases low-density cholesterol levels and increases high-density lipoprotein cholesterol, leading to lowering the risk of coronary heart diseases (Froyen and Burns-Whitmore, 2020; Ference et al., 2017; Manchanda and Passi, 2016). As reported by the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans, 2010, MUFAs are beneficial as they increase esterification of cholesterol in the liver and thereby reducing the free cholesterol pool and increasing receptor mediated uptake of LDL cholesterol, thus resulting in a decrease in blood cholesterol levels (Pattnaik et al., 2020). It can be seen from the studies that higher consumption of PUFA has an adverse impact on the health by weakening the capability of the antioxidants in the human body to tackle free radicles, thereby increasing the risk of aging, cardio-related issues, diabetes, and cancer (Choudhary and Grover 2013; WHO 2008; Vani, Laxmi, and Sesikeran 2002). Therefore, intake of balanced fatty acids are very important.

Vegetable oils, in general differ in fatty acid composition. The nutritional value of edible oils depends on the fatty acid profile, degree of unsaturation, arrangement of fatty acid in triglyceride structure. There is no natural lipid that directly provides all fatty acid in the optimum amounts as required (Mozaffarian et al., 2010). Tailoring the fatty acid composition and transforming them into various forms depending on the end use is seeking more attention these days. Lack of balanced fatty acids in the diet, especially polyunsaturated fatty acids

(PUFA), is one of the significant nutritional problems globally (Menina et al., 2018, Nayana et al., 2021). According to the Food and Nutrition Board's Committee on diet and health, the recommended ratio of SFA (saturated fatty acid)/MUFA (monounsaturated fatty acid)/PUFA (polyunsaturated fatty acid) in edible oils is 1/2/1, and that of omega-3/omega-6 is 0.05 (National Research Council 1989). According to WHO, the total fat intake should be 30 –35 %, to meet the total energy as SFA <10%, MUFA 10%–14%, PUFA 6%–11% (WHO 2008). However, to maintain good heart health, ω-6 and ω-3 ratio must be between 1:1 and 4:1 (Mishra and Manchanda 2012). A balanced ratio of MUFA, PUFA as well as essential fatty acids like ω-6 and ω-3 is essential to maintain a modulated lipid profile in the human body.

Most vegetable oils have limited technological application in their original forms because of their specific chemical and physical properties. Oxidative rancidity during transportation and storage, the nutritional quality and fatty acid composition, content of antioxidant bioactive, thermal stability are the major factors that determines the acceptability of vegetable oils. Food processing industries demand lipids with desirable and technological properties for lipids that can impart unique textural and sensorial properties to the processed food products. The search for customized lipids for various food, health and nutritional application with enhanced nutritional profile, technological properties, storage stability and convenience, has led to the research and development of blended oil, oil powders, or gels etc.

1.2 Limitations linked with oils and fats during storage and transportation

The limitation of oils and fats during storage, transportation and handling is due to their normal physical state which makes it inconvenient to handle during storage and transportation. Spillage, wastage are some issues faced by the industries during transportation (EPA, 1999). Most of the oils are unstable under processing and storage conditions due to their sensitivity to light and heat, which limits their application in the food industry. Different

cooking methods and temperatures cause deterioration of the oils due to oxidative stress (Gosh et al., 2019). Auto oxidation, photo oxidation and hydrolysis may cause the stability changes in oils and fats on storage (Roiaini et al., 2015). Hence, it is necessary to protect the oils to improve their stability during these handling, processing, and storage conditions (Mohammed et al., 2020) .

The major thermal degradation products of lipoids are alkanes, alkenes, aldehydes, ketones, alcohols, and aromatic compounds. According to the studies, poly unsaturated fatty acids are more prone to thermal degradation (Fortes and Baugh, 2004; Santos et al., 2004; Nik et al., 2005). Intermittent heating and cooling of oils cause degradation faster than continuous heating (Das et al., 2013).

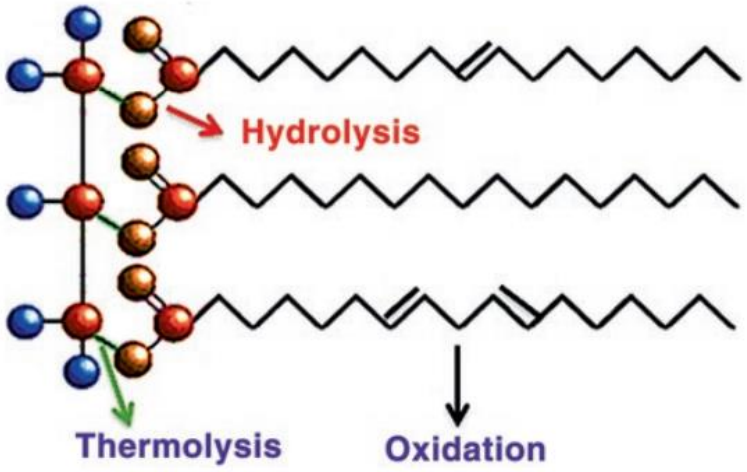


Figure: 1.2 Schematic representation of thermal and oxidative vulnerable positions of vegetable oils (Gnanasekaran and Chavidi, 2018)

Oxidation is the most important reaction of vegetable oils, resulting in chemical and physical properties. Oxidative stability is known as the resistance to oxidation under defined conditions. The presence of unsaturation in the triglyceride molecule due to C=C bonds functions as the active sites for various oxidation reactions. Therefore the fatty acid

composition gives an indicative information about oxidative stability of oil (Gnanasekaran et al., 2018). The formation of oxidation products follows a chain reaction consisting of initiation, propagation, branching and termination. Oil degradation process initiate with primary oxidation that yields hydro peroxides as primary oxidation products. These primary oxidation products degrade to secondary oxidation products (Gomna et al., 2019).

The different steps in oxidative degradation:

Initiation or induction	$RH \rightarrow R\bullet + H\bullet$
Propagation	$R\bullet + O2 \rightarrow ROO\bullet$
	$ROO\bullet + RH \rightarrow ROOH + R\bullet$
Branching	$ROOH \rightarrow RO\bullet + \bullet OH$
	$RO\bullet + RH + O2 \rightarrow ROH + ROO\bullet$
	$\bullet OH + RH + O2 \rightarrow H2O + ROO\bullet$
Termination	$ROO\bullet + ROO\bullet \rightarrow ROOR + O2$
	$ROO\bullet + R\bullet \rightarrow ROOR$
	$R\bullet + R\bullet \rightarrow R\text{-}R$

The main oxidation of lipids occurs through a free radical chain propagation reaction, in which peroxides and hydroperoxides are formed from fatty acids and oxygen, which is known as the auto-oxidation process. These compounds are quite unstable, so they can be broken, giving rise to more free radicals and generating a chain reaction. During this initiation lipid free radicals are formed (R•). Due to the presence of catalysts the free radicals react with oxygen to form peroxy radical (ROO•). This peroxy radical react with another lipid molecule and form hydro peroxides (ROOH + R•). This hydro peroxides propagate oxidation

process and react with more oxygen to form new hydro peroxides (RO• + •OH). Hydro peroxides are also shown to act as pro-oxidants (Porter et al., 1995; Pawlak and Mucha, 2003; Lubis et al., 2015; Walallawita et al., 2016; Gomna et al., 2019).

Lipid oxidation lead to the formation of unhealthy compounds such as free radicals and reactive aldehydes and reduces the nutritional value of lipids. However, lipid oxidation will also result in significant changes in the sensory properties including odour, flavour, colour and texture (Jacobsen et al., 2019). Therefore as an industrial point of view cost effective and efficient techniques to improve the shelf stability of lipids are in demand.

1.3 Methods for shelf life enhancement

Lipid oxidation is highly complex process that depends on various factors including light, temperature, and concentration of oxygen. The presence of phospholipids, anti-oxidants, pro-oxidants, metal ions, enzymes, mechanical processes, and processing conditions were some other factors that influence the rate of the reaction (Singh and Gandhi, 2018). Vegetable and marine oils oils rich in PUFA are prone to oxidative deterioration due to the presence of unsaturation (Bakry et al., 2016). Various techniques that were attempted to address the quality issues associated with edible oils include usage of antioxidants (both synthetic and natural), bio actives, blending, encapsulation, and usage of different packaging materials. Blending and encapsulation are two technologies which need more focus as they are more practical, economical and efficient. Blending of oils in appropriate proportions could result in a product with improved fatty acid composition, enriched nutritional content and improved shelf stability. At the same time microencapsulation is the process of enveloping one substance, for controlled release, increased stability, improved nutritive value, and better appearance of products (Pattnaik and Mishra, 2021). Conversion of liquid to easy-to-handle dry powders, gels, or beads are the inspirations for the utilization of microcapsules. The

different types of microcapsules and microspheres are produced from a wide range of wall materials like carbohydrates, proteins, and gums (Adelmann et al., 2012; Bakry, et al., 2016; Albert et al., 2017; Pattnaik et al., 2021).

Here, the use of antioxidants, blending and encapsulation is discussed as mean to improve the desired properties

1.3.1 Antioxidants

Antioxidants are the primary agents used by manufacturers to provide acceptable quality to edible oils. Quite a number of natural and synthetic preservatives are available for food preservation. Fat soluble vitamins act as a natural antioxidant preservatives, in which vitamin-A, tocopherols and tcorienols are reported to be effective in preventing the oxidative damage. Plant polyphenols in edible oil preservation is a new area which is showing a great potential to bring a healthier and greener future for edible oil (Fan et al., 2015).

1.3.1.1 Synthetic antioxidants

Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) and Propyl gallate (PG) and Tertiary Butyl Hydroquinone (TBHQ) have been permitted as synthetic antioxidant preservatives in edible oils and other different food products that contain oil or fat (Tapera, 2019). Synthetic antioxidants have been tested for safety and approval for use in food at low concentrations on the basis of complex toxicity studies as required by the regulatory authorities. An antioxidant should have two conditions to be considered as safe: its LD_{50} must not be less than 1,000 mg/kg body weight, and the antioxidant should not have any significant effect on the growth of the experimental animal in long-term studies at a level 100 times greater than that proposed for human consumption (Lehman et al., 1951; Mostafa et al., 2013). The Acceptable Daily Intake (ADI) levels of synthetic antioxidants are mentioned in Table- 2

Table- 1.2 Regulation of synthetic antioxidants and their Acceptable Daily Intake levels (ADI)

Antioxidant	**E- number**	**Max level (%)**	**ADI (**mg/kg body weight)
BHA	320	0.02	0–0.5
BHT	321	0.02	0–0.3
PG	310	0.01	0–1.4
TBHQ	319	0.02	0–0.7

*Mikova et al., 2001; Shahidi, 2005; The Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2003.

Although BHT, BHA and TBHQ are known to be effective preservatives, several adverse health reactions have been reported to be associated with BHA, BHT and TBHQ (Race, 2009; Tapera et al., 2019). It is reported that the synthetic antioxidants are associated with dermatitis, vasomotor rhinitis, headache, flushing, asthma, conjunctival suffusion, allergies and angioedema. Haas and Levin (2012) noted that BHT irritates the liver and kidneys in humans. According to the studies of Lanigan and Yamarik (2002), BHT induces carcinogenesis on rat's liver, kidney and lung. Some studies have proved BHA and BHT to be cytotoxic and induce carcinogenicity (Saito et al., 2003; Verhagen et al., 1991; Sarafian et al., 2002; Farag et al. 2003). Therefore, the consumers are looking for natural antioxidants in place of synthetic antioxidants for application in edible oil to improve oxidative stability during storage, transportation and processing.

1.3.1.2 Natural antioxidants

Use of natural plant extracts with antioxidant activities are advocated in various edible oils to overcome the adverse effects associated with synthetic antioxidants. The antioxidant activity is determined by the number of hydroxyl groups and aromatic ring substituents present in the phenolic compounds (Olszowy, 2019).

Tocopherols (Vitamin E) are plant derived antioxidants that are in wide use (Tapera et al., 2019). α-Tocopherol at about 100 ppm concentration exhibits the highest antioxidant effect in oils (Martin-Rubio et al., 2018; Mishra et al., 2020). Carotenoids were found to retard oil oxidation, 20 ppm of beta-carotene can enhance oxidative stability effectively (Martin-Rubio et al., 2018; Mohanan et al., 2018; Eggersdorfer & Wyss, 2018). Sesame oil has a special and strong antioxidant called sesamol, which was widely used for the preservation of many edible oils (Mostafa et al., 2013). Oryzanol in rice bran oil is reported to be an excellent antioxidant (Bumrungpert et al., 2019)

There are quite a variety of alternative oxidative inhibitor compounds derived from plant polyphenols. The major categories of polyphenols include phenolic resin acids, flavonoids, stilbenes and lignans (Tan et al., 2017). Phenolic compounds of plant origin have attracted considerable attention due to their antioxidant and antimicrobial activity (Bubonja-Sonje et al., 2011). Abd-Elghany et al., (2010), used olive waste cake extract to improve oxidative stability of sunflower oil. Taghvaei et al., (2015), encapsulated the olive leaf extract and used in soyabean oil as a natural antioxidant. In another study Al-Bandak et al., (2011) used Majorana syriaca extract to protect bulk corn oil against oxidation. A recent study by Urbancic et al., (2014) and Samotyja and Malecka (2010) showed the usage of Rosemary extracts were efficiently delay both primary and secondary oxidative changes in soybean oil. Subsequent research by Kreivaitis, Gumbyte, Kazanav, Padgurskas, and Makarevieiene

(2013) investigated sage and thyme extracts as natural antioxidants for rapeseed oil and it effectively slowed down the deterioration of sunflower oil during deep-frying of potatoes. Myricetin, Catechin, Genistein , and Caffeic acid were used in Linseed oil by Michotte et al., (2011). These phenolic compounds exhibited superior antioxidant properties in edible oils during storage.

Spice essential oils and some of its components have been reported to possess very good antioxidant activities. The ginger essential oil components b-bisabolene, zingiberene, a-curcumene and b-caryophyllene, d-limonene, b-pinene, sabinene present in black pepper essential oil is reported to exhibit antioxidant potential (Amorati et al., 2013). The study done by Chandran et al., (2017), reveals that essential oil from black pepper and ginger could be promoted as a natural antioxidant, to improve the shelf stability of coconut oil. Yang et al., (2016), suggested that the antioxidant potential of rosemary extract may help in extending the oxidative stability of oil.

The major drawback that are reported with natural antioxidants are their sensitive nature to processing temperatures, availability and cost effectiveness. Therefore, the possibility of usage of alternative stable methods are welcomed. Blending is such a technique which uses the potential of two or more edible oils, and can be used as an alternate technology to increase the shelf life.

1.3.2 Blending

Blending is considered as a natural method for shelf life enhancement of edible oils (Hashempour-Baltork et al., 2016). Edible vegetable oil blends represents an admixture of any two or more edible vegetable oils where the proportion by weight of any edible vegetable oil used in the admixture is not less than 20 per cent (FSSAI, 2020). Though edible vegetable oil blends are regulated internationally, neither specific standards nor any prohibitions are

imposed for blended edible vegetable oil (USFDA, CODEX STAN 33-1981, Rev. 1-1989). Vegetable oils have their own set of unique non-glyceride component (ex: rice bran oil has oryzanol and olive oils have polyphenols) and blend of two or more vegetable oils is recommended in order to get all the fatty acids in a balance & synergy of minor non glycoside component. Nutrition of two oils can be incorporated in one as the benefits of minor components and better balance of saturated to unsaturated fatty acids is incorporated in single oil (CODEXSTAN 33-1981, Rev. 1-1989). The blending of edible oils is considered as the least expensive approach that results in desired fatty acid composition (Srivastava et al., 2016). PUFA is susceptible to oxidative degradation due to its high degree of unsaturation, thus blending of PUFA rich oils to a saturated oil will increase the stability (Priol et al., 2019).

1.4 Methods for blending of edible oils

Vegetable oil blending has been a common acceptable practice in many countries. Blending of vegetable oils have been attempted to improve oxidative stability, thermal stability and optimum fatty acid composition. Blending can be practiced with two or more edible oils. The blending done with two edible oils is known as binary blending and blending done with three edible oils is known as ternary blending (Figure- 3) (Rabail et al., 2021). The blending ratio varies according to the oil type and desired quality (Grover et al., 2021). Blending can be done either by mechanical mixing or by hand blenders. The different methods used for designing binary and ternary blends was represented in Table -3.

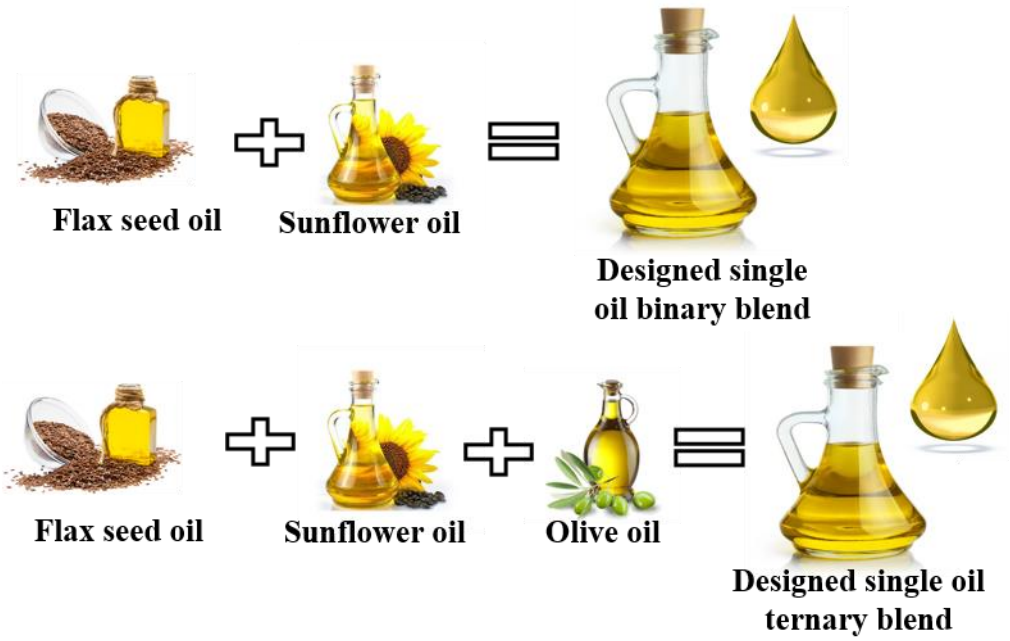


Figure 1.3 Designing of binary and ternary blending

Table- 1.3 Different methods used for designing binary and ternary blends is listed below

Sl. No	Oils blended	Method used	Reference
1	Coconut oil, olive oil, soybean oil, mustard oil, linseed oil, and sunflower oil	Magnetic stirring	Grover et al., 2021
2	Red palm oil, flax seed oil	Overhead stirrer	Nayana et al., 2021
3	Moringa oleifera oil with sunflower and soya bean oil	Mixing at room temperature (25-27 ^{0}C)	F. Anwar et al., 2007
4	Palm oil with sesame oil	Mixing at 60 ^{0}C	Abdulkarim et al., 2010
5	Coconut, peanut, palm and ground nut oils	Mixed in a blender for 1h	Sura et al., 2020
6	Soya bean oil blended with, sea buckthorn oil, Camelia oil, rice bran oil, sesame oil, and pea nut oil	Mixing at 60 ^{0}C	Yang et al., 2014
7	Flax seed oil, tomato seed oil, rice bran oil, olive oil and soya bean oil	Mechanical stirrer at 180 rpm- 15 min	Ghosh et al., 2019
8	Palm stearin and patawa oil	Homogenised for 10 min	Oliveira et al., 2017
9	Eucalyptus citriodora extract blended with canola, rapeseed and sunflower oils	Stirred at 100 ^{0}C for 30 min	Ali et al., 2016
10	Palm oil and macadamia oil	Magnetic stirrer at 50 ^{0}C for 3 min	Sara and Mohammed, 2019
11	Sunflower oil blended with pomegranate seed oil and blanched pomegranate seed oil	Mixed at room temperature (25-27 ^{0}C)	Kaseke et al., 2021
12	Canola and olive oil blends	Magnetic stirrer at 40 ^{0}C	Roiaini et al., 2015
13	Perilla seed oil with virgin olive oil	Magnetic stirrer equipped with nitrogen bubbling device for 15 min	Torri et al., 2019

1.4.1 Blending for improved fatty acid composition

Dietary fats provide desirable physical, nutritional, organoleptic properties to the food. Vegetable oils used as the cooking medium in different food preparation are the chief source of fat in the diet. The nutritional value of edible oils depends on the fatty acid profile, degree of unsaturation, arrangement of fatty acid in triglyceride structure. Deficiency due to lack of balanced fatty acids in the diet, especially polyunsaturated fatty acids (PUFA), is one of the significant nutritional problems globally (Domain et al., 2017). According to the Food and Nutrition Board's Committee on diet and health, the recommended ratio of SFA/MUFA/PUFAin edible oils is 1:2:1 and that of omega-3/ omega-6 is 0.05 (NRC (National Research Council), 1989). To improve the stability, shelf life, utility and nutritional value, fats and oils could be modified by blending (Dhyani et al., 2018).

Roiaini et al., (2015), increased the stability and modified the fatty acid composition by blending canola, olive and palm oil respectively. Upadya et al., (2015) blended rice bran oil and safflower oil to improve the fatty acid composition. Likewise Umesha et al., (2015) blended rice bran and garden cress oil and sunflower plus flax seed oil to improve the ω-3 as well as ω-6 fatty acid content. A study done by Adeyemi et al., (2016) proved that canola oil and palm oil, blend enhanced the ω-3 fatty acid content. Jan et al., (2016), blended olive oil plus sunflower oil and olive and soya bean oil, for providing higher amounts of MUFA. Perilla seed oil blended with extra virgin olive oil to increase the ratio of ω-3 and ω-6 (Torri et al., 2019). Nayana et al., 2021 established that blending redpalm oil and flaxseed oil improved the fatty acids which are deficient in individual oils. Recent studies on blending of oils for improved fatty acid profile and stability are summarised in Table 4.

1.4.2 Blending for thermal stability

Various types of edible oils are available for use in frying applications. Numerous chemical changes can take place in edible oils and fats during processing, storage, and frying operations. Mid-oleic and high-oleic vegetable oils (monounsaturated fat is around 70% or higher (Olive, canola oil), it is a high-oleic oil and 50% and 65%, it is a mid-oleic oil (sunflower oil)) are exceptionally suitable for frying applications. The oils with more saturation is more thermally stable. Palm oil is reported to be best suited for frying operations due to its higher smoke point reported (235 °C). Unsaturated oils are reported to be less stable to higher temperature, the unsaturation (rather than chain length) led to significant effects on thermooxidative degeneration (Naghshineh et al., 2010; Choe and Min, 2007). The studies related to oil blends and thermal stability are tabulated in table 4. In a study done by Mba et al., (2015) in the blends of canola and palm oil showed that, the tocopherols, tocotrienols and carotenoids in palm oil and refined canola oil exhibited better stability in deep-fat frying conditions. Coconut oil blends with sunflower and rice bran oil showed less peroxide formation and greater oxidative stability than the individual oils (Bhatnagar et al., 2009). At the same time blending of seinat seed (*Cucumis melo* var. tibish) oil with peanut oil improved the stability and tocopherols content (Siddeeg et al., 2015). Roiaini et al., (2015), proved that canola, palm and olive oil blends in the ratio 80: 20 with 20% palm olein is suitable for deep frying. Similar results were observed in blend of palm olein and canola oil suffered minimum losses of antioxidant and polyunsaturated fatty acid (PUFA) during repeated frying (Mohammed Al-Khusaibi et al., 2012; Dhyani et al., 2018). Study on the mixture of rice bran oil and soybean oil showed that, blended oil improved oxidative stability of soybean oil and retard the rancidity in fried product during storage (Abbas Ali et al., 2019). Thus, blending can reduce these undesirable reactions and improve their nutritional and sensory quality.

1.4.2 Blending for improved shelf life

Blending of vegetable fats/oils with different properties is one of the simplest methods to create new products with desired textural and oxidative properties. Different fats/oils have different physical and chemical characteristics. Use of single vegetable oil can have low physical, chemical and nutritional properties and also poor oxidative stability (Rabail et al., 2021). In an investigation by Choudhary et al., (2015), different rice bran oil blends were prepared with traditional oils and analysed for physicochemical, antioxidant and fatty acid profile. It was found that rice bran oil & groundnut oil blend (70:30) and rice bran & olive oil blend (70:30) are more stable than all other individual oils. So, blending of non-conventional oil with traditional oils can also be done to obtain stable cooking oil to reduce the demand and cost of traditional oils. Soybean oil has been blended with sea buckthorn oil, camellia oil, rice bran oil, sesame oil and peanut oil to find out the impact on oxidative stability and radial scavenging activity the results suggested that the blending improved the antioxidant profile of the oils that can contribute to prevention of chronic diseases associated to oxidative stress, such as in cancer and coronary artery disease (Yang et al., 2014). The blend of 80:20 of canola and olive with 20% palm olein is recommended for deep-frying and can be kept longer owing to the increased proportion of SFA in the blends (Roiaini et al., 2015). Ghosh et al; (2019) studied the different ratios of sun flower oil and sesame oil (80:20, 50:50) blends for chemical and oxidative stability and found that mixture of the unsaturated oils with saturated will give better stability. The results showed blending resulted in improved stability parameters and is an effective strategy to address consumer demands for oils containing only natural products as well as sources of ω-fatty acids (ω6, 9). According to a study conducted by Sura et al., (2020), blending of oils was suggested as the solution to reduce the oxidative damage, maximize fatty acids, and increase storage stability. The work was conducted to investigate the quality parameters of blended oils of coconut, palm, pea nut, and ground nut

oils at a proportion of 50:50 were used for the preparation of snacks. Acceptability studies of the snack foods shows better sensory parameters when compared to individual oil.

The study done by Oliveira et al., (2017) sought to develop lipid bases from blends between patawa oil and palm stearin at different ratios aiming to obtain a product with better physical and chemical properties. Findings provided by Pereira et al., (2019), demonstrated that fats and oils and their blends may be suitable for developing new fatty products in the food industry. According to Ghosh et al., (2019) blending of oils can be regarded as an effective strategy to address consumer demands for oils containing only natural products as well as sources of ω-fatty acids. These specialty oils can be used directly or blended with more readily available and cost-effective conventional vegetable oils. Flax seed oil (FO) is very important oil both from a nutritional or medicinal point of view due to its high content of omega-3 and omega-6 fatty acid. Unfortunately, its high content of PUFA makes it very susceptible to oxidation and hence limits its use. Such a problem could be solved through blending with other oils rich in natural antioxidants like Nigella sativa oil (Bhardwaj et al., 2015). According to Kaseke et al., (2021), blending sunflower oil (SO) with both pomegranate seed oil (PSO) and blanched pomegranate seed oil (BPSO) significantly improved TPC, TCC, α, and δ-tocopherols content of sunflower oil. incorporating PSO and BPSO in SO significantly increased palmitic acid, stearic acid, SFAs, MUFAs, MUFA:PUFAs index but did not significantly change the levels of PUFAs. Blended oils exhibited better oxidative stability than sunflower oil. According to a study done by Gulla and Waghray, (2011), consumer acceptance trials done for blended oil and control sample like sesame oil, corn oil, cottonseed oil, rice bran and soybean oil, indicated no strong rejection of an oil blend in comparison with the control. Nevertheless, the quality, composition, bioactive properties and oxidative stability of all the studied oils were greatly

affected by the storage time. Thus, a long-term storage of any of these oils should be avoided (Yang et al., 2014).

The oxidative and thermal stability of vegetable oils is one of the key factors in determining its use in foods and their applicability in food industries. There were a lot of challenges faced by oils in industries, the convenience of handling, altering the physical state of oil with temperature due to the presence of saturation and unsaturation, transportation inconvenience etc

Table—1.4 Various oil blends and their role in fatty acid, thermal and oxidative parameters

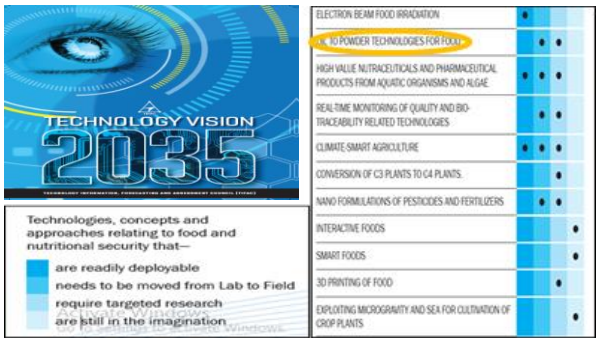
Blended oil	Effect on fatty acid composition	Effect on oxidation	Effect on thermal stability	Reference
Canola oil & palm oil	Enhancement of ω-3 fatty acid	Improved oxidative stability		Adeyemi et al., 2016
Rice bran oil & safflower oil	Moderating fatty acid composition	--	--	Upadya et al., 2015
Olive oil & soybean oil	Providing higher amounts of MUFA	--	--	Jan et al., 2016
Rice bran oil & garden cress oil	Increasing n6/n3	--	--	Umesha et al., 2015
Sunflower oil & flaxseed oil	Balanced fatty acids (n6/n3)	--	--	Umesha et al., 2015
Olive oil & sunflower oil	Providing higher amounts of MUFA.	--	--	Jan et al., 2016
Rice bran oil, groundnut oil and FSO	--	provided substantially higher oxidative stability	--	Pattnaik and Mishra, 2021
Olive oil ,sunflower Oil and cress oil	Balanced MUFA, PUFA & essential fatty acids	Improved the functional characteristics, thermal and oxidative stability of individual oils		Nehdi et al. (2019)
Palm oil & canola oil	Fatty acid composition improvement		Improved frying temperature	Choi, H., Lee, E., & Lee, K. G, (2014).
Canola oil and palm oil/ sunflower oil	--	--	Frying stability of the blended oils	El-Reffaei et al., (2016)
Soybean Oil and camellia oil	--	--	Thermal and frying stability	Wang et al., (2016)
Canola oil and olive oilþpalm oil	Increased stability by modifying the fatty acid composition	--	--	Roiaini et al., 2015
Soybean and sesame oil	--	Better oxidative stability at high temperatures	--	Li et al., 2014
Rice bran and olive oil	--	--	High smoke point and frying temperature	Choudhary and Grover 2013
Rice bran and flaxseed oil	--	Possessed good oxidative stability	--	Reddy et al., 2013

		over the storage time		
Palm oilþolive oil	--	Better oxidative stability	--	De Leonardis and Macciola, 2012
Canola oil and palm oil	--	--	Better frying stability	Enrıquez-Fernandez et al., 2011
Red palm oil and flax seed oil	Improved fatty acid composition lowering the	--	--	Nayana et al.,2021
Sesame oil and rice bran oil	blood pressure and modulating lipid profiles	--	--	Devarajan et al., 2016
Coconut oil and vegetable oils(palm , rice bran, sunflower, ground nut, and soybean oil)	Improved MUFA and PUFA	--	--	Bhatnagar et al., 2009
Coconut oil + rice bran oil sesame oil	Enhanced PUFA	--	--	Reena et al., 2007
Palm oil, soya bean oil, sun flower oil	--	--	Better frying stability	Rudzinska et al., 2017
Perilla seed oil+ extra virgin olive oil	High ω-3 /ω-6 ratio	Better oxidative stability	--	Torri et al., 2019
Flax seed oil+ palm olein	High ω-3 /ω-6 ratio	--	--	Bhardwaj et al., 2015
Refined palm oil & refined olive oil/sunflower oil	--	High thermo-oxidative stability in repeated deep-frying	High thermo-oxidative stability in repeated deep-frying	Akram et al., 2016
Canola oil & palm olein & virgin olive oil & bene kernel oil	--	Higher ant oxidative effects	Improved frying stability	Sharayei and Farhoosh, 2016

1.5 Oil to powder technology for shelf stable oil powder for food application

Edible oils can be converted in to powder using appropriate techniques for microencapsulation, to improve handling, storage stability and application, besides facilitating easy transport. Therefore, converting oil to powder can be an innovative and consumer-friendly approach to improve the nutritional and oxidative stability of oils and to widen their applications in the culinary/health/functional food and nutraceutical sectors. Various encapsulation techniques have been employed for converting oils into powder form by utilizing a wide range of carrier or wall materials.

Trapping of oils and fats into a matrix to mask its aroma, flavor, color, increase its oxidative stability, control its release, and increase its bioavailability can be termed as encapsulation of oil/fats (Pattnaik and Mishra, 2021; Sagiri et al., 2016; Adelmann et al., 2012; Tonon et al., 2011). Hence, depending upon the desirability of the product, the liquid oil is either converted into gels, beads, or powder. Industries are looking for food products with excellence in convenience, transportation, shelf life, and also improved delivery of nutritional parameters. There are lot of demands for oil to powder technologies and it also projected as one of the need for delivering convenience and nutritional products, hence Technology Information, Forecasting and Assessment Council (Technology vision 2035, TIFAC, 2015) emphasises the need for targeted research in this area for technology development, and 'Oil to powder technologies for food' is one of the areas identified under vision 2035.



"Microencapsulation is a method in which tiny particles or droplets are surrounded by a coating wall, or are embedded in a homogeneous or heterogeneous matrix, to form small capsules" (Gharsallaoui et al., 2007; Calvo et al., 2011; Bakry et al., 2016). At the same time microencapsulation is the process of

enveloping one substance, for controlled release, increased stability, improved nutritive value, and better appearance of products (Pattnaik and Mishra, 2021). Marine oil is one of the most encapsulated oils that have been applied in food products, followed by some vegetable oils such as flaxseed oil. Studies carried out by Ye et al., (2009) demonstrated that microencapsulation of fish oil and incorporating it into processed cheese during processing results in less oxidation of long-chain ω-3 PUFA. Each method of microencapsulation had several advantages and disadvantages on different aspects (Reis et al., 2022). However, selection of the microencapsulation process is mainly related with the thermosensitivity and solubility of the active compounds. Some of the limitations include, poor control of particle size, irregular morphology, degradation and loss of biological activity of thermo-sensitive compounds, low encapsulation efficiency and low precipitation yield. Therefore, the selection of encapsulation technique should be decided considering the factors such as the solubility, heat sensitivity of bioactive compounds, chemical structure, nature of the wall material, presence of other components such as sugars, proteins, economic values and time aspects (Sagiri., 2015; Ozkan et al., 2018).

1.5.1 Conversion of oil to powder using different technologies

Microencapsulation can be defined as a process of building a functional barrier between the core and the wall material to avoid chemical and physical reactions and to maintain the biological, functional, and physicochemical properties of the core materials (Bakry et al., 2016). Commonly used microencapsulation techniques are: emulsification, spray-drying, coaxial electrospray system, freeze-drying, coacervation, *in situ* polymerization, extrusion, fluidized-bed-coating, and supercritical fluid technology (Devi and Maji 2009; Anwar et al., 2010; Quispe-Condori et al., 2011; Sanchez-Navarro et al., 2011; Liu et al., 2012; Almeida et al., 2013; Soliman et al., 2013; Botrel et al., 2014; Sutaphanit and Chitprasert 2014; Tatar et

al., 2014; Wang et al., 2014; Bakry et al., 2016). In addition, wall composition and microencapsulation techniques may also determine functional properties and potential applications of encapsulated components. Microencapsulation of fish oil, rosemary oil, flax seed oil, Gac oil, soyabean oil, grape seed oil and various essential oils are already been done by Garcia- Encina, et al., (2016), Avramenko et al., (2016), Boger et al., (2018), Chuyen et al., (2019), Mori et al., (2019) by various techniques. Various techniques used to convert the oils into a variety of forms and their area of application is mentioned in Table -- 5.

Table-1.5 Various techniques used for encapsulation procedure of oils

Techniques for encapsulation	Oil type	Area of application	References
Complex coacervation	Tuna oil	Food	Wang et al.,2014
Spray drying	Anchovy oil	Food	Tatar et al., 2014
Spray drying	Fish oil	Food	Botrel et al., 2014
Spray drying	Tuna oil, tributyrin, & resveratrol	Food	Sanguansri et al., 2013
Complex coacervation	Fish oil	Food	Tamjidi et al., 2013
Spray drying	Fish oil	Food	Patrick et al., 2013
Spray drying	Fish oil	Food	Aghbashlo et al., 2013
Spray drying	Tilapia oil	Food	Huang et al., 2014
Complex coacervation	Fish oil, tributryin, & resveratrol	Food	Augustin et al., 2011
Spray drying	Fish oil & olive oil	Food	Polavarapu et al., 2011
Spray granulation & fluid bed film coating	Fish oil	Food	Anwar et al., 2010
Complex coacervation	Micro algal oil	Food & pharmaceutical	Zhang et al., 2012
Spray drying	Flaxseed oil	Food	Tontul and Topuz, 2013
Spray drying	Flaxseed oil	Food	Gallardo et al., 2013
Freeze-drying	Flaxseed oil	Food	Karaca et al., 2013

Freeze drying & spray drying	Flaxseed oil	Food	Tonon et al., 2011
Freeze drying	Olive oil	Agri-food	Calvo et al., 2012
Spray drying	Soy oil	Food	Liu et al., 2014
Supercritical solvent impregnation	Oregano oil	Food	Almeida et al., 2013
Non foamed & foamed spray drying	Sunflower oil	Food & pharmaceutical	Lewandowski et al., 2012
Coaxial electrospray system	Peppermint oil	Food	Koo et al., 2014
Spray drying	Blend of red palm olein & refined, bleached deodorized palm stearin	Food	Dian et al., 1996
Complex coacervation	Vanilla oil	Food	Yang et al., 2014
Spray drying	Gac seed oil	Food	Kha et al., 2014
Spray drying	Flax seed oil + redpalm oil	Food	Nayana et al., 2021
Spray drying	Sesame oil	Food	Alpizar-Reyes et al., 2020
Spray drying	Rice bran oil	Food	Atta et al., 2020; Benito- Rom Sanz, and Beltran, 2020
Spray drying	Soybean oil	Food	Silva James et al., 2019
Spray drying	Tailored PUFA rich oil	Food	Velez-Erazo, Consoli, and Hubinger, 2020
Microwave drying - Coacervation	PUFA rich oil blend	Food	Pattnaik and Mishra, 2021
Freeze drying	Sunflower oil	Food	Domian et al., 2014

1.5.2 Encapsulation by spray drying

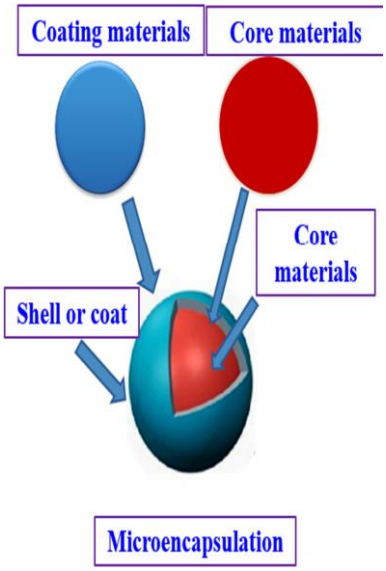
Microencapsulation is a process that involves covering a liquid, solid or gaseous substance in surrounding material (carrier) (Chranioti et al., 2015; Domian et al., 2015). Encapsulation of vegetable oils (essential oils and nonessential oils) in different formulations was proved to be successful in improving antibacterial, antifungal, and antiviral properties (Sagiri et al, 2016). According to Bakry et al., (2016) microencapsulation has the ability to enhance the oxidative stability, thermos ability, shelf-life, biological activity of oils, it can also be helpful in controlling the volatility and release properties of various bio active agents.

There are numerous microencapsulation methods that can be used, but before choosing the best one several factors must be taken into consideration: different chemical and physical properties of the encapsulated substance and core material, non-reactivity with core material, its stability for a long time and future usage (Janiszewska-Turak., et al 2017). Spray drying is one of the oldest and currently the most often used industrial friendly technique for the entrapment of various compounds including polar and nonpolar compounds (Chranioti et al., 2015). This method allows to obtain desired product by changing the parameters like temperature, carrier matrix, and pre- treatment of the raw material. The versatility of this technique made it more reliable and user friendly. Encapsulation by spray drying appears to be an effective way for those compounds in the powder form because of its excellent properties of the protection of core material, stabilization, solubility and controlled release of the bioactive compounds (Pattnaik and Mishra, 2021; Bakry et al., 2016; Zuidam and Henrich, 2010). Spray drying is a flexible, continuous, but more important an economical operation, it is the oldest and the most widely used encapsulation technique in the food

industry sector. The ease of scale-up, flexibility of the process, good powder quality, and cost-effectiveness of this method make it a popular one (Kaushik et al., 2015).

The liquid oil can be converted into stable powders by following few steps viz.:

- Dispersion of core material into the wall material solution
- Formation of a homogenous emulsion
- Transferring into the feeding pump if the spray dryer
- Solvent evaporation is used to remove the solvents used in the solution
- Atomization of spray through pressure nozzles
- collection of the dried powder microcapsules



The effectiveness of the process depends on the encapsulation efficiency (%EE). Selection of wall materials is based on the emulsifying activity, stability, solubility, properties of the core, and desired final product (Albert et al., 2017). They actively influence the physico-chemical properties of the microcapsule viz. encapsulation efficiency, particle size, powder morphology, and lipid oxidation. The commonly used wall materials can be broadly classified into carbohydrates, proteins, gums, and wax.

1.5.3 Different wall materials used for oil encapsulation

Different types of encapsulating agents have been used for spray drying; these include polysaccharides (starches, maltodextrins, corn syrups and arabic gum), lipids (stearic acid, mono and di glycerides), and proteins (gelatin, casein, milk serum, soy and wheat) (Saenz et al., 2009). Generally wall materials are chosen from a range of proteins, carbohydrates, lipids and waxes, which may be used alone or in combination. Polysaccharides provide stability to emulsion by forming a network in the continuous phase (Kumar et al. 2020). The polysaccharides like GA and gelatin have emulsifying capacity due to interfacial properties

but possess low encapsulation efficiency (Mahdavi et al., 2016). A combination of maltodextrin with protein or gums has been observed to show better results (Pattnaik and Mishra, 2021; Mahdavi et al., 2016). The different types of microcapsules and microspheres are produced from a wide range of wall materials like carbohydrates, proteins, and gums (Adelmann et al., 2012; Bakry et al., 2016; Albert et al., 2017; Pattnaik and Mishra, 2021).

The eligibility of proteins as wall materials is derived from their amphiphilic properties, ability to self-associate and interact with a variety of different types of substances, large molecular weight, and molecular chain flexibility. Proteins also have excellent functional properties such as solubility, viscosity, emulsification, and film-forming properties, all of which renders them capable of being used for encapsulation. Whey protein, sodium caseinate and gelatin are some of the proteinaceous wall materials used for food bioactive compounds, such as essential fatty acids (Karthik and Anandharamakrishnan, 2013), probiotics (Rajam et al., 2012) and volatiles (Rosenberg and Sheu, 1996). Goyal et al., (2015) encapsulated flaxseed oil using whey protein concentrate (WPC) or sodium caseinate (SC) in combination with lactose, whereas Takeungwongtrakul et al., (2015) tried to use the mixture of WPC and SC combining with gum Arabic, glucose syrup or maltodextrin to entrap shrimp oil. Hee et al., (2015) designed 1:3 core-to-wall microcapsule formulations of SC and maltodextrin mixing with gelatin, WPC, or gum Arabic for the encapsulation of virgin coconut oil. Because of advanced water solubility over a wider pH range, flexible structure to rapidly diffuse to the oil–water interface, and smaller molecular mass (Can Karaca et al., 2015), sodium caseinate was mostly used to develop microcapsules. Tamm et al., (2016) successfully designed microcapsules with pea protein isolate (PPI) and glucose syrup to entrap 20% rapeseed oil.

The choice of the optimal coating materials is one of the most important and critical steps in food microencapsulation. Determination of the best combination seems to be necessary**.** In the study conducted by Pourashouri et al., (2014) combinations of fish gelatin, chitosan, and maltodextrin were considered and found that microencapsulation was effective against oxidative stability of oils containing gelatin based encapsulates.

1.5.4 Emulsion formation

Emulsion formation is the first stage in the fabrication of microencapsulation by spray drying. 'Emulsions are colloidal dispersions in which a liquid is dispersed in a continuous liquid phase of different composition' (Laurier L. Schramm, 2014). In another way it can be termed as: 'droplets of one liquid dispersed in another, the two liquids being immiscible' (Vaclavik and Christian, 2003). The droplets are termed the dispersed phase and the surrounding medium containing them is the continuous phase. Practical emulsions may well contain droplets that exceed the classical size range limits given earlier, sometimes ranging upwards to tens or hundreds of micrometres. Emulsions can be of two types: *oil-in-water* (where oil is the dispersed phase) and *water-in-oil* (where water is the dispersed phase). The oil droplets dispersed in an aqueous phase are known as oil-in-water (O/W) emulsions. The water droplets dispersed in oil are called the water-in-oil (W/O) emulsions. The O/W emulsions can be used for the delivery of hydrophobic active substances, and W/O emulsions are used for the delivery of hydrophilic compounds.

With respect to the emulsions for microencapsulation by spray drying, the dispersed phase usually consists of the active compound to be encapsulated (core), while the aqueous dispersion of wall material serves as the continuous phase. An emulsion may also include an emulsifier (or surfactant), which coats the emulsion droplets and prevents their *coalescence* or aggregation with each other. The necessity for emulsifier(s) depends on the nature of the wall material used, since some of these (e.g. whey protein) inherently possess emulsifying

properties. The primary purpose of the emulsification step in the microencapsulation process is to bring together the substrate for encapsulation (core) and the encapsulating agent (wall) in the feed preparation before spray drying to form the microencapsulate. The rationale for the requirement of an emulsion, and not just a simple suspension or solution of the core compound for the purpose of encapsulation by spray drying, is the subject of debate, but it can be justified from different perspectives. Spray drying often involves drying of aqueous preparations, while encapsulation normally deals with lipophilic food bioactives (e.g. β-carotene). Thus, it is essential that the lipophilic compound is stable and primarily miscible in the aqueous medium of the feed before being subjected to drying. The instability of the core in the feed emulsion used for spray drying leads to poor encapsulation efficiency and the physical properties of the encapsulated powder (Danviriyakul et al., 2002). Obtaining a stable emulsion is therefore critical in the formation of stable microencapsulates by the subsequent spray drying step. The lipophilic ingredients, such as carotenoids and fat-soluble vitamins, are encapsulated within the hydrophobic core of the liquid droplets, where they may be protected from degradation during storage (McClements and Rao, 2011; McClements et al., 2007). On the other hand, hydrophilic ingredients such as polyphenols are encapsulated in either a water-in-oil emulsion (W/O) or double emulsions (W/O/W) (Appelqvist et al., 2007). A study done by Shy-Kai Ng et al., (2014), in their study of kenaf seed oil, showed that emulsion total solids have direct effect on the oxidative stability of core components. The similar aspects were proposed by Anandharamakrishnan et al., (2015), the solid content, viscosity, of emulsions were related to the drying properties of spray drying operation. The stability of emulsions before spray drying is also an important measure which contributes the particle size, encapsulation efficiency, and stability of micro particles (Ezhilarasi et al., 2013; Mohammed et al., 2021). Thus formation of a stable emulsion is an inevitable concept in the spray drying process.

1.5.5 Mechanism of spray drying

The different stages of the mechanism of the spray-drying process are atomization, droplet-to-particle conversion and particle collection. A solution is pumped to an atomizer, breaking up the liquid feed into a spray of fine droplets and the droplets are ejected into a drying gas chamber where the moisture vaporization occurs, resulting in the formation of dry particles. Finally, the dried particles are separated from the drying medium, being then collected in a tank (Santos et al., 2018).

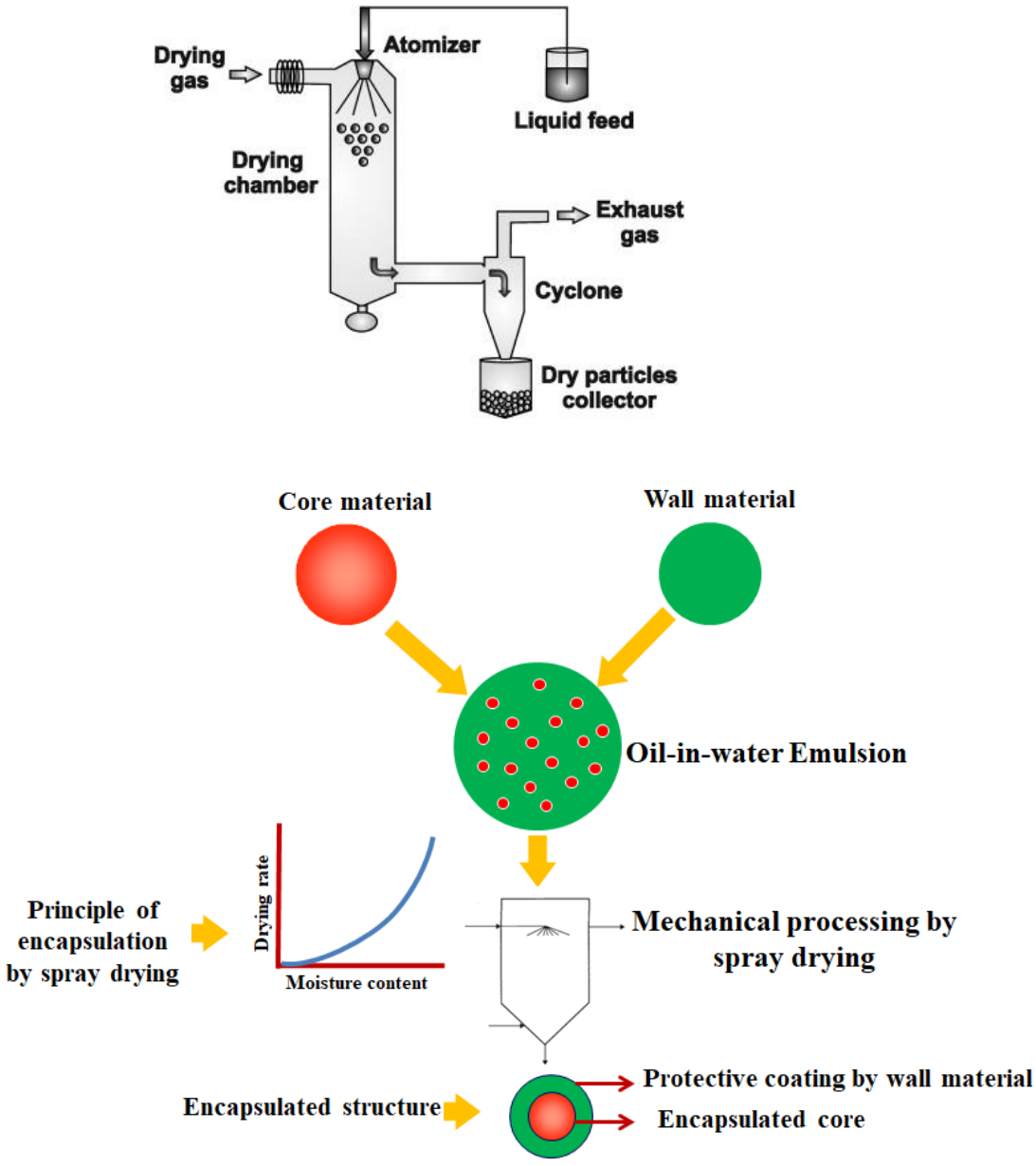


Figure – 1.4 Explaining the mechanism of spray drying

1.5.6 Encapsulation of oil for improved oxidative stability.

Vegetable oils are encapsulated to mask its aroma, flavor, color, increase its oxidative stability, control its release, and increase its bioavailability (Sagiri et al., 2016; Adelmann et al., 2012; Tonon et al., 2011). The selection of wall materials is based on the emulsifying activity, stability, solubility, properties of the core, and desired final product. They actively influence the physico-chemical properties of the microcapsule viz. encapsulation efficiency, particle size, powder morphology, and lipid oxidation (Kumar et al. 2020). A study done by Carneiro et al., (2013), aimed at evaluating the potential of maltodextrin (MD) in combination with different wall materials for microencapsulation of flaxseed oil by spray drying, in order to maximize the encapsulation efficiency and minimize lipid oxidation. A combination of maltodextrin and whey protein concentrate as the wall material exhibited best protection against lipid oxidation. Depending on the physico-chemical properties of the core, and wall composition, the purpose and the function of the encapsulates can be manipulated for various end use. The polysaccharides like gum arabic and gelatin have emulsifying capacity due to interfacial properties but possess low encapsulation efficiency (Mahdavi et al. 2016). Modified starches, like maltodextrin, have poor emulsifying activity and low oil retention. Conversely, sodium caseinate has excellent emulsifying activity and stabilizes the emulsion but lacks in entrapping liquid oil. However, a combination of maltodextrin with protein or gums has been observed to show better results (Pattnaik and Mishra 2021; Mahdavi et al. 2016). Furthermore, the addition of a surfactant like a lecithin or caseinate to the above solution might provide a better result (Salminen et al. 2014). Milk-based proteins is often be used as encapsulating material as they show good functional and film-forming properties (Gharsallaoui et al. 2007).

Besides the wall materials, the particle size of the microcapsules also governs the oxidation process. Sanchez et al., (2016) affirmed that surface to volume ratio is the main factor influencing the oxidation, because of exchange surface area. Linke et al., (2020) observed higher oxidation in smaller particles than larger ones, as the surface to volume ratio was smaller that caused a higher particle-air interface. The larger particle-air interface exposed the surface to oxygen and led to more oxygen diffusion resulting in detrimental changes by reacting with the oil droplets. However, by modifying the encapsulation procedure, the particle properties (porosity, density, size) can be changed which might affect the oxidative stability of oil powders. Also the fabrication of a stable emulsion prior to spray drying ensures uniform distribution of the dispersed phase in the encapsulation matrix. Proper selection of feed rate and drying temperature based on the glass transition temperature of the feed components results in efficient encapsulation and powder properties that results in better oxidative stability.

1.6. Future prospects and conclusion

The consumer preference for healthier lipids in the diet offers opportunities for the researchers to develop innovative lipids with enhanced stability, fatty acid profile, nutritional and bioactive compounds and convenience. Many studies have proven that blending is an economical and acceptable method to provide oil with balanced fatty acid profile, greater stability and also improved content of antioxidant and bioactive compounds. Though blending offers a cost effective and practical solution to enhance the above said attributes, it lacks the oxidative and thermal stability on long duration of shelf life storage. Blending of less exploited oils with nutritional and phytochemical enriched oils such as RPO, flax seed oil, rice bran oil, olive oil, etc. needs to be exploited to enhance the aforesaid qualities. The use of ternary blending should be explored more than binary blending since they deliver

better characteristics than the latter. Blending is a simple, feasible and economical method to alter the physicochemical and nutritional properties of the oil in a good way.

Powdered oil/fats can provide better stability and it offers a promising platform to develop functional lipids by incorporating bioactive compounds targeting various health benefits including preventive health management. The gradual shift of the population toward vegan products has urged the food industries to look for alternative vegetable oils rich in omega fatty acids than marine oils. Marine oils (mostly fish) have long-chain fatty acids such as EPA and DHA, while vegetable oils are endowed with short-chain fatty acids like linoleic and linolenic acid. The susceptibility of such PUFA enriched vegetable oils to oxidative degradation has beseeched the technologists toward its preservation through encapsulation.

In spray drying, the improvisation of pressure nozzles to withhold high viscosity without creating lumps can be attempted. The modification of nozzles successively can support a higher oil load. Nonetheless, microwave drying favours high oil load; a future suggestion would be to model this process as a continuous one. Spray cooling is generally preferred for encapsulation of probiotics, essential oils, or bioactive compounds; hence, future research can be conducted on vegetable oils. In this way, a better powder product can be obtained without compromising the other properties. Despite the methods described above, the use of supercritical fluid (specifically carbon dioxide) for the production of atomized spray particles can be explored in the near future. This supercritical carbon dioxide will not only eliminate the solubility issue but also produce particles of low temperature. It would be worthwhile to explore the use of biopolymers prepared from industrial or agricultural waste products as utilized as carrier material.

Based on the above gathered information and gaps identified, the present work was carried out with a hypothesis that

A. Blending of regularly consumed vegetable oil with bioactive rich with higher saturation enhances the shelf stability and thermal stability

B. Oil to powder technology can be adopted for formulating healthy lipid powders for food application as butter replacer

C. The inconvenience in handling associated with oils/fat due to its physical states can be addressed if it can be converted in to powder form and can improve convenience for usage in food preparations, and improve the shelf stability.

D. Development of powdered oils/fat (vegetable oils, butter, ghee, and red palm olein, etc.) by microencapsulation using different combinations of wall materials. The resulting encapsulated products can be used for regular culinary purpose, as well as, as an ingredient in various food products, for providing nutritional benefits and functionality.

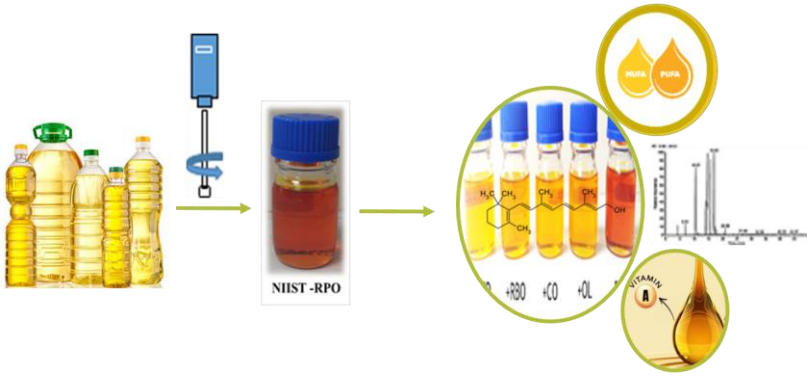
Objectives

The broad objective is to develop process for the production of blended edible oil as well as powdered edible oils/fats for food, nutritional and functional food applications. The detailed objectives are as follows:

1. Development of vegetable oil blends with optimum fatty acid profile for nutritional/ functional food products.
2. Encapsulation of vegetable oil blends with balanced fatty acid composition as a carrier of oil soluble vitamins for nutritional/ functional food products.
3. Development of stable emulsion with suitable wall material for evaluating higher oil payload.
4. Process development for powdered lipids (butter/ghee) for food applications.

Chapter 2

Development and Stability of Vegetable Oil Blends with Red Palmolein for nutritional applications.



2.1 Introduction

The amount of fat consumed daily is a topic of controversy though oils and fats play a vital role in daily diet. Oils and fat also play a significant role in food industry by attributing sensorial characteristics to most of the food products and also as a medium of heat transfer. Different types of vegetable oils are consumed globally for industrial and culinary applications, the major ones include palm oil, sunflower oil, flax seed oil, olive oil, rice bran oil, which differ in their fatty acids composition. Dietary lipids are the main source of essential fatty acids (EFA) such as linoleic（18:2), linolenic acid（18:3), EPA (Eicosapentaenoic acid) （20:5）and DHA (Docosahexaenoic acid)（22:6). The nutritional value of edible oils depends upon the fatty acid profile, degree of unsaturation, arrangement of fatty acid in triglyceride structure. The physical and chemical properties of fats and oils will affect the sensorial and nutritional attributes of foods (Endo, 2018). The dietary fats are the sole source of fatty acids viz., SFA (Myristic acid, Lauric acid, Palmitic acid, and Stearic acid), MUFA (Oleic acid), PUFA (Linoleic acid , Arachidonic acid, Eicosapentaenoic acid, Docosahexaenoic acid, Alpha Linolenic acid) etc, and balanced supply of these fatty acids are required for the healthy functioning of the body. There is no single edible oil available which has the desired fatty acid profile, oxidative stability and functional properties that satisfies nutritional and technological requirements.

Quality and stability of vegetable oil are the main factors that influence its acceptability and market value (Hashempour-Baltork et al., 2016). Oils undergo quality deterioration and become inedible by oxidation or by hydrolysis of fatty acids. This condition is generally termed as rancidity (Hammond, 2011). Rancidity results in the development of pungent and offensive off-flavour and lead to the deterioration of nutritive compounds including vitamins (A, D, E, K and C), essential fatty acids, chlorophylls, carotenes, amino acids, proteins or

enzymes by the production of toxic or physiologically reactive compounds (Gardner, 1979). The stability of fats and oils upon heating and frying temperature is an important measure of the thermal quality of oils. Antioxidants play a vital role in extending the stability of lipids. Antioxidants can be classified into synthetic as well as natural. Natural antioxidants present in food play a vital role in preventing the oil from deteriorative changes during storage and processing at higher and elevated temperatures. In the present scenario, consumption of healthy oil is a topic of concern and according to World Health Organization (WHO, 2008), the healthy oil should have saturated, mono and polyunsaturated ratio of 1:1.5:1. The ratio of essential fatty acid, linoleic acid (omega- 6): linolenic acid (omega - 3) should be 5- 10:1 and the presence of antioxidants. Lipids enriched with bioactive phytochemicals with antioxidant properties and optimum fatty acid profile with shelf stability is of great interest. Oxidation of polyunsaturated food lipids often affects the development of unpleasant odours, and tastes (Yang et al., 2014; Navarro et al., 2012). Lipid oxidation can directly reflect shelf life and also, oxidation induce chemical changes of the oils that may affect quality directly (Bansal et al., 2010; Cabiscol et al., 2010). Saturated oils are less prone to oxidation as compared to the unsaturated oils, however consumption of saturated oils are not recommended due to the adverse health effects reported (Vieira et al., 2015). The kind of oils and their oxidative stability are very important indicators of the shelf stability of lipids.

In this regard blending of oils is suggested as an alternative to overcome these adverse effects and also to improve the shelf and thermal stability (Dhyani et al., 2018). Blending of two or more oils has been suggested as a promising operation that can balances fatty acid composition with good functional and nutritional value. Most of the commonly consumed oils are deficient in essential fatty acids, omega -3 and omega - 6 which are very important in many of the metabolic pathways in the body (Tini et al., 2020). EFAs are also known to play a major role in the prevention and management of Alzheimer's, cancer and cardiovascular

diseases (CVDs) (Simonetto et al., 2019). Unsaturated fatty acids rich oils are good for human health as compared to saturated fatty acids, but they are highly unstable at high temperature and easily undergo oxidation (Sharma et al., 2013). Thus, blending is commonly used practice to achieve stable oil with the ideal fatty acid profile. Combining sunflower oil with canola oil or palm oil (De Marco et al., 2007; Farag et al., 2010), and mixing soybean oil with hydrogenated soybean oil or mixing corn oil with high-oleic sunflower oil (Abdulkarim et al., 2010; Naghshineh et al., 2010) are some examples. Blending of common edible oils with unconventional oils such as rice bran oil has been recently approved to achieve nutritional improvement, cost reduction processing and storage stability (Choudhary et al., 2015). Blending of oils can change odour profiles (Ravi et al., 2005) and moderate colour (Wang et al., 2016). A study by Goyal et al., (2018), reported that the blended ground nut and sunflower oil improved the content of β-carotene from zero to 18 ppm and 24 ppm respectively. Blending of oils with high stability and good nutritional properties is a good choice to decrease the rate of oxidation, without hydrogenation and formation of trans fatty acids.

Palm oil is the worlds most produced, consumed and traded vegetable oil with almost 50-50 composition of saturated and unsaturated fatty acids, which makes it suitable for numerous food applications (Mba et al., 2015). The edible food industry utilizes about 90% of palm oil, while the remaining 10% finds application in soap and oleo chemical manufacturing (Oil World, 2013). Under the Technology Mission on Oil seeds and Pulses (TMOP), the Agro processing division of CSIR-NIIST (Council for Scientific and Industrial Research- National Institute for Interdisciplinary Science and Technology) had taken up a major programme to develop palm oil extraction technology tailored to the Indian farming conditions. Further efforts to make value added products from palm oil was attempted and technology for the production of red palm olein was developed and commercialized. The red palm oil (RPO)

already available in Malaysia makes use of the principle of expensive molecular distillation technique and therefore, the end product is prohibitively costly. CSIR-NIIST process for the production of natural carotene and Vitamin E rich red palm oil is through the normal refining process under optimized controlled conditions (Figure- 1) so that even after refining more than 75% of the micro nutrients are retained in the end product. Unlike other processes this process is cost effective at the same time product is at par with international product available in terms of carotene and tocopherol/ tocotrienol contents (Mayamol et al., 2009).

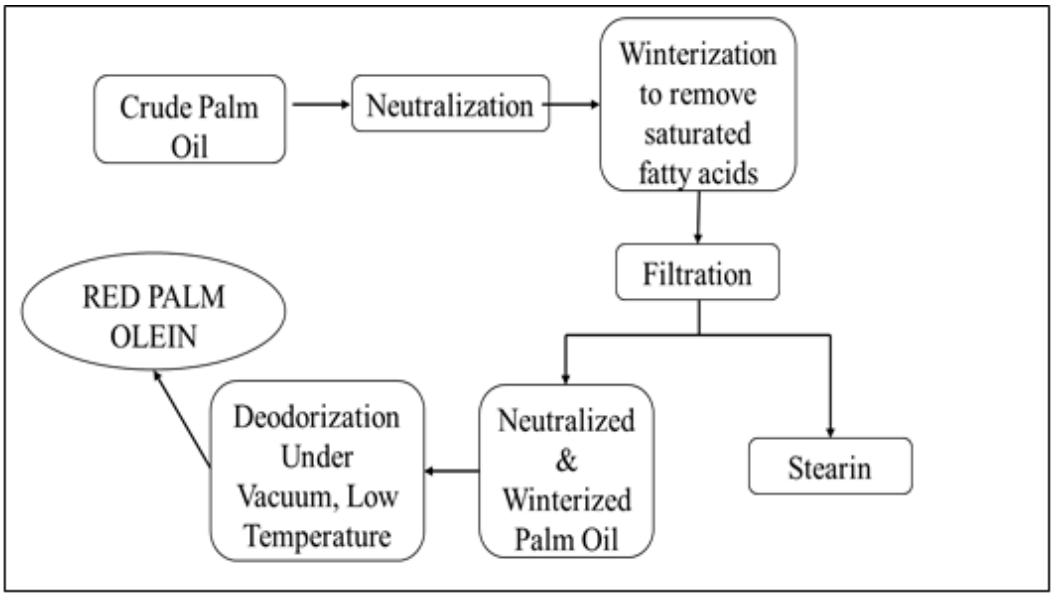


Figure -2.1 CSIR-NIIST process for the production of red palm oil

Red palm oil (RPO), which is produced from crude palm oil by using minimal processing conditions is very rich source of natural, carotenoids and tocotrienols. It's a good source of provitamin-A (β- carotene). It contains fairly good amounts of β- carotene (500-700ppm), tocopherol (255.342 ppm) and tocotrienol (744.143 ppm) in it (Nagendran et al., 2000; Gourichon, 2013; Chong et al., 2018). Red palm oil has a higher bioavailability of antioxidant nutrients than any other vegetable sources and it is particularly an important vegetable oil as

excellent vitamin –E supplement (Loganathan et al., 2017). It is nature's one of the richest source of carotenoids, containing 15 times more carotenoids than carrots and 30 times more than tomatoes (Scrimshaw, 2000). Carotenoids are extremely powerful anti-oxidant nutrients and is reported to enhance immune system and reduce the risk of cancer, heart disease, and cataract. Tocotrienols are another form of Vitamin E and have significant cardio-protective properties (Chong et al., 2018; Loganathan et al., 2017). Palm oil has high oxidative stability with low levels of essential fatty acids and high amounts of saturated fatty acids (Hashempour-Baltork et al., 2016). However, palm oil is deficit in MUFA and PUFA. A study done by Valantina et al., (2014), proved that blending palm oil with rice bran oil containing high levels of oryzanol and sitosterol, which are strong antioxidants, can reduce the rate of oxidation. At the same time blending RPO with flax seed oil is reported to improve the fatty acid profile with enriched beta-carotene content (Nayana et al., 2021).

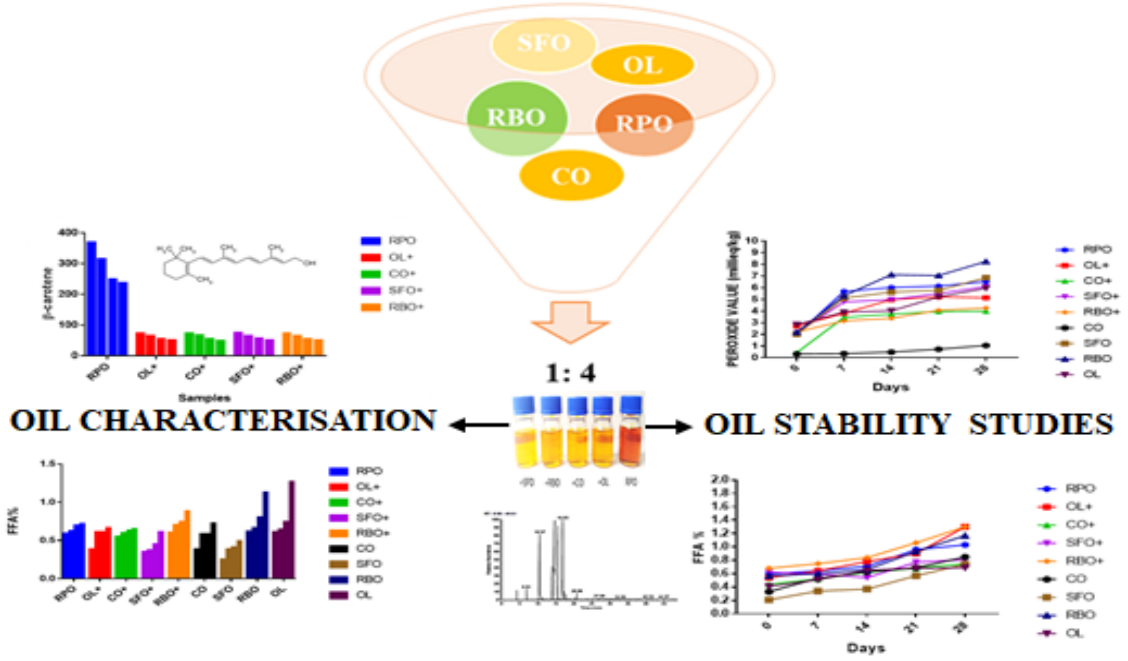
Healthy and stable oil with a high functional value always is a debatable idea because of changing lifestyle and dietary pattern. As per Household Consumer Expenditure (HCE) Survey, NSSO report 2011, consumption of oil is reasonably high, about 20-30g / person / day and is consumed by all population groups. Evident to this report, edible lipids can be taken as a medium for the delivery of certain bio actives, and functional compounds. Therefore the present study aimed at developing blends of commonly used edible vegetable oils viz, coconut oil, rice bran oil, sunflower oil and olive oil with minimally processed red palmolein (RPO). These blends were investigated for the stability during accelerated storage conditions as well as thermal conditions.

2.1.1 Objective

The overall objective of this chapter is to understand the effect of blending of natural antioxidant enriched RPO with other commonly consumed vegetable oils such as coconut oil, rice bran oil, sunflower oil and olive oil on the thermal and oxidative stability in comparison with individual oils

2.2 Materials and methods

The Experimental design for the development and stability of vegetable oil blends with Red Palmolein.



2.2.1 Materials

Coconut oil (CNO), sunflower oil (SFO), rice bran oil (RBO), olive oil (OL), and flax seed oil (FSO) were selected as the base oils and were collected from authorized source /brands. Crude red palm oil was procured from the M/s Godrej Industries, Trichy, Tamil Nadu, India.

Refined red palm oil (RPO) was produced using the NIIST technology in the pilot plant (Mayamol et al., 2009). Blending of oils were done in the ratio 1: 4, with red palm oil and base oils, as per government regulations (FSSAI, 2020), and ratio was finalized with the help of GC-MS/MS. All the chemicals and reagents used in the study were analytical grade / HPLC grade from Merck, Germany.

2.2.2 Methods

2.2.2.1 Blending of oils

Blending of two or more oils is generally carried out to share the advantages and disadvantages according to the blending ratio (Iftikhar, 2019). Based on the well-established data on the fatty acid composition of the commonly consumed vegetable oil, the blending of oils were done in the ratio 1:4 of red palm oil and base oils. The fatty acid composition of individual oils and blends calculated based on the reported fatty acid composition is represented in Table-1. The base oils were sunflower oil (SFO), rice bran oil(RBO), olive oil (OL), and coconut oil (CO) and the blends of these oils with RPO are represented as, RBO+, SFO+, OL+, and CO+. Blending RPO with other base oils were done using an overhead stirrer (Remi, RQ126/D, with 40V, Mumbai, India), based on the procedure of Guiotto et al., (2014), with slight modifications.

Table – 2.1 Fatty acid composition of individual oils and blends

*Fatty acid Composition	Percentage of fatty acid present in individual and blended oils								
	RPO	CO	RBO	SFO	OL	CO+	RBO+	SFO+	OL+
Caprylic acid (C8:0)	--	6.21	--	--	--	4.9	--	--	21.6
Capric acid (C10:0)	--	6.15	--	--	--	4.9	--	--	2.84
Lauric acid (C12:0)	--	51.02	--	--	--	40.81	--	--	61.52
Myristic acid (C14:0)	--	18.94	0.35	--	--	15.15	0.28	--	15.12

Palmitic acid (C16:0)	42	8.62	19.34	6.52	16.5	13.07	23.87	13.61	--
Stearic acid (C18:0)	5	1.94	2	1.98	2.3	2.55	2.6	2.58	--
Oleic acid (C18:1)	42	5.84	43.42	45.39	66.4	13.07	43.13	44.71	--
Linoleic acid (C18:2)	10	1.28	32.04	46.02	16.4	3.02	27.63	38.81	--
Linolenic acid (C18:3)	--	--	0.59	0.12	--	--	0.47	0.12	--

*The fatty acid percentage was taken from the literatures Chowdhury et al., (2007), Montoya et al., (2014), Orsavova et al., (2015), Dorni et al., (2018), Rabil et al., (2021).

2.2.2.2 Physico chemical characterisation of oils

2.2.2.2.1 Free fatty acid (FFA)

The acidity is frequently expressed as the percentage of FFA in the sample. Sample (2 g) was weighed into a stopped conical flask. 30 ml methanol was added to the weighed sample and the contents were boiled till the appearance of first bubble. Two drops of phenolphthalein indicator were added and titrated against 0.1N standardized alkali taken in burette. Titration was repeated for concordant values. The end point of titration is the appearance of persistent pink colour. The percentage of FFA in most oils and fats is calculated on the basis of oleic acid; although in coconut oil it is often calculated as lauric acid, in sunflower oil it is linoleic acid and in palm oil in terms of palmitic acid (ISO, 1996). The calculation is given below:

$$FFA\% = \frac{M \times N \times V}{10 \times W} \quad (1)$$

Where,

M – Molecular weight of the main triglyceride

N -- Normality of NaOH

V – Volume of NaOH (ml)

W -- Weight of sample (g)

2.2.2.2.3 Peroxide value (PV)

Peroxide value is an indication of the extent of oxidation of the oil. Sample (2-5g) was taken into 250 ml glass stoppered flask. 30 ml acetic acid – chloroform reagent was added. Shake until to dissolve the sample. To this 0.5 ml of saturated potassium iodide solution was added and mixed. Keep the mixture in dark for few minutes, followed by the addition of 30 ml of distilled water. 0.1N sodium thiosulphate was used for titration using freshly prepared starch as indicator. End point is the disappearance of blue color. Conduct blank (must be less than 0.1 ml 0.1 N sodium thiosulphate) (AOAC, 2000). Peroxide value expressed as milliequivalent of peroxide oxygen per kg sample (meq/kg):

$$PV = \frac{(S - B)N \times 1000}{W} \qquad (2)$$

Where,

B – Volume in mL of standard sodium thiosulphate solution required for the blank (ml)

S -- Volume in mL of standard sodium thiosulphate solution required for the sample (ml)

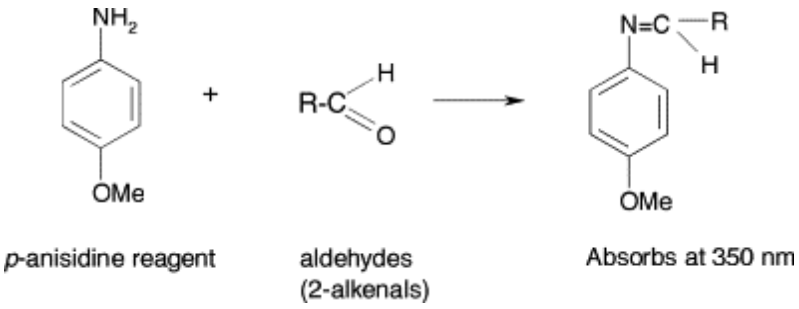
N -- Normality of sodium thiosulphate solution

W -- Weight of sample (g)

2.2.2.2.4 Para anisidine value (PaV)

Secondary oxidation products were measured by determining the p-anisidine value. Aldehydic compounds in fats and oils react with p-anisidine, in the presence of acetic acid, to form yellowish reaction products. According to the method the intensity of the yellowish compounds is not only related to the amount of aldehydic compounds present, but also to their structure. A double bond in the carbon chain conjugated with the carbonyl double bond increases the molar absorbance four to five times. This is why 2-alkenals and dienals will contribute substantially to the value. This determines the quantity of aldehydes (principally 2-

alkenals and 2,4-dienals) present in fats and oils. The AOCS Method Cd 18-90 (AOCS, 1997) was used. P-Anisidine was recrystallised according to the method and used for two consecutive days only, after which fresh p-anisidine was prepared. *Para*-anisidine is a reagent that reacts with aldehydes to give products that absorb at 350 nm. The *p*-anisidine value is defined as the absorbance of a solution resulting from the reaction of 1g fat in isooctane solution (100 ml) with *p*-anisidine (0.25% in glacial acetic acid). The products formed by reaction with unsaturated aldehydes (2–alkenals) absorb more strongly at this wavelength, and consequently the test is particularly sensitive to these oxidation products. Although the test does not distinguish between volatile and nonvolatile products, the palate is generally more sensitive to unsaturated volatile aldehydes than to saturated volatile aldehydes, so the test is a reasonable way to assess secondary oxidation products.



$$PaV = \frac{25 \times As - Ab}{m} \qquad (3)$$

Where,

As – absorbance of fat solution after reaction with p-anisidine

Ab – absorbance of fat solution

m -- Weight of sample (g)

Measurements of *p*-anisidine value are commonly used together with peroxide value measurements in describing the total extent of oxidation by the Totox value, which equals the sum of the *p*-anisidine value plus twice the peroxide value. However, the Totox value is an empirical parameter since it corresponds to the addition of two parameters with different units. The Totox value was calculated from the peroxide value and the p-anisidine value with the formula as follows,

$$Totox\ value = 2PV + AV \quad (4)$$

2.2.2.2.5 β-carotene estimation by spectrophotometric method

The β-carotene content of the RPO, and blended oils were estimated by the method (BS 684, section 2.2:1977). The oil (0.5-1 g) was dissolved in hexane and made up to known volume. The absorbance was read at 446 nm using the spectrophotometer (Shimadzu UV-2600, Japan). The carotene content was calculated using the Eq. (6) and expressed in ppm (Dian et al., 1996).

$$Carotene = \frac{V \times 383 \times (As - Ab)}{1000 \times W} \quad (5)$$

Where,

V is the volume of oil made-up with hexane (mL),

W is the weight of the sample (g),

A_s is the sample absorbance, and

A_b is the blank absorbance.

2.2.2.2.3 Stability studies

2.2.2.2.3.1 Accelerated Storage studies

The accelerated stability test (Schaal oven test) was performed to evaluate the oxidative stability of the individual and blended oils. The oils were stored at 60 ± 2 °C in a hot air oven

(Globe Tex, Digital Laboratory Hot Air Oven, Ghaziabad, India) for a period of 28 days in darkness. The oxidation reaction was accelerated at 60 ^{0}C in the oven at the said aforesaid conditions. Samples were removed on seven-day interval for analysing the extend of lipid oxidation and β- carotene evaluation. Each experiment was performed in triplicate (n = 3). Chemical and physical parameters of oils namely free fatty acid (FFA), peroxide value (PV), p-anisidine values (PaV), and β- carotene were measured according to the methods described earlier in section 2.2.2.2.5.

2.2.2.2.3.2 The thermal stability studies

The thermal stability of the RPO and blended oils were done to assess the stability of oils during frying. According to Majchrzak et al., (2017), oils incubated at 140 °C have a short oxidation timeand therefore not suitable as a frying medium. Therefore we took 140 °C as a benchmark for the thermal stability studies and heating oils at 80 °C, 100 °C, 120 °C and 140 °C were done for elongated time that is 20 minutes. These time temperature combinations were matching with common culinary preparation methods. The thermal stability studies were performed with the help of an oil bath (JULABO ME-4, Heating Circulator, Germany). The samples were analysed for their physico- chemical parameters like FFA, PV, P-anisidine and β- carotene, which follows the methodology described in the earlier sections.

2.2.3 Statistical analysis

All the measurements were performed in triplicates, and the results were expressed as mean ± standard deviation (SD). The significance of the difference between the means of all the parameters was examined by, using EXCEL^{TM} 2010 (Microsoft, USA) and one way ANOVA with using the software GraphPad Prism 7.00, at a confidence level of 95%. The level of significance was set at $p<0.05$.

2.3 Results and discussion

2.3.1 Quality analysis of red palm oil and their blends

Shelf stability of vegetable oils is an important parameter, as in the case of any food commodity that decides its acceptability, quality and market value (Kua et al., 2014). Oxidation of oil is an autocatalytic reaction generating hydro peroxides from unsaturated acylglycerols. Blending of different vegetable oil can also improve the content of antioxidant and bioactive that in turn can enhance the nutritional, thermal and storage stability of vegetable oils (Abdel-Razek et al., 2011, Valantina et al., 2014).

In the present study, RPO produced using the indigenous technology developed at CSIR-NIIST, was blended with commonly used edible oils, like sun flower oil, rice bran oil, olive oil, and coconut oil, to improve its fatty acid profile and antioxidant bioactive such as β-carotene. The carotene content of RPO and its blends were quantified and were found to be 374.76 ± 0.08, 73.72 ± 0.27, 73.25± 0.08, 73.97±0.001, and 72.71±0.60 for RPO, CO+, RBO+, SFO+, and OL+ respectively. Red palm oil is highly effective in improving vitamin A status amongst populations at risk of vitamin A deficiency as it contain 300-500 ppm of β-carotene (Mba et al., 2015, Sivan et al., 2001). Rice et al., (2010), stated that increased dietary intake of Red palm oil ensures supply of abundant amount of β-carotene. However, the consumer's acceptance of RPO was poor due to the deep red colour owing to the high level of β-carotene. As the new generation consumers are more convinced about the nutritional value rather than the visual appeal, the demand for RPO is on a rise in the global market (Radhika et al., 2003; Delisle et al., 2001). In the present study, the individual oils are

deficient in carotene content. Blending of individual oils with red palm oil offers a possibility for enriched β-carotene content and improved fatty acid profile.

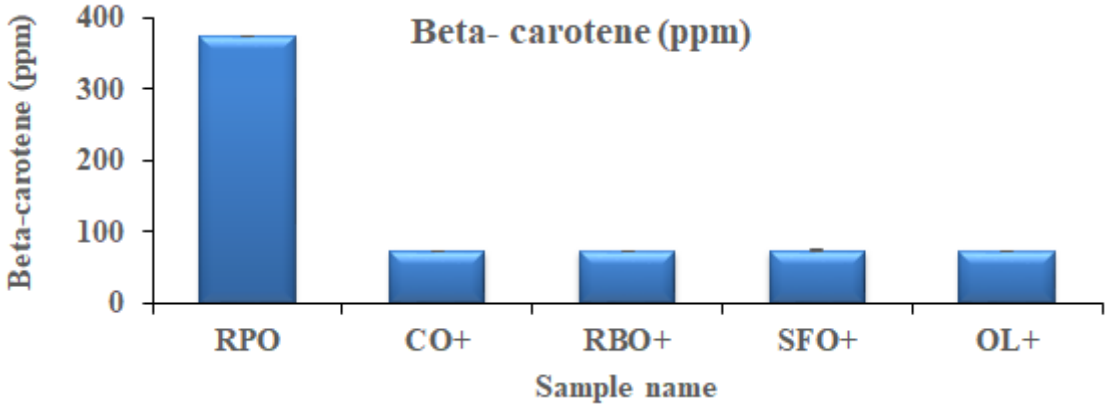


Figure 2.2: Beta carotene (ppm) content of RPO and its blends
Each value represents the mean ± standard deviation (SD) from triplicate measurements.

The oxidative stability of the individual oil and its blends before the storage and thermal studies evaluated in terms of free fatty acids, formation of primary (peroxide value) and secondary oxidation products (p-Anisidine value). The results are presented in Table- 2. As per Codex Alimentarius (CODEX STAN 210-1999), the FFA values should be less than 2 % and PV of should not exceed 15 meq/kg. Generally, peroxide values of fresh oils are less than 15 meq/kg, but the rancid taste becomes noticeable in oils when PV ranged between 30-40 meq/kg (Kamsiah and Yosof,, 2012; Choe and Min, 2006). Oils with high peroxide values are unstable and easily become rancid. In the present study, the freshness of both individual oil and its blends were confirmed by the values for FFA and PV, well below the upper limits indicated by the regulations. RPO showed PV of 1.97 ± 0.01 meq/kg. The highest PV was reported with SFO (2.84 ± 0.040 meq/kg), as the higher PUFA content of SFO may initiate

the formation of hydro peroxides (Bhatnagar et al., 2009; Kozłowska and Gruczyńska, 2018). The para- anisidine value is a measures the secondary oxidation products. The anisidine value measures high molecular weight saturated and unsaturated carbonyl compounds in triacylglycerol (Moigradean et al., 2012) and the maximum permissible limit is 25 (Ismail et al., 2016; Maszewska et al., 2018). SFO showed the highest PaV value, 23.87 ± 0.11. The results confirmed that all the oxidative parameters were within the permissible range. According to Bhatnagar et al., (2009), stability of oils can be increased by blending MUFA containing oil, this can be taken as a reason for the increased stability of coconut oil blends with other oils.

Table-2.2 Free fatty acid, Peroxide, and para-anisidine value of samples

Samples	FFA (%)	PV	PaV
RPO	0.60 ± 0.007^{a}	1.97 ± 0.01	05.88 ± 0.46
CO+	0.43 ± 0.014^{b}	0.44 ± 0.01^{d}	11.38 ± 0.82^{f}
CO	0.32 ± 0.007^{a}	0.33 ± 0.005^{c}	03.33 ± 0.36^{e}
RBO+	0.67 ± 0.014^{b}	2.21 ± 0.01^{c}	06.44 ± 0.17^{f}
RBO	0.55 ± 0.021^{a}	2.18 ± 0.005^{c}	10.02 ± 0.28^{e}
SFO+	0.63 ± 0.021^{b}	2.84 ± 0.040^{c}	20.71 ± 0.54^{f}
SFO	0.23 ± 0.028^{a}	2.72 ± 0.030^{c}	23.87 ± 0.11^{e}
OL +	0.54 ± 0.007^{b}	2.13 ± 0.015^{d}	10.22 ± 0.75^{f}
OL	0.42 ± 0.014^{a}	2.01 ± 0.015^{c}	12.08 ± 0.01^{e}

All values are shown as mean±standard deviations. Small letter ‘a –f ’ denotes the significance difference between blended and individual oils, with the p value < 0.05

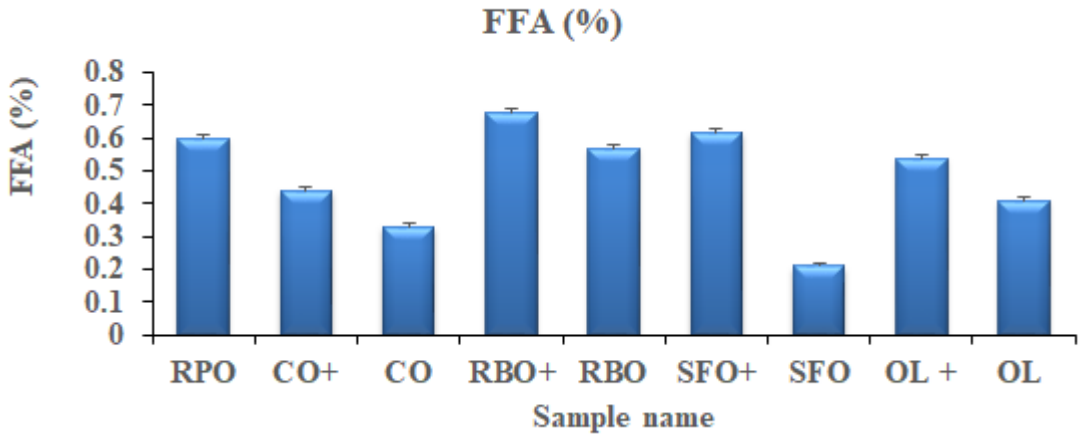


Figure -2.3: FFA% of RPO and its blends

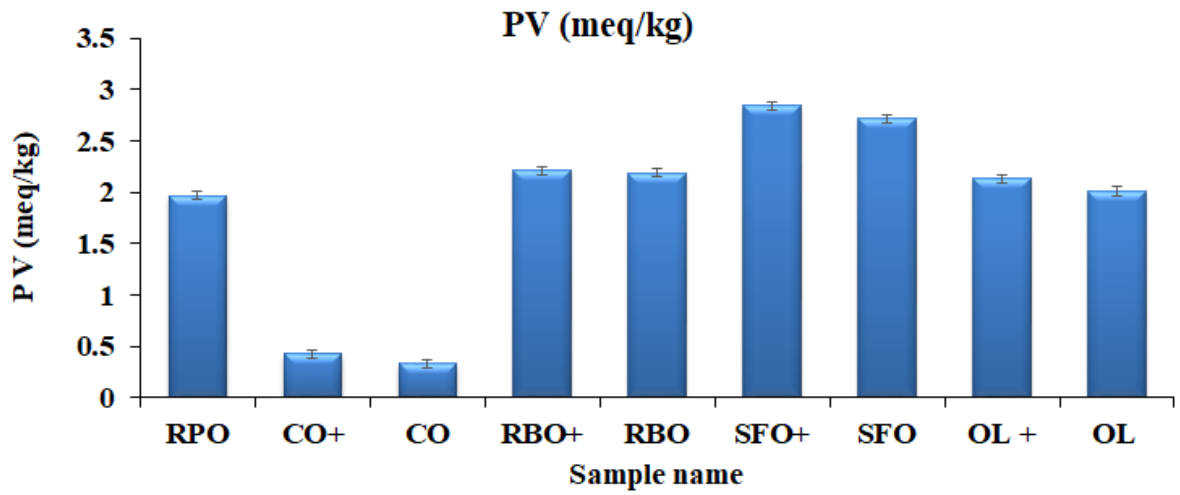


Figure- 2.4: PV of RPO and its blends

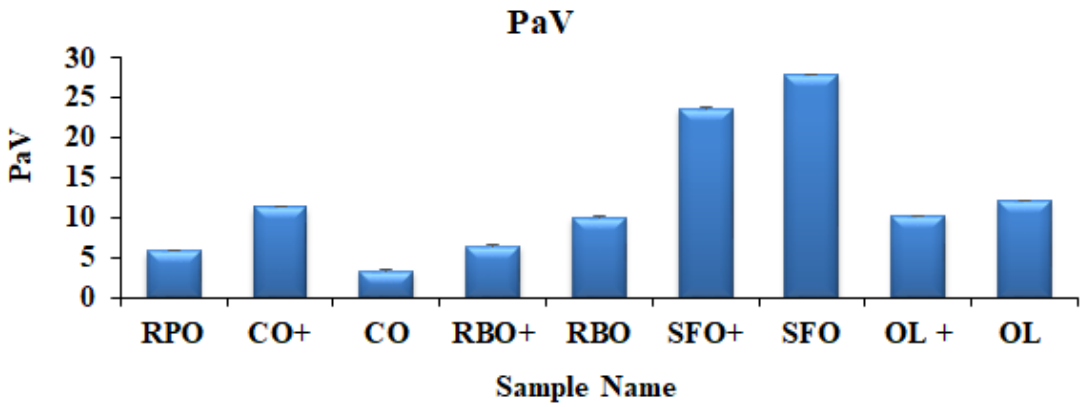


Figure- 2.5: PaV of RPO and its blends

2.3.2 Accelerated Storage studies

2.3.2.1 Oven stability studies

The accelerated stability test (Schaal oven test) was performed to evaluate the oxidative stability of RPO and its blends during storage. The stability studies were done at 60 ±2 °C for 28 days at 7 days interval. Apart from the oxidative parameters, the β-carotene content was also evaluated for each week interval time. The results demonstrated that, β-carotene content of RPO and the blends decreased over the storage period. The highest reduction was seen in SFO+, up to 30% (52.22 ± 0.31 ppm), after the twenty eighth day of storage (Figure -6). The result Table -3, indicated that ≈75 % of carotene was retained in all the oils even under accelerated conditions.

Free fatty acids increased during the accelerated storage, however it was within the regulatory limits (CODEX STAN 210-1999). The FFA content of oil blends were significantly less than the base oils ($p < 0.05$), except CO and CO+. Mai et al., (2012) reported that the increased proportion of unsaturated acids in the blends results in higher FFA during storage, as observed in the blend of 80:20 canola: olive (0.15%) as compared to 20:80 blends (0.32%).

This indicate that, the presence of unsaturated fatty acids will leads to increase in FFA on storage due to greater susceptibility of the same towards oxidative rancidity. Which is in agreement with earlier reports (Koohikamali and Alam, 2019; Nadeem et al., 2015). In the present study, the blending of RPO rich in saturated fatty acids with other oils with unsaturated oils resulted in lower oxidation. This could also be attributed to the presence of β-carotene, which is a strong antioxidant.

Higher oxidative stability, the lower will be the rate of generation of hydro peroxides (Kaseke et al., 2021). Figure-7, represented the PV during storage. As can be seen, PV value of RPO after 28 days of storage was 6.73±0.29 meq/Kg. The results were in agreement with the study conducted by Kaseke et al., (2021). The PV of all the blends were within the permitted limits after 28 days of storage and the stability of the blends were significantly higher than the individual oils, except for OL. This may be due to the presence of higher levels of bioactive compounds which can scavenge the free radicals formed (Reference). There is a possible slowdown of oxidative degradation if the blend containing a higher amount of MUFA or oleic acid than PUFA. That may be the reason why CO+ showed comparatively less PV than the other blended oils that is (3.97 ± 0.007 meq/Kg). Although PV is a valuable biomarker in the early stages of lipid oxidation, it is not a sufficient indicator to estimate the extent of oil stability at elevated temperatures. Due to their instability, peroxides degrade into secondary oxidation products such as aldehydes, ketones, and alcohols, which are responsible for the off flavour in the oxidised edible oils (Koohikamali and Alam, 2019). Hydro peroxides are less heat stable than aldehydes; therefore, p-AV is considered a more reliable indicator of advanced oxidative rancidity in oils (Choe and Min, 2006). Figure 8, represents the changes in the p-AV of RPO and its blends after twenty-eight days of storage at accelerated conditions. The p-AV value for SFO, SFO+, OL, OL+, RBO and RBO+ were 51.01± 0.01, 44.10± 1.01, 37.28 ± 0.38, 28.12 ± 0.17, 48.24 ± 0.01 and 37.28 ± 0.06 respectively. A

significant reduction in p-AV was observed in blended oils, except SFO+. The p-AV values of individual oils were much higher than those of blends over storage up to 28th day, indicating that lipid oxidation was less extensive oil blends which may be correlated with their higher β-carotene as well as with reduced levels of polyunsaturated fatty acids. A study conducted by Yang et al., (2014), showed that the blends of rice bran oil with soyabean oil showed reduced PaV when compared to the oil without blending. On blending with RPO which is a good source of saturated fatty acids like Palmitic acid (Nayana et al., 2021; Montoya et al., 2014 and Bayrak et al., 2010), the proportion of unsaturation comes down that help in minimizing the oxidative deterioration in blended oils. The accelecated studies suggests that the RPO blends are more stable to oxidation than the individual oils, which may be attributed to the increase in saturation as well as the presence of antioxidant β-carotene.

Table -2.3 β-carotene content of RPO and the blended oils during accelerated storage

Days	RPO	OL+	CO+	SFO+	RBO+
0	374.38 ± 0.53	71.85 ± 1.20	73.21 ± 0.29	73.48 ± 0.68	73.13 ± 0.16
7	320.11 ± 0.16	67.26 ± 0.36	66.17 ± 0.24	68.37 ± 0.52	66.02 ± 0.00
14	282.02 ± 0.03	62.26 ± 0.36	62.23 ± 0.33	64.01 ± 0.01	62.24 ± 0.33
21	267.26 ± 0.36	58.27 ± 0.38	57.11 ± 0.16	56.25 ± 0.36	55.92 ± 1.30
28	267.15 ± 0.29	54.07 ± 0.81	53.48 ± 0.68	52.22 ± 0.31	54.07 ± 0.10

All values are shown as mean±standard deviations. Significance difference between blended and individual oils, with the p value < 0.0002 which is $p < 0.05$.

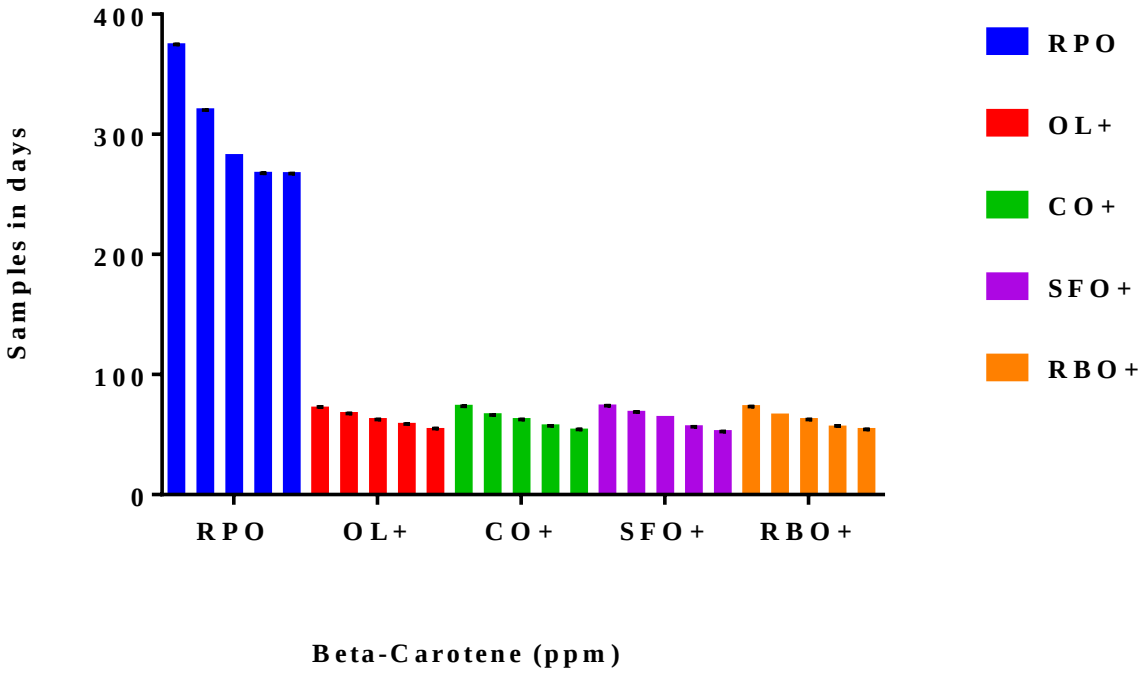


Figure- 2.6: Beta carotene (ppm) content RPO and its blends during accelerated storage.

***p=0.002, p*value ≤ 0.05**

Table -2.4 FFA content of RPO and the blended oils during accelerated storage

Days	RPO	CO	CO+	SFO	SFO+	RBO	RBO+	OL	OL+
0	$0.55 \pm 0.07^{*}$	$0.33 \pm 0.00^{a*}$	0.44 ± 0.00^{b}	$0.56 \pm 0.01^{c*}$	0.68 ± 0.007^{d}	$0.21 \pm 0.007^{e*}$	0.62 ± 0.00^{f}	$0.41 \pm 0.00^{g*}$	0.52 ± 0.02^{h}
7	$0.63 \pm 0.01^{*}$	$0.525 \pm 0.007^{a*}$	0.52 ± 0.00^{b}	$0.60 \pm 0.00^{c*}$	0.73 ± 0.02^{d}	$0.32 \pm 0.02^{e*}$	0.60 ± 0.00^{f}	$0.50 \pm 0.007^{g*}$	0.62 ± 0.02^{h}
14	$0.71 \pm 0.00^{*}$	$0.62 \pm 0.014^{a*}$	0.61 ± 0.007^{b}	$0.66 \pm 0.00^{c*}$	0.83 ± 0.007^{d}	$0.36 \pm 0.007^{e*}$	0.54 ± 0.007^{f}	$0.63 \pm 0.02^{g*}$	0.75 ± 0.04^{h}
21	$0.98 \pm 0.02^{*}$	$0.68 \pm 0.007^{a*}$	0.64 ± 0.05^{b}	$0.97 \pm 0.05^{c*}$	1.03 ± 0.03^{d}	$0.55 \pm 0.02^{e*}$	0.77 ± 0.007^{f}	$0.67 \pm 0.01^{g*}$	0.94 ± 0.05^{h}
28	$1.03 \pm 0.00^{*}$	$0.845 \pm 0.007^{a*}$	0.76 ± 0.02^{b}	$1.12 \pm 0.06^{c*}$	1.25 ± 0.07^{d}	$0.73 \pm 0.01^{e*}$	0.81 ± 0.007^{f}	$0.68 \pm 0.01^{g*}$	1.16 ± 0.19^{h}

All values are shown as mean ± standard deviation. Small letter 'a –h' denotes the significance difference between blended and individual oils, with the p* value < 0.05, *RPO is significantly different from the individual oils, $p < 0.05$.

Table -2.5 PV content of RPO and the blended oils during accelerated storage

Days	RPO	CO	CO+	SFO	SFO+	RBO	RBO+	OL	OL+
0	1.95 ± 0.02*	0.33 ± 0.00*^{a}	0.43 ± 0.007^{b}	2.32 ± 0.44*^{c}	2.14 ± 0.00^{d}	2.18 ± 0.007*^{e}	2.23 ± 0.02^{f}	2.86 ± 0.02*^{g}	2.75 ± 0.04^{g}
7	5.62 ± 0.08*	0.34 ± 0.007*^{a}	3.44 ± 0.007^{b}	5.22 ± 0.17*^{c}	4.77 ± 0.007^{d}	5.32 ± 0.02*^{e}	3.40 ± 0.33^{f}	3.92 ± 0.03*^{g}	3.84 ± 0.04^{g}
14	6.11 ± 0.13*	0.45 ± 0.04*^{a}	3.76 ± 0.02^{b}	5.65 ± 0.04*^{c}	4.98 ± 0.007^{d}	7.18 ± 0.08*^{e}	3.68 ± 0.42^{f}	4.11 ± 0.13*^{g}	4.53 ± 0.63^{g}
21	6.36 ± 0.31*	0.72 ± 0.007*^{a}	3.59 ± 0.50^{b}	5.88 ± 0.15*^{c}	5.35 ± 0.20^{d}	7.19 ± 0.21*^{e}	4.10 ± 0.06^{f}	5.27 ± 0.09*^{g}	5.12 ± 0.14^{g}
28	6.73 ± 0.29*	1.05 ± 0.00*^{a}	3.97 ± 0.007^{b}	6.84 ± 0.02*^{c}	6.07 ± 0.03^{d}	8.45 ± 0.28*^{e}	4.32 ± 0.09^{f}	5.96 ± 0.02*^{g}	5.345 ± 0.29^{g}

All values are shown as mean ± standard deviation. Small letter 'a –h' denotes the significance difference between blended and individual oils, with the p* value < 0.05,
OL and OL+ are not significant to each other, $p=0.53$, $p \geq 0.05$
*RPO is significantly different from the individual oils, $p < 0.05$.

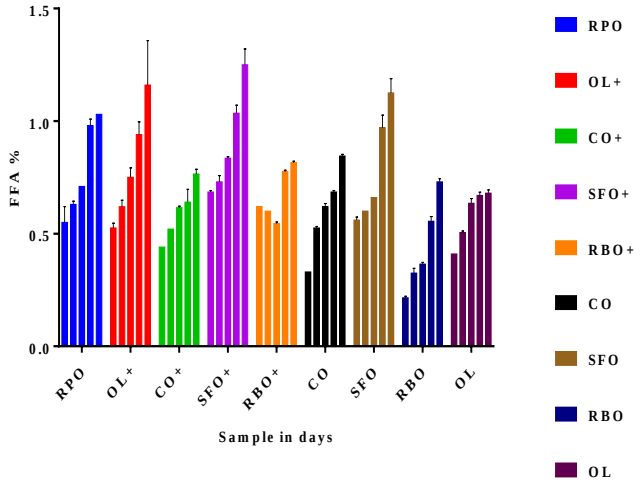


Figure- 2.7: FFA content of RPO and blends after 28 days of storage ***p=0.0028, p*value ≤ 0.05**

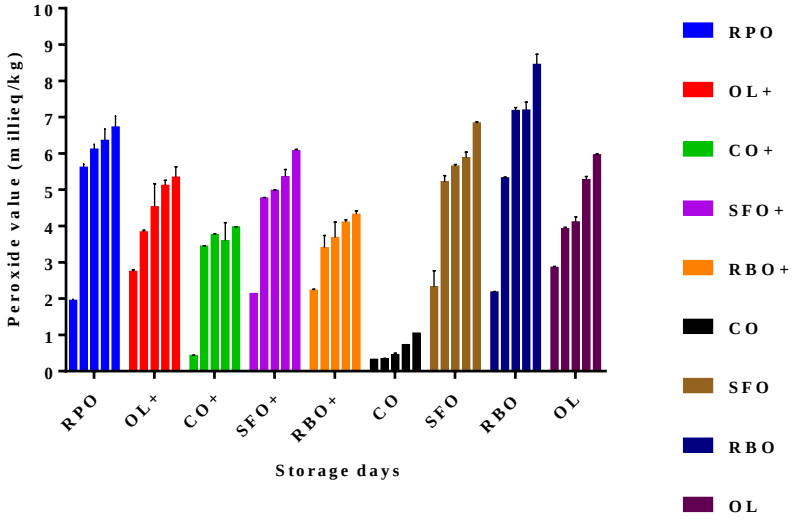


Figure- 2.8: Peroxide value of RPO and its blends after 28 days of storage

***p=0.0022, p*value ≤ 0.05**

Table -2.6 PaV content of RPO and the blended oils during accelerated storage

Days	RPO	CO	CO+	SFO	SFO+	RBO	RBO+	OL	OL+
0	5.72 ± 0.22*	3.32 ± 0.01*^{a}	11.35 ± 0.04^{b}	23.63 ± 0.11*^{c}	27.87 ± 0.007^{c}	10.02 ± 0.00*^{e}	6.38 ± 0.08^{f}	18.57 ± 0.48*^{g}	8.93 ± 0.02^{h}
7	10.23 ± 0.02*	4.14 ± 0.19*^{a}	14.77 ± 0.29^{b}	28.60 ± 0.53*^{c}	30.21 ± 0.007^{c}	17.22 ± 0.03*^{e}	10.82 ± 0.38^{f}	22.01 ± 0.04*^{g}	14.77 ± 0.69^{h}
14	15.92 ± 0.40*	5.76 ± 0.22*^{a}	20.27 ± 0.08^{b}	34.41 ± 0.20*^{c}	34.10 ± 0.14^{c}	24.11 ± 0.19*^{e}	22.17 ± 0.26^{f}	28.01 ± 0.007*^{g}	20.80 ± 0.26^{h}
21	20.62 ± 0.53*	6.405 ± 0.27*^{a}	22.34 ± 0.06^{b}	50.36 ± 0.52*^{c}	37.14 ± 0.23^{c}	35.22 ± 0.02*^{e}	29.19 ± 0.19^{f}	32.98 ± 0.007*^{g}	25.19 ± 0.30^{h}
28	30.55 ± 0.48*	6.23 ± 0.01*^{a}	24.63 ± 0.06^{b}	51.01 ± 0.01*^{c}	44.10 ± 1.05	48.24 ± 0.01*	37.28 ± 0.06^{f}	37.25 ± 0.38*^{g}	28.12 ± 0.17^{h}

All values are shown as mean ± standard deviation. Small letter 'a –h' denotes the significance difference between blended and individual oils, p* value < 0.05,
SFO and SFO+ with the p=0.40, not significant, p≥ 0.05.
*RPO is significantly different from the individual oils, p= 0.003, where p < 0.05.

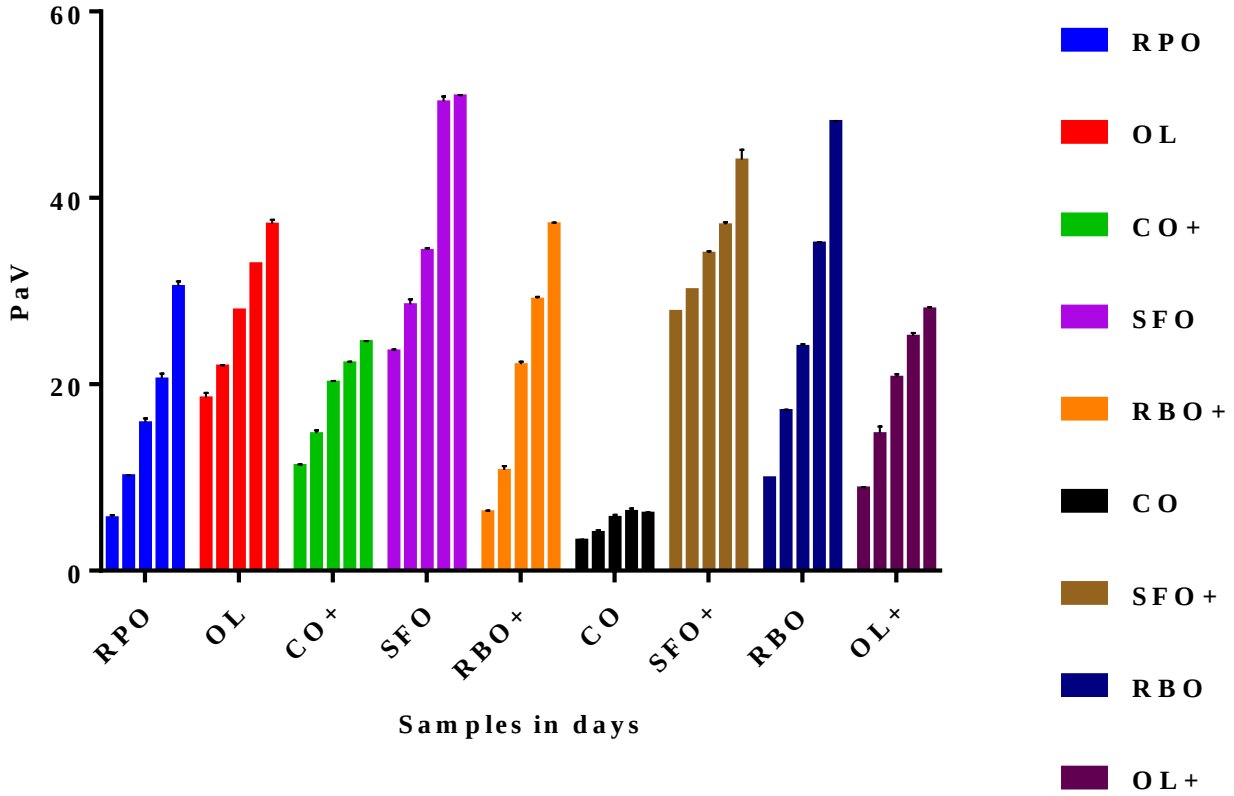


Figure- 2.9: PaV value of RPO and its blends after 28 days of storage

***p=0.0038, p*value ≤ 0.05**

2.3.2.2 Thermal stability studies

Oils are the medium for frying for large number of food products, it is very important that these oils must be thermally stable at the frying temperatures. The stability of fats and oils upon heating and frying temperature is an important measure of oil quality for its application in food industry. The natural antioxidants present in food play a vital role in preventing the oil from deteriorative changes from higher and elevated temperature exposures. The blending of different oils is reported to show better thermal and oxidative stability (Siddique et al., 2015; Siddeeg and Xia 2015; Chugh and Dhawan, 2014).

Thermal degradation of oils at frying temperature results in a number of chemical reactions which includes hydrolysis, oxidation, thermal decomposition and polymerization (Melton et al., 1994). Prolonged heating also reduces the organoleptic and nutritive quality of oils. The main thermal degradation products of vegetable oils are straight chain alkanes, alkenes, aldehydes, alcohols, aromatic compounds, and carboxylic acids (Majchrzak et al., 2017). Fortes and Baugh, (2004) reported that temperature, time, and type of atmosphere influence the stability of vegetable oils. Therefore in order to evaluate the thermal stability, the individual oils and the blends were subjected to heating at 80, 100, 120, and 140 °C for 20 min (Gomna et al., 2019; Fortes and Baugh., 2004) and were evaluated in terms of retention of beta-carotene, FFA, PV, and p-AV, values. As showed in Figure-9, it was found that, at 140 °C of heating, 60% of the carotenes were retained. Here in the present study with RPO blends, retention of beta-carotene a strong antioxidant (Loganathan et al., 2017) as well as the higher smoke point of RPO (Chew et al., 2021) (Tarmizi and Ismail, 2014), might help in the stability for frying operations. According to Chugh and Dhawan, (2014), it was noted that blending of mustard oil improved the polymorphic stability of palm oil as mustard oil is a rich source of antioxidants and vitamin A. A study conducted by Tiwari et al., (2014),

showed increased stability of blends of palm oil with sesame to thermal and oxidative deterioration even up to 180 °C of heating.

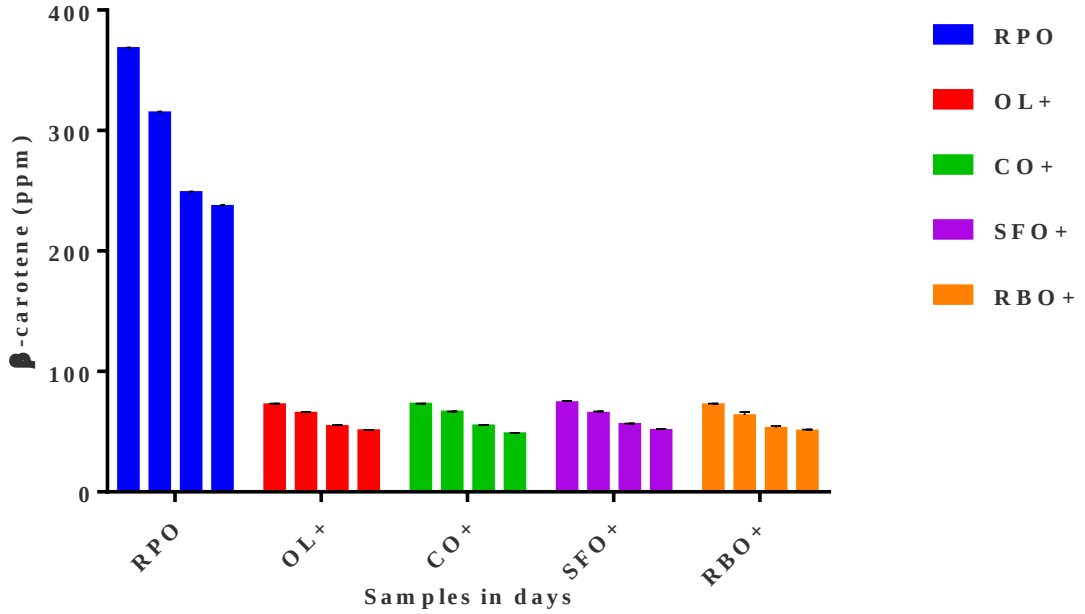


Figure- 2.10: Beta carotene content of RPO and its blends, analyzed at 80, 100, 120, 140 °C.
***p=0.0028, p*value ≤ 0.05**

FFA's are formed by the breakdown of triacyl-glycerols on hydrolytic or autoxidation. It has been reported that on thermal processing, hydrolysis occurs more in oils with short chain fatty acids than those with long chain saturated fatty acids. RPO, which is known source of saturated fatty acids (Mba et al., 2015), when blended with the unsaturated fatty acid containing RBO, SFO, OL, and CO, the extend of rancidity can be reduced when compared with the individual oils (Koohikamali and Alam, 2019). As can be send from Figure-10, the FFA of individual and blended oils were less than 1 %. It was mentioned that FFA affects the smoke point and thus, oils with higher FFA content are known to have lower smoke points (Gunstone, 2011). In the present study higher temperature heating does not alter the

FFA content much, the individual oils showed significant difference among the FFA content when compared with blended oils, thus indicating better thermal stability.

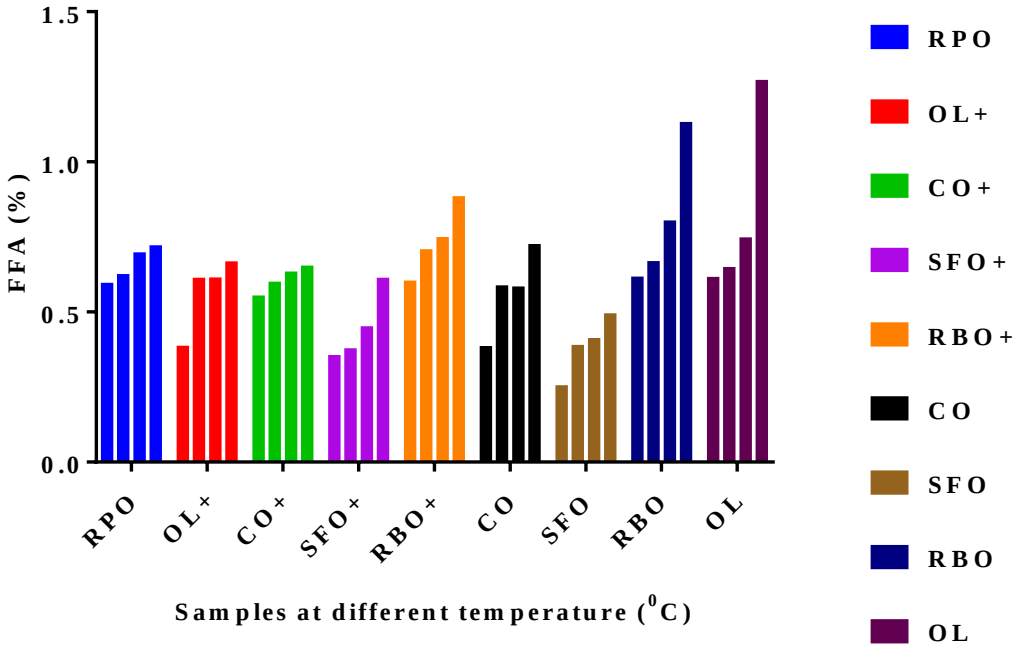


Figure -2.11: Free fatty acid of RPO and its blends, analyzed at 80, 100, 120, 140 °C for 20 min.
***p=0.003, p*value ≤ 0.05**

Blending tailors and improves frying properties of oil by fine tuning the fatty acid composition and antioxidant balancing. The high degradation rate of frying oils raise concerns about the safety of used oils in restaurants therefore blending of oil can be adopted to reduce the complex chemical reactions and use of synthetic antioxidants (Ramroudi et al., 2022). The peroxide and p-AV of oil samples after heating is shown in Table 7 and 8 respectively. The RPO blends showed the least values for peroxide and p-AV as compared to individual oils. As can be seen, though PV is within the permissible limits, the p-AV exceeds the limit, except of RBO, RBO+, CO and C+. It indicates the secondary stage of oxidation

prevails leading to the formation of aldehydes which are more heat-stable than the hydroperoxides (Koohikamali, and Alam, 2019). Palm oil is an excellent medium for frying as it is rich in saturated fatty acids (Tiwari et al., 2014). Thus blending of polyunsaturated oils with highly saturated oils reduces the content of linoleic and linolenic acids to the desirable level where the effect is similar to partial hydrogenation without worrying about the formation of *trans* fatty acid isomers (Hoffmann et al., 2002; Naghshineh and Mirhosseini, 2010; Tiwari et al., 2014).

Different researchers have evaluated the frying characteristics of blends of different vegetable oils such as canola oil, soybean oil, and sesame oil with palm olein and found that the improved fatty acid profile upon blending helped to improve the the thermal stability (Naghshineh and Mirhosseini, 2010; Del Carmen FloresÁlvarez et al., 2012; Kupongsak and Kansuwan, 2012; Tiwari et al., 2014). Roiaini et al., (2015) reported that blending of canola and olive oil with palm olein, which is most stable against rancidity and oxidation, enhanced the stability. De Leonardis and Macciola, (2012) showed that thermal stability of virgin olive oil greatly increased when blended with palm oil. A study done by Siddiquie et al., (2010), showed that palm olein-canola blend was found to have more resistance against oxidation, followed by palm olein-soybean and palm olein-sunflower blends. As observed in the present study, primary and secondary oxidation parameters during the accelerated storage and thermal stability the blended oils indicated stability against oxidative and thermal degradation when compared with the values of individual oils. Oxidation, which is accelerated at the high temperature as used in deep frying, creates rancid flavours and reduces the organoleptic characteristics of fried food thus blending is a good choice to ensure the product quality. The food value of the oils and blends can also be predetermined to provide the food with improved fatty acid profile to the consumers.

Table -2.7. Peroxide value of RPO and its blends, analyzed at 80, 100, 120, 140 °C

Temperature (°C)	RPO	OL	OL+	CO	CO+	SFO	SFO+	RBO	RBO+
80	3.22 ± 0.31*	7.02± 0.02*^{b}	4.5± 0.05^{c}	0.57± 0.007*^{a}	0.21± 0.007^{d}	8.05± 0.014*^{e}	1.96± 0.014^{f}	13.56± 0.01*^{g}	8.72± 0.007^{h}
100	3.55± 0.14*	8.43± 0.02*^{b}	4.93± 0.02^{c}	0.63± 0.014*^{a}	0.36± 0.014^{d}	9.17± 0.028*^{e}	2.62± 0.085^{f}	14.25± 0.007*^{g}	9.39± 0.07^{h}
120	3.59± 0.07*	9.23± 0.12*^{b}	5.11± 0.18^{c}	0.97± 0.00*^{a}	0.54± 0.007^{d}	10.13± 0.15*^{e}	3.46± 0.29^{f}	16.67± 0.02*^{g}	10.60± 0.08^{h}
140	3.97± 0.02*	9.51± 0.07*^{b}	8.435± 0.62^{c}	1.01± 0.014*^{a}	0.85± 0.021^{d}	11.45± 0.17*^{e}	3.96± 0.13^{f}	17.29± 0.41*^{g}	11.20± 0.09^{h}

All values are shown as mean ± standard deviation, *RPO is significantly different from the individual oils, $p < 0.05$, All values are shown as mean ± standard deviation. Small letter 'a –h' denotes the significance difference between blended and individual oils, p* value < 0.05

Table -2.8. PaV of RPO and its blends, analyzed at 80, 100, 120, 140 ˚C.

Temperature (°C)	RPO	OL	OL+	CO	CO+	SFO	SFO+	RBO	RBO+
80	3.44 ± 0.04*	28.34 ± 0.14*^{a}	13.55 ± 0.14^{b}	21.26 ± 0.01*^{c}	4.11 ± 0.01^{d}	41.37 ± 0.20*^{e}	11.08 ± 0.05^{f}	33.5 ± 0.05*^{g}	5.15 ± 0.12^{h}
100	9.25 ± 0.00*	36.5 ± 0.53*^{a}	16.76 ± 0.10^{b}	26.19 ± 0.06*^{c}	6.38 ± 0.27^{d}	49.21 ± 0.00*^{e}	15.4 ± 0.09^{f}	43.24 ± 0.00*^{g}	9.13 ± 0.02^{h}
120	15.35 ± 0.12*	42.69 ± 0.14*^{a}	19.49 ± 0.07^{b}	29.38 ± 0.07*^{c}	11.34 ± 0.14^{d}	57.38 ± 0.23*^{e}	18.39 ± 0.21^{f}	50.56 ± 0.45*^{g}	15.72 ± 0.23^{h}
140	20.38 ± 0.16*	51.16 ± 0.02*^{a}	21.2 ± 0.21^{b}	31.39 ± 0.21*^{c}	24.19 ± 0.19^{d}	64.50 ± 0.06*^{e}	22.36 ± 0.12^{f}	56.39 ± 0.21*^{g}	19.25 ± 0.00^{h}

All values are shown as mean ± standard deviation. *RPO is significantly different from the individual oils, $p < 0.05$, All values are shown as mean ± standard deviation. Small letter 'a –h' denotes the significance difference between blended and individual oils, p* value < 0.05

2.4 Summary and Conclusion

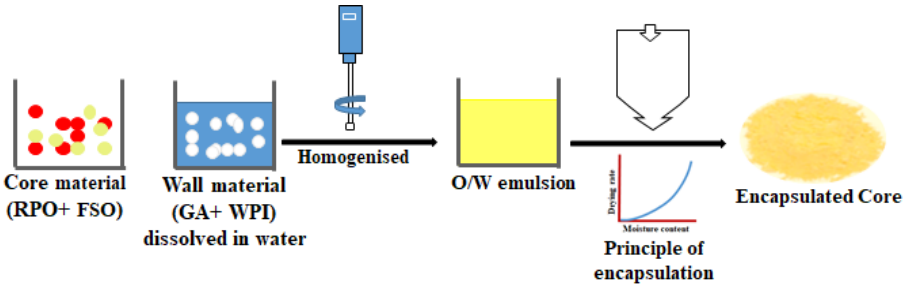
Oils and fats play a vital role in daily life. Red palm oil is a rich source of β-carotene. The blending of β-carotene rich RPO with different edible vegetable oils such as SFO, RBO, OL, and CO in the ratio of 20:80 resulted in improved fatty acid profile with a noticeable amount of beta carotene content. The thermal, oxidative and storage stability of these blended oils in comparison with the base oils has been characterized on the basis of β-carotene content, FFA, PV, and PaV. Blending with RPO improved the carotene content to base oils which other was absent in them. It was found that the blended oils were retaining carotene up to 70 % during accelerated storage and frying conditions. The oxidative and thermal stability analysis showed that the blending with RPO helped the base oils to withstand oxidative and thermal conditions by improving the saturated fatty acid content. As the blended oils exhibited greater stability to oxidation and temperature, blending can be opted as a method for reducing the oxidation levels. Blending of oils rich in unsaturation with other oils rich in phytochemicals and saturated fatty acids in an optimum level offers enhanced nutritional quality as well as shelf and thermal stability. Blending of less exploited oils with nutritional and phytochemical enriched oils with commonly consumed oils. Needs to be exploited more to enhance the nutritional and technological properties further.

The key findings were summarized below:

- Red palm oil blend with other oils improved the shelf life and thermal stability.
- Blending of RPO with other oils could be used as a medium to deliver beta carotene (20 % RDA) and thus can be used as Vitamin-A supplement in culinary applications.
- Blending can be opted as a method for reducing the oxidative rancidity of oil.

Chapter 3

Process Development of Red Palm Oil/ Flax Seed Oil Blends with Balanced Fatty Acid Composition and Application in Food Products for Nutrient Delivery



3.1 Introduction

Tailoring the balance of fatty acids in edible oils and transforming them into various household and industrial applications are gaining more interest. Deficiency due to the lack of balanced fatty acids in the diet, especially polyunsaturated fatty acids (PUFA), is one of the significant nutritional problems globally (Karthik and Anandharamakrishnan, 2013; Menina et al., 2018). According to the Food and Nutrition Board's Committee on diet and health, the recommended ratio of saturated fatty acid (SFA)/ monounsaturated fatty acid (MUFA)/ polyunsaturated fatty acid (PUFA) in edible oils is 1/2/1, and that of omega-3/omega-6 is 0.05 (National Research Council, 1989). The blending of edible oils is considered as the least expensive approach that results in desired fatty acid composition (Guiotto et al., 2014; Srivastava et al., 2016). For instance, palm oil blending with canola and olive oil has been found to enhance the essential fatty acid composition (Roiaini et al., 2015). Palm oil is known for its saturated fatty acid composition, which gives good stability during frying and storage (Hashempour-Baltork et al., 2016).

Red palm oil (RPO) is a minimally processed palm oil that naturally contains tocopherols and tocotrienols (500-1000 ppm), and carotenoids (500-700 ppm) (Lee et al., 2018; Mba et al., 2015). Its primary constituent fatty acids are palmitic acid (42%) and oleic acid (42%). Despite its various benefits, RPO is deficient in PUFA. Flaxseed oil (FSO) has gained wide acceptability as a vegan source of essential fatty acids, alpha-linolenic acid (50–60%), vitamin E (tocopherols ranging from 20 to 70 mg/100 g), and vitamin A (carotenoids: ~57 ppm) (Goyal et al., 2015; Mohanan et al., 2018). Therefore, the blending of RPO and FSO in appropriate proportions could result in a product with improved fatty acid composition and enriched β-carotene content. However, PUFA is susceptible to oxidative degradation due to its high degree of unsaturation (Ramadan and Wahdan, 2012).

Using the microencapsulation approach, edible oils can be converted into powder form using appropriate techniques to improve handling. Moreover, encapsulation promotes stability of oils and fats through long period of storage, besides facilitating easy transport. Therefore, converting oil to powder can be an innovative and consumer-friendly approach to improve the nutritional and oxidative stability of oils and to widen their applications in the culinary/health/functional food and nutraceutical sectors (Campos et al., 2019). Microencapsulation of RPO has been reported to improve nutrient availability in piglets (Ren et al., 2020). FSO has been successfully microencapsulated by spray drying using different wall materials. The resultant oil encapsulates exhibited good dissolution and reconstitution behavior and good storage stability at room temperature (Goyal et al., 2015; Sharif et al., 2017). However, there are no reports on the microencapsulation of RPO and FSO blends until now.

Various encapsulation techniques have been employed for converting oils into powder form by utilizing a wide range of carrier or wall materials. The commonly used microencapsulation techniques include emulsification, spray-drying, and freeze-drying (Liu et al., 2004). Among the above, spray drying (SD) is considered as an industry-friendly drying-cum-encapsulation technique. Spray drying is a practical approach to improve the oxidative stability of oils (Carneiro et al., 2013; Priol et al., 2019). Choosing an ideal wall material is the key to achieve optimal encapsulation efficiency; the general traits expected of a wall material include, but are not limited to, bland flavor, high solubility, emulsification ability, film-forming, and drying properties. Diverse wall materials have been used for encapsulating oils, including gums (gum arabic (GA), xanthan), proteins (whey protein (WP), soy protein), polysaccharides, and modified starches (Leyva et al., 2018). Among the hydrolyzed starch-based wall materials, maltodextrin was used extensively by different authors for the encapsulation process. However, maltodextrin lacks emulsifying capacity

and thereby leads to poor emulsion stability and lower retention of volatiles and oils (Kenyon, 1995). Consequently, use of maltodextrin as wall material for the encapsulation of lipids often demands an additional emulsifying agent.

In the above context, whey protein is a type of wall material which exhibits good encapsulation properties due to its gelling behavior and ability to entrap both volatile and non-volatile core substances (Gad et al., 2011; Edris and Langrish, 2016). Gum arabic exhibits heat-resistant properties (Salar-Behzadi et al., 2013; Bucurescu et al., 2018). The protective effect of gum arabic has been attributed to the stabilization of the phospholipid membrane by hydrogen bonding (Anandharamakrishnan et al., 2015).

Blending studies in Chapter 2 indicated that, though blends of RPO has improved β-carotene content and demonstrated better oxidative shelf and thermal stability, the blends were lacking ω3 and ω6 fatty acids. Thus, the aim of present work is to microencapsulate the RPO-FSO blend by spray drying using different combinations of whey protein and gum Arabic as wall materials. The resultant product is expected to have enhanced shelf-stability, in addition to an improved fatty acid profile and enriched β-carotene content. The physical and structural properties of the developed RPO-FSO microencapsulate were evaluated besides its encapsulation efficiency and β-carotene content. Further, the potential applications of the resultant oil encapsulate as a healthy fat substitute and β-carotene fortificant were assessed, using cupcakes as the model food product.

3.2 Objectives

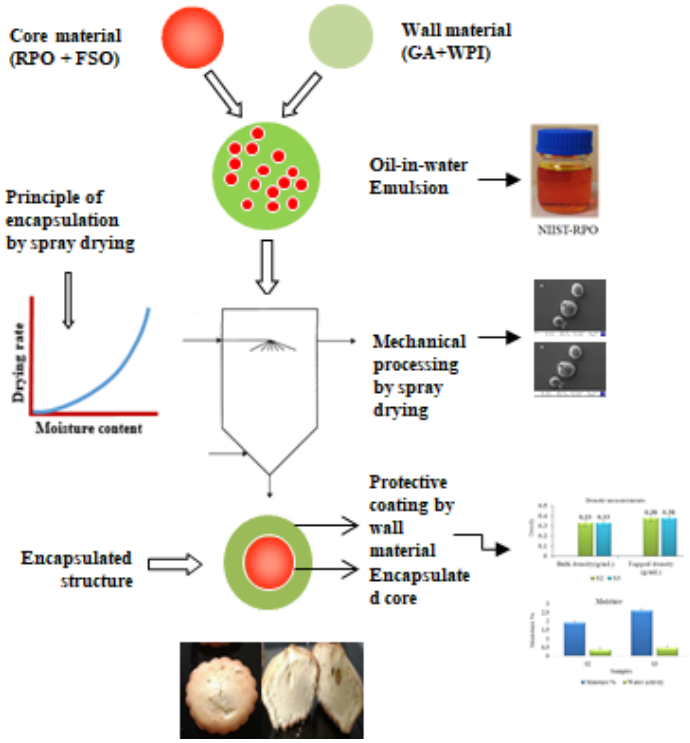
- Process development for the production of spray dried oil encapsulate using RPO-FSO blend with a varying combination of wall materials

- To fabricate a product with improved fatty acid profile and enriched β-carotene content

- To deliver the RPO-FSO encapsulate as a healthy fat substitute and β-carotene fortificant in model food product

3.3 Materials and methods

The Experimental design for Process development of Red Palm oil/ Flax Seed oil blends with balanced fatty acid composition and application in food products for nutrient delivery is represented below.



3.3.1. Materials

Crude palm oil was procured from M/s Godrej Industries, Tamil Nadu. The RPO used in this study was produced from crude palm oil, using the minimal processing technology

developed by CSIR-NIIST, Thiruvananthapuram, India (Mayamol et al., 2009). Flaxseed oil (FSO) was purchased from the local market in Thiruvananthapuram. Whey protein isolate (>95% purity and having a molecular weight of 18.3 k Da) was obtained from ACE International LLP, New Delhi, India. Gum arabic, xanthan gum, and guar gum, maltodextrin were bought from Premia Food Additives, Mumbai, India. Sodium bicarbonate, sodium hydroxide, anhydrous sodium sulfate, Potassium iodide (>99.5% purity), and solvents including hexane, methanol, glacial acetic acid, diethyl ether (>99.9% purity) were procured from Merck Limited, Mumbai, India**.** All the chemicals were of analytical grade. The ingredients for the preparation of cupcakes, including self-rising flour, sugar, butter, and eggs, were procured from the local market of Thiruvananthapuram.

3.3.2 Methods

3.3.2.1 Preparation and characterization of blends

The RPO and FSO were blended in two different ratios, 70:30 and 60:40, to obtain oil blends with an improved fatty acid profile. The RPO and FSO were mixed thoroughly using an overhead stirrer (Remi, RQ126/D, with 40V, Mumbai, India), based on the procedure of Guiotto et al., (2014), with slight modifications. GC-MS analysis of the blended oil was performed to confirm the fatty acid composition.

3.3.2.2 Fatty acid profiling

For the characterization, fatty acids were subjected to esterification to form fatty acid methyl esters (FAMEs) (AOCS, 2017; Method Ce 2-66). Briefly, 1 mL of 2 % methanolic sulphuric acid was added to 0.5 g of oil and refluxed at 55 °C for 3 h. Thin-layer chromatography of the sample was done using hexane, diethyl esters, and glacial acetic acid in the ratio 80:20:1, ensuring the completion of methylation. Proper methylation is indicated by the formation of a single band of methyl ester below the solvent front in a paper chromatogram, which was

developed with the help of hexane: di-ethyl ether: glacial acetic acid. After refluxing, the cooled oil sample was washed with 2 mL hexane 2-3 times, then with 2% sodium bicarbonate for 2-3 times, and finally with water. The aqueous layer was then drained off, and the top layer was filtered through cotton, which contained anhydrous sodium sulfite. The FAMEs (Fatty acid methyl esters) were then analyzed using GC-MS/MS (GC-MS/MS, Model: Thermo scientific TRACE 1310/TSQ 8000) equipped with TR-FAME (Thermoscientific) 30 m × 0.25 mm × 0.25 um capillary column. The carrier gas was helium at a flow rate of 1.5 mL/min. The initial column temperature was set at 140 °C with a holding time of 4 min. Subsequently, the temperature was increased up to 230 °C at the rate of 4 °C/min, followed by a holding time of 20 min. MS (TSQ 8000) with triple quadrupole was used for the identification of FAME. Qualitative analysis of each sample was performed using the NIST library (National institute for standers and technology) by comparing with standard curves derived for each of the major fatty acids under investigation.

3.3.2.3. Emulsion preparation

Oil-in-water emulsions of the RPO-FSO blends (core) were prepared initially, prior to encapsulation by spray drying. Whey protein isolate (WPI) and gum arabic (GA) at different proportions were used as the wall material (Table 1). WPI and GA also act as drying aid and surfactant/emulsifier (Fuchs et al., 2006; Kha et al., 2014; Chuyen, 2019). Preliminary trials were carried out to find out the most efficient combination of WPI and GA that resulted in maximum product yield (Table 1). The wall materials in the optimized proportions were entirely dispersed in the required amount of water, followed by oil addition. The core-to-wall ratio was fixed at 1:2. The mixture was homogenized using a rotor-stator homogenizer (T25 ULTRA-TURRAX digital; dispersing tool: S 25 N - 8G; IKA India Private Limited, Bengaluru), at 5000 rpm for 10 min at room temperature (28 ± 2 °C). The emulsion thus obtained was immediately taken for spray drying.

Table 3.1: Effect of different composition of gum arabic and whey protein isolate on the yield

Trails	Wall material	Composition (w/w)	Yield (%)
S1	GA:WPI	2:1	40.90 ± 0.12
S2	GA:WPI	1:1	55 ± 0.04
S3	GA:WPI	1:2	62.07 ± 0.10

Significant at *p-value* = 2.23×10^{-7}*; p≤ 0.05*

3.3.2.4. Microencapsulation by spray drying

Immediately after the emulsion preparation, the emulsion was converted to encapsulated powder by spray drying using a mini spray dryer (LABULTIMA, Process technologies Pvt Ltd., Mumbai, India, Model LU 228 ADVANCED), operating in a co-current configuration. A two-fluid nozzle having an orifice diameter of 1.0 mm was used for atomization. Spray drying was carried out at various inlet and outlet air temperatures, at a constant feed flow rate and aspirator rate. The air pressure was held steady at 1.5 kg/cm^2. The emulsion was fed at 60 °C into the main drying chamber at a feed flow rate of 1.2 mL/s, controlled by a peristaltic pump. And the airflow rate was set at 73 m^3/h (1.2 m^3/min). The samples of oil encapsulate were stored in Duran bottles until further analysis

3.3.2.5 Characterization of spray-dried encapsulates

3.3.2.5.1 Water activity (a_w)

A digital water activity meter (Rotronic HygroPalm23-AW-A, Switzerland*)* was used to measure the water activity of the spray-dried oil encapsulate. The sample was placed in a sample cup of 14 mm depth, completely covering the bottom of the cup. A sealed container was formed by placing the probe above the sample cup, and the value of water activity was recorded from the digital display of the water activity meter.

3.3.2.5.2 Moisture content

The moisture content of the oil encapsulate was determined using a Moisture analyzer (HC 103 Mettler Toledo, India). Oil encapsulate (0.5 g) was placed in the analyzer set at a temperature of 105 °C until a constant weight was reached. The value of moisture content (in percentage, %) was recorded from the digital display of the moisture analyzer.

3.3.2.5.3 Color analysis

The color of the oil encapsulate was determined using Hunter lab, ColorFlex EZ (Hunter Associate Laboratory Inc., Reston) Port up, or Port forward dual-beam spectrophotometer. The results were expressed as Hunter color values of L*, a*, b*, where L denotes lightness/ darkness, a* denotes redness and greenness, and b* denotes yellowness and blueness. The total color difference or change in color between the two samples was calculated using the below formula.

$$\Delta E = \sqrt{(L^*_o - L^*)^2 + (a^*_o - a^*)^2 + (b^*_o - b^*)^2} \qquad (1)$$

Where,

L_o*, a_o*, and b_o* are the color values of the standard reference type;

L*, a*, and b* are the color values of the test sample (oil encapsulate).

3.3.2.5.4 Bulk density

Bulk density (g/mL) was determined by the method proposed by Goula and Adamopoulos, (2004), with slight modifications. Oil encapsulate (2 g) was gradually added into an empty 10 mL graduated cylinder. The bulk density was calculated as the volume occupied by the mass of the powder added to the cylinder (Eq. 2).

$$Bulk\ density = \frac{Mass\ of\ the\ powder}{volume\ of\ the\ powder} \qquad (2)$$

3.3.2.5.5 Tapped density

The tapped density of the sample was determined using the protocol described by by Chinta et al., (2009) with some modifications. Oil encapsulate (2 g) was gradually added into an empty 10 mL graduated cylinder. After tapping the cylinder for ten times, the volume occupied by the sample was noted. The tapped density was calculated using the below equation.

$$Tapped\ density = \frac{Mass\ of\ the\ powder}{volume\ of\ the\ powder\ after\ tapping} \qquad (3)$$

3.3.2.5.6 Flowability

Flowability was evaluated in terms of the Carr compressibility index (CI) and Hausner ratio (HR) (Fitzpatrick, 2005), which were calculated from the bulk and tapped densities of the powders using the following equations:

$$HR = \frac{\rho_T}{\rho_B} \qquad (4)$$

$$CI = \left(\frac{\rho_T - \rho_B}{\rho_T}\right) \text{x } 10 \qquad (5)$$

Where ρ_T and ρ_B are tapped and bulk density, respectively. Table 4 presents the correlation between the values of the Carr index, Hausner ratio, and powder flowability.

3.3.2.5.7 Particle (true) density

For the determination of particle density, approximately 1 g (m_0) of the sample was filled in a burette containing toluene. The rise in toluene level (V1) was measured and calculated as true particle density. Toluene was used because of its ability to penetrate the finest external pores connected to the surface without dissolving the material (Premi and Sharma, 2017)

$$Particle\ density = \left(\frac{m_0}{V_1}\right) \qquad (6)$$

3.3.2.5.8 Product yield

The product yield was calculated as the ratio of the amount of powder collected after every spray-drying experiment to the initial amount of solids in the feed solution. The dry weight of the oil encapsulate obtained by deducting the weight of residual moisture present in it, which was estimated using the moisture analyzer. Then, the product yield was calculated using the below expression.

$$Y = \frac{(W2-W1)-Xwb(W2-W1)}{FvTs} \times 100 \qquad (7)$$

Where,

Y is the powder yield (%),

X_{wb} is the moisture content (w.b),

F_v is the feed volume (mL),

T_s is the total solid content (mg/L), and

W_1 and W_2 are the weight of the powder bottle before and after spray drying (g), respectively.

3.3.2.5.9 Solubility

Solubility is expressed as the percentage of dried supernatant with the amount of powder originally added (Chew et al., 2018). Solubility was determined according to the methods of Eastman and Moore, (1984) and Cano-Chauca et al., (2005), with some modifications. 100 mL of distilled water was transferred into a blender jar. The powder sample (1g, dry basis) was carefully added into the blender operating at high velocity for 5 min. The solution was placed in a tube and centrifuged at 3000xg for 5 min. An aliquot of 25 mL of the supernatant was transferred to pre-weighed Petri dishes and immediately oven-dried at 105 $^{\circ}$C for 5 h. Then the solubility (%) was calculated by weight difference.

3.3.2.5.10 Wettability

Wettability is expressed as time in seconds, necessary for a given amount of powder to penetrate the quiet surface of the water, i.e., the ability of a powder to absorb water on the surface and become wet. The wettability test was carried out according to the procedure proposed by Fuchs et al., (2006). A powder sample of 0.1g was sprinkled over 100 mL distilled water at 20°C without agitation. The time (in seconds) taken for all the powder particles to submerge was recorded as wettability.

3.3.2.5.11 Determination of carotenoid content of microencapsulated oil encapsulate

0.5-1 g of oil was weighed into a 25 mL volumetric flask. The oil was dissolved in hexane and made up to the mark. The absorbance of the oil solution was recorded at 446nm in a spectrophotometer (BS 684, section 2.2:1977; Dian et al., 1996).

$$Carotene = \frac{V \times 383 \times (As - Ab)}{1000 \times W} \qquad (8)$$

Where,

V is the volume of oil made-up with hexane (mL),

W is the weight of the sample (g),

A_s is the sample absorbance, and

A_b is the blank absorbance.

3.3.2.5.12 Microencapsulation efficiency

Total oil content in microcapsules was quantified using the AOAC Official Method 925.32 (2012). Briefly, 1 g of powder was transferred to a fat-extraction tube, and 10 mL HCl was added slowly. The tubes were kept in a boiling water bath. After cooling to room temperature (25°C), 25 mL of ethyl ether and 25 mL of petroleum ether were added, and the tubes were vigorously shaken for a minute. The ether solution (supernatant) was separated and filtered through packed cotton. The remaining aqueous phase was further extracted

twice with 15 mL of ethyl ether and 15 mL of petroleum ether. The solvent was evaporated in a rotary evaporator (Hei-VAP-Value Digital (G3), Heidolph Instruments, Schwabach, Germany), and the oil was dried in a vacuum oven at 100 °C to constant weight. Extractable oil, usually referred to as surface oil (EO), was determined according to the methodology of Davidov-Pardo et al., (2008). This non-encapsulated oil is defined as the fraction that can be easily extracted with organic solvents without disrupting the solid matrix. Briefly, 4 g microcapsule powder was drip washed with 75 mL of ethyl ether for 15 min at 25°C. The suspension was filtered through a Whatman No. 1 filter paper, and the powder on the filter was rinsed three times with ethyl ether. The solvent was dried and rota-evaporated to obtain the surface oil mass. Encapsulation efficiency (EE %) was calculated from the following equation:

$$EE\% = \left\{\frac{TO-EO}{TO}\right\}100 \qquad (9)$$

Where TO be the total oil content in microcapsules, and EO is the extractable oil content determined as previously described.

3.3.2.5.13 Scanning Electron Microscopy (SEM)

Morphology and particle size of spray-dried oil encapsulate were determined using a Scanning Electron Microscope (Carl Zeiss EVO-18, Germany) and using a software Digimizer Version 4.6.1 (Digimizer Version 4.6.1, Copyright © 2005-2016, MedCalc Software, Belgium; pixel per mm of the image was 9.7 units). For SEM, the samples were transferred to a cryo-preparation chamber, which was maintained at a constant temperature of −10 °C using a "Peltier- cooling" stage. The prepared samples were mounted on an aluminium stub using carbon tape. The samples were examined under vacuum using an accelerating beam at a voltage of 10 kV. The micrographs were recorded at a magnification of 1000-3000X.

3.3.2.5.14 Chemical quality analysis of encapsulated and individual oils

The free fatty acid content of the RPO and FSO samples, blended oils, and spray-dried encapsulates were estimated using AOCS Ca5a-40 (1989). Briefly, the sample (2 g) was dissolved in methanol (30 mL). The contents were boiled until the first bubble's appearance and titrated against 0.1 N standardized alkali using phenolphthalein as an indicator.

Peroxide value was estimated by following AOCS Cd8-53 (1998). Sample (2-5 g) was mixed with 30mL acetic acid – chloroform reagent in a 250 mL glass stoppered flask and shaken until the sample was dissolved. Then, 0.5 mL of saturated potassium iodide solution was added to the above mixture and kept under dark conditions for a few minutes. After mixing with 30 mL distilled water, the mixture was titrated against standardized 0.1N sodium thiosulphate using freshly prepared starch solution as an indicator. The endpoint is the disappearance of blue color. The peroxide value of oils was expressed in units of milliequivalents of peroxide per kilogram oil. A blank sample was set up as reagent control.

The stability of oil in the microencapsulated powders was evaluated by extracting the oil from the encapsulated powders using the procedure described by Lee et al., (2018). 20 mL of aqueous ethanol (85 mL ethanol/ 100 mL) was poured into sample followed by adding 50 mL of petroleum ether. The samples were then stirred with a magnetic stirrer for 30 min. Phase separation was observed after the stirring stopped and the top layer was extracted into a tared round bottom flask. Petroleum ether (5 mL) was used to re-extract the remaining ethanol solution and the procedure was repeated microcapsules turned white. The oil thus obtained was used for further analysis like β-carotene content.

3.3.2.5.14.1. β-carotene estimation by spectrophotometric method

The β-carotene content of the RPO, FSO, and the spray-dried oil encapsulate was estimated using a spectrophotometer according to the method (BS 684, section 2.2:1977). The oil (0.5-1 g) was dissolved in hexane and made up to known volume. The absorbance was read at 446 nm using the spectrophotometer (Shimadzu UV-2600, Japan). The carotene content was calculated using the Eq. (9) and expressed in ppm. The encapsulate oil was extracted using the procedure described by Lee et al., (2018). Aqueous ethanol (85%, 20 mL) was added to 2 g of the sample, followed by the addition of 50 mL of petroleum ether. The samples were then stirred using a magnetic stirrer at 1200 rpm (IKA, RCT basic, 220V) for 30 min. After 30 min of stirring, the top layer that separated was carefully decanted into a tared round bottom flask. Petroleum ether (5 mL) was used to re-extract the remaining ethanol solution, and the procedure was repeated until the yellowish RPO microcapsules turned white. The oil content was calculated after solvent evaporation. And, this oil was further used for the determination of β-carotene content, using the methodology described in section 2.5.12.

3.3.2.5.15 Product formulation and characterization

To evaluate the potential of the spray-dried oil encapsulate as a replacer of hard stock fat (e.g., butter), product development studies were carried out by replacing 40% of butter in the formulation of cupcakes with the oil encapsulate. The control and test cupcakes were prepared using the formulations given in Table 2. The ingredients were then mixed and blended using an electric whisk until a creamy consistency was attained. The batter was transferred into the cupcake mold and baked in a preheated oven (Bajaj OTG, 4500 TMCSS, Mumbai, India) for 12 minutes at 180 °C (until the crumb turned golden brown). The product was cooled at room temperature for 3 hours. The sensory and texture characteristics of the control and test cupcakes were compared.

Table 3.2. Ingredient composition of cupcakes

Ingredients	***T1***	***T2 (40% replacement with oil powder)***
Flour	100 g	100 g
Sugar	75 g	75 g
Butter	64 g	39 g
Oil powder	Nil	25.6 g
Vanilla essence	2.5 g	2.5 g
Egg	2 no.s	2 no.s
Salt	To taste	To taste
Baking powder	5 g	5 g

3.3.2.5.16 Cupcake characterization

3.3.2.5.16.1 Sensory evaluation

A hedonic test was performed to determine the acceptability and any significant differences in the sensory attributes between the control and test cupcakes. Care was taken to avoid interference from other sources. The samples were presented to ten semi-trained panelists familiar with the techniques of sensory analysis. They were asked to score the product for appearance, color, texture, taste, and overall acceptability with a scale representing quality grade description given in Table 3.

Table 3.3. Hedonic scale grade description

Preference	Grade
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

3.3.2.5.16.2 Color and texture profile analysis

The color of the control and test cupcakes was determined using the methodology mentioned in section 2.5.3. A texture analyzer equipped with a 50 N load cell (TA1, AMTECK, Lloyd instrument) was used to determine the texture profile of cupcakes. The force required to compress cupcakes by 50% was measured with a rounded bottom stainless steel probe at a speed of 10 mm/s. Texture measurements were performed in triplicates for each sample, and the mean values were reported. Before the test, the sample was placed centrally under the probe to avoid irregular areas of the crust regions. The Nexygen MT software program was used to quantify the parameters of interest in this work: hardness (N), cohesiveness (TPA), springiness, chewiness (N), adhesiveness (TPA), and gumminess (N).

3.3.2.5.17 Statistical analysis

All the measurements were performed in triplicates, and the results are expressed as mean ± standard deviation. The significance of the difference between the means of all the parameters was examined by the one-way analysis of variance (ANOVA) at a confidence level of 95%, using the EXCEL^{TM} 2010 (Microsoft, USA).

3.4 Results and discussion

3.4.1 Quality analysis of red palm olein, flaxseed oil, and their blends

Initially, the quality of RPO and FSO was assessed in terms of their free fatty acid (FFA) content, peroxide value (PV), and β-carotene content. The FFA content of RPO and FSO was found to be 0.647 ± 0.04%, and 0.740 ± 0.18%, respectively, and the corresponding peroxide values were 2.75 ± 0.636 millieq/kg and 1.432 ± 1.04 millieq/kg. These values are in alignment with the values reported by Teh and Birch, (2013) (2.04 millieq/g) and Domian et al., (2017) (1.45 millieq/g). PV of oil is an indication of the amount of hydroperoxides present in it. Hydroperoxides are compounds that arise from lipid oxidation. Hence, PV is a

measure of oil quality. Generally, peroxide values of fresh oils are less than 10 millieq/kg, and the rancid taste is noticeable in oils with PV ranging between 30-40 millieq/kg (Choe and Min 2006; Kamisah and Yosof, 2012). Thus, in this study, the freshness and quality of both RPO and FSO is confirmed as their peroxide values were less than 10 millieq/kg.

β-carotene content of the RPO and FSO were estimated to be 390 ± 0.08 ppm and 34.4 ± 0.02 ppm, respectively. Earlier studies have also reported a β-carotene content of 370–700 ppm in red palm oil (Liu et al., 2008; Manorama et al., 1993; Mba et al., 2015). Further, the carotene content of the blend was found to be 270.029 ± 0.12 ppm.

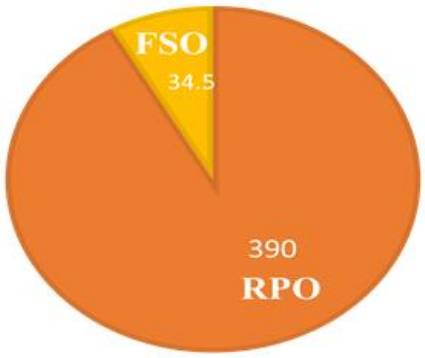


Fig 3.1: Beta carotene content of individual oils (RPO, FSO)

The fatty acid profile of individual oils based on the published literature and the value obtained with GC-MS/MS (Fig.1) for blended oils are provided in Table 4. Notably, there was a considerable improvement in the α-linolenic acid content of blended oil. Moreover, the blended oil had an omega-3 to omega-6 ratio of 0.61. This is relevant as the RPO is inherently devoid of polyunsaturated fatty acid. Thus, blending of RPO and FSO in the ratio of 70:30 showed a balanced fatty acid profile with enhanced content of PUFA, compared to pure oils.

Table 3.4: Fatty acid composition of individual and blended oils

Sl. no	Fatty acid composition	*Percentage of fatty acids in RPO (Red Palm Oil)	**Percentage of fatty acids in FSO (Flaxseed Oil)	Percentage of fatty acids in RPO-FSO blends by GC-MS/MS
1	Palmitic acid	42	6	21.57
2	Stearic acid	5	2.5	8.61
3	Oleic acid	42	19	30.95
4	Linoleic acid	10	24.1	14.05
5	Alpha-linolenic acid	--	47.4	8.61

*(Montoya et al., 2014)

**(Bayrak et al. 2010)

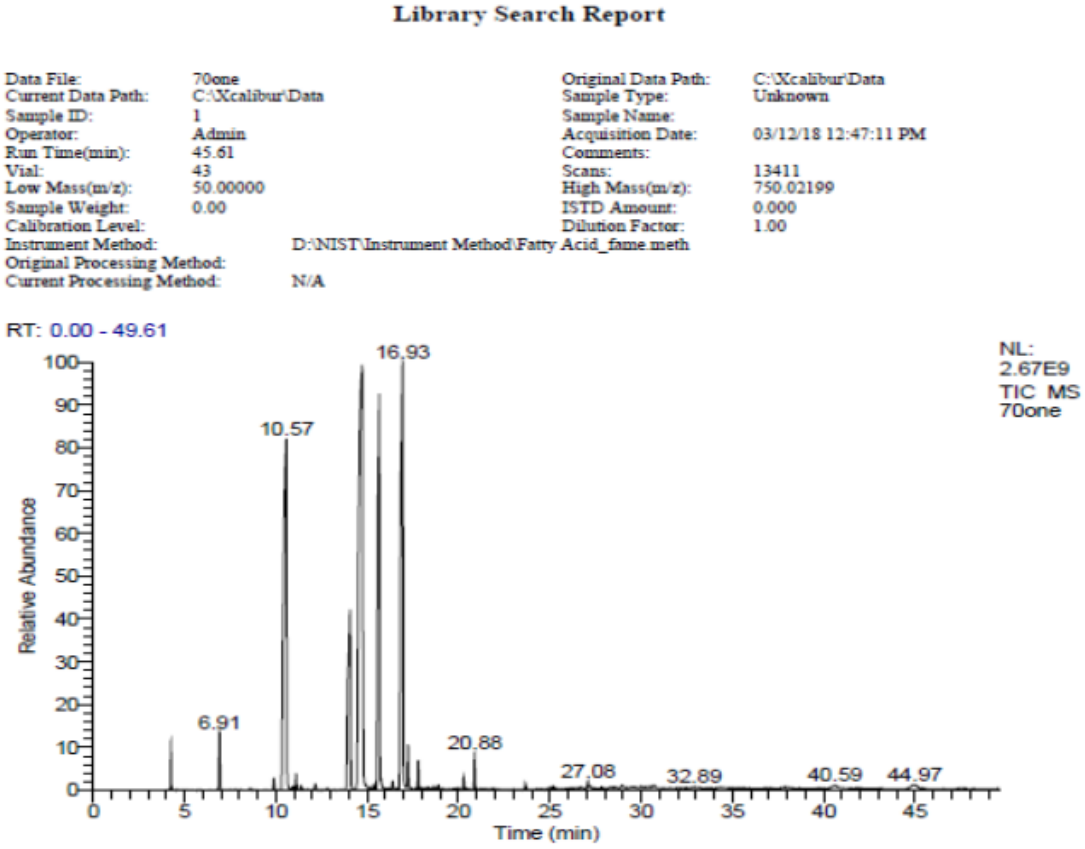


Fig 3.2. GC MS/MS profile of blended oil (RPO + FSO)

3.4.2 Optimization of wall material composition and spray drying conditions for the microencapsulation of RPO-FSO blend

The inlet temperature of spray drying is reported to play a major role in determining the end product's quality (Anandharamakrishnan and Ishwarya, 2015; Koc et al., 2015). In this study, an inlet/outlet temperature combination of 180 ± 5/80 ± 5 °C was selected for the microencapsulation of the RPO-FSO blend. This inlet temperature is within the range of 160–200°C reported in earlier investigations on the spray drying encapsulation of highly unsaturated oils (Davidov-Pardo et al., 2008; Tonon et al., 2011; Carneiro et al., 2013; Wen et al., 2017).

To determine the best effective combination of wall materials, the spray-dried RPO-FSO encapsulates were prepared using different proportions of gum arabic, whey protein, and maltodextrin (Table 5), at a constant oil payload of 34%. Product yield and encapsulation efficiency were determined for each trial. Based on the results, whey protein isolate (WPI) and gum arabic (GA) in the ratio of 1:2 was selected as the wall material for further studies, as this combination resulted in maximum product yield (62.07%) (Table 1) and highest encapsulation efficiency (40%) among all the other wall material compositions (Table 2**)**. The superiority of WPI and GA as encapsulating agents for oils has been demonstrated in several studies. For instance, Kha et al., (2014) encapsulated Gac oil using a combination of GA and WPI as wall materials. The efficiency of WPI has been attributed to its skin-forming behavior that results in a particle surface without any pores and cracks after spray drying. The antioxidant activity of WPI (Gad et al., 2011) is an added advantage of using it as a wall material for the spray drying encapsulation of unsaturated oils (Ramakrishnan et al., 2014**)**.

Table 3.5. Influence of wall material composition on the yield and encapsulation efficiency of spray-dried oil encapsulates

Trail	Wall material	The Proportion of wall materials (w/w)	Core-to-wall ratio	Temperature of spray-drying (Inlet/outlet) (°C)	*Encapsulation efficiency (%)	#Yield %
1	MD+WPI	1:2	1:2	181/75 ± 5	12.5 ± 0.21	20.0 ± 0.14
2	MD+GA	1:2	1:2	181/80 ± 5	14.2 ± 0.10	15.0 ± 0.07
3	MD+GA+WPI	1:2:1	1:2	180/75 ± 5	18.4 ± 0.04	32.1 ± 0.14
4	GA+WPI	2:1	1:2	181/80 ± 5	6.8 ± 0.50	40.9 ± 0.12
5	GA+WPI	1:1	1:2	181/80 ± 5	20.0 ± 0.021	55.0 ± 0.04
6	GA+WPI	1:2	1:2	181/85 ± 5	40.0 ± 0.04	62.1 ± 0.10

* Significant at *p-value* = 1.52×10^{-10}*; P≤0.05* #Significant at p-*value* =2.23×10^{-7}*; P≤0.05*

Similarly, GA's effectiveness for oil encapsulation is due to its excellent emulsifying property, viscoelastic film-forming ability, high solubility, and low viscosity in aqueous systems (Matsumura et al., 2000). Generally, proteins are not easily soluble in water; therefore, a common approach is to combine the proteins and gums to utilize their emulsifying, film-forming/matrix-forming ability (Labuschagne, 2017). Sunflower oil protected by whey protein isolate and maltodextrin depicted similar characteristics (Xu et al., 2013). In the present study, incorporation of a combination of gum arabic with whey protein as wall material improved the barrier properties of the wall and this is because incorporation of polysaccharides in minor quantities will improve the encapsulation efficacy of whey protein (Anandharamakrishnan and Ishwarya, 2015). However, WPI and GA, in combination with maltodextrin (MD), resulted in lower product yield and encapsulation efficiency. This may be because of maltodextrin's poor emulsification capacity, which led to low oil retention (Fernandesa et al., 2014).

It is also noted from Table 1, that the yield increased with an increase in the proportion of WPI at a constant inlet temperature. When the ratio of GA: WPI was increased from 1:1 to 1:2, the product yield and encapsulation efficiency increased from 55% to 62% and 20 to

40%, respectively. However, the encapsulation efficiency was lower than that reported in previous studies. This may be due to the high atomization pressure (147.1 kPa) and high airflow rate (1.2 m^3/min) used in this study. High atomization pressure leads to smaller droplet size and thereby low moisture content (moisture in small droplets under rapid evaporation than in big ones, due to the enhanced surface area of smaller droplets). Low moisture results in cracks on the surface of particles that can lower the encapsulation efficiency. Further, the drying air flow rate bears an inverse relationship with the encapsulation efficiency. Increasing aspiration rate offers more mass flow rate of drying air, which leads to higher moisture evaporation from feed droplets due to higher heat and mass transfer values (Aghbashlo et al., 2013; Carmona et al., 2018). Huang et al., (2014) also reported a decrease in the encapsulation efficiency of tilapia oil at atomization pressure greater than 100 kPa and airflow rate beyond 0.67 m^3/min.

The carotene content of the oil encapsulates from the S2 and S3 trials were 30.0 ± 0.12 ppm and 84 ± 0.03 ppm, respectively corresponding to carotene retention of 74.5% and 77.8% after spray drying, with respect to the initial carotene content of the oil blend before encapsulation. Therefore, the thermal stability of encapsulated β-carotene and the protective effect of the wall materials on the core is evident. The carotene retention thus obtained is in line with the studies of De Paz et al., (2012), who reported 70% of carotene retention. Spray drying with carbohydrate and protein-based wall materials such as those used in this study has been reported to improve the stability of β-carotene (De Paz et al., 2012; Kha et al., 2014; Donhowe and Kong, 2014).

3.4.3 Morphology of encapsulates

The SEM images of the oil encapsulate revealed spherical-shaped particles with surface dents (Fig. 3). Based on digital image processing, the mean radius of S3 oil encapsulate produced at the inlet temperature of 180/80 ± 5 °C was found to be 8.741 ± 3.551 μm, and that of S2 oil encapsulate was 11.658 ± 0.553 μm. Encapsulate S3 showed more uniform and smooth surface with no fissures. As discussed in section 3.2 on encapsulation efficiency, the aspirator flow rate and the feed flow rate play a major role in determining the particle morphology. An increase in concentration of whey protein and gum might have helped in the film formation and lowering the permeability of encapsulates to gases. Further, smooth surface morphology of S3 may be responsible for its low surface oil content and, thereby, high encapsulation efficiency (Hundre et al., 2014). As S3 showed an optimal morphology, and a higher encapsulation efficiency and yield, further physicochemical properties of the encapsulate were carried out only with S3.

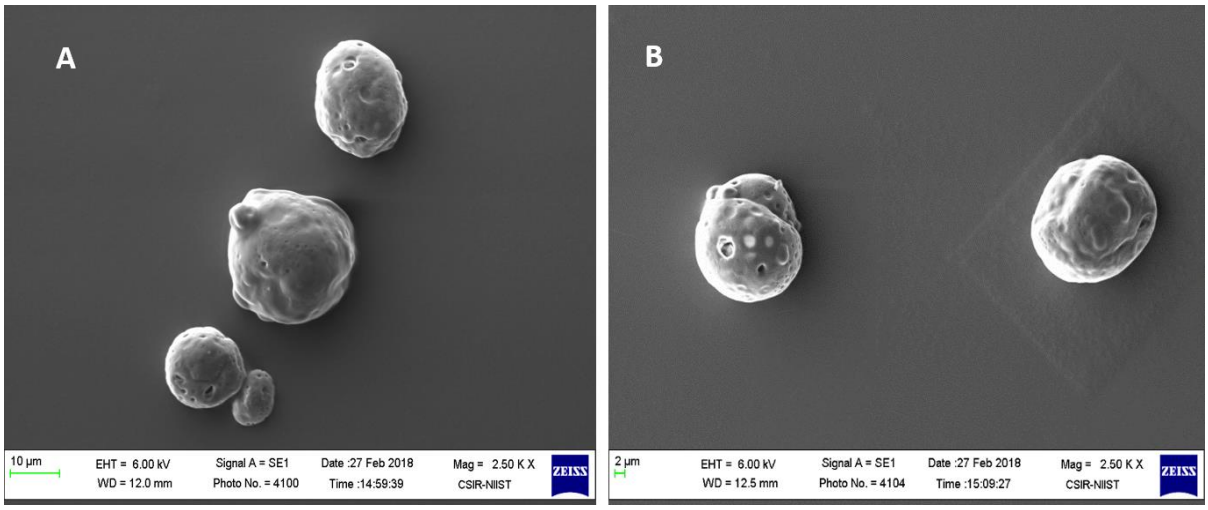


Fig 3.3: SEM images of encapsulated powders S3 (A) and S2 (B).

3.4.4 Powder properties

3.4.4.1 Moisture content and water activity

Low moisture content and water activity of microcapsules are preferable as high moisture content accelerates the oxidation of fat and decreases the powder flow (Turchilui et al., 2014). The moisture content and water activity of the oil encapsulate obtained in this study are given in Table 6. Water activity is a measure of the available water present in the microorganisms for their growth. Restriction of water content in foods will always lead to the possibility of a reduction in microbial spoilage and, consequently, to an increase in the shelf life of foods. Effect of water activity on conjugated linoleic acid's physical properties in microencapsulated samples using different matrices showed that the ideal storage condition prevails at a water activity of 0.5 or below (Jiménez et al., 2009). Lipid oxidation is delayed in dried products having water activity in the range of 0.2 - 0.4 (Labuz, 1968). The value of moisture content (2.6%), and water activity (0.46) of encapsulate obtained in the study fall within the above range.

Table 3.6: Comparison of results obtained by changing the proportion of GA: WPI

Sample	GA: WPI (wt/wt)	[a]Bulk density (g/mL)	[b]Tapped density (g/mL)	Flowability	[c]Total colour change	[d]Moisture %	[e]Water activity
S2	1:1	0.33±0.21	0.38 ± 0.14	Good	73.23 ± 1.10	1.90 ± 0.04	0.35 ± 0.00
S3	1:2	0.33±0.12	0.38 ± 0.32	Good	73.96 ± 0.40	2.60 ± 0.16	0.46 ± 0.01

[a]Significant at *p-value = 0.97; p≥0.05,*
[b]Significant at *p-value = 1.00; p≥0.05*
[c]Significant at *p-value* = 1.2 x 10^{-7}*; p≤0.05*
[d] Significant at *p-value = 0.02; p≤0.05,*
[e]Significant at *p-value = 0.01; p≤0.05,*

3.4.4.2 Bulk density and tapped density

Density is an important parameter in powders when packed or stacked in bulk. By definition, density decreases as volume increases for a constant mass. Therefore, similar relationship between the bulk density of the powder and the diameter of the particles is expected. The bulk density of the oil encapsulate was 0.335±0.12 g/mL (Table 6). The bulk density of spray-dried flaxseed oil encapsulate produced using MD/GA and MD/WP as wall materials were 0.28 g/mL and 0.40 g/mL, respectively (Carniero et al., 2013). At 20 % oil payload, Tonon et al., (2011) obtained flaxseed oil microcapsules with a bulk density of 0.303 g/mL and 0.473 g/mL, using whey protein concentrate and gum arabic as wall material, respectively. In another study that used a combination of GA and WPI for Gac oil encapsulation, the bulk density ranged from 0.24 to 0.33 g/mL (Kha et al., 2014). Thus, the value of bulk density obtained in this study is within the range reported by similar studies. The complementing film-forming property of GA and WP might have resulted in rapid crust formation around the oil core during the constant rate period of spray-drying. As a result, air occlusion within the particles may have been restricted, leading to higher bulk density. A low amount of occluded air prevents lipid oxidation. Further, powders with higher bulk density are advantageous as they can be stored in large quantities in smaller packages, relative to products with lower densities (Carniero et al., 2013).

Tapped density indicates the weight and amount of powder that can fit in a container. The value of the tapped density of a powdered product is always higher than its bulk density (Chew et al., 2018). The above findings hold good in this study, as well. The oil encapsulates exhibited higher tapped density (0.385±0.32 g/mL) than its bulk density (0.335±0.12 g/mL). Flaxseed oil encapsulate prepared with whey protein as wall material showed tapped density in the range of 0.454 - 0.498 g/mL (Goyal et al., 2015). The reason for the low tapped density of encapsulate in this study can be attributed to its high bulk

density due to the reasons explained above. When subjected to tapping, small particles roll between the particle voids and reach the densest packing condition (Ishwarya and Anandharamakrishnan, 2015). High bulk density of particles implies a low amount of occluded air (voids) between the particles (Carniero et al., 2013). This justifies the low tapped density of oil encapsulates observed in this study.

3.4.4.3 Flowability

The flowability of powder was evaluated based on the Hausner ratio (HR) and Carr's compressibility index (Carr, 1965), which was calculated from the loose and tapped bulk densities of the encapsulate. In this study, the oil encapsulate showed an HR and CI of 1.149 and 12.987, respectively. The FSO encapsulated in whey protein concentrate at 35% oil load was reported to have an HR and CI of 1.55 and 33.82, respectively (Goyal et al., 2015). From Tables 6 and 7, it is apparent that the oil encapsulate obtained in this study exhibits superior flowability than that reported in the above study. The surface composition of the powder would affect the flowability so as to overcome the surface interactions among the particles (Chew et al., 2018). Thus, the low Hausner ratio of the oil encapsulates obtained in this study is indicative of its less cohesive nature and superior flowability (Table 7). From the results, it is evident that the low moisture content (discussed in section 3.3.1), high inlet temperature and the wall material composition played a major role to attain a good flowability of the encapsulate even at a high proportion of oil in the feed (34% on a dry basis with respect to the wall materials).

Table 3.7. Correlation between powder flowability, Hausner ratio, and Carr index (Turchiuli et al., 2005)

Carr's index	Flowability	Hausner ratio
≤10	Excellent	1.00–1.11
11.0–15.0	Good	1.12–1.18
16–20	Fair	1.19–1.25
21–25	Passable	1.26–1.34
26–31	Poor	1.35–1.45
32–37	Very poor	1.46–1.59
>38	Awful	>1.60

3.4.4.5. Solubility

Microencapsulation enhances the solubility of oil in water (Mohammed et al., 2017). The solubility of the RPO-FSO oil encapsulate was found to be 60 %. This value is either comparable to or higher than the solubility of spray-dried encapsulates reported in other studies. Fernandes et al., (2013) reported solubility in the range of 55.75 to 67.75% for rosemary essential oil encapsulated within gum arabic. The solubility of encapsulates is strongly influenced by the wall material composition (Fernandes et al. 2013). Whey protein, which is present in a higher proportion in the wall material composition as used in this study, is reported to impart better solubility (Goyal et al., 2015) depending on whether it is in its native or denatured state (Pelegrine and Gasparetto, 2005; Anandharamakrishnan et al., 2008). Anandharamakrishnan et al., (2008) reported that at inlet and outlet temperatures in the range of 160-190°C and 65-90°C, respectively, there is less chance of whey protein

denaturation. As the inlet/outlet temperature used in this study falls within the range mentioned above, the high solubility of oil encapsulate is justified.

3.4.4.6 Wettability

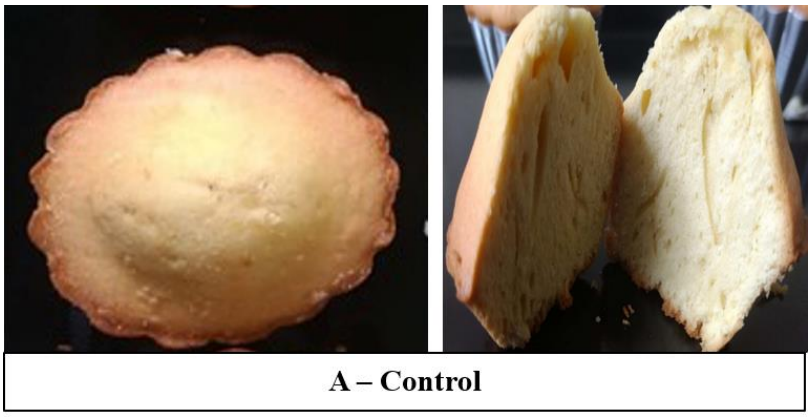
The wettability of microcapsules indicates their ability to absorb water related to the powder reconstitution. Shorter the dissolution time in water, the better the physical attributes in food processing (Chew et al. 2018). The wettability varied from 543-550 seconds for the oil encapsulates (S3). The low moisture content of particles (2.6%) at 180 °C - inlet temperature may be the reason for good wettability. Microencapsulated flax seed oil formulations prepared using WP as wall material showed similar dissolution behavior and were completely dissolved in less than 15 min (Goyal et al., 2015). Gum arabic is highly soluble in water due to its high number of hydrophilic hydroxyl groups. Gum arabic helps to increase the adherence capacity of water molecules on the surface of microcapsules, thus shortening the instantizing time through greater interaction with water (Fernandes et al., 2014; Edris et al., 2016).

3.4.4.7. Color

The color of any food product is affected by the ingredients used in its formulation. The color of encapsulated powder was measured using HUNTERLAB colorimeter and reported as L*, a*, b* values. The L*, a*, b* values of the encapsulates were 82.95 ± 0.08, 2.95 ± 0.008, and 33.37 ± 0.008, respectively. In this work the core material was a combination of two vegetable oil fractions, which contain flaxseed oil and red palm oil. Thus, it could be inferred from the data that the wall material significantly affects the color of the encapsulated powder. The results of present work in agreement with the values reported in earlier studies: L*, a*, and b* values for microencapsulated flax seed oil powder with whey protein were 88.60, 0.06, and 13.56 (Goyal et al., 2015) and fish oil encapsulate showed color values varying from L* = 78.01 to 82.76, a* = 1.37 to 2.54 and b* = 18.97 to 24.23 respectively when encapsulated with gum Arabic (Binsi et al., 2017).

3.4.5 Spray-dried RPO-FSO microcapsules as a functional ingredient in cupcakes

As evident from the results, the encapsulates are enriched with essential fatty acids and micronutrients and therefore, it was used as a fat replacer and β-carotene fortificant in cupcakes to improve the nutritional qualities. The control cupcakes were prepared with butter as the source of fat, and the test cupcakes were formulated by replacing 40% of butter with the oil encapsulate shown in Fig. 4A and Fig. 4B. The test cake's external appearance differed from that of the control cupcakes with a slightly shrunken structure. This observation may be attributed to the replacement of butter with oil encapsulate, reduced the moisture content of the batter for test cupcake. This may affect the volume of the product during baking, thus affecting the appearance of test cupcake.



A – Control

B – Test sample enriched with encapsulated oil as a replacement of butter at 40%

Fig 3.4A and 3.4B: Images of control and test cupcakes

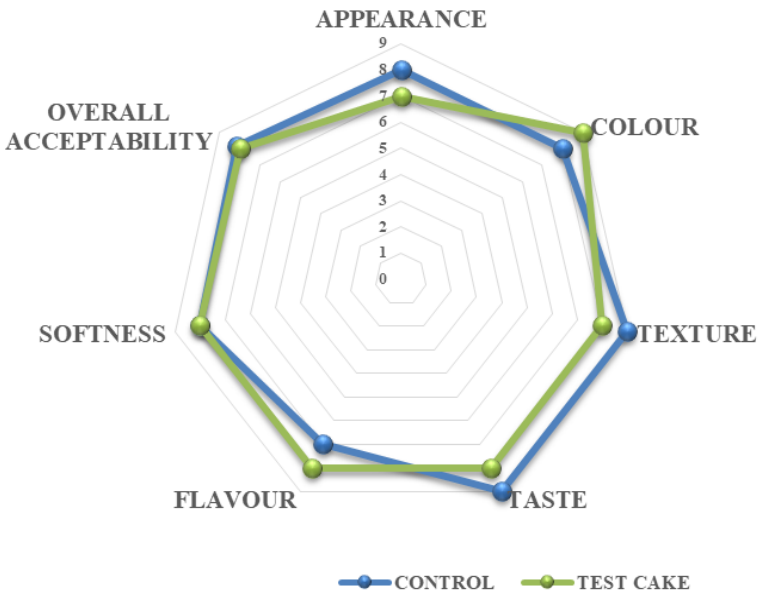


Fig 3.5: Sensory analysis of S3 (GA: WPI-1:2) and control cake

The sensory evaluation results showed (Table 8) that there was no significant difference in the overall acceptability values between the control and test cupcakes. Although flax seed oil is known for its fishy smell, due to its high PUFA content. The test cupcakes with oil encapsulate masked the smell, there for it was liked by panelists. This may be due to the presence of whey protein, which is reported to interact with the compounds responsible for off-flavor compounds and masks the off-taste (Maehashi and Huang, 2009). Moreover, encapsulation by spray drying using gums and whey protein concentrate as wall materials has been demonstrated to mask the off-taste. Indeed, enhanced palatability of bioactive compounds with unfavorable sensory characteristics is an important functional property of spray-dried encapsulates that permits their inclusion in different food products (Anandharamakrishnan and Ishwarya, 2015).

Table 3.8. Sensory evaluation

Parameters	Control	Test Cake
Appearance	8.5 ± 0.5	7.5 ± 0.5
Colour	8.1 ± 0.7	8.7 ± 0.4
Texture	8.5 ± 0.7	7.3 ± 0.4
Taste	8.8 ± 0.3	8.5 ± 0.5
Flavour	7.3 ± 0.4	8.7 ± 0.4
Softness	8.5 ± 0.5	8.3 ± 0.4
Overall Acceptability	8.2 ± 0.4	8 ± 0.6

Significant at *p-value = 0.01; P ≤ 0.05*

The texture profile analysis results showed that the test cupcake samples exhibited similar textural quality as that of control, except for chewiness and springiness, which were significantly lower for the test sample (Table 9). The lower values for springiness and chewiness of the oil encapsulate-fortified cakes may be attributed to the weaker and less elastic structure of the cakes caused by the polyphenols present in the oil core (RPO and FSO). A similar effect of polyphenols on cake springiness and gumminess has been reported by Pasukamonset et al., (2018). Springiness is a measure of the elasticity, which is observed as the degree to which the sample recovers between the first and second compression during the texture profile analysis (Santhanam et al., 2014). Chewiness and springiness are directly related as the former is obtained by multiplying gumminess and springiness. And chewiness is the amount of energy required to disintegrate food for swallowing (Ghaboos and Aradabili, 2018). Thus, the reduction of chewiness and enhancement of springiness is desirable for the test cake. Springiness is indication of the cupcake's ability to entrap gases. Thus increase in chewiness is desirable for test cakes.

Table 3.9. Texture profile analysis of cupcake samples

Texture parameters	Control cake	Test cake
Adhesiveness (TPA)	1.34 ± 0.02	1.13 ± 0.15
Cohesiveness (TPA)	0.51 ± 0.05	0.53 ± 0.003
Springiness (TPA) (mm)	17.71 ± 0.26	28.42 ± 0.29
Chewiness (N)	4.98 ± 0.51	0.55 ± 0.05
Hardness (N)	2.49 ± 0.05	2.37 ± 0.01
Springiness	3.29 ± 0.30	0.48 ± 0.01
Firmness (N)	2.58 ± 0.05	2.37 ± 0.01
Gumminess (N)	1.51 ± 0.146	1.15 ± 0.06

Significant at *p-value = 0.01; P ≤0.05*

Further, the results of texture profile analysis exhibited a good correlation with the inferences derived from sensory analysis. A study done by Santhanam et al., (2014) also revealed that fish oil encapsulates could be successfully used as a fortificant in cakes without much alteration in the textural and sensory qualities. The present study yet again confirmed that oil encapsulates can be easily incorporated in the formulation of baked foods without much alteration in the textural and sensory qualities.

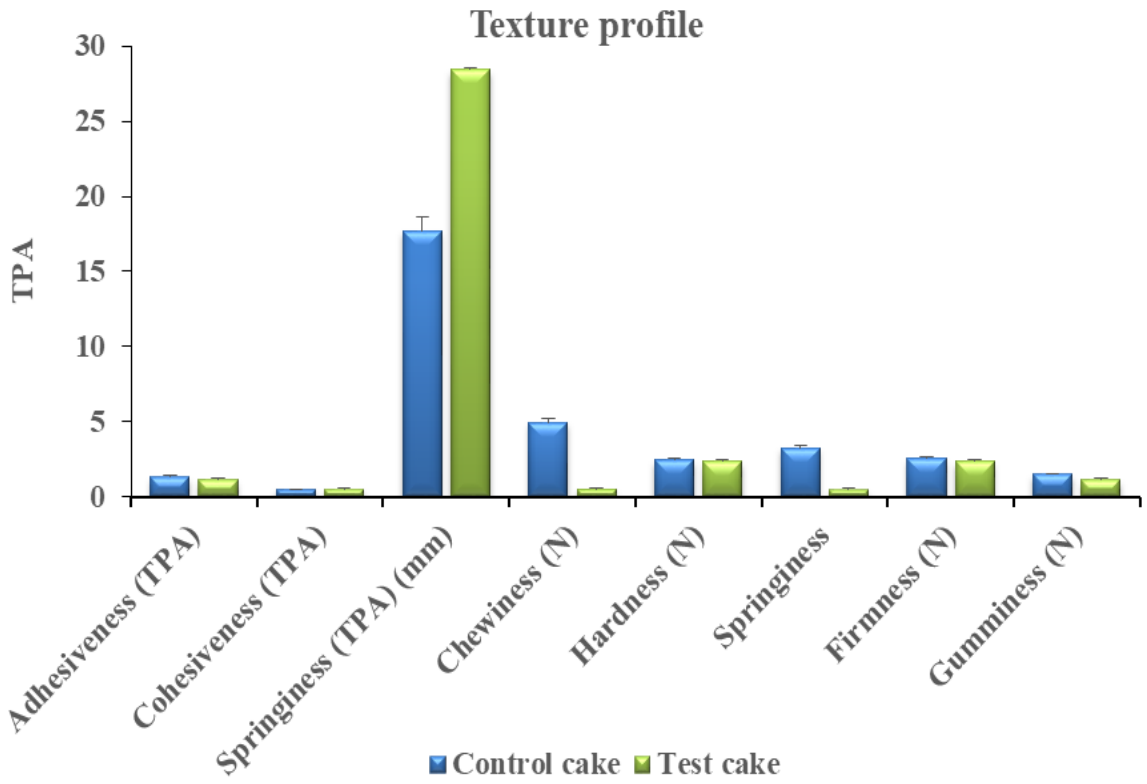


Fig 3.6. Texture profile analysis of S3 (GA: WPI-1:2) and control cup cake

The β-carotene content of the test cake was 208.32 ± 0.02 ppm. Therefore, consumption of one test cupcake (90 g) enriched with the RPO-FSO oil encapsulate at 40% replacement of butter (fat) will meet 36.87% of recommended dietary allowances (RDA) requirement of β-carotene proposed by the regulating agencies (FSS Act, 2019). According to this regulation, a person should gain 4800 μg of β-carotene per day (ICMR, 2010). Thus, encapsulation of blended edible oil containing RPO: FSO as core ingredient can be opted as a good enrichment method of Vitamin A, especially β-carotene. At the same time, there was a considerable improvement in the α-linolenic acid content and RPO, which is inherently devoid of RPO. Thus, blending of RPO and FSO in the ratio of 70:30 showed improved fatty acid profile with enhanced content of PUFA, compared to pure oils.

3.5 Summary and Conclusions

Thus, this work demonstrated the possibility of offering a blend of red palm olein and flaxseed oil as core material for encapsulation by spray drying. The composition and proportion of wall materials was found to exert a substantial influence on the spray-dried oil microcapsules' yield and entrapment efficiency. After microencapsulation, the oil encapsulates retained >70% of the carotene content of its blended-oil core. The use of gum arabic and whey protein as wall materials for the encapsulation of oil enabled the incorporation of oil encapsulates in the cupcake formulation at 40% replacement of conventional fat (butter) with desirable sensory appeal and textural characteristics of the final product. Moreover, one-third of the RDA of β-carotene was met by replacing butter in the cupcake formulation with the oil encapsulate. Also, encapsulation mitigated the off-flavor typical of flaxseed oil and thereby retained the sensory quality of the cupcakes fortified with the oil encapsulate, on par with the control cakes. Hence, the potential application of the spray dried encapsulate of RPO- FSO blend developed in this study is in formulating functional foods with nutraceutical benefits.

The key findings were summarized below:

- Blends of RPO & FSO in ratio of 70 & 30 were optimized to provide balanced fatty acid profile/ improved fatty acid content for encapsulation by spray drying
- GC- MS/MS profiling confirmed the presence of improved fatty acid composition
- The conditions for spray drying were optimised in terms of
    - Wall material composition -
    - core–to-wall ratio -- 1:2 (GA: WPI)
    - oil payload -- 34 %
    - Inlet temperature -- 180°C

- Product development by replacing 40 % of butter with encapsulated oil blend showed 36.87 % of the recommended dietary allowance (RDA) for β- carotene
- Encapsulation mitigated the off-flavor typical of flaxseed oil and thereby retained the sensory quality of the cupcakes fortified with the oil encapsulate
- Also improved the nutritional quality with the presence of essential fatty acids

Chapter 4

Fabrication of fat encapsulates (ghee) for food and health care applications

4.1 Introduction

The society and its consumers are more and more aware of the relationship between food and well-being. Consumers are demanding safe and healthy food products at the same time as natural as possible. Many people have now turned to diet-based approaches to prevent or ease the diseases (Medium chain triglyceride powders), and food with functional ingredients/nutrients incorporated is becoming popular. Unfortunately, a lot of natural nutrients (e.g., vitamins, carotenoids, and ω-3 polyunsaturated fatty acids) are liable to degrade when subjected to light, oxygen, or heat, and they usually have poor water solubility. Therefore, researchers have been designing edible delivery systems for these nutrients to overcome the disadvantages to fullfill their potential health benefits. Development of safer and healthier foods is the focal point of food industries and is a growing trend. The new trends in the food market are heading towards more rational use of food ingredients, such as preservatives, minimizing the concentration of synthetic additives, or even replacing them with natural substances. Recently the food industry has shown growing interest in encapsulated functional foods due to increase in consumers' desire for a ‘healthier’, ‘easier’ lifestyle with enhanced nutritional and therapeutic values (Moumita et al., 2018). Encapsulation is bursting into the food sector with several applications since it offers new insights to develop safer and healthier foodstuffs. “The technique used to entrap an active compound within a stable, protective wall to produce encapsulates of varied size and functional properties”, is known as encapsulation. The active ingredient is usually termed the “core”, and the enclosing matrix as “wall”. According to Saifullah et al., (2019), the technique creates a coating to the active material for the protection and/or preservation of these bio actives, volatile compounds from biochemical and thermal deterioration process. The sensory properties of foods has been enhanced by this

process with the homogenous distribution of ingredients and masking undesirable tastes/flavours in the product (Aryee and Boye, 2015). The importance of encapsulation, as an efficient method towards its complex properties, such as delayed release, stability, thermal protection and suitable sensorial profile was aimed to achieve in this chapter.

The different encapsulation technologies their advantages and disadvantages were discussed in the previous chapters (chapter-1). Spray-drying is the most commonly employed microencapsulation technology in the food industry (Franco et al., 2017). This drying technique has long been used in the food industry for the production of powder ingredients, such as dairy powders (Drapala et al., 2017), and its use was later extended to the field of microencapsulation. Since then, spray-drying has been used for the encapsulation of a wide range of food ingredients, including carotenoids (Robert et al., 2003; Deng et al., 2014), phenolic compounds (Mahdavi et al., 2014; Paini et al., 2015), vegetable oils, marine oils, essential oils, polyunsaturated fatty acids (Kolanowski et al., 2004; Klinkesorn et al., 2005; Shaw et al., 2007; Encina et al., 2016) and probiotics (Arslan et al., 2015; Liu et al., 2015). It is estimated that about 80-90 per cent of the microcapsules produced in the food industry are obtained by spray drying. These powders with low water activity, convenient for handling, transport and storage as well as ensuring microbiological stability (Gouin, 2004). Spray-drying is a commonly used and low-cost encapsulation technology and with a wide use in food industry. Spray-drying involves atomization of the feed emulsion containing active material is atomised using a pressure nozzle into a hot air medium, resulted the rapid evaporation of water and transformation of feed emulsion from a liquid form into powder (Zuidam and Shimoni, 2010). Opting a suitable wall material is an important factor. In addition to the type of wall material, another important factor in the microencapsulation of oils is the stability of the feed emulsions from which

encapsulated particles are produced, which is related to some emulsion properties such as droplets size, total soluble solids and viscosity. In general, the greater the emulsion stability, the greater is the encapsulation efficiency (Chan, 2011).

Accumulated reports showed that emulsions itself are good delivery systems to protect nutrients from degradation, to make them easily soluble, to control their release, and finally to improve their bioavailability (Mao et al., 2015). They are thermodynamically unstable systems consisting of at least two immiscible liquid phases one of which is dispersed in the other liquid phase stabilized by a third substance called emulsifiers (Tadros et al., 2004; McClements et al., 2007; Acosta, 2009). Oil and water are the commonly used liquids to form emulsion. The oil droplets dispersed in an aqueous phase are known as oil-in-water (O/W) emulsions. The water droplets dispersed in oil are called the water-in-oil (W/O) emulsions. The O/W emulsions can be used for the delivery of hydrophobic active substances, and W/O emulsions are used for the delivery of hydrophilic compounds. Emulsions are categorized as coarse emulsions, microemulsions and nanoemulsions based on their droplet size and stability (Komaiko and McClements, 2016).

Increasing the oil pay load from 50 % to 70 %, with fat as the core component has been less addressed. Therefore an attempt has been made to find out the wall material composition and homogenisation conditions to form a kinetically stable emulsion. And the emulsions were characterised for the droplet size, structure and other physical propertirs, to make them perfect for the delivary of higher payload core component (ghee). O/W emulsions of the ghee (core) and natural stabilizers, were prepared, by increasing the oil load (dry basis). Oil load was increased from 50% to 70% and optimized for stable emulsion in terms of homogenization speed, time and matrix

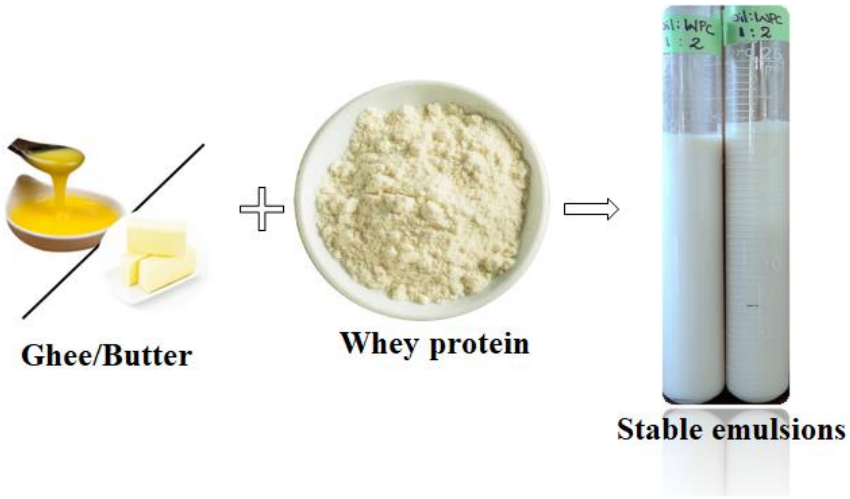
composition. Fat encapsulation is also a less attempted area, conversion of the higher oil payload emulsions into a powder has also not been attempted so far. Formation of encapsulated powder by optimising the process conditions of spray drying, for its better yield and efficiency was attempted. In order to confirm the fundamental characteristics, the formulated encapsulated fat were assessed for product yield, process efficiency, acceptability, physico-chemical characterization, shelf life & nutritional benefits (efficacy studies).

This chapter deals with the optimization of process conditions for fabricating fat encapsulates (ghee) for food and health care applications. In order to develop a fat substitute in the form of a powder, it is important to fabricate a stable emulsion without affecting the inherent properties of the fat.

Hence, this chapter is divided in to two subchapters **4a** and **4b**, where in **4a** focussed on developing a stable emulsion with higher oil payload and **4b** dealt with conversion of the stable emulsion to encapsulated powder. The process parameters were standardized, based on pilot scale experiments. Process efficiency, product quality, yield, powder properties, morphology, thermal analysis, in vitro release studies, shelf life (oxidative stability), product formulation and its characterisation were done. Further, the potential applications of the resultant oil encapsulate as a healthy fat substitute is evaluated. Conversion of solid fats to powder-form is expected to improve their functional properties and convenience of handling as a B2B ingredient in the bakery and health product sectors.

Chapter 4a

Optimization of Conditions for Developing Stable Ghee Emulsions with Higher Oil Pay Load



4a.1. Objectives

- Fabrication of stable emulsion, by optimising the wall material composition, oil payload and homogenisation conditions.
- Increasing the oil pay load from 50 % to 70 %, with fat as the core component
- Characterisation of the stable emulsions on the basis of physical, structural, thermal and electrical properties.

4a.2. Materials and methods

4a.2.1. Materials

Ghee was purchased from a local market in Thiruvananthapuram, India. Whey protein concentrate (WPC- 95%) was procured from ACE International LLP, New Delhi, India. The reagents and solvents were of >99.9% purity, and were procured from Merck Limited, Mumbai, India. All the chemicals were of analytical grade.

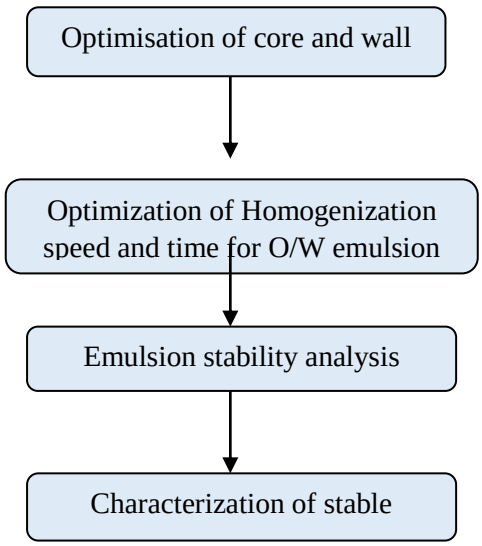


Figure 4a 1. Schematic representation of experimental design

4a.2.2 Methods

4a.2.2.1 Emulsion preparation

O/W emulsions of the ghee (core) and whey protein concentrate (WPC), were prepared, at different proportions by increasing the oil load (in dry basis). At a constant total soluble solids (TSS) content of 30%, the oil load was increased from 50% to 70% on dry basis. Preliminary trials were carried out to find out the suitable combination of ghee and WPC that resulted in maximum physical stability in terms of phase separation. The proportion of oil verses WPC is given in (Table-1). The wall material solution (WPC) was prepared by dispersing in water followed by mixing under magnetic stirring until complete dissolution (1500 rpm for 10 min). After the hydration of the wall material, oil phase (ghee) was added until complete mixing. They were dispersed completely to form a coarse emulsion. The coarse emulsion was then, homogenized using a rotor-stator homogenizer (T25 ULTRA-TURRAX digital; dispersing tool: S 25 N - 8G; IKA, China), at 15000 rpm for 45 min at room temperature (28 ± 2 °C). Initially, Speed and time of the homogenization was varied from 15000 rpm to 20000 rpm for 10, 15, 20, 25, 30, 40, 45 mins respectively. Soon after the emulsions were prepared, it was gradually poured into 15 ml graduated test tube (15x150 mm).The physical stability or phase separation of the prepared emulsions was observed after 24 hour of storage, at room temperature (indicate temperature). From the obtained results, room temperature stability and the optimized emulsion conditions were standardized at 15000 rpm for 45 min. The emulsion thus obtained was immediately taken for further characterization and stored (-20 $^{\circ}$C) for further analysis.

4a.2.3. Characterization of oil in water emulsion.

4a.2.3.1 Creaming index

About 10 ml of the prepared emulsion was poured into a graduated test tube with glass cap and stored at 25°C for 24 hr. The emulsion stability was measured by the percentage of separation calculated using Eq (1).

$$\%\text{separation} = \frac{H_0}{H_1} \times 100 \quad (1)$$

Where H_0 is the initial emulsion height, and H_1 is the height of upper phase (Tonon, Pedro, Grosso, & Hubinger, 2012; Reddy et al., 2019).

4a.2.3.2 Droplet size and Zeta- potential

The droplet size and zeta potential of emulsions was analyzed using the Malvern Zeta sizer (Zeta Nano-ZS; Malvern Instruments, UK), which works on the principle of dynamic light scattering (DLS). Water (refractive index: 1.33) was used as the dispersant. 20µl of emulsion was diluted to 5 mL with deionized water before analysis for mean droplet diameter and surface charge. Measurements were done in triplicates (three runs for each test) for both analysis (Iceu Agustinisari, Kamarza Mulia and Mohammad Nasikin., 2020, Mohammed et al., 2021).

4a.2.3.3 Conductivity

Measurement of emulsion electrical conductivity was carried out in triplicate, using, Labman benchtop conductivity meter (Labman- LMCM20 Scientific Instruments Pvt. Ltd. Chennai, India.). The conductivity values were measured by inserting the electrode directly into the emulsion sample at 25°C.

4a.2.3.4 pH

The pH of emulsions was determined at room temperature (temperature) using a pH meter, Oakton pH 700 (Benchtop Meter, Oakton Instruments, USA). Prior to analysis electrode was calibrated with buffer solution having pH 7.

4a.2.3.5 Color analysis

The color parameters (L*, a*, b) of ghee, WPC and emulsions were determined using Hunter lab, ColorFlex EZ (Hunter Associate Laboratory Inc., Reston, US) Port up, or Port forward dual-beam spectrophotometer. Where L* denotes lightness/ darkness, a* denotes redness (+) and greenness (-), and b* denotes yellowness (+) and blueness (-). The total color difference or change in color between two samples was calculated using the below formula.

$$\Delta E = \sqrt{(L^*_o - L^*)^2 + (a^*_o - a^*)^2 + (b^*_o - b^*)^2} \quad (2)$$

Where,

L_o*, a_o*, and b_o* are the color values of the standard reference type;

L*, a*, and b* are the color values of the test sample (oil encapsulate).

4a.2.3.6 Bulk density

Bulk density (g/mL) was determined by the method proposed by Goula & Adamopoulos, (2004), with slight modifications. 2 ml of oil encapsulate was gradually added into an empty 10 mL graduated cylinder (15.3 cm × 1.55 cm). The value of Bulk density was calculated as the volume occupied by the mass of the powder added to the cylinder (Eq. 3).

$$\text{Bulk density} = \frac{\text{Mass of the powder}}{\text{Volume of the powder}} \quad (3)$$

4a.2.3.7 Fourier transform infrared spectroscopy (FTIR)

The Fourier transform infrared (FTIR) spectra of emulsions, ghee, and WPC powder were recorded using an FTIR-ATR spectrometer (Perkin Elmer, Spectrum Two, US), equipped with ATR accessory with a diamond crystal at incidence angle of 45° at room temperature (25 $^{\circ}$C) (Mohammed et al., 2021). Prior to analysis the crystal was cleaned using acetone (99.9% purity). The absorbance data were averaged from 32 scans recorded at 4 cm^{-1} resolution, in the region from 4000 to 400 cm^{-1}. The spectra were obtained from the instrument software (OPUS v. 7.5, Bruker Optik GmbH., Ettlingen, DE).

4a.2.3.8 Thermal Behaviour of emulsions (DT-TGA)

The thermal behaviour of the emulsion was determined using a Perkin Elmer, ST6000 TG-DTA. Samples of about 5 mg were scanned from 30 to 160 ^{o}C at a rate of 10 ^{o}C/ min under a nitrogen purge flow at 50 mL/min (Xuran Cai et al., 2019).

4a.2.3.9 Viscosity of emulsion

The flow behavior and viscosity of emulsions were determined from the steady-shear flow curves obtained using a controlled stress rheometer (MCR 102 Rheometer, Anton Paar GmbH, Ostfildern-Scharnhausen, Germany). Parallel plate geometry (25 mm diameter; 0.105 mm gap) was used for the study. Flow curve measurement was performed in the shear rate range from 0 to 300 s^{-1}, at a set temperature of 25 °C. Viscosity/apparent viscosity was estimated by calculating the slope of the flow curve and expressed in units of Pascal second (Pa s) (Reddy et al., 2019; Mohammed et al., 2021).

4a.2.3.10 Mechanical stability

A centrifugation test was used to find out the mechanical stability of emulsion, 10 ml emulsion was centrifuged at 3500 RPM for 10 minutes. The oil separated out was measured to find out the mechanical stability of emulsion (Restu et al., 2015).

4a.2.3.11 Surface tension analysis of emulsions

Surface tension of emulsions was determined by the pendant drop method using a drop shape analyser (Model: DSA30E, KRÜSS GmBH, Hamburg, Germany; with the KRUSS ADVANCE Software 1.7.0.8, Version 15). The analysis was carried out at a temperature of 20 ± 4 °C. The test was performed in triplicates, and the mean and standard deviation were calculated.

4a.2.3.12 Microstructure of emulsion droplets by Fluorescence's microscopy

The morphology of dispersed phase of emulsion was observed using a fluorescence microscope Olympus IX83 (Olympus Corporation of the Americas, Center Vally, PA, USA) with Evolve delta 512 EMCD Camera (photometrics, USA). The Nile red fluorochrome (Sigma –Aldrich, St. Louis, USA) was used to observe fluorescence of the retained oil with an excitation and emission wavelength of 560 and 630 nm (Jarpa-Parra, Tian, Temelli, Zeng, & Chen, 2016).

4a.2.13. Statistical analysis

All the measurements were performed in triplicates, and the results are expressed as mean ± standard deviation. Significance of difference between the means of all the parameters was examined by the one-way analysis of variance (ANOVA) at a confidence level of 95%, using the EXCEL^{TM} 2010 (Microsoft, USA).

4a.3. Results and discussion

4a.3.2 Characterization of emulsion

4a.3.2.1 Creaming index

O/W emulsions of the ghee (core) and WPC, were prepared, at different proportions by increasing the oil load (in dry basis) at a constant TSS content of 30%. The oil load was increased from 50% to 70% (dry basis). The emulsions prepared were observed during a

period of 24 h. The emulsions were found to be kinetically stable as the percent of phase separation was found to be zero, even after 24 h of storage at room temperature. It is reported that the stable emulsions for 24 h, is found to give a very efficient spray drying purpose (Salimi et al., 2018). The observed results were exhibited in Table-1 and Figure- 2. The observed emulsion stability can be attributed to the emulsifying/surfactant property of WPC (Kha et al., 2014; Chuyen et al., 2018), as well as the increasing oil fraction. Because of the increase in packing fraction of oil droplets creaming is reduced. According to a study conducted by Sun et al., (2009) even at 40% (v/v) O/W emulsions did not show any creaming regardless of WPI concentration, which indicated that oil-phase volume fraction played a dominant role in the creaming of emulsions.

Table 4a.1. Creaming index (CI) percentage of different trials

% Of oil payload	Oil (g)	Gum (g)	Protein (g)	Water (g)	CI %
50	4.5	0.00	4.50	21	0.00
	4.5	2.25	2.25	21	14
	4.5	1.50	3.00	21	0.00
	4.5	0.75	3.75	21	0.00
60	5.4	0.00	3.60	21	0.00
	5.4	1.80	1.80	21	15
	5.4	1.20	2.40	21	0.00
	5.4	0.60	3.00	21	0.00
70	6.3	0.00	2.70	21	0.00
	6.3	1.35	1.35	21	18
	6.3	0.90	1.80	21	0.00
	6.3	0.45	2.25	21	0.00

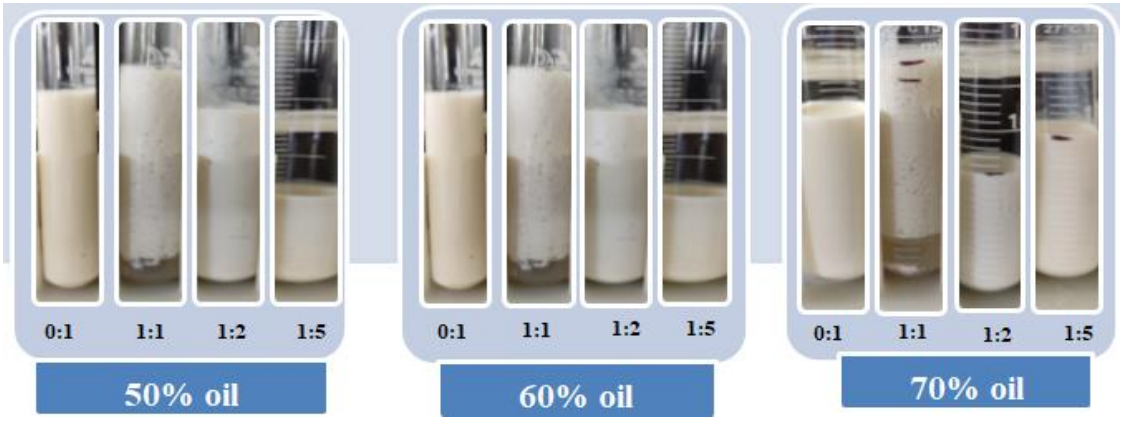


Figure 4a.2. Creaming index percentage of 50, 60, and 70% trails after 24h of storage at ambient temperature

4a.3.2.2 Droplet size

The droplet size of the emulsions were estimated using droplet size analyzer (section **4a.2.3.2**) , and the mean droplet diameters were found to be 385.1 ± 131.0 nm, 404 ± 87.39 nm, 410.6 ± 269.9 nm, respectively for 50, 60, and 70% respectively ($p=0.02$, $p<0.05$). The corresponding polydispersity indices (PDIs) were 0.124 ± 0.00, 0.032 ± 0.00, and 0.227 ± 15.35. The value of PDI ranges between 0 and 1, where 0 signifies monodisperse droplet size distribution, and 1 implies a polydisperse dispersion of droplets in the emulsion in other words, PDI >0.7 indicates more heterogeneous nature and wide distribution of particles (Goyal et al., 2015). The excellent emulsifying, surface binding activities of WPC at oil/water interfaces (Bae & Lee 2008) made the emulsion stable with smaller particle sizes. In the present study, the smallest particle size was observed in the emulsion containing higher amount WPC, similar was also reported earlier by Bae & Lee (2008), for microencapsulation of Avocado oil. When comparing the influence of oil concentration on the average droplet size, Taneja et al., (2013) observed a reduction of average soybean oil droplet size when the WPI concentration was increased in the emulsion. Dluzewska et al., (2006), when studying the stability of essential oil and WPI emulsions, observed no significant increase in

droplet size over time. Therefore, from the above observations it is evident that all the emulsions are stable and indicates more homogeneous emulsion.

4a.3.2.3 Zeta potential

Zeta potential is an important physicochemical parameter related to the stability. Zeta potential is influenced by the electric charge of the interface (Agustinisari et al., 2020). When the charge on the emulsion droplet increases, the stability against droplets aggregation will be enhanced (McClements et al., 2005, Sukhotu et al., 2016). The zeta potential values for 50, 60 and 70% emulsions in the present study were -28.1 ± 4.42, -17.2 ± 6.28, and -26.0 ± 6.19 mV, respectively ($p=0.016$, $p<0.05$). Strongly negative values make the emulsion highly stable because of the high electrostatic repulsion (Rosa et al., 2016). Higher surface electric charge (+ / −) indicates potential stability of the system (Espinosa Solis et al., 2021). The negative zeta potential values signify the negative charges on the surface of starch particles (Wei et al., 2014). A higher zeta potential reduces the Van der Waals force because of electrostatic repulsion between the particles (Ahmad et al., 2020; Schafer et al., 2010). Dai et al. (2018) reported that higher zeta potential leads to less tendency for particle agglomeration and hence lead to higher particle stability. There are two types of emulsion stabilization processes: steric stability, when the emulsifier molecule adheres to the oil droplet surface, maintaining it stable; and electrostatic stability, when there is repulsion among the droplets due to the high surface charge, hindering the droplet agglomeration (Jayme et al., 1999 and Sari et al., 2015). The zeta potential is the most commonly used parameter to determine the electrostatic contribution to emulsion stability. Surface charges control possible system droplet–droplet interactions and contribute to the emulsion stability. Proteins are known to be easily absorbed in the water-oil interface, promoting greater emulsion kinetic

stability. The electrostatic repulsion of protein produce surface charge concentration (Campelo et al., 2017).

4a.3.2.7 Colour

The values of colour parameters (L*, a*, and b*) are expressed as total colour change (ΔE). ΔE of the different emulsions in the study was 78.01, 80.34, and 80.46 for 50%, 60% and 70% emulsions which indicated that ΔE increased with oil payload, which is expected. Tabaszewska and Najgebauer, (2016), reported that L* increased with increase in WPC in the emulsion. In the present study when the oil pay load increased WPC concentration decreased that resulted in the increase in ΔE for higher oil load emulsion. The colour values of ghee, WPC and individual emulsions were represented in Table 3.

Table 4a.2. Total colour change (ΔE) of emulsion

Parameters	50%	60%	70%
*ΔE	78.164 ± 0.22	80.332 ± 0.01	80.569 ± 0.14
ϕYellowness index (b*)	13.475± 0.09	13.655± 0.00	13.660± 0.16
♣Lightness index (L*)	87.955± 0.37	87.445± 0.02	87.330± 0.55

**p value = 0.82, ϕp value = 0.75, ♣p value = 0.76* where $p < 0.05$

4a.3.2.12 Mechanical stability

Mechanical stability by centrifugation at a 3500 rpm for 10 minutes time, is reported to be equivalent to gravity for ±1 year (Restu et al., 2015). The phase separation is correlated with the stability of the emulsion. Lower the separation, the higher will be the stability. The results from the present study is represented in Fig.4. From the results it's

clear that emulsions are all the emulsions under study were stable at the higher centrifugal pressure. Thus, the role of WPC as a stabilizing agent is very critically established here.

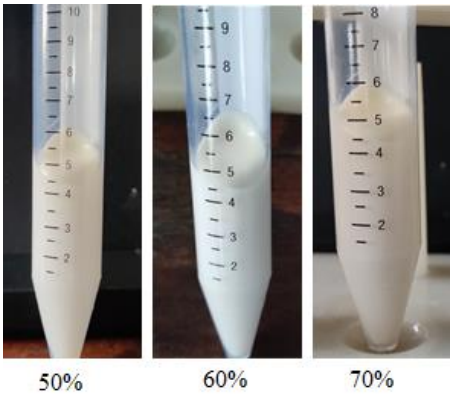


Figure 4a.3. Mechanical stability showing 50%, 60%, and 70% emulsions

4a.3.2.6 Bulk Density

By definition, density decreases as volume increases for a constant mass, and we, therefore, expect a similar relationship between the bulk density and the droplet diameter. The bulk density of Ghee was found to be 0.9403 g/mL, and that of whey protein solutions alone was found to be 0.998±0.007, 0.986±0.004, and 0.949±0.01 g/mL for the three concentrations respectively without ghee. The bulk density of the emulsions were 1.007±0.12, 1.04±0.02, and 0.97±0.21 g/mL for 50, 60, and 70% oil pay load. Which is found without any significant difference, $p=0.95$, $p>0.05$. According to a study reported by Hebishy et al., (2017), density can be correlated to emulsion stability. Emulsions with lower oil contents, the droplets are far apart and the inter-droplet interactions are relatively weaker. As oil content increases, the droplets are closer and the number density of droplets (number of droplets per unit volume of emulsions at a given dispersed phase volume fraction) increases. According to Stoke's law High-pressure homogenization also improves stability of emulsions by decreasing

droplet size, which in turn affects the density of droplets and concomitantly the viscosity of the emulsion, slowing down the droplet movement (Hebishy et al., 2017; Sims et al., 1979). Thus in the present study these two factors may be the reason for its stability as well as the relatively stable density values.

4a.3.2.10 Emulsion flow behavior and viscosity

The emulsion viscosity as a function of shear rate is shown in Figure 3. The viscosity of the present emulsions was found to be 0.0509 Pa s, 0.0498 Pa s, and 0.0301 Pa s respectively for 50, 60, and 70% emulsions ($p= 0.02; p\leq 0.05$). As can be seen, the viscosity decreased with increase in oil pay load. Generally, the viscosity of the continuous phase increases the overall emulsion viscosity (Alliod et al. 2019). Newtonian behaviour was also observed in other emulsions produced with WPI and whey protein concentrate in combination with oils such as avocado oil (Bae & Lee, 2008) and flaxseed oil (Tonon et al.,2012), respectively. The viscosity of coffee oil emulsions were found to be 0.0034 Pa s (3.4 cP) and 0.0009 Pa s (0.9 cP), respectively (Reddy et al., 2019). Yamauchi et al. (1980) reported emulsions that contained 40% oil and 8% whey protein, the stability was highest due to high viscosity. An emulsion with higher apparent viscosity is considered to have good structure, enhancing its suitability for potential food applications such as shortening/fat replacement (Moriano and Alamprese 2020; Mohammed et al., 2021).

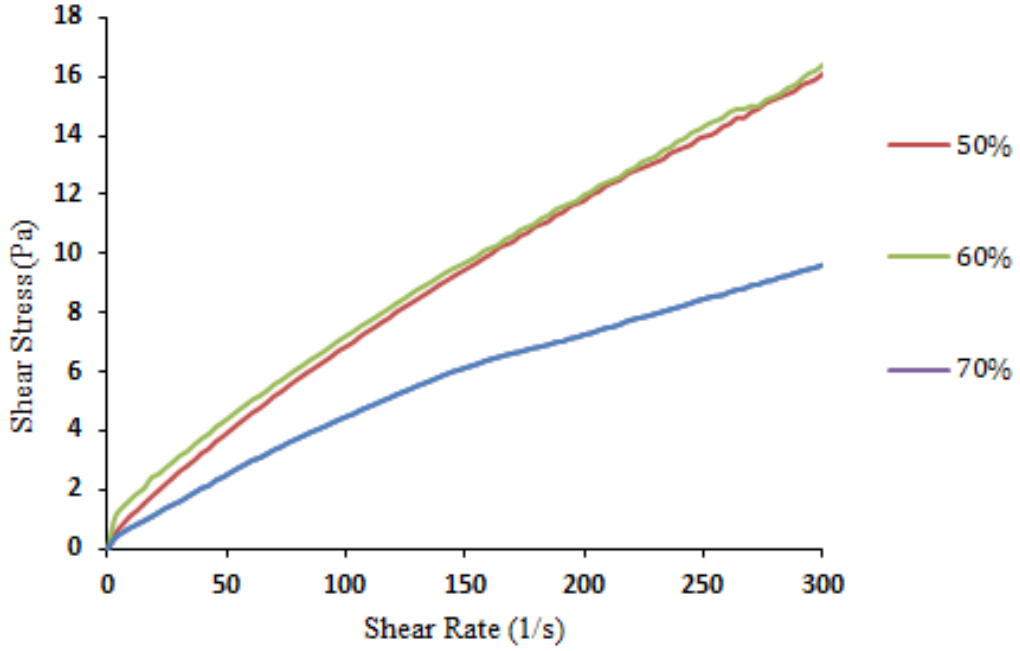


Figure 4a.4. Rheology of 50%, 60%, and 70% emulsions

4a.3.2.11 Microscopy

Fluorescence microscopic imaging was employed to visualize the distribution of oil in the emulsions using Nile red and Nile blue as the stain. The distribution of the oil droplets in the emulsions at different concentrations and the merged images showing encapsulation efficiency were shown in Figure -5. The fluorescence microscope images indicate the size as well as the distribution of oil droplets when the concentration of the oil is increased. Fluorescence microscopy of the freshly prepared emulsions were stained with Nile blue and Nile red. The figure clearly shows the encapsulation efficiency of the emulsions, the powders were dispersed in the stains and then imaged under the microscope with the help of a coverslip. After imaging the primary images are merged to form the image which describes the efficiency of emulsion entrapment. Nile red is reported to stain lipids (core-ghee) and Nile blue to proteins (wall -WPC). Figure -5 clearly demonstrate that the interface of, protein (green colour) entrapped ghee within the inner phase (red) which clearly depicts the entrapment of oil by the WPC as wall material.

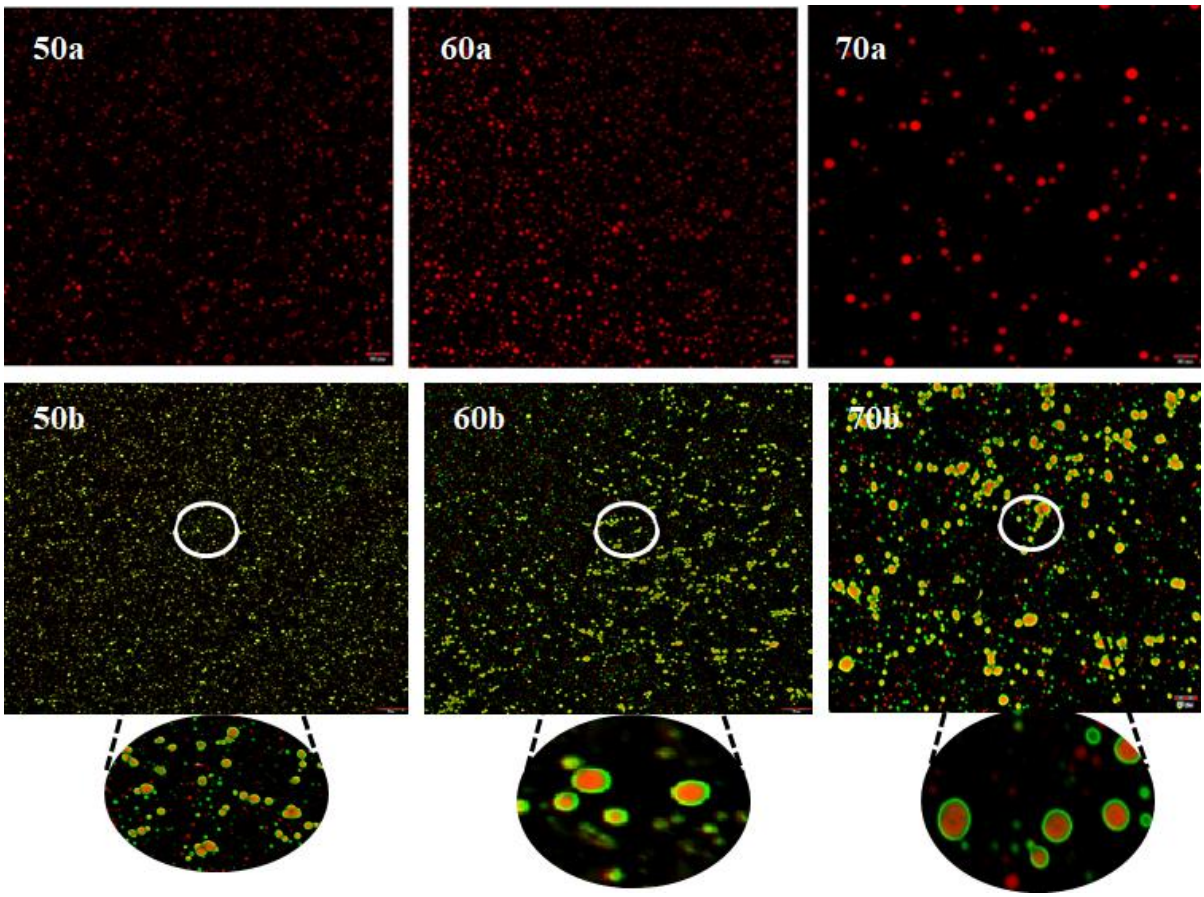


Figure 4a.5. Fluorescent microscopic images of 50, 60, and 70 % emulsions at 40X magnification, with Nile red and Nile blue as the stain

4a.3.2.4 Conductivity

The conductivity of emulsions was found to be 13.385 ± 0.17, 13.245 ± 2.20, and 12.77 ± 4.27 μs for 50, 60 and 70% emulsions. The results showed that the electrical conductivity of O/W emulsion was affected by oil volume fraction and the wall concentration. The larger the oil volume fraction, the less the electrical conductivity of the emulsion (Zhang et al., 2008). The concentration of the emulsifier also affect the electrical conductivity. Conductivity value decreased only at the highest oil concentration. This behaviour can be explained by the poor conductive characteristic of the oil. When the oil concentration is increased there was a gradual decrease in conductivity because of the influence of higher oil load. In the present study, the

stability of emulsion at higher pay load may be the reason for slight difference in conductivity. In the study when the oil concentration is increased the wall material (WPC) concentration is gradually decreased, this inference can be taken for minute difference in conductivity in the present study (Al-Malah, K. 2000, Campelo et al., 2017).

4a.3.2.5 pH

pH is another significant parameter, a change in its value may suggest chemical changes of the components present in the formulation. Here in the present study, immediately after homogenization the pH of the emulsions were noted, and it was 5.93 ± 0.007, 5.82 ± 0.03, 5.80 ± 0.007 for 50, 60 and 70 emulsions respectively. Thus, it can be inferred that, the components of the emulsion did not alter significantly over the analysed time period the emulsion formed are stable. Food emulsions generally have pH in the range of 2.5 to 7.5 (McClements, 2016). Emulsions were prone to droplet flocculation near the isoelectric point of the proteins (pI –WPI ≈ 4.8) but were stable at a higher and lower pH. When the pH values of oil body suspension are far away from the isoelectric point, electrostatic repulsion should be much greater than that at isoelectric point. The pH values were close to those found by Silva et al., (2015) it was around 5.93. Showing that pH is not influenced by the oil content, but by the polysaccharides or proteins used (Campelo et al., 2017). The amount of surfactant is an important parameter which affects the stability as well as pH of emulsion system (Daaou et al., 2012).

4a.3.2.13 Surface tension analysis

The interfacial tension is a measure of the forces trying to keep the two phases separate, the goal in preparing emulsions must be to reduce the interfacial tension to promote a more intimate blending of the two phases (Stephen F. Masucci & Chris Little., 2017). Based on Gibbs thermodynamics theory, surface tension is directly related to the droplet

size. Reduced surface tension may be favourable in achieving higher emulsion stability. Although the effects of whey proteins on surface tension are well known (Xu, Howes, Adhikari, & Bhandari, 2012) the surface-active behaviour of milk proteins has rarely been reported in relation to encapsulation behaviour. WPC significantly reduces the surface tension. Some reports have implied that surface-active substances in liquid formulations might be useful to encapsulate and protect sensitive proteins or enzymes (Elversson & Millqvist-Fureby, 2006; Jayasundera, Adhikari, Aldred, & Ghandi, 2009). In this context, the surface tension values of emulsions were determined, and these are shown in Table 4. The surface tension of water at room temperature is 72 mN/m, and that of edible oil is 34 mN/m. The present study stipulated that, the emulsifier concentration when reduced in emulsions, the surface tension increased.

Table 4a.3. Characterisation of emulsions

Oil concentration (dry weight basis) (%)	WPC concentration (dry weight basis) (%)	pH	Conductivity (μs)	Bulk density (g/mL)	Surface tension
50	50	5.93 ± 0.007	13.385 ± 0.17	1.007	40.902 ± 0.06
60	40	5.82 ± 0.03	13.245 ± 2.20	1.04	41.972 ± 0.05
70	30	5.80 ± 0.007	12.77 ± 4.27	0.97	42.600 ± 0.31

4a.3.2.8 FTIR

The emulsion was characterized using Fourier transform infrared spectroscopy (FTIR) to understand the chemical interactions, if any, by measuring the vibrational properties of functional groups. In the infrared (IR) fingerprint region of whey protein, the vibration at 1700 cm^{-1} to 1600 cm^{-1} (Fig.1a) corresponds to the primary amide region of whey proteins, and the one at 1600 cm^{-1} to 1500 cm^{-1} relates to the secondary amide

region (Kher et al. 2007; Mohammed et al., 2018) and 1500 cm^{-1} to 1600 cm^{-1} corresponds to tertiary amide region (Andrade et al., 2019). In lipids, the most abundant bond participating in IR absorption is the C–H bond present in CH_2 groups in the fatty acyl chain and its terminal CH_3 group, as well as C–H and CH_2 present in the glycerol moiety. The next most abundant bond is for C=O of ester linkages (Antony et al., 2017). Overall, the IR pattern for ghee (Fig.1b) was in close agreement with the patterns reported by Bency Antony et al. (2018). The band in 2922 cm^{-1} represents the CH_2-CH_3 fatty acid chains and 1744 cm^{-1} represents the C-O ester linkage. The IR pattern of emulsion (Fig.1c) the water content of the emulsion showed the presence bands from 3700–3100 (Hydroxyl region)(Kiefer et al., 2016; Samar Daoud et al., 2019). The presence of fingerprint regions of both ghee and WPC also confirms from the present spectrum. Thus, the results of FTIR analysis confirmed the absence of chemical interactions between the emulsion constituents such as ghee and WPC. And it is clear that WPC as a wall material conserved ghee, after encapsulation within the emulsion system. The absorption bands corresponding to the main functional groups typical of ghee, and whey protein concentrate were retained in all the three emulsions.

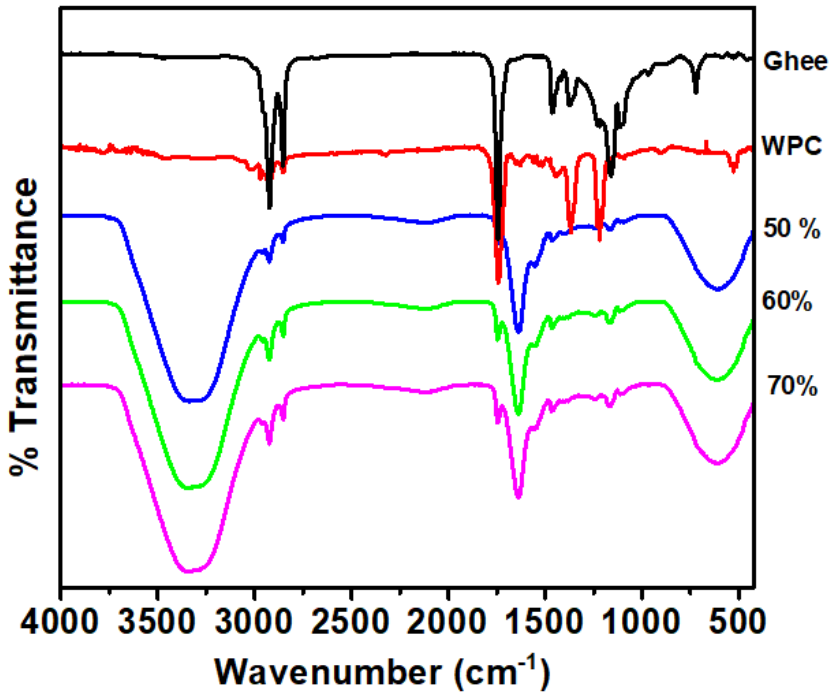


Figure 4a.6. FT-IR spectra of 50%, 60%, and 70% emulsions

4a.3.2.9 Thermal analysis

The efficiency of emulsions to protect the core component with whey protein as wall material was evaluated using DT-TGA (Figure. 9). The denaturation temperature (Td) of whey protein was reported to be 62–66 °C (Anandharamakrishnan, 2008). The denaturation peak of WPC was absent in the three emulsions. The melting temperature of ghee was found to be 40-45 °C. In the present thermogram (Figure.9 and Figure 10) there was no endothermic peak observed. A crystallization peak observed at 110 °C-112 °C, in both thermograms. This may be due to the phase change of emulsions to an amorphous state. The weight reduction was found only as 72, 62, and 73% for the three emulsions with oil pay load 50, 60, and 70% respectively, at the particular temperature. It is attributed to the decompositions and depolymerisation of wall materials

constituents at 110 °C- 112 °C, such as the cleavage of S–S, O–N, and O–O linkages and consequently the breakdown of covalent peptide bonds in WPI (Azizi et al. 2018, Tavares et al., 2019). As the sample is an emulsion, another possible factor for weight loss is mainly due to the evaporation of water at the 110 °C- 112 °C. Importantly, the first stage of mass loss of the TGA curves (between 50 and 110 °C) refers to the loss of moisture from the material, while the second stage (above 110 °C) corresponds to the decomposition processes of the particle constituents (Fritzen-Freireet al., 2012), such as proteins and carbohydrates (Macêdo, de Moura,Souza, & Macêdo, 1997; Carmo et al., 2018).

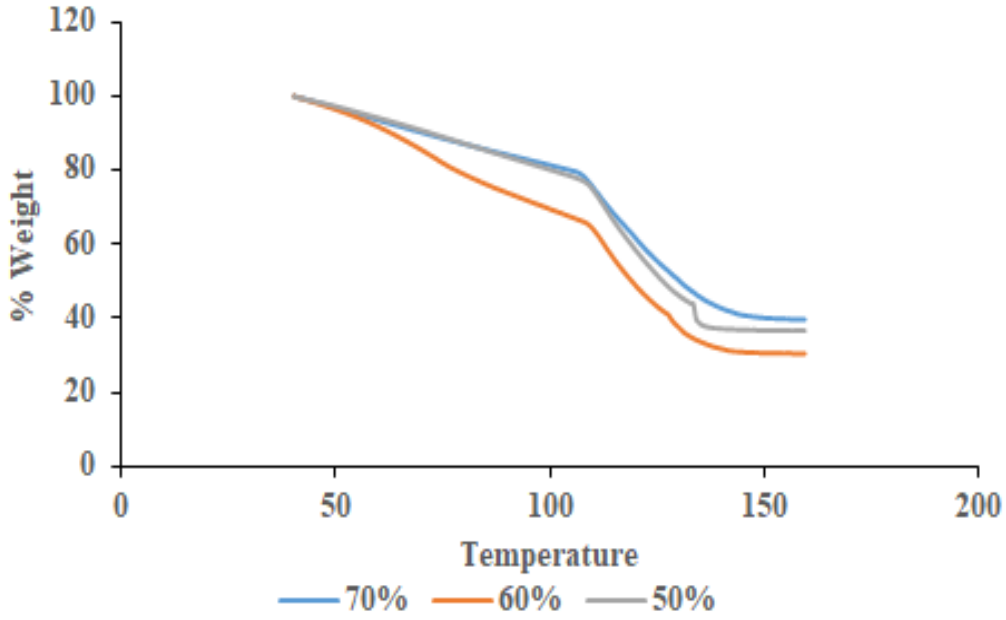


Figure4a. 4a.7. TGA thermogram for of 50%, 60%, and 70% emulsions

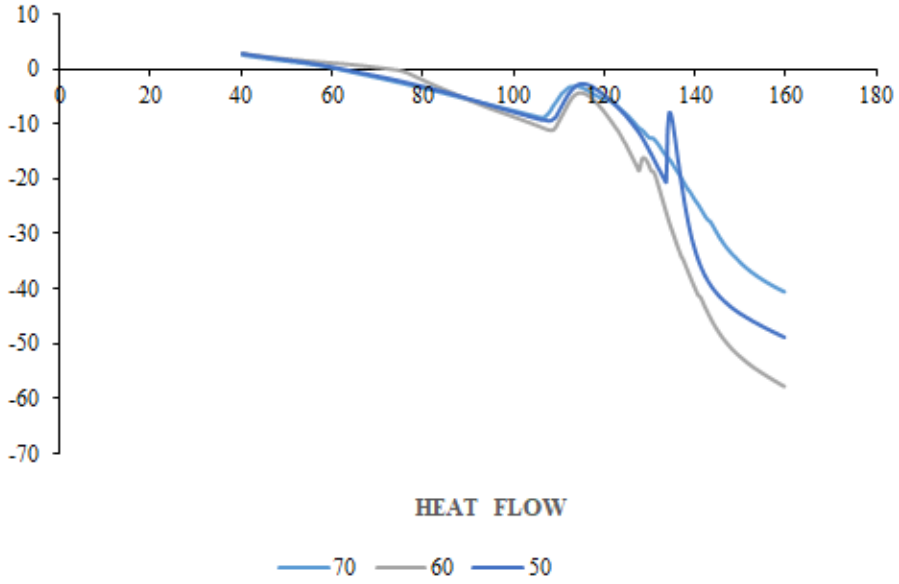


Figure 4a.8. DTA of 50%, 60%, and 70% emulsions

4a.4. Summary and Conclusion

The optimization was carried out in terms of oil payload, wall material composition and homogenization conditions. The oil load was increased from 50 % to 70 % (dry basis) at a total soluble solids (TSS) of 30 %. The optimized emulsions were kinetically stable even after 24 h of storage at ambient conditions (29 to 35 °C). Bulk density of the emulsions were 1.007 g/mL, 1.04 g/mL, and 0.97 g/mL for 50 %, 60 % and 70 % oil payloads, respectively; whereas the corresponding viscosities were 50.9 cP, 49.8 cP, and 30.1 cP. The mean droplet diameters were 385.1 ± 131 nm, 404 ± 87.39 nm, and 410.6 ± 269.9 nm with zeta potential values -28.1 ± 4.42 mV,-27.2 ± 6.28 mV, and -26.0 ± 6.19 mV for 50%, 60% and 70% emulsions, which indicated high stability. Thermal analysis (DT-TGA) and FTIR of the emulsions indicated that core component

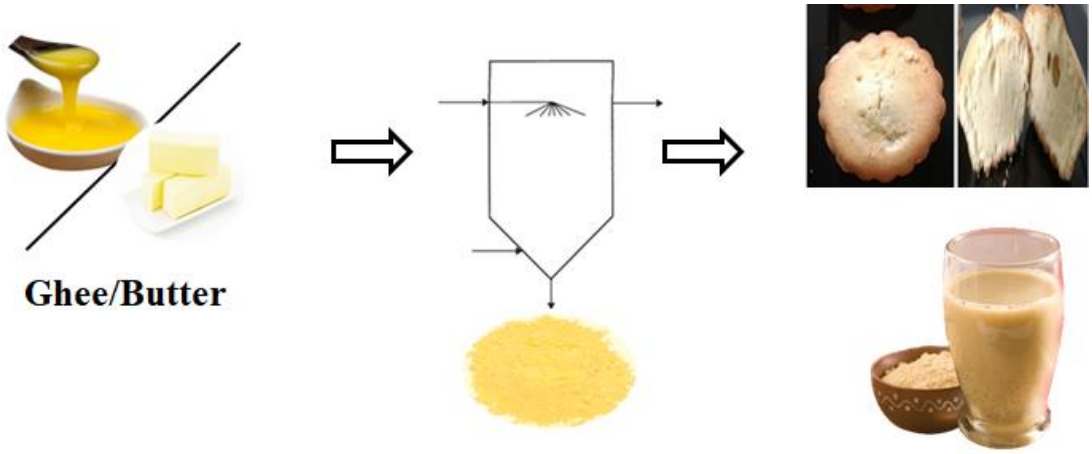
(oil) was well protected when whey protein was used as wall material. This thermally and kinetically stable emulsions were further taken for encapsulation studies.

The key findings were summarised below

- The oil load increased from 50% to 70% (dry basis).
- The emulsions -kinetically stable.
- Optimised conditions for stable emulsion:
    - Homogenization speed:- 15000 rpm
    - Time :- 45 min
    - TSS % :- 30-45
    - GUM :WPC ratio :- 0:1
- Better stability of the core component as evident from thermal analysis and FTIR spectra.
- This thermally and kinetically stable emulsions were further taken for encapsulation studies.

Chapter 4b

Fabrication of encapsulated butter/ghee (Lipids) powder and application in food formulation



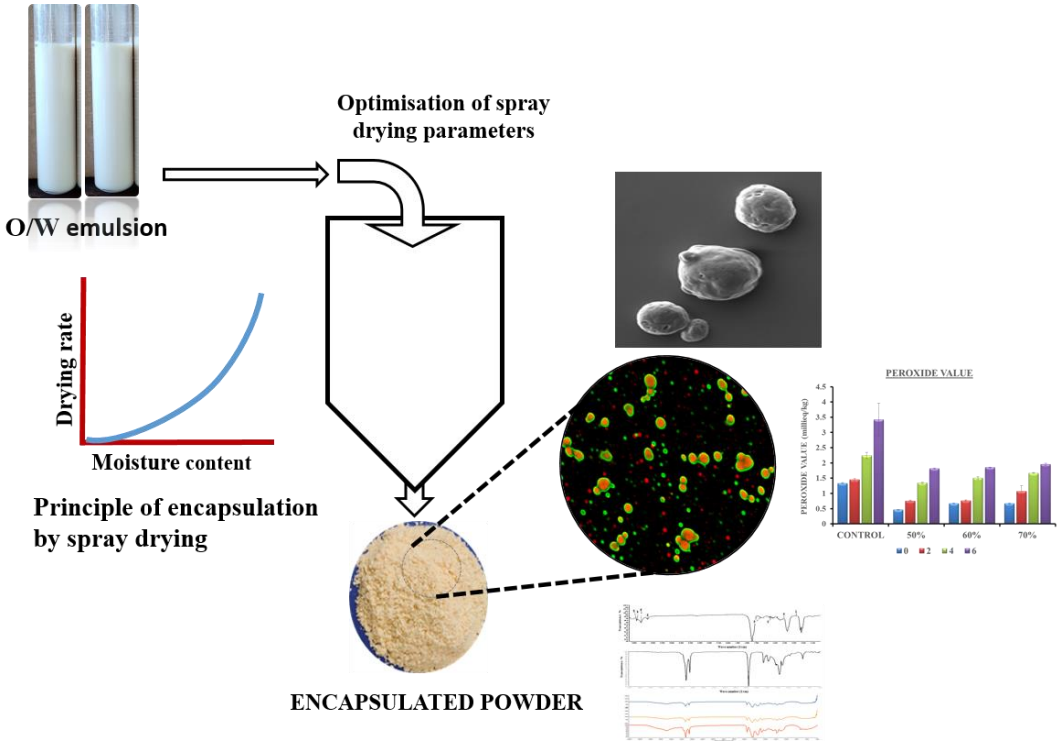
4b.1 Objectives

- Process parameter optimisation of spray drying conditions with higher oil load emulsions in terms of better encapsulation efficiency and yield
- Characterisation of encapsulates on the basis of physichochemical, structural and thermal properties
- Stability studies of encapsulates to confirm the shelf stability
- Simulation studies of encapsulates to find out the release pattern – Thermal and gastro intestinal
- Application studies of the oil encapsulates as replacer of ghee in food applications.

4b.2. Materials and methods

4b.2.1. Materials

Ghee and Whey protein concentrate (WPC- 95%) was procured as detailed in chapter 4a 2.1. The reagents and solvents were of >99.9% purity, and were procured from Merck Limited, Mumbai, India. All the chemicals were of analytical grade. The ingredients for the preparation of ghee cakes, including self-rising flour, sugar, milk, and eggs, were procured from the local market of Thiruvananthapuram, India.



Schematic representation of experimental design

4b.2.2 Methods

4b.2.2.1 Microencapsulation by spray drying

Immediately after the emulsion preparation (explained in previous Chapter- 4a), the emulsion was converted to encapsulated powder by spray drying using a mini spray dryer (LABULTIMA, Process technologies Pvt Ltd., Mumbai, India, Model LU 228 ADVANCED), operating in a co-current configuration. The process conditions for spray drying was optimized by conducting many trails in the spray dryer. The experimental trails of spray drying was optimized using nozzle of 0.7 mm, which is used for atomization. Spray drying was carried out at various inlet and outlet air temperatures from 110 to 120 °C, at a varying feed flow rate (0.8 to 1 mL/min) and aspirator rate of 110 m^3/h. The air pressure was

held steady at 1.12 kg/cm^2. The emulsion was fed at 60 °C into the main drying chamber, controlled by a peristaltic pump. The vacuum and the airflow rate was set at -198 and 73 m^3/h respectively. The oil encapsulate were stored in Duran bottles under ambient temperature and immediately taken for further analysis**.**

4b.2.2.2.10 Product yield

The product yield was calculated as the ratio of the amount of powder collected after every spray-drying experiment to the initial amount of solids in the feed solution. The dry weight of the oil encapsulate obtained by deducting the weight of residual moisture present in it, which was estimated using the moisture analyzer. Then, the product yield was calculated using the below expression.

$$Y = \frac{(W2-W1)-Xwb(W2-W1)}{FvTs} \times 100 \qquad (7)$$

Where,

Y is the powder yield (%),

X_{wb} is the moisture content (w.b),

F_v is the feed volume (mL),

T_s is the total solid content (mg/L), and

W_1 and W_2 are the weight of the powder bottle before and after spray drying (g), respectively.

4b.2.2.2.11 Microencapsulation efficiency

Total oil content in microcapsules was quantified using the AOAC Official Method 925.32 (2012). Briefly, 1 g of powder was transferred to a fat-extraction tube, and 10 mL HCl was added slowly. The tubes were kept in a boiling water bath. After cooling to room temperature (28 ± 2 °C), 25 mL of ethyl ether and 25 mL of petroleum ether were added, and the tubes were vigorously shaken for a minute. The ether solution (supernatant) was separated and filtered through packed cotton. The remaining aqueous phase was further extracted twice

with 15 mL of ethyl ether and 15 mL of petroleum ether. The solvent was evaporated in a rotary evaporator (Hei-VAP-Value Digital (G3), Heidolph Instruments, Schwabach, Germany), and the oil was dried in a vacuum oven at 100 °C to constant weight. Extractable oil, usually referred to as surface oil (EO), was determined according to the methodology of Davidov-Pardo, Roccia, Salgado, Leon & Pedroza-Islas (2008). This non-encapsulated oil is defined as the fraction that can be easily extracted with organic solvents without disrupting the solid matrix. Briefly, 4 g microcapsule powder was drip washed with 75 mL of ethyl ether for 15 min at 25°C. The suspension was filtered through a Whatman No. 1 filter paper, and the powder on the filter was rinsed three times with ethyl ether. The solvent was dried and rota-evaporated to obtain the surface oil mass. Encapsulation efficiency (EE %) was calculated from the following equation:

$$EE\% = \left\{\frac{TO-EO}{TO}\right\}100 \qquad (8)$$

Where TO be the total oil content in microcapsules, and EO is the extractable oil content determined as previously described.

4b.2.2.2 Physiochemical characterization spray dried encapsulates

4b.2.2.2.1 Water activity (a_w)

A digital water activity meter (Rotronic HygroPalm23-AW-A, Switzerland) was used to measure the water activity of the spray-dried oil encapsulate. The sample was placed in a sample cup of 14 mm depth, completely covering the bottom of the cup. A sealed container was formed by placing the probe above the sample cup, and the value of water activity was recorded from the digital display of the water activity meter.

4b.2.2.2.2 Moisture content

The moisture content of the oil encapsulate was determined using a Moisture analyzer (HC 103 Mettler Toledo, India). Oil encapsulate (0.5 g) was placed in the analyzer set at a

temperature of 105 °C until a constant weight was reached. The value of moisture content (in percentage, %) was recorded from the digital display of the moisture analyzer.

4b.2.2.2.3 Bulk density

Bulk density (g/mL) was determined by the method proposed by Goula & Adamopoulos, (2004), with slight modifications. 2 mL of oil encapsulate was gradually added into an empty 10 mL graduated cylinder (15.3 cm X 1.55 cm). The value of Bulk density was calculated as the volume occupied by the mass of the powder added to the cylinder (Eq. 3).

$$\text{Bulk density} = \frac{\text{Mass of the powder}}{\text{Volume of the powder}} \quad (1)$$

4b.2.2.2.4 Tapped density

The tapped density of the sample was determined using the protocol described by by Chinta et al., (2009) with some modifications. Oil encapsulate (2 g) was gradually added into an empty 10 mL graduated cylinder. After tapping the cylinder for ten times, the volume occupied by the sample was noted. The tapped density was calculated using the below equation.

$$Tapped\ density = \frac{Mass\ of\ the\ powder}{volume\ of\ the\ powder\ after\ tapping} \quad (2)$$

4b.2.2.2.5 Flowability

Flowability was evaluated in terms of the Carr compressibility index (CI) and Hausner ratio (HR) (Fitzpatrick, 2005), which were calculated from the bulk and tapped densities of the powders using the following equations:

$$HR = \frac{\rho_T}{\rho_B} \quad (3)$$

$$CI = \left(\frac{\rho_T - \rho_B}{\rho_T}\right) \text{x } 10 \quad (4)$$

Where ρ_T and ρ_B are tapped and bulk density, respectively. Table 1 presents the correlation between the values of the Carr index, Hausner ratio, and powder flowability.

Table 4b.1. Correlation between powder flowability, Hausner ratio, and Carr index (Turchiuli et al., 2005)

Carr's index	Flowability	Hausner ratio
≤10	Excellent	1.00–1.11
11.0–15.0	Good	1.12–1.18
16–20	Fair	1.19–1.25
21–25	Passable	1.26–1.34
26–31	Poor	1.35–1.45
32–37	Very poor	1.46–1.59
>38	Awful	>1.60

4b.2.2.2.6 Particle (true) density

For the determination of particle density, approximately 1 g (m_0) of the sample was filled in a burette containing toluene. The rise in toluene level (V1) was measured and calculated as true particle density. Toluene was used because of its ability to penetrate the finest external pores connected to the surface without dissolving the material (Premi & Sharma, 2017)

$$Particle\ density = \left(\frac{m_0}{V_1}\right) \quad (5)$$

4b.2.2.2.7 Particle size and Zeta- potential

The particle size and zeta potential of encapsulates was analyzed using the Malvern Zeta sizer (Zeta Nano-ZS; Malvern Instruments, UK), which works on the principle of dynamic light scattering (DLS). Water (refractive index: 1.33) was used as the dispersant. The encapsulate

was diluted to 5 mL with deionized water before analysis for mean droplet diameter and surface charge. Measurements were done in triplicates (three runs for each test) for both analysis (Iceu Agustinisari, Kamarza Mulia and Mohammad Nasikin., 2020, Mohammed et al., 2021).

4b.2.2.2.8 Solubility

Solubility is expressed as the percentage of dried supernatant with the amount of powder originally added (Chew et al. 2018). Solubility was determined according to the methods of Eastman & Moore (1984) and M. Cano-Chauca et al., (2005), with some modifications. 100 mL of distilled water was transferred into a blender jar. The powder sample (1g, dry basis) was carefully added into the blender operating at high velocity for 5 min. The solution was placed in a tube and centrifuged at 3000xg for 5 min. An aliquot of 25 mL of the supernatant was transferred to pre-weighed Petri dishes and immediately oven-dried at 105 $^{\circ}$C for 5 h. Then the solubility (%) was calculated by weight difference.

4b.2.2.2.9 Color analysis

The color parameters (L*, a*, b) of ghee, WPC and encapsulates were determined using Hunter lab, ColorFlex EZ (Hunter Associate Laboratory Inc., Reston, US) Port up, or Port forward dual-beam spectrophotometer. Where L* denotes lightness/ darkness, a* denotes redness (+) and greenness (-), and b* denotes yellowness (+) and blueness (-). The total color difference or change in color between two samples was calculated using the below formula.

$$\Delta E = \sqrt{(L^*_o - L^*)^2 + (a^*_o - a^*)^2 + (b^*_o - b^*)^2} \quad (6)$$

Where,

L_o*, a_o*, and b_o* are the color values of the standard reference type;

L*, a*, and b* are the color values of the test sample (oil encapsulate).

4b.2.2.2.12 Morphology of encapsulates by Scanning Electron Microscopy (SEM)

Morphology and particle size of spray-dried oil encapsulate were determined using a Scanning Electron Microscope (Carl Zeiss EVO-18, Germany). For SEM, the samples were transferred to a cryo-preparation chamber, which was maintained at a constant temperature of −10 °C using a "Peltier- cooling" stage. The prepared samples were mounted on an aluminium stub using carbon tape. The samples were examined under vacuum using an accelerating beam at a voltage of 10 kV. The micrographs were recorded at a magnification of 3000X, 10000X and 25000X .

4b.2.2.2.13 Microstructure of spray dried encapsulates by Fluorescence's microscopy

The morphology of encapsulates was observed using a fluorescence microscope Olympus IX83 (Olympus Corporation of the Americas, Center Vally, PA, USA) with Evolve delta 512 EMCD Camera (photometrics, USA). The Nile red fluorochrome (Sigma –Aldrich, St. Louis, USA) and Nile blue fluorochrome (Sigma –Aldrich, St. Louis, USA) was used to observe fluorescence of the retained oil and protein in the encapsulates. The encapsulated powder was mixed with both the stains for one minute, then the samples were dropped down to a glass slide and covered with a glass slide cover. An excitation and emission wavelength of 560 and 630 nm (Jarpa-Parra, Tian, Temelli, Zeng, & Chen, 2016) and 631 nm-660 nm respectively.

4b.2.2.2.14 BET surface area analysis

For this, powders were taken for physiosorption study in a sample tube and an isothermal jacket was used during sample analysis. Surface area analyzed based on an N2 adsorption–desorption process with nuance (Du et al., 2013; Jadhav & Vavia, 2017; Sannya and Nisha, 2022). The analysis was carried out on a TriStar II 3020 instrument (Micromeritics Instrument Corporation, GA, USA). The system was operated at pressure (P/P0) range of 0.1 to 1.0. The samples were degassed at 100 °C overnight under vacuum before adsorption and

the temperature was maintained at 77 K. The surface area was calculated using Brunauer–Emmett–Teller (BET) method.

4b.2.2.2.15 Accelerated Stability studies of encapsulates

The accelerated stability test (Schaal oven test) was performed to evaluate the oxidative stability of the encapsulated oils. The encapsulates were stored at 60 ± 2 °C in a hot air oven (Globe Tex, Digital Laboratory Hot Air Oven, Ghaziabad, India) for a period of 12 days in darkness. The oxidation reaction was accelerated at 60 °C in the oven at the said aforesaid conditions. Samples were removed on third day interval for analysing the extend of lipid oxidation. Each experiment was performed in triplicate (n = 3). Chemical and physical parameters of oils namely peroxide value (PV), p-anisidine values and totox value were evaluated.

The stability of oil in the microencapsulated powders was evaluated by extracting the oil from the encapsulated powders using the procedure described by Lee et al. (2018). 20 mL of aqueous ethanol (85 mL ethanol/ 100 mL water) was poured into sample followed by adding 50 mL of petroleum ether. The samples were then stirred with a magnetic stirrer for 30 min. Phase separation was observed after the stirring stopped and the top layer was extracted into a tared round bottom flask. Petroleum ether (5 mL) was used to re-extract the remaining ethanol solution and the procedure was repeated microcapsules turned white. The oil thus obtained was used for further analysis like peroxide value, para-anisidine value and Totox value content.

4b.2.2.2.15.1 Peroxide value (PV)

Peroxide value is an indication of the extent of oxidation of the oil. Sample (2-5g) was taken into 250 ml glass stoppered flask. 30 ml acetic acid – chloroform reagent was added. Shake

until to dissolve the sample. To this 0.5 ml of saturated potassium iodide solution was added and mixed. Keep the mixture in dark for few minutes, followed by the addition of 30 ml of distilled water. 0.1N sodium thiosulphate was used for titration using freshly prepared starch as indicator. End point is the disappearance of blue color. Conduct blank (must be less than 0.1 ml 0.1 N sodium thiosulphate) (AOAC, 2000). Peroxide value expressed as milliequivalent of peroxide oxygen per kg sample (meq/kg):

$$PV = \frac{(S - B)N \times 1000}{W} \qquad (9)$$

Where,

B – Volume in mL of standard sodium thiosulphate solution required for the blank (ml)

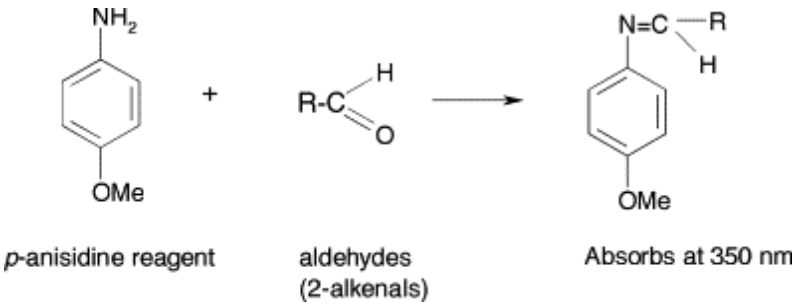
S -- Volume in mL of standard sodium thiosulphate solution required for the sample (ml)

N -- Normality of sodium thiosulphate solution

W -- Weight of sample (g)

4b.2.2.2.15.2 Para anisidine value (pAV)

The AOCS Method Cd 18-90 (AOCS, 1997) was used to determine pAV. P-Anisidine was recrystallised according to the method and used for two consecutive days only, after which fresh p-anisidine was prepared. *Para*-anisidine is a reagent that reacts with aldehydes to give products that absorb at 350 nm. The *p*-anisidine value is defined as the absorbance of a solution resulting from the reaction of 1g fat in isooctane solution (100 ml) with *p*-anisidine (0.25% in glacial acetic acid). The products formed by reaction with unsaturated aldehydes (2–alkenals) absorb more strongly at this wavelength, and consequently the test is particularly sensitive to these oxidation products. Although the test does not distinguish between volatile and nonvolatile products, the palate is generally more sensitive to unsaturated volatile aldehydes than to saturated volatile aldehydes, so the test is a reasonable way to assess secondary oxidation products.



$$PaV = \frac{25 \times As - Ab}{m} \quad (10)$$

Where,

As – absorbance of fat solution after reaction with p-anisidine

Ab – absorbance of fat solution

m -- Weight of sample (g)

Measurements of *p*-anisidine value are commonly used together with peroxide value measurements in describing the total extent of oxidation by the Totox value, which equals the sum of the *p*-anisidine value plus twice the peroxide value. However, the Totox value is an empirical parameter since it corresponds to the addition of two parameters with different units. The Totox value was calculated from the peroxide value and the p-anisidine value with the formula as follows,

$$Totox\ value = 2PV + AV \quad (11)$$

4b.2.2.2.16 Heat induced core release simulation studies of encapsulates

The simulation studies of the oil encapsulates were done to find out the oil release from the encapsulated powders to the outer surface under different environments to assess its stability and further use in food applications. According to T. Majchrzak et al., (2017), oils incubated at 140 °C have a short oxidation time and therefore not suitable as a frying medium. Therefore we took a maximum temperature of 180 °C as a benchmark for the thermal

stability studies and heating encapsulates at 40 °C, 80 °C, 100 °C, 120 °C 160 °C and 180 °C were done for elongated time that is 30, 60, and 90 min. These time temperature combinations were matching with common culinary preparation methods, industrial and baking applications. The thermal stability studies were performed with the help of an oil bath (JULABO ME-4, Heating Circulator, Germany).

4b.2.2.2.17 In-vitro release behaviour of encapsulates under gastro intestinal conditions

In-vitro release behaviour of oil encapsulates was investigated using a simulated gastrointestinal model according to the method given by Pan et al., (2019), with slight modifications. Simulated gastric fluid (SGF) was prepared by dissolving 2.0 g of NaCl and 7.0 ml of 36% HCl in 900 ml of water. After the addition of 5.0 g of pepsin, the pH of the solution was adjusted to 1.2 with 0.1 M HCl and the final volume was made up to 1000 ml with water. Simulated intestinal fluid (SIF) was prepared by dissolving 6.8 g of Dipotassium phosphate (K_2HPO_4) in 800 ml of water. To this solution, 77 ml of 0.2 M NaOH and 100.0 g of pancreatin was added followed by stirring overnight at 4 °C. Solution pH was adjusted to 6.8 with 1.0 M NaOH or 1.0 M HCl and the final volume was made up to 1000 ml with water. The release studies were done as per the following procedure

Mouth phase: For sequential exposure to mouth, mucin solution (0.03 g/mL) to encapsulates were taken at 1:1. The pH value of mixture was adjusted to 6.8. The mixture was placed in an incubator shaker (37 °C) at 130 rpm for 10 min.

Stomach phase: 20 mL of the sample from the mouth phase was mixed with 20 mL of gastric fluid and pepsin and incubated at 37 °C for 2 h. The pH value of mixture was adjusted to 1.2 and the system was incubated at 37 °C, at 130 rpm for 2 h.

Small intestine phase: 30 mL of reactant from stomach phase was mixed with the small intestine phase which was simulated by adding 1.5 mL of CaCl2 (36.7 mg/mL) and NaCl (219.1 mg/mL) and 1.8 g of bile salts. The pH of the system was adjusted to 6.8 and 1.8 g of pancreatin was added. The mixture was incubated in an incubator shaker (37 °C) at 130 rpm for 2 h.

The contents were continuously mixed by using magnetic beads and magnetic stirrer. Released oil was extracted using 30 mL petroleum ether and 30 mL di-ethyl ether in a separating funnel. The extraction of oil was repeated two times with 40 mL of mixture of petroleum ether and di-ethyl ether (1:1). Solvent was evaporated at 80 °C and extracted oil was dried in hot air oven maintained at 100±2°C for 30 min and the quantity of released oil was determined gravimetrically. Total oil in the prepared powder was 50, 60 and 70 % on dry weight basis, it is measured by the method described in section 4b.2.2.2.11.

4b.2.2.2.18 Fourier transform infrared (FTIR) spectra

The FTIR of encapsulated powders, ghee, and WPC were recorded using an FTIR-ATR spectrometer (Perkin Elmer, Spectrum Two, US), equipped with ATR accessory with a diamond crystal at incidence angle of 45° at room temperature (25 °C) (Sannya and Nisha, 2022). Prior to analysis the crystal was cleaned using acetone (99.9% purity). The data were averaged from 32 scans recorded at 4 cm^{-1} resolution. Transmittances were recorded at wave numbers between 4000 and 400 cm−1.

4b.2.2.2.19 Thermal Behaviour of emulsions (DT-TGA)

The thermal behaviour of the emulsion was determined using a Perkin Elmer, ST6000 TG-DTA. Samples of about 5 mg were scanned from 30 to 160 °C at a rate of 10 °C/ min under a nitrogen purge flow at 50 mL/min (Xuran Cai et al., 2019).

4b.2.2.3 Product formulation and characterization

To evaluate the potential of the spray-dried oil encapsulate as a replacer of conventional ghee, in the product development studies were carried out by replacing 100% of ghee in the formulation of cakes with the oil encapsulate. The control and test cakes were prepared using the formulations given in Table 2. The ingredients were then mixed and blended using an electric whisk until a creamy consistency was attained. The batter was transferred into the cake mold and baked in a preheated oven (Bajaj OTG, 4500 TMCSS, Mumbai, India) for 40 minutes at 180 °C (until the crumb turned golden brown). The product was cooled at room temperature. The sensory and texture characteristics of the control and test cupcakes were compared.

Table 4b. 2. Ingredient composition of cupcakes

Ingredients	***T1***	***T2 (replacement with oil powder)***
Flour	100 g	100 g
Sugar	60 g	60 g
Ghee	70 g	--
Oil powder	--	70 g
Vanilla essence	1.5 g	1.5 g
Egg	2 no.s	2 no.s
Salt	To taste	To taste
Baking powder	3.5 g	3.5 g
Milk	50mL	50mL

4b.2.2.3.1 Sensory evaluation

A hedonic test was performed to determine the acceptability and any significant differences in the sensory attributes between the control and test cakes. Care was taken to avoid interference from other sources. The samples were presented to ten semi-trained panelists familiar with the techniques of sensory analysis. They were asked to score the product for appearance, color, texture, taste, and overall acceptability with a scale representing quality grade description given in Table 3.

Table 4b.3. Hedonic scale grade description

Preference	Grade
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

4b.2.2.3.2 Texture profile analysis

The texture profile analysis of control and test cakes was determined using the, texture analyzer equipped with a 50 N load cell (TA1, AMTECK, Lloyd instrument) was used to determine the texture profile of cupcakes. The force required to compress cupcakes by 50% was measured with a rounded bottom stainless steel probe at a speed of 10 mm/s. Texture measurements were performed in triplicates for each sample, and the mean values were reported. Before the test, the sample was placed centrally under the probe to avoid irregular areas of the crust regions. The Nexygen MT software program was used to quantify the parameters of interest in this work: hardness (N), cohesiveness (TPA), springiness, chewiness (N), adhesiveness (TPA), and gumminess (N).

4b.2.2.4 Statistical analysis

All the measurements were performed in triplicates, and the results are expressed as mean ± standard deviation. Significance of difference between the means of all the parameters was

examined by the one-way analysis of variance (ANOVA) at a confidence level of 95%, using the EXCEL^{TM} 2010 (Microsoft, USA).

4b.3. Results and discussion

4b.3.1 Optimisation of spray drying conditions for the encapsulation of ghee

The spray drying conditions for the stable emulsions with oil payload 50-70%, were fabricated in the previous chapter, were optimised by controlling various parameters of the instrument. By altering the aspirator flow rate, the amount of heated drying air entering the spray chamber can be regulated. Outlet temperature correlates with the final moisture content and surface topography of the final product (Maas *et al.,* 2011). Stickiness is considered to be the major process challenge in spray drying. It leads to product agglomeration and poses problems of caking and lumping of the product during packaging of the spray dried products (Rahman, 1995). Initial trials revealed that the temperature below 110 ^{o}C, results in encapsulates that are tacky and adhered to the walls of the spray dryer, decreasing the final yield of powder. Therefore the fabricated emulsions with oil payload 50, 60 and 70% (db) were further studies for the spray drying at inlet and outlet temperatures of 110 ± 5 & 65 ± 5 ^{o}C and 120 ± 5 & 75 ± 5 ^{o}C respectively, by changing the flow rate of feed emulsion, aspiration and vacuum as mentioned in section 4b.2.2.1 also varied. As can be seen from Table 5, the outlet temperature of 120 °C demonstrated better yield and efficiency for all the trials. With this optimized conditions trials were repeated with inlet temperature 120 °C, nozzle size of 0.7 mm, flow rate 0.8 ml/min, with atomisation pressure as 1.12 kg/cm^2. The TSS of feed emulsion was 30 % as explained in the previous chapter (TSS was adjusted to result an oil pay load of 50, 60 and 70% on dry wight basis). Table-6 summarizes the optimized process parameters employed for spray drying to obtain the powdered ghee. The oil payload achieved in the present study had not been reported earlier for any of the edible oil so far (Pattnaik and Mishra, 2021).

Table 4b. 4. Optimized conditions results of yield and efficiency

Oil load	Sample name	Inlet (° C)	Outlet (° C)	Oil (gm)	WPC (gm)	water (gm)	TSS %	SSL %	EE %	YIELD %
50% trials	L	110±5	58±5	7.5	7.5	35	30	0.5	79 ±0.70	65±0.10
	M	120±5	68±5	7.5	7.5	35	30	0.5	79±0.50	73±0.23
60% trials	O	110±5	60±5	9	6	35	30	0.5	70.5±0.01	65±0.02
	P	120±5	65±5	9	6	35	30	0.5	82±0.25	62±0.07
70% trials	Q	110±5	61±5	10.5	4.5	35	30	0.5	68.57±0.70	54.5±0.31
	R	120±5	67±5	10.5	4.5	35	30	0.5	60.80±0.11	63±0.22

Table 4b.5. Final Optimized conditions of spray drying

% Oil payload (gb)	Sample name	Inlet (° C)	Outlet (° C)	Nozzle (mm)	Flow (ml/min)	Aspiration (m3/h)	Atomisation pres. (kg/cm^2)	Vacuum	Oil (gm)	WPC (gm)	Water (gm)	TSS %	YIELD %	EE %
50	Mr	120	73	0.7	0.8	110	1.19	-198	7.5	7.5	35	30	73±0.70	70.05±1.35
60	Pr	120	65	0.7	0.8	110	1.11	-196	9	6	35	30	65±0.70	64.23±2.48
70	Rr	120	67	0.7	0.8	110	1.12	-198	10.5	4.5	35	30	62±2.82	58.23±1.22

4b.3.1.1 Product yield and Encapsulation efficiency

Presence of surface oil and microencapsulation efficiency (EE) of wall materials affect the physico-chemical stability of dry powders. Surface oil leads to the aggregation of the powder particles and increases the rate of oxidation. The surface oil in the present study was 29.95 ± 0.35, 35.77± 0.28, and 41.77± 0.54 % for 50, 60 and 70% oil encapsulates, which was used for calculating EE. The process yield for ghee encapsulates at 50, 60, 70% payload were 73.42 ± 0.70, 65.23 ± 0.70 % and 62 ± 2.82 % and the encapsulation efficiency was 70.05 ± 1.35, 64.23 ± 2.48 and 58.23 ± 1.22 respectively.

The film forming ability of whey protein helped in getting finer yield and efficiency even at higher concentrations of oil load (Sogut, 2020). The superiority of WPI as encapsulating agents for oils has been demonstrated in several studies. For instance, Kha et al. (2014) encapsulated Gac oil using a combination of GA and WPI as wall materials. The efficiency of WPI has been attributed to its skin-forming behaviour that results in a particle surface without any pores and cracks after SD. The antioxidant activity of WPI (Gad et al., 2011) is an added advantage of using it as a wall material for the SD encapsulation of unsaturated oils (Ramakrishnan et al., 2014).

4b.3.2 Physiochemical characterization spray dried encapsulates

4b.3.2.1 Moisture content and Water activity (a_w)

The moisture content of 50, 60, and 70 % encapsulates were 3.02 ±0.16, 4.25±0.07 and 4.9± 0.13 % respectively and the water activity of the aforementioned samples showed 0.31 ±0.09, 0.41 ±0.04, and 0.48 ±0.09. The moisture contents and a_W are very important parameters that play significant roles in the assessment of powder quality, shelf life and retention of core component (Liang, Huang et al., 2013). Lower moisture content and water activity would prevent microbial growth and caking issues, which could improve the physical stability,

chemical stability and total acceptability (Goyal et al., 2015). According to chew et al., (2018), the moisture content of the refined kenaf seed oil ranged from 2.7 to 3.9 % and the water activity of the same ranged between 0.2-0.3. Nayana et al., (2021), in a study done by blends of palm and flax seed oil showed that the moisture content was 2.6 % for core oil of 40 %. The water activity and moisture content of the encapsulates in the present study indicates its stability against lipid oxidation (Shariff et al., 2017).

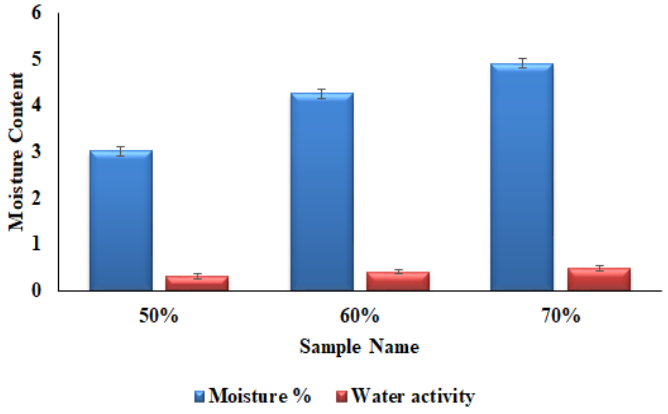


Figure-4b.1 Moisture content and water activity of 50, 60, and 70% ghee encapsulate (p= 0.0014, p≤0.05)

4b.3.2.2 Particle size and Zeta- potential

Particle size plays a significant role in physical characteristics and encapsulation efficiency. The average particle size of a powder influences transportation, handling, and storage related characteristics (Correa-Filho, et al., 2019). Table 7, depicts average particle size and Polydispersity Index (PDI). Particle size of 50, 60, 70 % oil payloads were 1.92 ± 126 μm, 2.26 ± 255 μm and 3.036 ± 142 μm respectively. The poly dispersity index (PDI) ranges from 0.387 ± 0.00, 0.390 ± 0.00, and 0.388 ± 15.35 respectively. Particle size can also influence the stability of core materials that are sensitive to light, humidity, and temperature. According to Sun et al., (2019), the average particle size of carvacrol encapsulated in a pectin/sodium

alginate matrix ranged from 2.58-5.30 μm. The lowest PDI is most entertained as it attributes to high stability (Álvarez et al., 2020). The value of PDI ranges between 0 and 1, where 0 signifies monodisperse droplet size distribution, and 1 implies a polydispersion of droplets in the emulsion in other words, PDI > 0.7 indicates more heterogeneous nature and wide distribution of particles (Goyal et al., 2015). The results of the present study indicated that even at higher concentration of oil load, the encapsulates demonstrated better zeta potential values of -34.1 ± 4.42, -27.8 ± 6.28 and -28.4 ± 6.19 mV respectively. In a dispersion of particles, higher surface electric charge above ±25 mV (+/−) indicates potential stability of the system (Espinosa Solis et al., 2021). The negative zeta potential values signifies that negative charges on the surface of protein particles (Wei et al., 2014).

4b.3.2.3 Bulk density (BD) and Tapped density (TD)

Bulk density is crucial during storage of powders into containers/vessels and their transportation. Density is an important parameter in powders when packed or stacked in bulk. By definition, density decreases as volume increases for a constant mass. Therefore, similar relationship between the bulk density of the powder and the diameter of the particles is expected. The bulk density values of the encapsulates ranged from 0.22 ± 0.02, 0.24 ± 0.003 to 0.35 ± 0.03 g/mL, with the increasing order of oil concentration that is 50%, 60% and 70%. Even if the particle size of 70% oil higher for a given volume, the amount of oil is also high, which contributed to the increasing order for BD. Sheriff et al., (2017), found BD between 0.27 and 0.32 g/cm^3 in fresh powders of β-carotene- eugenol co encapsulates, which is similar to our results.

Tapped density indicates the weight and amount of powder that can fit in a container. When subjected to tapping there is chance of rolling small particles in the voids and reach the densest packing condition. The value of the tapped density of a powdered product is always

higher than its bulk density (Chew et al., 2018). The above findings hold good in this study, as well. The oil encapsulates exhibited higher tapped density (0.24 ± 0.01, 0.27 ± 0.01 and 0.40 ± 0.03 g/mL) for 50, 60 and 70% oil payload, than its bulk density (0.22 ± 0.02 g/mL, 0.24± 0.003 g/mL and 0.35 ± 0.03 g/mL).

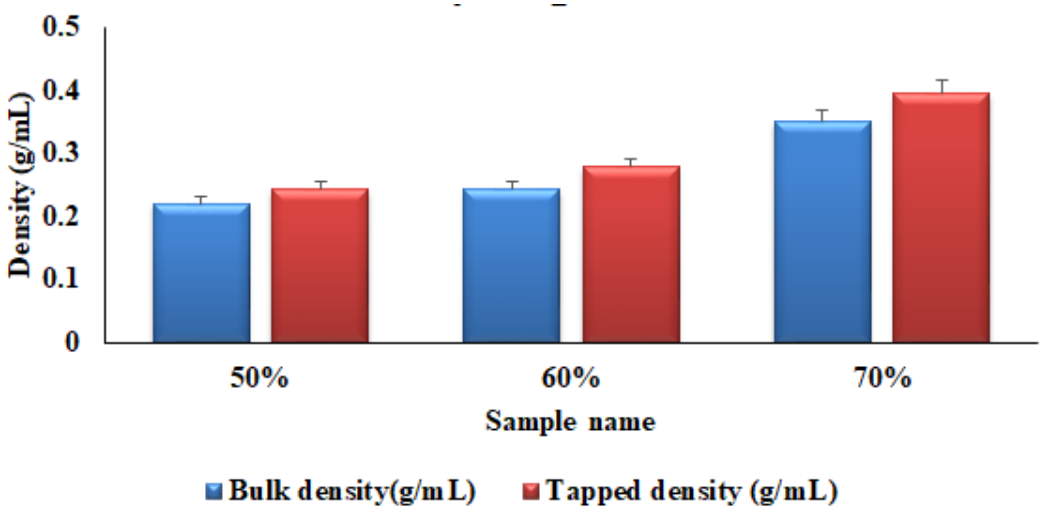


Figure-4b.2 Graphical representation of densities of 50, 60, and 70% encapsulates ($p \leq 0.05$)

4b.3.2.4 Flowability

The flowability of powder was evaluated on the basis of Hausner ratio (HR) and Carr's compressibility index (Carr, 1965), which was calculated from the loose and tapped bulk densities of the encapsulated powders (Table 7). The ghee encapsulates exhibits superior flowability with HR and CI of 0.91 & 8.3, 1.12 & 11.11, and 1.14 & 12.5 for 50, 60 and 70% oil pay load respectively. These results showed excellent flowability for the 50, 60, and 70% powders which differs significantly ($p \leq 0.05$). Goyal et al., (2015) reported that the flax seed oil encapsulated with whey protein concentrate at 35% oil load had HR and CI of 1.55 and 33.82, respectively. The surface composition of the powder affects the flowability so as to overcome the surface interactions among the particles (Chew et al., 2018). Thus, the low

Hausner ratio of the oil encapsulates obtained in this study is indicative of its less cohesive nature and superior flowability, especially for 50% oil loaded powders.

4b.3.2.5 Particle (true) density

Particle density is the real solid density, where space between the particles is not considered. Particle density of 50, 60 and 70% oil loaded powders varied from 0.66 ± 0.02, 0.82± 0.11 to 1.85± 0.13 g/cm^2. Particle density of the powders was significantly lower. However, the size of the particles may affect the particle density. Smaller size particles creates higher compression among the particles thereby forming dense packing and leads to increased particle density. Here 50 % powders showed the lowest particle size when compared to 60 and 70% powders (Table 7). The particle size was found to increase gradually with oil load which was in order with the BD. Another factor that influenced particle density was the concentration of wall material, concentration of feed emulsion, and temperature used for drying. Particle density for rosemary essential oil microencapsulated by spray drying using gum arabic as encapsulant varied from 0.97 to 1.27 g/mL **(**Fernandes et al., 2013). Particle density ranged 1.15 to 1.34 g/cm^3, with different combination of carrier agents used in the encapsulation of drumstick oil powders (Premi and Sharma, 2017). The particle density values were matching with the reported values.

4b.3.2.6 Porosity

Another property of fundamental importance in food processing operations is porosity. Porosity (ε) is defined as the void fraction in the powder sample which plays an important role during reconstitution of powder (Krokida; Zogzas; and Maroulis, 1997; Premi and Sharma, 2017). Porosity of powdered ghee samples were 0.64 ± 0.007, 0.67 ± 0.03, and 0.77 ± 0.007g/cm^3, respectively for 50, 60 and 70% oil payload (Table 7) and the varying

proportion of WPC and oil may be the reason for the same. The emulsion droplet size that leads to formation of globule clusters during drying as the WPC. There will always a connection between true density and porosity. True density is the real solid density without ant spaces (Arepally et al., 2019). But the true density and porosity results obtained from the present study showed no significant difference ($p = 0.34$, $p \geq 0.05$) between true density and porosity. That establishes the lower void fraction present in the encapsulates. And thus it may lead to the chance of good storage stability.

4b.3.2.7 Solubility

Solubility is the last particle dissolution step and is a decisive factor for the quality of the powder to be used as ingredients for the food industry (Fernandes et al., 2014). Poorly soluble powders may cause processing difficulties especially economic losses. The results of this study showed solubility values of 83.72± 0.98 , 79.73 ± 1.6 , and 77.97 ± 1.5 % for 50, 60, 70 % ghee encapsulates (Table 7). The solubility of encapsulates is strongly influenced by the wall material composition (Fernandes et al., 2013). According to a study done by Chew et al., (2018), the protein based wall materials like betacyclodextrin, and sodium caseinate used to encapsulate the kenaf seed oil had improved its solubility to 85.6 to 90.2%. Whey protein concentrate, the wall material used in the present study, is reported to impart better solubility (Goyal et al., 2015) depending on whether it is in its native or denatured state (Pelegrine & Gasparetto, 2005). It is reported that outlet temperatures in the range of 60 – 90 °C could prevent, whey protein denaturation. The outlet temperature used in this study (65 ± 5 °C) falls within the range mentioned above, thus the solubility of oil encapsulate is justified.

4b.3.2.8 Color analysis

Color is an important parameter that indicates the product storage stability. The color of encapsulated powder was measured using HUNTERLAB colorimeter and reported as L*, a*, and b* values. The L* values of the encapsulates were 91.31 ± 0.20, 88.93 ± 0.04 and 85.22 ± 0.05 for 50, 60, 70 % encapsulates and b* values were 12.13 ± 0.11, 14.78 ± 0.09 and 18.8 ± 0.15, respectively. In this work, core material used was ghee. The L* and b* values of ghee was found to be, 60.14± 0.06 and 45.37± 0.15. And that of WPC was found to be 83.47± 0.02 and 20.56± 0.10. Thus, it could be inferred from the data that the wall material significantly affects the encapsulated powder's color. The colour of ghee was significantly reduced in the final encapsulates. The results of present work agree with the values reported in earlier studies: L* and b* values for microencapsulated flax seed oil powder with whey protein were 88.60 and 13.56 (Goyal et al., 2015) and fish oil encapsulate showed color values varying from L* = 78.01 to 82.76 and b* = 18.97 to 24.23, respectively, when encapsulated with GA (Binsi et al., 2017).

Table- 4b.7 Powder properties

Analysis	50%	60%	70%
[a]Solubility (%)	83.72± 0.98	79.73 ± 1.6	77.97 ± 1.5
[b]Porosity (g/cm^3)	0.64 ± 0.007	0.67 ± 0.03	0.77 ± 0.007
[c]Particle density (g/cm^2)	0.66 ± 0.02	0.82± 0.11	1.85± 0.13
[d]Particle size (nm)	1192 ± 126	2261 ± 255	3036 ± 142
PDI	0.387 ± 0.00	0.390 ± 0.00	0.388 ± 15.35
[e]Zeta potential (mV)	-34.1 ± 4.42	-27.8 ± 6.28	-28.4 ± 6.19

[f]**L***	91.31 ± 0.20	88.93 ± 0.04	85.22 ± 0.05
[g]**b***	12.13 ± 0.11	14.78 ± 0.09	18.8 ± 0.15
***Encapsulation efficiency (%)**	70 ± 1.35	64 ± 2.48	60 ± 1.22
[♣]**Surface area (**m^2/g**)**	0.12 ± 0.06	0.06 ± 0.00	0.02 ± 0.55
[h]**Yield (%)**	73 ± 0.70	65 ± 0.70	62 ± 2.82

All the analysis were done in triplicates and results were expressed as mean ± SD.
[a]p= 0.009 p < 0.05; [b]p= 0.34, p ≥ 0.05;
[c]p= 0.005 p < 0.05; [d]p= 0.01; p < 0.05;
[e]p= 0.08; p > 0.05 ; [f]p= 0.01; p < 0.05;
[g]p= 0.01; p < 0.05; *p= 0.017,p < 0.05;
[♣]p= 0.04; p < 0.05; [h]p= 0.02; p < 0.05

4b.3.2.9 BET surface area analysis

In order to determine the surface area, of the ghee powder, BET analysis was carried out (Table 7) and the results demonstrated that the surface area of the ghee powders were significantly different ($p<0.05$). The surface area values were 0.12 ± 0.06, 0.06 ± 0.00, and 0.02 ± 0.55 m^2/g for 50, 60 and 70% oil encapsulates respectively. The higher surface area is found in 50% samples, due to the lesser particle size compared to other samples.

4b.3.2.10 Morphology of encapsulates by Scanning Electron Microscopy (SEM)

The scanning electron microscopy, is a commonly used technique for imaging and characterization of microstructures. It was employed to observe the external (surface) morphology of encapsulates**.** Most of the microcapsules had spherical shape, but in the samples 60 and 70% encapsulate surface showed apparent fissures or crack. It may be due to the high amount of oil load in 60 and 70%. The presence of fissures can increase the permeability to gases, which can accelerate oxidative. However, better protection and core retention of the encapsulates were observed in all the images. Oil encapsulates with whey proteins showed comparatively fewer dents and imperfections due to faster film formation at

the drying stage as stated by Tonon et al. (2012). Imperfections or dents are generally formed due to uneven drying, when there is a slow process of film formation (Goyal et al., 2015). Our results are in line with the findings of earlier researchers who reported a similar kind of aggregation in microencapsulated oil powders with higher oil load (Kha et al., 2014; Sun et al., 2019)

4b.3.2.11 Microstructure of spray dried encapsulates by Fluorescence's microscopy

To confirm the surface morphology and the oil encapsulation, the fluorescence microscopy of the prepared encapsulates were carried out using nile blue for staining the wall and nile red for the core. Nile red is reported to stain lipids (core-ghee) and Nile blue to proteins (wall - WPC). The EE of most of 50% encapsulate is significantly high when compared to 60% and 70%. Figure -4 clearly demonstrate that ghee is completely enclosed in the microcapsule of protein (green colour) with inner oil phase (red). As can be seen, the particle size of the encapsulates increases with oil payload, confirming the discission sin the previous sections. The increase in particle size is visible in the image from 50-70% samples.

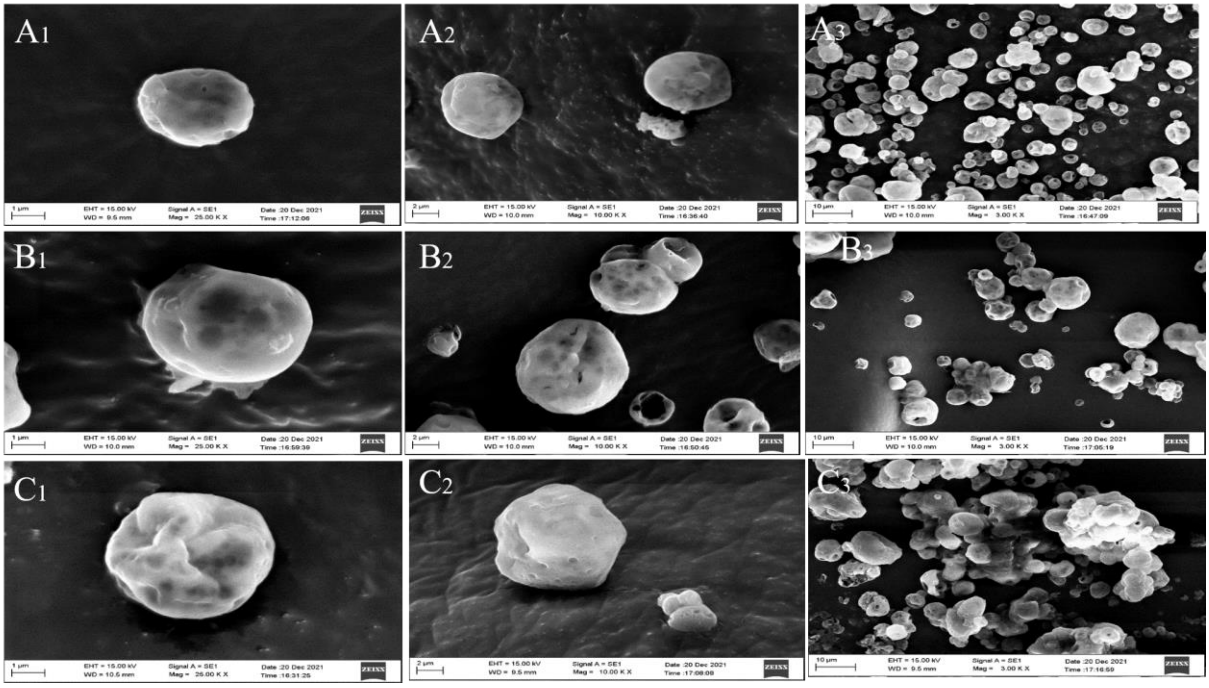


Figure-4b. 3 SEM images showing of 50 %, 60 %, and 70% encapsulates at 25000 X, 10000 X, and 3000 X magnification.

A_1, A_2, A_3 - 50 % encapsulate with 25000 X, 10000 X, and 3000 X magnification.

B_1, B_2, B_3 - 60 % encapsulate with 25000 X, 10000 X, and 3000 X magnification

C_1, C_2, C_3 - 70 % encapsulate with 25000 X, 10000 X, and 3000 X magnification

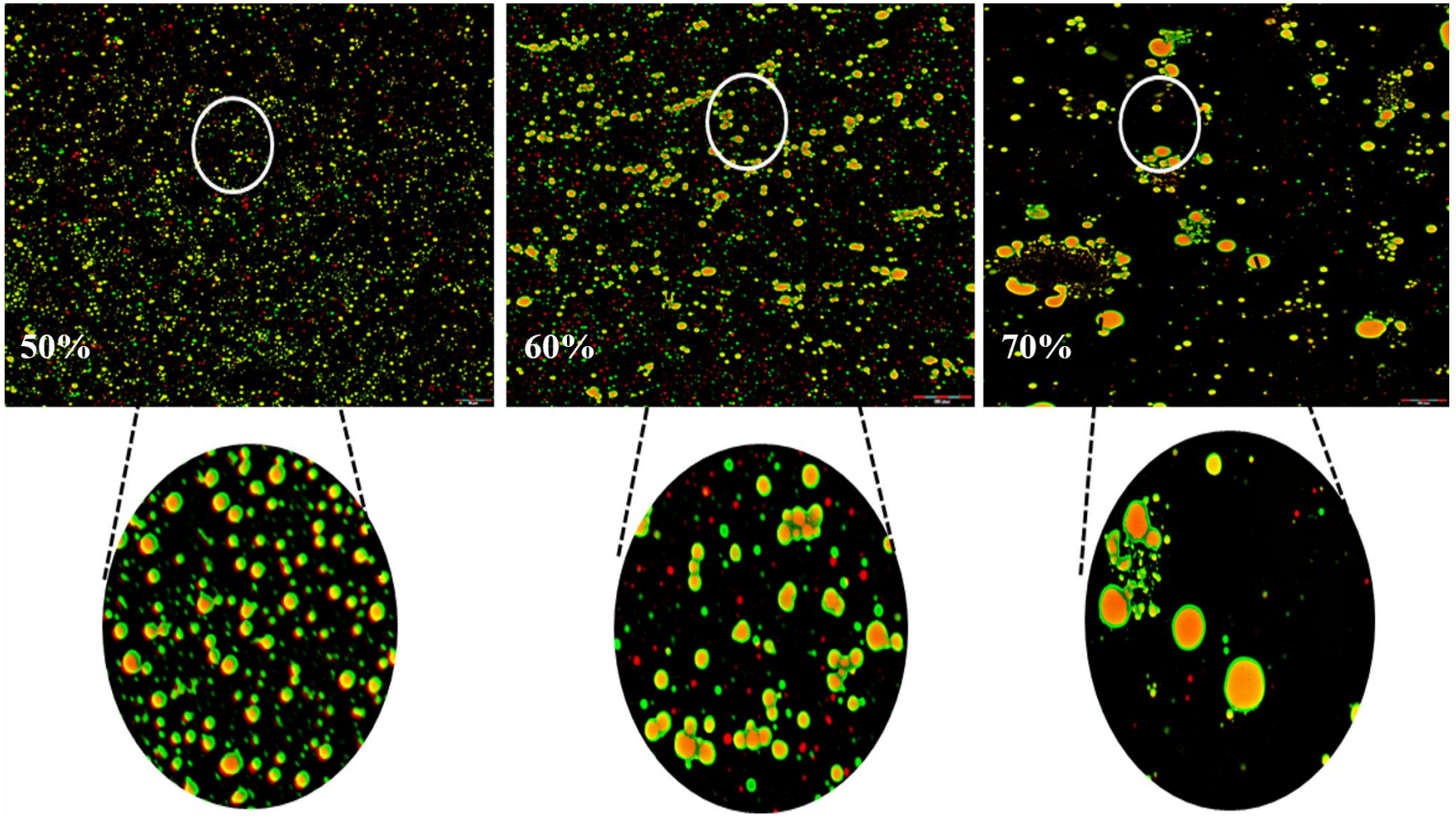


Figure- 4b.4 Merged Florescent microscopic images of 50 %, 60 %, and 70% encapsulates and their projected view in the circle. The red indicates oil and green the wall material, which is WPC.

4b.3.2.12 Accelerated Stability studies of encapsulates

The oxidative stability of ghee encapsulated into the WPC as wall material was evaluated by two different methods namely peroxide value, for primary oxidation products; para-anisidine value for secondary oxidation products and finally Totox value. During the 9 days of accelerated storage, at 65 ± 2°C. The samples were analysed in each 3rd day that is 0th, 3rd, 6th, and 9th days analysis. One day in Schaal oven is equivalent to one month at room temperature (Gaca et al., 2021) and therefore the 9 days of analysis in the present study corresponds to 9 months at ambient temperature.

4b.3.2.12.1 Peroxide value (PV)

Peroxide value (PV) indicates the extent of oxidation and formation of primary oxidation products. In the present study, peroxide value of different formulations were measured at an interval of 3rd day of storage for 9 days at the accelerated conditions. The PV of ghee encapsulates showed a gradual increase after each interval of analysis. The peroxide value of control ghee and ghee encapsulates were 0.663 ± 0.02, 0.669 ± 0.01, 0.67 ± 0.01, and 0.665 ± 0.02 meq peroxides/kg at zeroth day, which was increased gradually to 6.42 ± 0.53, 4.81±0.02, 5.85 ± 0.01, and 5.95 ±0.03 meq/kg at the end of 9 days of storage at accelerated conditions, which is represented in Figure- 5. It is evident from the figure that there was a significant difference ($p= 0.019$, $p<0.05$) amongst the PV of different ghee encapsulates with control ghee. The data suggested that the microencapsulation followed by spray drying had a protective effect on the ghee against oxidative damage and that the microencapsulation provides protection to the oil in the core of the microcapsules. This may also be correlated with the antioxidative and free-radical scavenging properties of whey proteins (Cervato, et al.,1999). In general, milk proteins showed free-radical scavenging properties due to various amino acids such as cysteine, tyrosine, tryptophan, phenylalanine and histidine (Singh, 2011)

and free sulphydryl groups. Present results are in good agreement with the findings of Tonon et al. (2011), who reported lowest PV (<1.5 meq peroxides/kg) in microencapsulated flaxseed oil prepared by using modified starch, WPC and gum arabic. Goyal et al., (2015) developed flaxseed oil emulsions and reported ~20.98% increase in PV after four weeks of low (4-7°C) temperature storage. Carneiro et al., (2013), found that WPC was more effective in the protection against lipid oxidation during the study done on oxidative stability of flax seed oil with different combination of wall materials. Therefore, in the present study, the antioxidant properties of WPC along with its protection offered to the ghee in the microencapsulates against oxidation could be correlated with the better stability of ghee in the encapsulates.

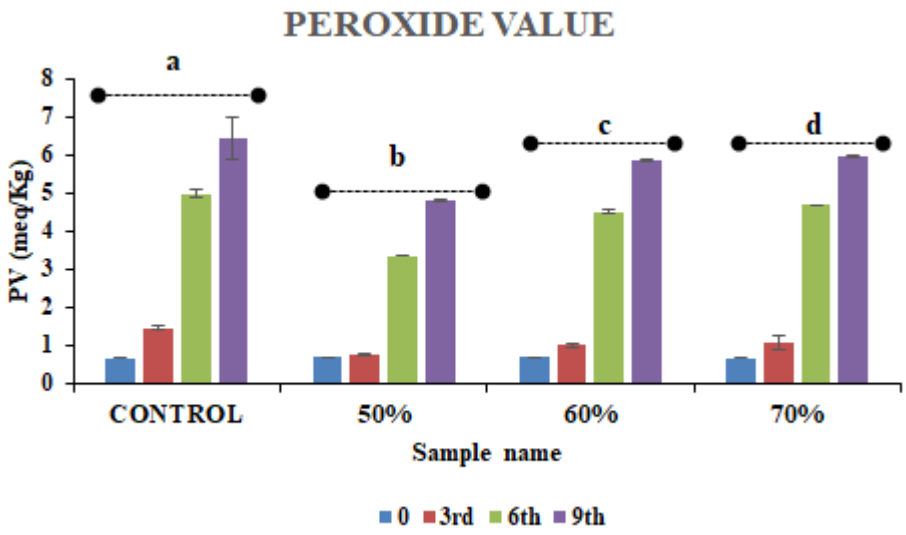


Figure- 4b.5 Peroxide value of 50 %, 60 %, and 70% encapsulates with significant values, where 'a-d' denotes the significant difference, with $p < 0.05$

4b.3.2.12.2 Para-anisidine value (p-AV)

The anisidine value measures high molecular weight saturated and unsaturated carbonyl compounds in triacylglycerol (Diana Moigradean et al., 2012) and the maximum permissible limit is 25 (CODEX STAN 210-1999; Ismail et al., 2016; Maszewska et al., 2017). The control ghee and encapsulated powders showed increase in p-AV on storage. The p-AV of control ghee was increased to 12.35 ± 0.56 from 1.02 ± 0.06 The ghee encapsulates also showed a gradual increase in PaV to 8.82±0.03, 11.25 ± 0.007, and 11.41 ± 0.12 for 50, 60, 70% encapsulates., which was less than the control. The control values were significantly different from the encapsulates ($p=0.0003$, $p<0.05$). According to a study done by Omar, Shan, Zou, Song, & Wang, (2009), used p-AV as a measure of stability of oxidation in spray dried ω3-PUFA with protein and gum as wall material showed increased p-AV value of 80, after two months of storage the encapsulates. Therefore gradual increase in p-AV values with time is already reported. But the present results showed that after the accelerated conditions of storage our values were still under the limit.

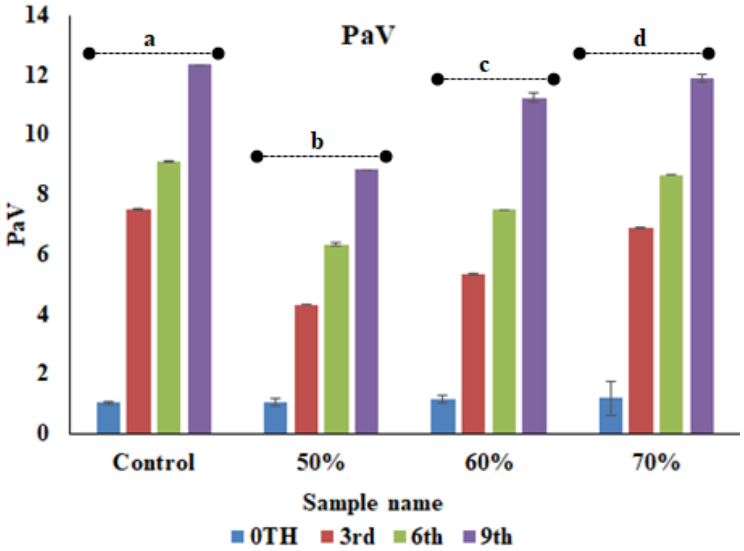


Figure-4b.6 Para-anisidine value of 50 %, 60 %, and 70% encapsulates with significant values, where 'a-d' denotes the significant difference, with $p < 0.05$

4b.3.2.12.3 Totox value

Totox value is the total oxidation rates and it can be seen from the Figure 4b.7, the rate of increase in totox values of fresh oil was apparently higher than the totox values of microcapsules. The totox value of 50, 60, and 70% encapsulates at 9th day of storage were 18.45±0.02, 22.95±0.07 and 23.32±0.07 respectively and that for control ghee was 25.19 ± 0.50. The values were significant different from control that p=0.0078, p≤0.05. This could be explained by the fact that the wall structure of microencapsulation surrounding the core material protected the material from oxidation. Hardas, Danviriyakul, Foley, Nawar, & Chinachoti (2000) and Partanen et al. (2002) reported in their studies that increase in totox value on storage will led to increase in permeability due to the change in glass transition temperature and decrease the stability of the structure. The reason of the decrease in oxidative stability of microcapsules during storage could be due to the increase in permeability of wall material. Encapsulation was found to be effective in retarding oxidation of wheat germ oil during storage at 45 °C (Meltem Karadeniz., 2015). Jimenez et al., (2004), observed WPC as an effective wall component in their study of microencapsulation and oxidative stability of conjugated linoleic acid.

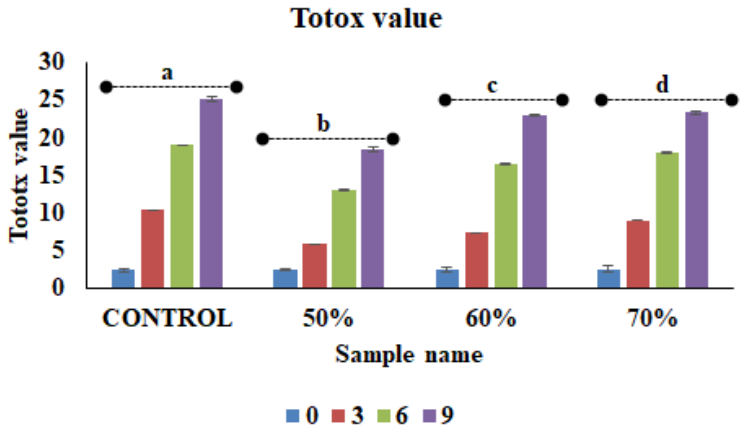


Figure- 4b.7 Totox value of 50 %, 60 %, and 70% encapsulates with significant values, where 'a-d' denotes the significant difference, with p < 0.05

4b.3.2.13 Heat induced core release studies

The heat induced core release done at 40, 80, 100, 120, 160, and 180°C for 30, 60, and 90 minutes. The study has been done to find out the release of core to the external environment at elevated temperature. At 180°C for the release of 50% powders was 45%, but that of 60% and 70% powders showed 65 % and 70% release respectively after heating at 90 min. The release profile of the powders followed a steady increase according to the oil payload. It is reported that oil payload and porosity of the powder decides the release pattern and it follows a zero order kinetics (Kha et al., 2014). In the present study, the porosity was higher for the higher oil payload capsules and that justify the trend.

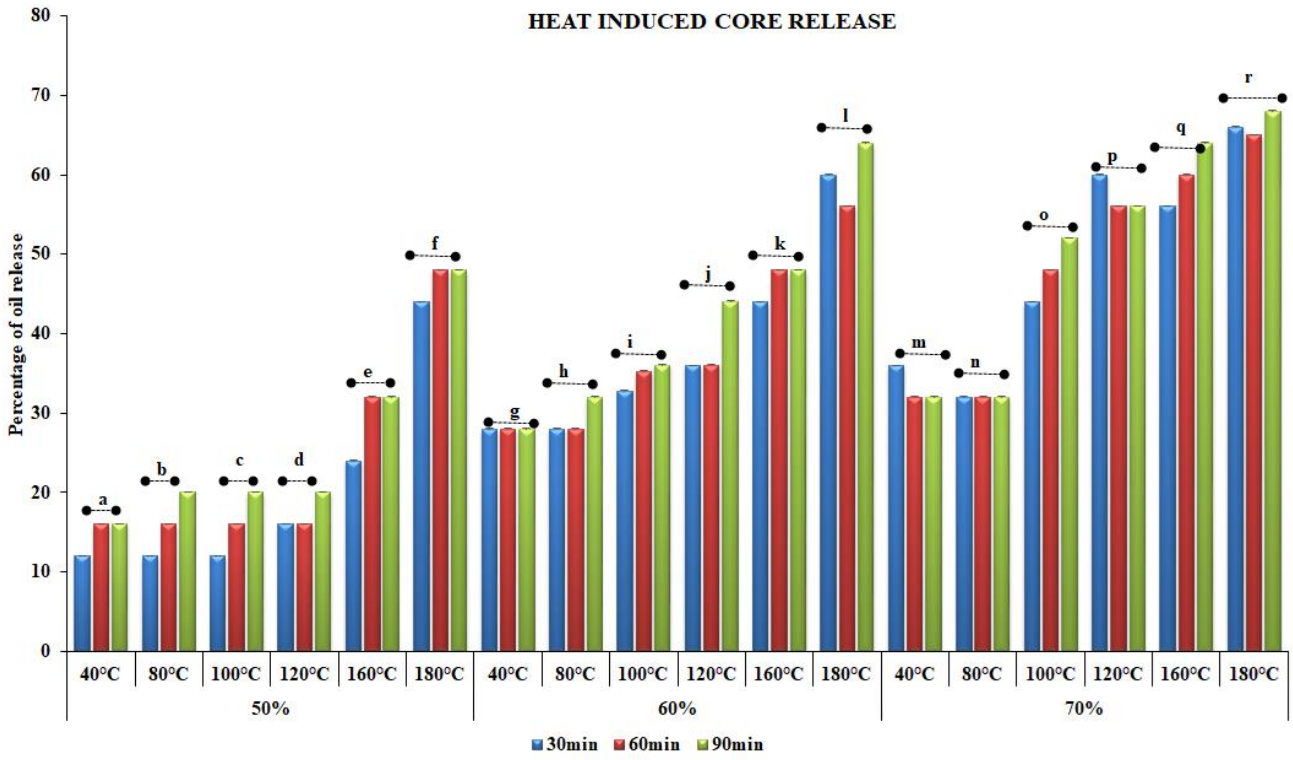


Figure-4b.8 Graphical representation of 50 %, 60 %, and 70% encapsulates at temperatures 40 °C, 80°C, 100°C, 120°C, 160°C, 180°C at 30 minutes,60 minutes and 90 minutes where 'a-r' denotes the significant difference, with $p < 0.05$, a,g,m; b,h,n; c,i,o; d,j,p; e,k,q; and f,l,r are significantly different to each other.

4b.3.2.13.1 *In-vitro* simulation studies under gastro intestinal conditions

Percent release of oil from the microcapsules of different ghee formulations exposed to different gastric conditions of mouth (6.8), gastric fluid (SGF: pH-1.2) and intestinal (SIF: pH- 6.8,) is shown in Figure 9. It was found that the oil released in the mouth phase was 10.01, 13.34 , and 20.32%, which is significantly different ($p<0.05$) from each other for the encapsulates with increase in oil payload which indicated that the release of ghee microcapsules in the mouth under the study conditions was found to depend on the oil load of powders that is in the order of 50 <60 < 70 %.

The percentage of release in the gastric conditions followed same trend as in the case of oral phase where the order was 50 (30.33 %) < 60 (44.23%) < 70% (50.91%) encapsulates. Whey proteins are highly resistant to peptic hydrolysis in their native state this could be attributed to the conformation of protection of the wall material giving under SGF conditions (Goyal et al., 2015). It was noticed that the percent of oil released is comparatively high in case of 70% powders. Our results are in agreement with the results of Pan et al. (2020), who reported smaller particle and higher oil load can be the reason for high release under the exposure of SGF especially for 70% (72.48 %). However, the encapsulates in the present study with the higher oil load made a sustained release of core in the intestinal conditions.

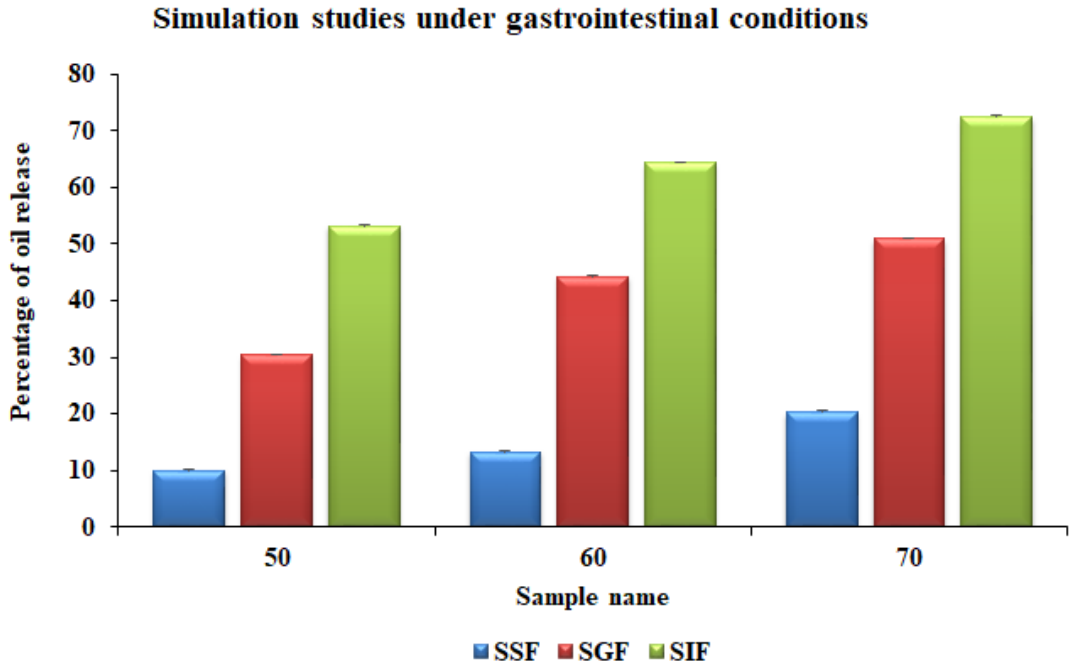


Figure- 4b.9 Simulation studies under gastro intestinal conditions of 50 %, 60 %, and 70% encapsulates with pH 1.2 and 6.8. Where 50, 60, 70 are significantly different with $p < 0.05$

4b.3.2.14 FTIR

Structure of system was analysed using Fourier transform infrared spectroscopy (FTIR) by measuring the vibrational properties of functional groups. To understand the nature of the interaction between wall and core in control ghee, WPC, and ghee encapsulates, FT-IR spectroscopic analysis of both the encapsulates were carried out (Figure 10). In the infrared (IR) fingerprint region of whey protein, a band around 1700 cm^{-1} to 1600 cm^{-1} was found, usually it corresponds to the primary amide region of whey proteins, and the one at 1600 cm^{-1} to 1500 cm^{-1} relates to the secondary amide region (Kher et al. 2007; Mohammed et al., 2018) and 1500 cm^{-1} to 1600 cm^{-1} corresponds to tertiary amide region.

In lipids, the most abundant bond participating in IR absorption is the C–H bond present in CH_2 groups in the fatty acyl chain and its terminal CH_3 group, as well as C–H and CH_2 present

in the glycerol moiety. The next most abundant bond is for C=O of ester linkages (Antony et al., 2017). Overall, the IR pattern for ghee was in close agreement with the patterns reported by Bency Antony et al. (2018). The band in 2922 cm^{-1} represents the CH_2- CH_3 fatty acid chains and 1744 cm^{-1} represents the C-O ester linkage. The presence of fingerprint regions of both ghee and WPC also confirms from the present spectrum. Thus, the results of FTIR analysis confirmed the absence of chemical interactions between the emulsion constituents such as ghee and WPC. And it is clear that WPC as a wall material conserved ghee, after encapsulation within the system. The absorption bands corresponding to the main functional groups typical of ghee, and whey protein concentrate were retained in all the three encapsulates. In the present study, it was evident from the FTIR profile that even though the emulsions were kinetically stable at ambient temperature, the elevated temperature and mechanical shearing during spray drying affected the behaviour of wall material is observed with the help of FT-IR.

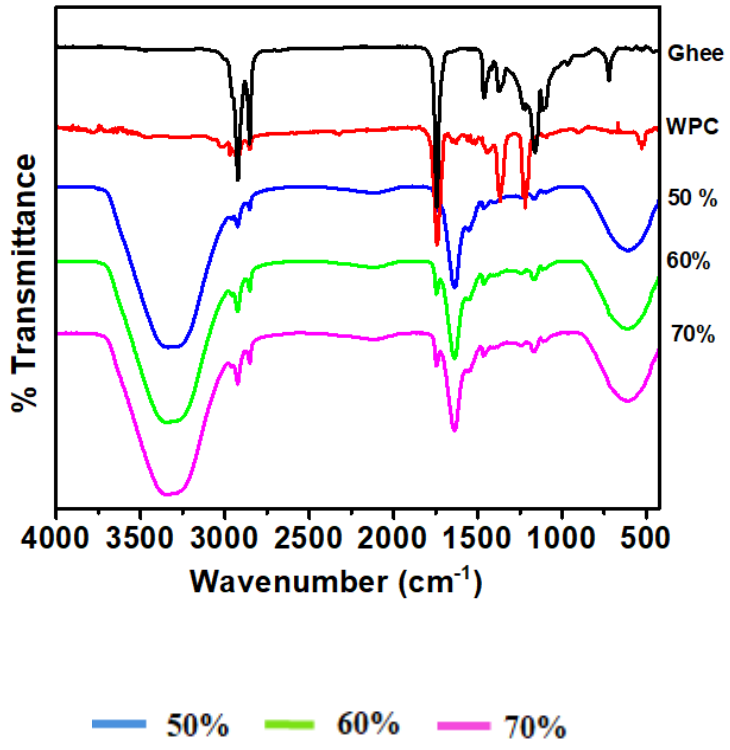


Figure- 4b.10 FTIR stacked spectrum of 50 %, 60 %, and 70% encapsulates.

4b.3.2.15 Thermal Behaviour of emulsions (DT-TGA)

Thermal behaviour is important with respect to storage stability of encapsulates (Laura, Gomez-Mascaraque et al., 2017). Thermal analysis of the encapsulates was done using DT-TGA to find out the efficiency of wall material to protect the core component. The results are represented in Figure 11a and 11b. The denaturation temperature (Td) of whey protein is reported to be 60–66 °C (Anandharamakrishnan, 2008). The melting temperature of ghee was found to be 40-45 °C and in the present thermogram there was no endothermic peak observed at this temperature range. Importantly, the first stage of mass loss of the TGA curves (between 50 and 110 °C) refers to the loss of moisture from the material, while the second stage (above 110 °C) corresponds to the decomposition processes of the particle constituents (Fritzen-Freireet al., 2012), such as proteins and carbohydrates (Macêdo, de Moura,Souza, & Macêdo, 1997; Carmo et al., 2018). It is also attributed to the decompositions and depolymerisation of wall materials constituents at 110 °C- 112 °C, such as the cleavage of S–S, O–N, and O–O linkages and consequently the breakdown of covalent peptide bonds in WPI (Azizi et al. 2018, Tavares et al., 2019).

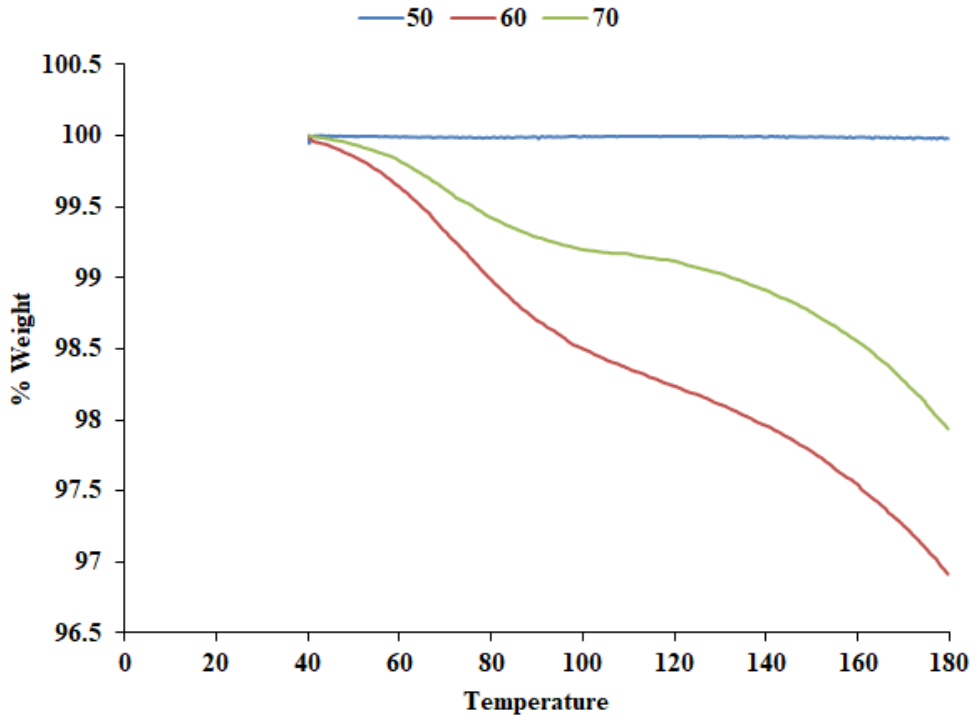


Figure- 4b.11a TGA thermogram of 50 %, 60 %, and 70% encapsulates

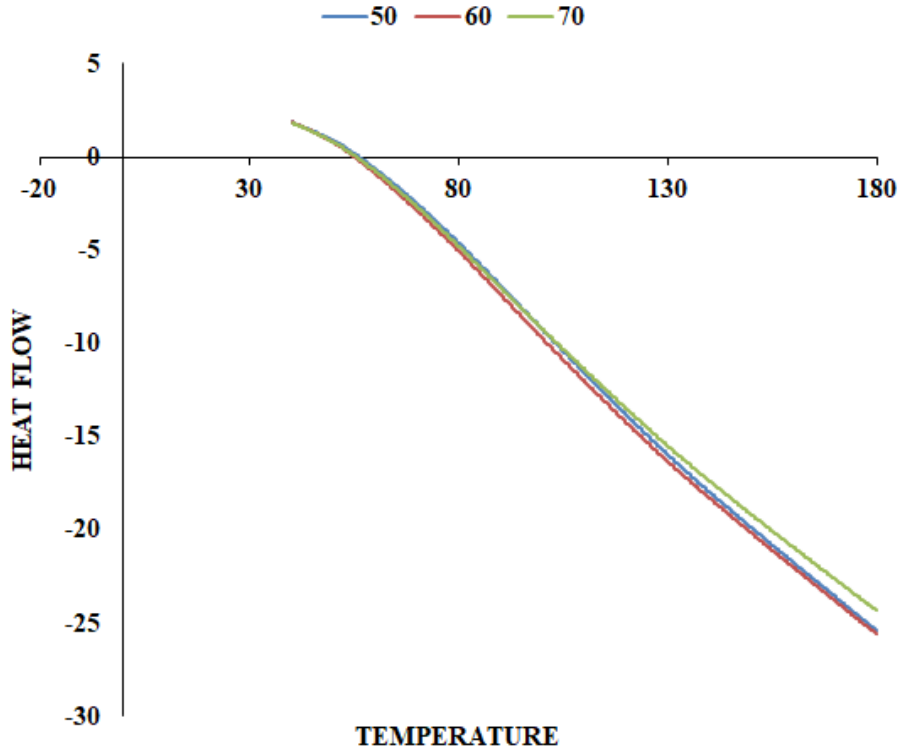


Figure- 4b.11b DTA of 50 %, 60 %, and 70% encapsulates

4b.3.3 Spray-dried ghee microcapsules for application in food products

The ghee encapsulates were used for preparation of ghee cake so as to evaluate its suitability in bakery products as ghee substitute and was compared with that of cake prepared with pure ghee. The control cupcakes were prepared with ghee as the source of fat, and the test cupcakes were formulated by replacing ghee with the oil encapsulate shown in Figure 13.

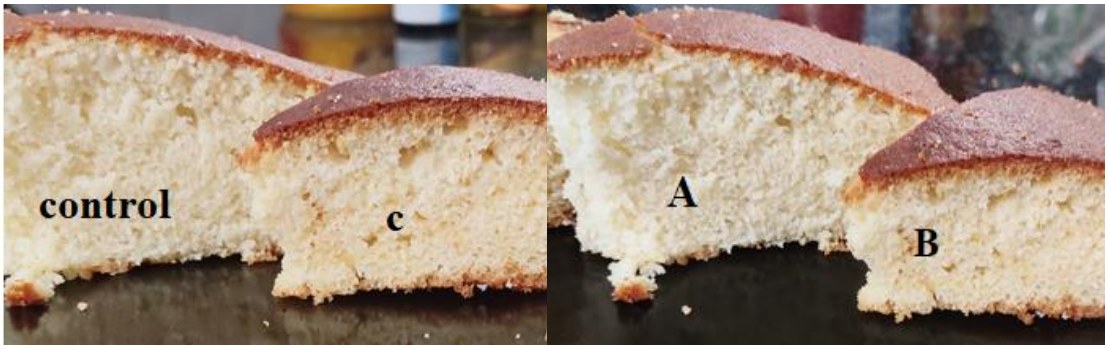


Figure- 4b.12 Photographs of cakes formulated with encapsulated oil as a replacement of A- with 70% of encapsulate, B- with 60% of encapsulate and C- with 50% of encapsulate.

The sensory evaluation results showed (Figure- 14) that there was no significant difference in the overall acceptability values between the control and test cupcakes. Encapsulation by spray drying using whey protein concentrate as wall material enhanced palatability of test cake sensory characteristics. The texture profile analysis results showed that the test cake samples exhibited similar textural quality as that of control. The springiness, cohesiveness, and resilience showed no significant difference among control and test cakes. Springiness is a measure of the elasticity, which is observed as the degree to which the sample recovers between the first and second compression during the texture profile analysis (Santhanam, Lekshmi, Chouksey, Tripathi & Gudipati 2014). A similar effect on springiness has been reported by Pasukamonset et al. (2018) with the addition of polyphenols. Chewiness and springiness are directly related, as the former is obtained by multiplying gumminess and springiness. And chewiness is the amount of energy required to disintegrate food for swallowing (Ghaboos, Aradabili & Kashaninejad 2018). Chewiness and gumminess showed a significant difference ($p \leq 0.05$). Chewiness for control cake was 381 N and that for 50%

encapsulated test was 1082 N, 60% was 893 N, and finally 70% was 751N. Gumminess for control cake was 423 N, and that for 70 60, and 50% oil payload was 920 N , 1009 N and 1189 N respectively. This may be due to the sustained release of encapsulate and the 70% showed better release and contain higher oil pay load. The presence of WPC which is a good emulsifier and the core (ghee) together helped in attaining better textural qualities.

Further, the results of texture profile analysis exhibited a good correlation with the inferences derived from sensory analysis. A study done by Santhanam et al. (2014) also revealed that fish oil encapsulates could be successfully used as a fortificant in cakes without much alteration in the textural and sensory qualities. The present study yet again confirmed that oil encapsulates can be easily incorporated in the formulation of baked foods without much alteration in the textural and sensory qualities.

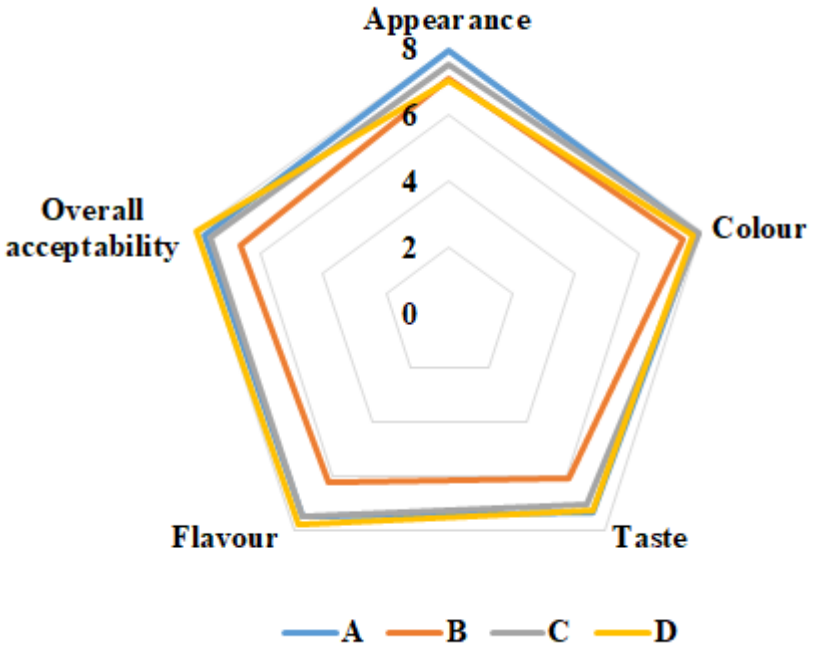


Figure- 4b.13 Sensory evaluation results of control ghee cake and test cake loaded with encapsulates

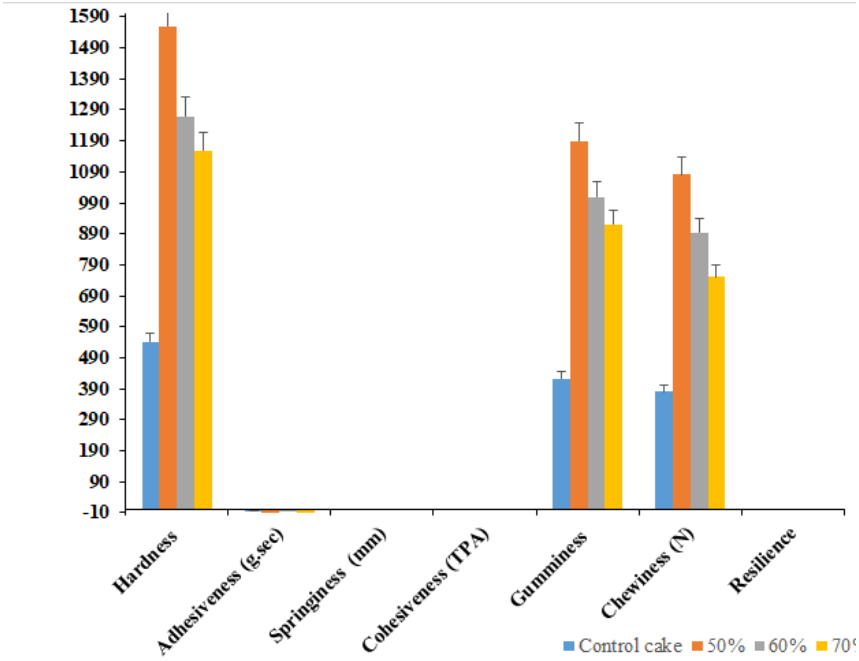


Figure- 4b.14 Texture profile results of control and test cakes

4b.4. Summary and conclusion

This chapter deals with development shelf stable and convenient encapsulated lipids (/ghee), for culinary and industrial applications. The stable emulsions fabricated using 50, 60 and 70 % of ghee (db), from the previous chapter was converted in to powder using spray drying conditions. The TSS % of 30 was kept constant. The process conditions were optimized as, inlet 120 °C, feed flow as 0.8 mL/min, nozzle size 0.7mm in terms of better encapsulation efficiency and yield. The fat encapsulates were characterized in terms of powder properties (flow-ability, moisture content, water activity, solubility etc.), and morphology (SEM, fluorescent microscopy). Encapsulation efficacy of encapsulates up to 58% and above confirmed better incorporation of lipids in the system. The moisture content of encapsulates were > 4.9% for all the encapsulates, with water activity was < 0.5. Particle size ranged from 1.92 to 2.26 μm with maximum zeta potential ranged from -34.1 ± 4.42 mV and exhibited a poly dispersity index (PDI) of 0.38, which indicated a stable encapsulates with excellent

powder properties. The core release studies showed sustained release, suggesting its application for delivery of temperature sensitive compounds. The in-vitro release of oil under stimulated gastric conditions ensured very low release kinetics in mouth and sustained release in gastro-intestinal fluids at different pH values. The morphological characterisation of emulsion using SEM and fluorescent microscopy showed better encapsulation properties. The applications studies of encapsulates in ghee cake suggested the huge potential of microencapsulated oils/lipids for industrial application where by the increased consumer demand for safe stable and convenient products can be addressed. The key finding of the present work is that we could increase the oil payload up to 70%, with an EE of 58.23%, reported so far for edible lipids, for the food application as a replacer of conventional solid fat. Further studies need to be explored to encapsulate other lipids for the aforesaid application. This will aim for the development of convenient and stable powder lipids that will revolutionize the edible lipid market.

The key findings were summarised below

- The process conditions optimized -in terms of better encapsulation efficiency and yield as

  Inlet temperature—120 °C.
  Nozzle size—0.7 mm
  Feed flow—0.8 ml/min
  Aspiration –110 m^3/h
  Atomisation pressure – 1.1 Kg/cm^2

- Better incorporation of lipids in the system with 70%, 64 % and 54 % for 50, 60 and 70% samples.

- Core release studies showed sustained release, by reduced release rate of 54% even in higher oil loaded sample (70%) suggesting its application for delivery of temperature sensitive compounds.
- The in-vitro release under stimulated gastric conditions ensured very low release kinetics in mouth and sustained release in gastro-intestinal fluids at different pH values.
- Morphological characterisation - SEM - fluorescent microscopy showed better encapsulation properties.
- Further investigation of encapsulates done for application study and found 50% encapsulate is the best for application in bakery products.

Chapter 5

SUMMARY & CONCLUSION

SUMMARY & CONCLUSION

Shelf stable edible lipids with tailored fatty acids enriched with nutritional and bioactive ingredients for various household and industrial applications are gaining more interest. The market indicates a steady increase in the demand for healthy oils with added health benefits e.g., optimum fatty acid profile and bioactive compounds, though the amount of fat that is consumed daily is a topic of controversy. Deficiency due to lack of balanced fatty acids in the diet, especially polyunsaturated fatty acids (PUFA), is one of the significant nutritional problems globally. According to the Food and Nutrition Board's Committee on diet and health, the recommended ratio of SFA (saturated fatty acid)/MUFA (monounsaturated fatty acid)/PUFA (PUFA) in edible oils is 1:2:1 and that of omega-3/ omega-6 is 0.05. The blending of edible oils is considered the least expensive approach that results in desired fatty acid composition. However, PUFA is susceptible to oxidative degradation due to its high degree of unsaturation. Various encapsulation techniques have been proposed for improving oxidative stability of PUFA, by converting oils in to powder, by encapsulating the oil using a carrier matrix. The oxidative stability of vegetable oils is one of the key factors in determining its use in foods and their applicability in industrial situations. Using the microencapsulation approach, edible oils can be converted in to powder using appropriate techniques to improve handling, storage stability and application, besides facilitating easy transport. Therefore, converting oil to powder can be an innovative and consumer-friendly approach to improve the nutritional and oxidative stability of oils and to widen their applications in the culinary/health/functional food and nutraceutical sectors. Various encapsulation techniques have been employed for converting oils into powder form by utilizing a

wide range of carrier or wall materials. The commonly used microencapsulation techniques include emulsification, spray drying, and freeze drying. Among the above, spray drying is considered as an industry-friendly drying-cum- encapsulation technique. Choosing an ideal wall material is the key to achieve optimal encapsulation efficiency. The general traits expected of a wall material include, but are not limited to, bland flavor, high solubility, emulsification ability, film-forming, and drying properties. Diverse wall materials have been used for encapsulating oils, including gums (gum arabic, xanthan), proteins (whey protein, soy protein), polysaccharides, and modified starches. **'Oil to powder technologies for food' is one of the areas identified under the 'Technology vision document 2035' prepared by Technology Information, Forecasting and Assessment Council (Technology vision 2035, TIFAC, 2015). The document emphasises the need for targeted research in this area for technology development**. With this back ground, we focussed on: (1) developing oil blends with optimum fatty acid profile enriched with bio actives, and (2) development of powdered oils/fat (vegetable oils, butter, ghee, and red palm olein, etc.) by microencapsulation using different combinations of wall materials. The resulting encapsulated products can be used for regular culinary purpose, as well as, as an ingredient in various food products, for providing nutritional benefits and functionality.

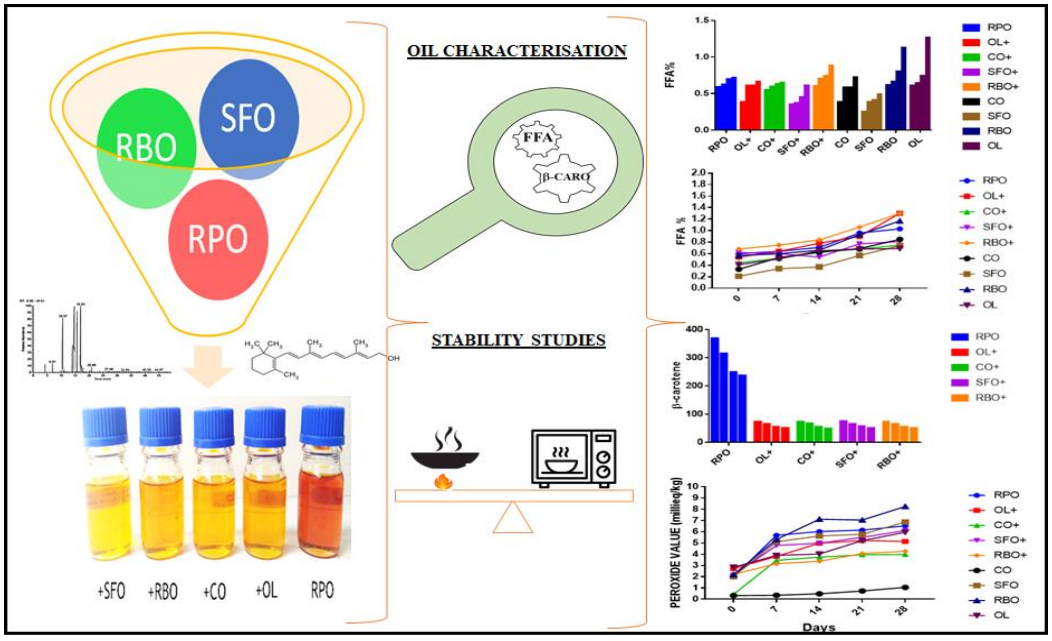
The present investigation entitled " **Encapsulation of Edible Lipids for Functional Food Applications: Process Development and Characterization**" encompassed three broad endeavours. Firstly development of vegetable oil blends with optimum fatty acid profile for nutritional/ functional food products. Secondly encapsulation of vegetable oil blends with balanced fatty acid composition as a carrier of oil soluble

vitamins for nutritional/ functional food products. And finally process development for powdered lipids (butter/ghee) for food applications.

Development and Stability of Vegetable Oil Blends with Red Palmolein for Nutritional Applications.

Oils and fats play a vital role in daily life. Red palm oil is a rich source of β-carotene. Base oils (coconut oil (CNO), sunflower oil (SFO), rice bran oil (RBO), olive oil (OL), and flax seed oil (FSO) were collected from authorized source /brands. Crude red palm oil was collected and refined to red palm oil (RPO) using the NIIST technology in the pilot plant. Blending of oils were done with red palm oil and base oils, as per government regulations. The blending was optimised to 20:80 ratio resulted in improved fatty acid profile with a noticeable amount of beta carotene content. The thermal, oxidative and storage stability of these blended oils in comparison with the base oils has been characterized on the basis of β-carotene content, FFA, PV, and PaV. Blending with RPO improved the carotene content of the base oils which was absent in base oils. It was found that the blended oils were retaining carotene up to 70 % during accelerated and frying conditions. The oxidative and thermal stability analysis showed that the blending with RPO helped the base oils to withstand oxidative and thermal conditions by improving the saturated fatty acid content. As the blended oils exhibited greater stability to oxidation and temperature, blending can be opted as a method for reducing the oxidation levels. Blending of oils rich in unsaturation with other oils rich in phytochemicals and saturated fatty acids in an optimum level offers enhanced nutritional quality as well as shelf and thermal stability. Blending of less exploited oils with nutritional and phytochemical enriched oils with commonly consumed oils

needs to be exploited more to enhance the nutritional and technological properties further.

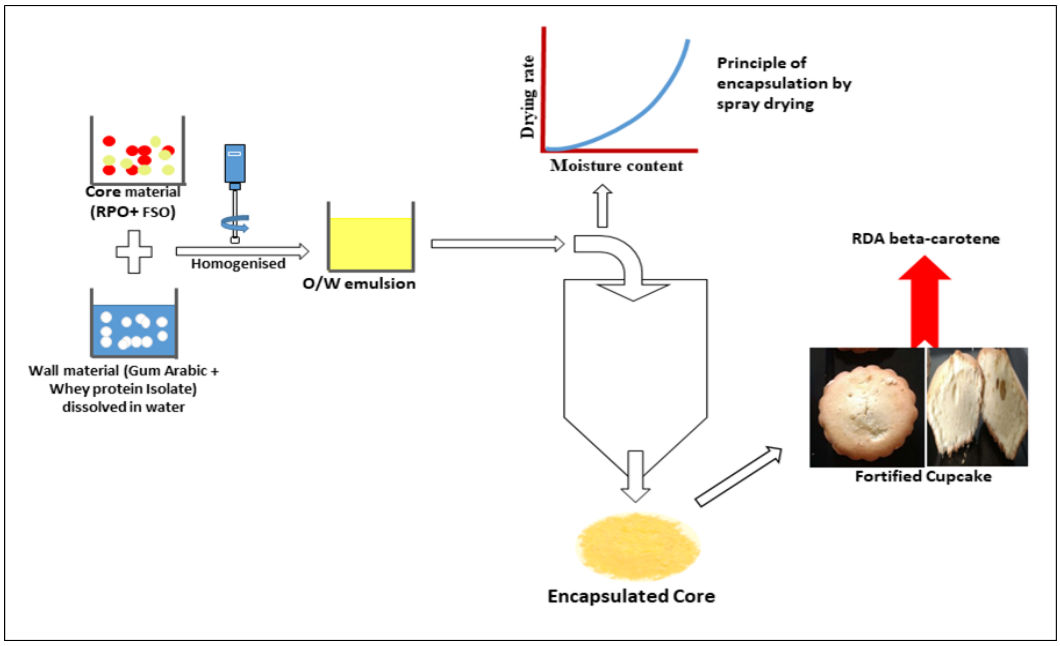


Graphical abstract of Stability of Vegetable Oil Blends with Red Palmolein.

Process Development of Red Palm Oil/ Flax Seed Oil Blends with Balanced Fatty Acid Composition and Application in Food Products for Nutrient Delivery

The RPO and FSO were blended in 70:30 and 60:40 ratios. GC-MS analyses of the blended oil was performed to confirm the fatty acid composition. Whey protein (WP) and gum arabic (GA) were the wall materials. The mixture was homogenized using a rotor-stator homogenizer (IKA). Stable emulsion was prepared and spray dried. Inlet temperature of spray drying was optimized to maximize the encapsulation efficiency. The blended oils were selected in such a way that it improved fatty acid composition, and enriched beta carotene content. The peroxide value (PV) and free fatty acids (FFA) values of the component oils, during the shelf

life studies, were less than 10.0 millieq/kg, and 1.0 % (government regulations) respectively, which indicated that quality of the oil was within the prescribed limits. The carotene content of the blend was found to be 270 ppm. The GC MS/MS profiling of the blend showed the presence of improved fatty acid composition. The core–to-wall ratio of 1:2, oil payload of 34 % and inlet temperature of 180 °C has been optimized. The developed encapsulate was employed as a partial fat substitute and carotenoid fortificant in bakery products which indicated that the developed oil powder could replace 40 % of butter without influencing sensorial properties, and also met 37 % of the recommended dietary allowance (RDA) for β- carotene.



Graphical abstract of encapsulation of red palm oil and flax seed oil blends for the development of nutritional products.

Fabrication of Fat Encapsulates (Ghee) for Food and Health Care Applications

This chapter deals with the optimization of process conditions for fabricating fat encapsulates (butter/ghee) for food and health care applications. In order to develop

a fat substitute in the form of a powder, it is important to fabricate a stable emulsion without affecting the inherent properties of the fat. Hence, this chapter is divided in to two subchapters **a** and **b**, where in **a** focussed on developing a stable emulsion with higher oil payload and **b** dealt with conversion of the stable emulsion to encapsulated powder.

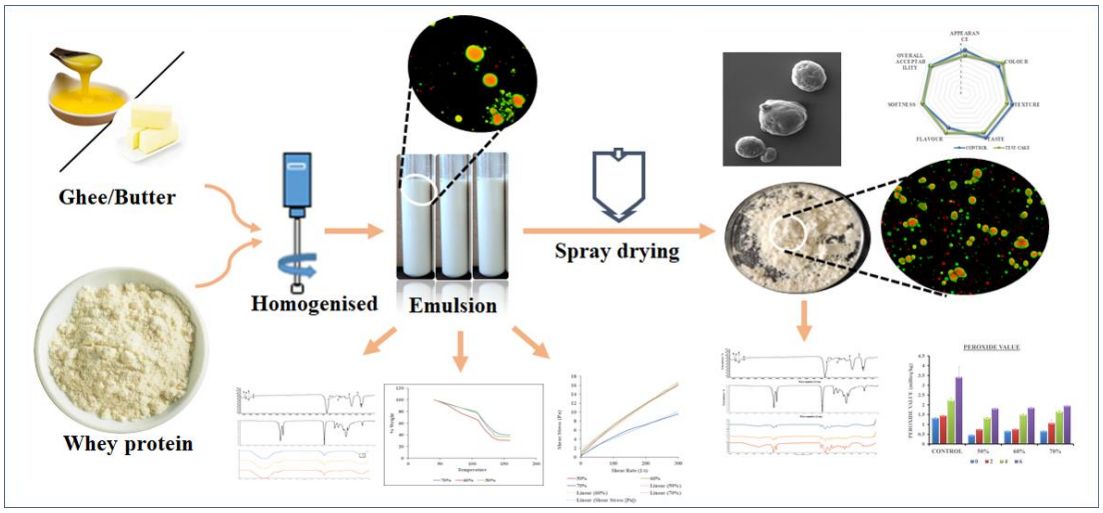
(a). Optimization of Conditions for Developing Stable Ghee Emulsions with Higher Oil Pay Load

The optimization was carried out in terms of oil payload, wall material composition and homogenization conditions. The oil load was increased from 50 % to 70 % (dry basis) at a total soluble solids (TSS) of 30 %. The optimized emulsions were kinetically stable even after 24 h of storage at ambient conditions (29 to 35 °C). Bulk density of the emulsions were 1.007 g/mL, 1.04 g/mL, and 0.97 g/mL for 50 %, 60 % and 70 % oil payloads, respectively; whereas the corresponding viscosities were 50.9 cP, 49.8 cP, and 30.1 cP. The mean droplet diameters were 385.1 ± 131 nm, 404 ± 87.39 nm, and 410.6 ± 269.9 nm and, corresponding zeta potential values were -28.1 ± 4.42 mV,-27.2 ± 6.28 mV, and -26.0 ± 6.19 mV for 50%, 60% and 70% emulsions, which indicated high stability. Thermal analysis (DT-TGA) and FTIR of the emulsions indicated that core component (oil) was well protected when whey protein was used as wall material. This thermally and kinetically stable emulsions were further taken for encapsulation studies.

(b). Fabrication of Encapsulated Butter/Ghee (Lipids) Powder and Application in Food Formulation

The stable emulsions fabricated using 50 %, 60 % and 70 % of ghee, from the previous chapter was converted in to powder using spray drying. The process

conditions were optimized for spray drying in terms of better encapsulation efficiency and yield. The fat encapsulates were characterized in terms of powder properties (flow-ability, moisture content, water activity, solubility etc.), and morphology (SEM, fluorescent microscopy). *In-vitro* release and core release studies were conducted under simulated environments. Besides this, stability (oxidative and thermal) and food application studies were also carried out. Encapsulation efficacy confirmed better incorporation of lipids in the system. The moisture content of encapsulates were < 5 %.Water activity was < 0.5 and, the density values were ≤ 0.4 g/mL, which showed less occluded air and hence, a chance of higher oxidative stability. Particle size ranged from 1000-3000 nm, zeta potential ranged from -34.1 ± 4.42 mV and exhibited a poly dispersity index (PDI) of 0.38. The core release studies showed sustained release, suggesting its application for delivery of temperature sensitive compounds. The *in-vitro* release of oil under stimulated gastric conditions ensured very low release kinetics in mouth and sustained release in gastro-intestinal fluids at different pH values. The morphological characterisation of emulsion using SEM and fluorescent microscopy showed better encapsulation properties. The encapsulates were then further investigated for various food applications.



Graphical abstract of development of encapsulated fat for product development and application level studies.

Conclusions

- Blending of red palm oil with other edible oils such as coconut oil, rice bran oil, sunflower oil, and olive oil could be used as a medium to deliver β-carotene (20 % RDA) and thus can be used as Vitamin-A supplement in culinary applications.
- Blending improves the oxidative rancidity and thermal stability of oil for high temperature applications such as frying, baking and processing.
- To address balance in fatty acid composition, flaxseed oil is blended with red palm oil and encapsulated via spray drying.
- The conditions for spray drying of red palm oil-flax seed oil blends were optimised for the delivery of nutritionally improved fatty acid profile. With a core to wall ratio 1:2 and inlet temperature of 180 °C, an oil payload of 34 % was achieved.

- Replacement of 40 % of butter with encapsulated red palm oil-flax seed oil blend in the cupcakes showed 36.87 % of the recommended dietary allowance (RDA) for β- carotene.
- Encapsulation mask the typical off-flavor of flaxseed oil hence improve its acceptability.
- Further investigations were carried out to optimise the fat (ghee) encapsulated emulsions to obtain the maximum oil payload.
- Emulsion of ghee was fabricated with an oil payload of 50 % to 70 % on dry weight basis which is experimented for the first time. The conditions were optimised at- total soluble solids (TSS) 30 % and homogenisation speed 15000 rpm for 45 min.
- This thermally and kinetically stable emulsions were taken forward for encapsulation studies. Stable emulsions fabricated using 50 % to 70 % of ghee were converted into powder by means of spray drying.
- The fat encapsulates were characterized in terms of powder properties, morphology, *in-vitro* core release studies and encapsulation efficacy. The different powdered encapsulates were then utilized as a solid fat replacer in sponge cake.
- Among the encapsulates, the one with 50% oil payload was discovered the finest in yield, encapsulation efficiency, powder properties, morphological, and shelf life characteristics.
- Edible lipids in the form of encapsulates as demonstrated in the study using ghee, offers a very promising platform in the convenient food sector.

Future aspects

- Scale up of the fabricated encapsulates into industrial level- This powdered oil could be used as conventional fat replacer.
- Health care application- Blending of edible oils rich in nutritionally important compounds and phytochemicals could be used to modify fatty acid profile, which can be fine-tuned to deliver the optimum fatty acid composition required for the maintenance of health.
- Application studies with different bioactive formulations- Blends in the form of encapsulated powder could be used for delivering lipids enriched with thermally unstable- health giving bioactives.

Bibliography

REFERENCES

Abd-ElGhany, M. E., Ammar, M. S., & Hegazy, A. E. (2010). Use of olive waste cake extract as a natural antioxidant for improving the stability of heated sunflower oil. *World Appl Sci J*, *11*(1), 106-113.

Abdel-Razek, A. G., El-Shami, S. M., El-Mallah, M. H., & Hassanien, M. M. M. (2011). Blending of virgin olive oil with less stable edible oils to strengthen their antioxidative potencies. *Australian Journal of Basic and Applied Sciences*, *5*(10), 312-318.

Abdulkarim, S. M., Myat, M. W., Ghazali, H. M., Roselina, K., & Abbas, K. A. (2010). Sensory and physicochemical qualities of palm olein and sesame seed oil blends during frying of banana chips. Journal of Agricultural Science, 2(4), 18.

Adelmann, H., Binks, B. P., & Mezzenga, R. (2012). Oil powders and gels from particle-stabilized emulsions. *Langmuir*, *28*(3), 1694-1697.

Adeyemi, K. D., Sazili, A. Q., Ebrahimi, M., Samsudin, A. A., Alimon, A. R., Karim, R., ... & Sabow, A. B. (2016). Effects of blend of canola oil and palm oil on nutrient intake and digestibility, growth performance, rumen fermentation and fatty acids in goats. *Animal Science Journal*, *87*(9), 1137-1147.

Aghbashlo, M., Mobli, H., Madadlou, A., & Rafiee, S. (2012). The correlation of wall material composition with flow characteristics and encapsulation behavior of fish oil emulsion. *Food Research International*, *49*(1), 379-388.

Aghbashlo, M., Mobli, H., Madadlou, A., & Rafiee, S. (2013). Influence of wall material and inlet drying air temperature on the microencapsulation of fish oil by spray drying. *Food and Bioprocess Technology*, *6*(6), 1561–1569.

Agustinisari, I., Mulia, K., & Nasikin, M. (2020). The effect of eugenol and chitosan concentration on the encapsulation of eugenol using whey protein-maltodextrin conjugates. *Applied Sciences (Switzerland)*, *10*(9).

Agustinisari, I., Mulia, K., & Nasikin, M. (2020, September). Physical and chemical properties of encapsulated eugenol using whey protein-maltodextrin mixed solution and chitosan as emulsifier and wall materials. In *AIP Conference Proceedings* (Vol. 2255, No. 1, p. 060031). AIP Publishing LLC.

Ahamad, T., Naushad, M., Al-Shahrani, T., Al-Hokbany, N., & Alshehri, S. M. (2020). Preparation of chitosan based magnetic nanocomposite for tetracycline adsorption: Kinetic and thermodynamic studies. *International journal of biological macromolecules*, *147*, 258-267.

Al-Bandak, G., Dermesonlouglou, E. K., Taoukis, P. S., & Oreopoulou, V. (2011). Antioxidant effect of Majorana syriaca extract in bulk corn oil and o/w emulsion after applying high hydrostatic pressure. *Food Chemistry*, *125*(4), 1166-1170.

Albert, K., Vatai, G., & Koris, A. (2017). Microencapsulation of Vegetable Oil-Alternative Approaches Using Membrane Technology and Spray Drying. *Hungarian Journal of Industry and Chemistry*, 29-33.

Ali, M. A., Islam, M. A., Othman, N. H., Noor, A. M., & Ibrahim, M. (2019). Effect of rice bran oil addition on the oxidative degradation and fatty acid composition of soybean oil during heating. *Acta Scientiarum Polonorum Technologia Alimentaria*, *18*(4), 427-438.

Ali, S., Chatha, S. A. S., Ali, Q., Hussain, A. I., Hussain, S. M., & Perveen, R. (2016). Oxidative stability of cooking oil blend stabilized with leaf extract of Eucalyptus citriodora. *International Journal of Food Properties*, *19*(7), 1556-1565.

Al-Khusaibi, M., Gordon, M. H., Lovegrove, J. A., & Niranjan, K. (2012). Frying of potato chips in a blend of canola oil and palm olein: changes in levels of individual fatty acids and tocols. *International journal of food science & technology*, *47*(8), 1701-1709.

Alliod, O., Messager, L., Fessi, H., Dupin, D., & Charcosset, C. (2019). Influence of viscosity for oil-in-water and water-in-oil nanoemulsions production by SPG premix membrane emulsification. *Chemical Engineering Research and Design*, *142*, 87-99.

Al-Malah, K. I., Azzam, M. O. J., & Omari, R. M. (2000). Emulsifying properties of BSA in different vegetable oil emulsions using conductivity technique. *Food Hydrocolloids*, *14*(5), 485-490.

Almeida, A. P., Rodríguez-Rojo, S., Serra, A. T., Vila-Real, H., Simplicio, A. L., Delgadilho, I., ... & Duarte, C. M. (2013). Microencapsulation of oregano essential oil in starch-based materials using supercritical fluid technology. *Innovative food science & emerging technologies*, *20*, 140-145.

Alpizar-Reyes, E., Varela-Guerrero, V., Cruz-Olivares, J., Carrillo-Navas, H., Alvarez-Ramirez, J., & Pérez-Alonso, C. (2020). Microencapsulation of sesame seed oil by tamarind seed mucilage. *International journal of biological macromolecules*, *145*, 207-215.

Amorati, R., Foti, M. C., & Valgimigli, L. (2013). Antioxidant activity of essential oils. *Journal of agricultural and food chemistry*, *61*(46), 10835-10847.

Anandharamakrishnan, C. (2008). *Experimental and computational fluid dynamics studies on spray-freeze-drying and spray-drying of proteins* (Doctoral dissertation, Loughborough University).

Anandharamakrishnan, C., & Padma, I. S. (2015). Selection of wall material for encapsulation by spray drying. In C. Anandharamakrishnan, & I. S. Padma (Eds.), spray drying techniques for food ingredient encapsulation (pp. 77–100). Oxford – UK: John Willey & Sons, Ltd.

Anandharamakrishnan, C., Rielly, C. D., & Stapley, A. G. F. Ã. (2008). Loss of solubility of a -lactalbumin and b -lactoglobulin during the spray drying of whey proteins. *LWT - Food Science and Technology*. *41*, 270–277.

Andrade, J., Pereira, C. G., de Almeida Junior, J. C., Viana, C. C. R., de Oliveira Neves, L. N., da Silva, P. H. F., ... & dos Anjos, V. D. C. (2019). FTIR-ATR determination of protein content to evaluate whey protein concentrate adulteration. *Lwt*, *99*, 166-172.

Antony, B., Sharma, S., Mehta, B. M., Ratnam, K., & Aparnathi, K. D. (2017). Study on FT-MIR spectra of ghee (anhydrous milk fat). *British food journal*.

Antony, B., Sharma, S., Mehta, B. M., Ratnam, K., & Aparnathi, K. D. (2018). Study of Fourier transform near infrared (FT-NIR) spectra of ghee (anhydrous milk fat). *International Journal of Dairy Technology*, *71*(2), 484-490.

Anwar, F., Hussain, A. I., Iqbal, S., & Bhanger, M. I. (2007). Enhancement of the oxidative stability of some vegetable oils by blending with Moringa oleifera oil. *Food Chemistry*, *103*(4), 1181-1191.

Anwar, S. H., Weissbrodt, J., & Kunz, B. (2010). Microencapsulation of fish oil by spray granulation and fluid bed film coating. *Journal of Food Science*, *75*(6), E359-E371.

AOAC, H. W. (2000). International A: Official Methods of Analysis of the AOAC International. *The Association: Arlington County, VA, USA*.

AOAC. (2012). Approved methods of the Association of Official Analytical Chemists, 925.32 (Fat in eggs. Acid hydrolysis method), (19th ed.). International, Gaithersburg : Association of Official Analytical Chemists.

AOCS, A. (1997). Official Method Cd 18-90. p-Anisidine Value. *Official Methods and Recommended Practices of the AOCS*, 1-2.

AOCS. (1989). Approved methods of the American Oil Chemists Society, Ca 5a-40 (4th ed.). Champaign : American Oil Chemists Society.

AOCS. (1998). Approved methods of the American Oil Chemists Society, Cd 8-53 (Peroxide Value), (5th ed.). Champaign, III : American Oil Chemists Society.

AOCS. (2017). Approved methods of the American Oil Chemists Society, Ce 2-66 (Preparation of Methyl Esters of Fatty Acids), (7th ed.). International Urbana, Il : American Oil Chemists Society.

Appelqvist, I. A., Golding, M., Vreeker, R., & Zuidam, N. J. (2007). Emulsions as delivery systems in foods. *Encapsulation and controlled release technologies in food systems*, 41-81.

Arepally, D., & Goswami, T. K. (2019). Effect of inlet air temperature and gum Arabic concentration on encapsulation of probiotics by spray drying. *Lwt*, *99*, 583-593.

Aryee, A. N., & Boye, J. I. (2015). Current and emerging trends in the formulation and manufacture of nutraceuticals and functional food products. *Nutraceutical and functional food processing technology*, 1-52.

Assadpour, E., & Jafari, S. M. (2019). Advances in spray-drying encapsulation of food bioactive ingredients: From microcapsules to nanocapsules. *Annual review of food science and technology*, *10*, 103-131.

Atta, N., Abo Khashaba, A., Shaltout, O., El–lateaf, A., Hisham, A., & El-Difrawy, E. (2020). Microencapsulation of rice bran oil by spray drying and its application in producing high quality yoghurt. *Alexandria Science Exchange Journal*, *41*(JANUARY-MARCH), 129-140.

Augustin, M. A., Abeywardena, M. Y., Patten, G., Head, R., Lockett, T., De Luca, A., & Sanguansri, L. (2011). Effects of microencapsulation on the gastrointestinal transit and tissue distribution of a bioactive mixture of fish oil, tributyrin and resveratrol. *Journal of Functional Foods*, *3*(1), 25-37.

Avramenko, N. A., Chang, C., Low, N. H., & Nickerson, M. T. (2016). Encapsulation of flaxseed oil within native and modified lentil protein-based microcapsules. *Food Research International*, *81*, 17-24.

Azizi, K., Moraveji, M. K., Hassanzadeh, H., & Najafabadi, H. A. (2018). Consideration of inclined mixers embedded inside a photobioreactor for microalgae cultivation using computational fluid dynamic and particle image velocimetry measurement. *Journal of Cleaner Production*, *195*, 753-764.

Azizi, M., Kierulf, A., Lee, M. C., & Abbaspourrad, A. (2018). Improvement of physicochemical properties of encapsulated echium oil using nanostructured lipid carriers. *Food chemistry*, *246*, 448-456.

Bae, E. K., & Lee, S. J. (2008). Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. *Journal of microencapsulation*, *25*(8), 549-560.

Bakry, A. M., Fang, Z., Ni, Y., Cheng, H., Chen, Y. Q., & Liang, L. (2016). Stability of tuna oil and tuna oil/peppermint oil blend microencapsulated using whey protein isolate in combination with carboxymethyl cellulose or pullulan. *Food hydrocolloids*, *60*, 559-571.

Baltork, F. H., Torbati, M., Damirchi, S. A., & Savage, G. P. (2016). Vegetable oil blending: A review of physicochemical, nutritional and health effects. *Trends in Food Science & Technology*, *57* 52e58.

Bansal, G., Zhou, W., Barlow, P. J., Lo, H. L., & Neo, F. L. (2010). Performance of palm olein in repeated deep frying and controlled heating processes. Food chemistry, 121(2), 338-347.

Bayrak, A., Kiralan, M., Ipek, A., Arslan, N., Cosge, B., & Khawar, K. M. (2010). Fatty acid compositions of linseed (Linum usitatissimum L.) genotypes of different origin cultivated in Turkey. *Biotechnology & Biotechnological Equipment*, *24*(2), 1836-1842.

Behzadi, S. S., Wu, S., Toegel, S., Hofrichter, M., Altenburger, I., Unger, F. M., Wirth, M., & Viernstein, H. (2013). Impact of heat treatment and spray drying on cellular properties and culturability of bifidobacterium bifidum BB-12. *Food Research International, 54, 93–101*.

Benito-Román, Ó., Sanz, T., & Beltrán, S. (2020). Microencapsulation of rice bran oil using pea protein and maltodextrin mixtures as wall material. *Heliyon*, *6*(4), e03615.

Bhardwaj, K., Verma, N., Trivedi, R. K., Bhardwaj, S., & Shukla, N. (2015). A novel approach for improvement of oxidative stability of flaxseed oil by blending with palm oil. *Int. J. Adv. Res*, *3*, 1399-1407.

Bhatnagar, A. S., Prasanth Kumar, P. K., Hemavathy, J., & Gopala Krishna, A. G. (2009). Fatty acid composition, oxidative stability, and radical scavenging activity of vegetable oil blends with coconut oil. Journal of the American Oil Chemists' Society, 86(10), 991-999.

Binsi, P. K., Natasha, N., Sarkar, P. C., Ashraf, P. M., George, N., & Ravishankar, C. N. (2017). Structural, functional and in vitro digestion characteristics of spray dried fish roe powder stabilised with gum arabic. *Food chemistry*, *221*, 1698-1708.

Böger, B. R., Acre, L. B., Viegas, M. C., Kurozawa, L. E., & Benassi, M. T. (2021). Roasted coffee oil microencapsulation by spray drying and complex coacervation techniques: Characteristics of the particles and sensory effect. *Innovative Food Science & Emerging Technologies*, *72*, 102739.

Böger, B. R., Georgetti, S. R., & Kurozawa, L. E. (2018). Microencapsulation of grape seed oil by spray drying. *Food Science and Technology*, *38*, 263-270.

Botrel, D. A., Fernandes, R. V. B., Borges, S. V., & Yoshida, M. I. (2014). Influence of wall matrix systems on the properties of spray-dried micro particles containing fish oil. *Food Research International, 62*, 344–352.

Bubonja-Sonje, M., Giacometti, J., & Abram, M. (2011). Antioxidant and antilisterial activity of olive oil, cocoa and rosemary extract polyphenols. *Food Chemistry*, *127*(4), 1821-1827.

Bucurescu, A., Blaga, A. C., Estevinho, B. N., & Rocha, F. (2018). Microencapsulation of Curcumin by a Spray-Drying Technique Using Gum Arabic as Encapsulating Agent and Release Studies. *Food and Bioprocess Technology*, *11*(10), 1795–1806.

Bumrungpert, A., Chongsuwat, R., Phosat, C., & Butacnum, A. (2019). Rice bran oil containing gamma-oryzanol improves lipid profiles and antioxidant status in hyperlipidemic subjects: a randomized double-blind controlled trial. *The Journal of Alternative and Complementary Medicine*, *25*(3), 353-358.

Burgar, M. I., Hoobin, P., Weerakkody, R., Sanguansri, L., & Augustin, M. A. (2009). NMR of microencapsulated fish oil samples during in vitro digestion. *Food Biophysics*, *4*(1), 32-41.

Cabiscol Català, E., Tamarit Sumalla, J., & Ros Salvador, J. (2000). Oxidative stress in bacteria and protein damage by reactive oxygen species. *International Microbiology, 2000, vol. 3, núm. 1, p. 3-8*.

Cai, X., Du, X., Cui, D., Wang, X., Yang, Z., & Zhu, G. (2019). Improvement of stability of blueberry anthocyanins by carboxymethyl starch/xanthan gum combinations microencapsulation. *Food Hydrocolloids*, *91*, 238-245

Calvo, P., Castaño, Á. L., Hernández, M. T., & González-Gómez, D. (2011). Effects of microcapsule constitution on the quality of microencapsulated walnut oil. *European Journal of Lipid Science and Technology*, *113*(10), 1273-1280.

Calvo, P., Castaño, Á. L., Lozano, M., & González-Gómez, D. (2012). Influence of the microencapsulation on the quality parameters and shelf-life of extra-virgin olive oil encapsulated in the presence of BHT and different capsule wall components. *Food Research International*, *45*(1), 256-261.

Campelo, P. H., Junqueira, L. A., Resende, J. V. D., Zacarias, R. D., Fernandes, R. V. D. B., Botrel, D. A., & Borges, S. V. (2017). Stability of lime essential oil emulsion prepared using biopolymers and ultrasound treatment. *International journal of food properties*, *20*(sup1), S564-S579.

Can Karaca, A., Low, N., & Nickerson, M. (2013). Encapsulation of flaxseed oil using a benchtop spray dryer for legume protein–maltodextrin microcapsule preparation. *Journal of agricultural and food chemistry*, *61*(21), 5148-5155.

Cano-Chauca, M., Stringheta, P. C., Ramos, A. M., & Cal-Vidal, J. (2005). Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. *Innovative Food Science & Emerging Technologies, 6(4), 420–428.*

Carmo, E. L., Teodoro, R. A. R., Félix, P. H. C., de Barros Fernandes, R. V., de Oliveira, É. R., Veiga, T. R. L. A., ... & Botrel, D. A. (2018). Stability of spray-dried beetroot extract using oligosaccharides and whey proteins. *Food chemistry*, *249*, 51-59.

Carmona, P. A. O., Garcia, L. C., Ribeiro, J. A. de A., Valadares, L. F., Marçal, A. de F., de França, L. F., & Mendonça, S. (2018). Effect of Solids Content and Spray-Drying Operating Conditions on the Carotenoids Microencapsulation from Pressed Palm Fiber Oil Extracted with Supercritical CO2. *Food and Bioprocess Technology*, *11*(9), 1703–1718.

Carneiro, H. C. F., Tonon, R.V., Grosso, C. R. F., & Hubinger, M. D. (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *Journal of Food Engineering*, 115, 443–451.

Carneiro, H. C., Tonon, R. V., Grosso, C. R., & Hubinger, M. D. (2011). Effect of different combination of wall materials on the encapsulation efficiency of flaxseed oil microencapsulated by spray drying. In *Embrapa Agroindústria de Alimentos-Artigo em*

anais de congresso (ALICE). In: INTERNATIONAL CONGRESS ON ENGINEERING AND FOOD, 11., 2011, Athens. Food process engineering in a changing world: proceedings... Athens: National Technical University of Athens, 2011.

Carr R. L. (1965). Evaluating flow properties of solids. *Chemical Engineering Journal 72*, 163–168.

Celli, G. B., Ghanem, A., & Brooks, M. S. L. (2015). Bioactive encapsulated powders for functional foods—a review of methods and current limitations. *Food and Bioprocess Technology*, *8*(9), 1825-1837.

Cervato, Roberta Cazzola, Benvenuto Cestaro, G. (1999). Studies on the antioxidant activity of milk caseins. *International Journal of food sciences and nutrition*, *50*(4), 291-296.

Chandran, J., Nayana, N., Roshini, N., & Nisha, P. (2017). Oxidative stability, thermal stability and acceptability of coconut oil flavored with essential oils from black pepper and ginger. *Journal of food science and technology*, *54*(1), 144-152.

Chang, C., & Nickerson, M. T. (2018). Encapsulation of omega 3-6-9 fatty acids-rich oils using protein-based emulsions with spray drying. *Journal of Food Science and Technology*, *55*(8), 2850-2861.

Chauca, M. C, Stringheta, P. C., Ramos, A. M., & Vidal, J. C. (2005). Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. *Innovative Food Science and Emerging Technologies,* 6, 420–428.

Chew, C. L., Ab Karim, N. A., Quek, W. P., Wong, S. K., Lee, Y. Y., & Chan, E. S. (2021). Aerobic-liquor treatment improves the quality and deep-frying performance of refined palm oil. *Food Control*, *126*, 108072.

Chew, S. C., Tan, C. P., & Nyam, K. L. (2018). Microencapsulation of refined kenaf (Hibiscus cannabinus L.) seed oil by spray drying using β-cyclodextrin/gum arabic/sodium caseinate. *Journal of food engineering*, *237*, 78-85.

Chinta, D. D., Graves, R. A., Pamujula, S., Praetorius, N., Bostanian, L. A., & Mandal, T. K. (2009). Spray-dried chitosan as a direct compression tableting excipient. *Drug development and industrial pharmacy*, *35*(1), 43-48.

Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. Comprehensive reviews in food science and food safety, 5(4), 169-186.

Choe, E., & Min, D. B. (2007). Chemistry of deep-fat frying oils. *Journal of food science*, *72*(5), R77-R86.

Choi, H., Lee, E., & Lee, K. G. (2014). Quality evaluation of noble mixed oil blended with palm and canola oil. *Journal of oleo science*, *63*(7), 653-660.

Chong, W. T., Tan, C. P., Cheah, Y. K., B. Lajis, A. F., Habi Mat Dian, N. L., Kanagaratnam, S., & Lai, O. M. (2018). Optimization of process parameters in preparation of tocotrienol-rich red palm oil-based nanoemulsion stabilized by Tween80-Span 80 using response surface methodology. *PLoS One*, *13*(8), e0202771.

Choudhary, M., & Grover, K. (2013). Effect of deep-fat frying on physicochemical properties of rice bran oil blends. *IOSR J. Nurs. Health Sci.(IOSR-JNHS)*, *1*, 1-10.

Choudhary, M., Grover, K., & Kaur, G. (2015). Development of rice bran oil blends for quality improvement. Food chemistry, 173, 770-777.

Chowdhury, K., Banu, L. A., Khan, S., & Latif, A. (2007). Studies on the fatty acid composition of edible oil. *Bangladesh Journal of Scientific and Industrial Research*, *42*(3), 311-316.

Chranioti, C., Chanioti, S., & Tzia, C. (2015). Microencapsulation of steviol glycosides (Stevia rebaudiana Bertoni) by a spray drying method–Evaluation of encapsulated products and prepared syrups. *International Journal of food studies*, *4*(2).

Chugh, B., & Dhawan, K. (2014). Storage studies on mustard oil blends. *Journal of food science and technology*, *51*(4), 762-767.

Chuyen, H. V., Roach, P. D., Golding, J. B., Parks, S. E., & Nguyen, M. H. (2019). Encapsulation of carotenoid-rich oil from Gac peel: Optimisation of the encapsulating process using a spray drier and the storage stability of encapsulated powder. *Powder Technology*, *344*, 373-379.

Ciamponi, F., Duckham, C., & Tirelli, N. (2012). Yeast cells as microcapsules. Analytical tools and process variables in the encapsulation of hydrophobes in S. cerevisiae. *Applied microbiology and biotechnology*, *95*(6), 1445-1456.

Codex Alimentarius Commission. (2005). Codex Standard for Named Vegetable Oils CODEX STAN 210-1999. Roma: FAO/WHO.

Codex Alimentarius Commission. (2005). Codex Standard for Named Vegetable Oils CODEX STAN 210-1999. Roma: FAO/WHO.

Corrêa-Filho, L. C., Moldão-Martins, M., & Alves, V. D. (2019). Advances in the application of microcapsules as carriers of functional compounds for food products. *Applied Sciences*, *9*(3), 571.

da Silva James, N. K., Castro, L. P. S., Freitas, S. P., & Nogueira, R. I. (2019). Increasing energy efficiency in microencapsulation of soybean oil by spray drying. *Brazilian Journal of Development*, *5*(7), 8082-8095.

Daaou, M., & Bendedouch, D. (2012). Water pH and surfactant addition effects on the stability of an Algerian crude oil emulsion. *Journal of Saudi Chemical Society*, *16*(3), 333-337.

Dai, L., Zhu, W., He, L., Tan, F., Zhu, N., Zhou, Q., ... & Hu, G. (2018). Calcium-rich biochar from crab shell: an unexpected super adsorbent for dye removal. *Bioresource technology*, *267*, 510-516.

Danviriyakul, S., McClements, D. J., Decker, E., Nawar, W. W., & Chinachoti, P. (2002). Physical stability of spray-dried milk fat emulsion as affected by emulsifiers and processing conditions. *Journal of Food Science, 67*(6), 2183-2189.

Daoud, S., Bou-Maroun, E., Dujourdy, L., Waschatko, G., Billecke, N., & Cayot, P. (2019). Fast and direct analysis of oxidation levels of oil-in-water emulsions using ATR-FTIR. *Food chemistry, 293,* 307-314.

Das Purkayastha, M., Das, S., Manhar, A. K., Deka, D., Mandal, M., & Mahanta, C. L. (2013). Removing antinutrients from rapeseed press-cake and their benevolent role in waste cooking oil-derived biodiesel: conjoining the valorization of two disparate industrial wastes. *Journal of agricultural and food chemistry*, *61*(45), 10746-10756.

Davidov-Pardo, G., Roccia, P., Salgado, D., León, A. E., & Pedroza-Islas, R. (2008). Utilization of different wall materials to microencapsulate fish oil evaluation of its behavior in bread products. *American Journal of Food Technology, 3,* 384–393.

de Barros Fernandes, R. V., Borges, S. V., & Botrel, D. A. (2014). Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. *Carbohydrate polymers*, *101*, 524-532.

De Leonardis, A., & Macciola, V. (2012). Heat-oxidation stability of palm oil blended with extra virgin olive oil. *Food chemistry*, *135*(3), 1769-1776.

De Marco, E., Savarese, M., Parisini, C., Battimo, I., Falco, S., & Sacchi, R. (2007). Frying performance of a sunflower/palm oil blend in comparison with pure palm oil. *European Journal of Lipid Science and Technology*, *109*(3), 237-246.

De Paz, E., Martin, A., Estrella, A., Rodriguez-Rojo, S., Matias, A. A., Duarte, C. M. M., & Cocero, M. J. (2012). Formulation of β- carotene by precipitation from pressurized ethyl acetate-on water emulsions for application as natural colorant. *Food Hydrocolloids*, 26(1), 17–27.

Del Carmen Flores-Álvarez, M., Molina-Hernández, E. F., Hernández-Raya, J. C., & Sosa-Morales, M. E. (2012). The effect of food type (fish nuggets or French fries) on oil blend degradation during repeated frying. *Journal of Food Science*, *77*(11), C1136-C1143.

Delisle, H., Zagré, N., & Ouedraogo, V. (2001). Marketing of red palm oil as a food source of vitamin A in Burkina Faso: a pilot project involving women's groups. *Food and Nutrition Bulletin*, *22*(4), 388-394.

Deshmukh, R., Wagh, P., & Naik, J. (2016). Solvent evaporation and spray drying technique for micro-and nanospheres/particles preparation: A review. *Drying technology*, *34*(15), 1758-1772.

Determination of carotene in vegetable oils. *British standard Methods of Analysis – BS, 684,* Section 2.20:1977.

Devarajan, S., Singh, R., Chatterjee, B., Zhang, B., & Ali, A. (2016). A blend of sesame oil and rice bran oil lowers blood pressure and improves the lipid profile in mild-to-moderate hypertensive patients. *Journal of clinical lipidology*, *10*(2), 339-349.

Devi, N., & Maji, T. K. (2009). Preparation and evaluation of gelatin/sodium carboxymethyl cellulose polyelectrolyte complex microparticles for controlled delivery of isoniazid. *Aaps PharmSciTech*, *10*(4), 1412-1419.

Dhyani, V., & Bhaskar, T. (2018). A comprehensive review on the pyrolysis of lignocellulosic biomass. *Renewable energy*, *129*, 695-716.

Dhyani, V., Mahantashetti, N. S., Ganachari, M. S., Kambar, S., & Ghatnatti, V. (2018). Awareness of gestational diabetes mellitus among pregnant women attending a tertiary health center. *Indian Journal of Health Sciences and Biomedical Research (KLEU)*, *11*(1), 51.

Dhyani, V., Mahantashetti, N. S., Ganachari, M. S., Kambar, S., & Ghatnatti, V. (2018). Awareness of gestational diabetes mellitus among pregnant women attending a tertiary health center. *Indian Journal of Health Sciences and Biomedical Research (KLEU)*, *11*(1), 51.

Dian, N. L. H. M., Sudin, N. A., & Yusoff, M. S. A. (1996). Characteristics of microencapsulated palm-based oil as affected by type of wall material. Journal of the Science of Food and Agriculture, 70(4), 422-426.

Dłużewska, E., Stabiecka, A., & Maszewska, M. (2006). Effect of oil phase concentration on rheological properties and stability of beverage emulsion. *Acta scientiarum polonorum technologia alimentaria*, *5*(2), 147-156.

Do Carmo, M. M. R., Sarmento, U. C., Cavalheiro, L. F., Fernandes, A., Filiú, W. F. D. O., Gielow, K. D. C. F., ... & Dos Santos, E. F. (2018). Intake of polydextrose alters hematology and the profile of short chain fatty acids in partially gastrectomized rats. *Nutrients*, *10*(6), 792.

Domian, E., Cenkier, J., Górska, A., & Kopytowska, A. B. (2017). Effect of oil content and drying method on bulk properties and stability of powdered emulsions with OSA starch and linseed oil. *LWT- Food Science and Technology*.

Domian, E., Sułek, A., Cenkier, J., & Kerschke, A. (2014). Influence of agglomeration on physical characteristics and oxidative stability of spray-dried oil powder with milk protein and trehalose wall material. *Journal of Food Engineering*, *125*, 34-43.

Donhowe, E. G., & Kong, F. (2014). Beta-carotene: Digestion, Microencapsulation, and In Vitro Bioavailability. *Food and Bioprocess Technology*, *7*(2), 338–354.

Đorđević, V., Balanč, B., Belščak-Cvitanović, A., Lević, S., Kalušević, A., Kostić, I., ... & Nedović, V. (2015). Trends in encapsulation technologies for delivery of food bioactive compounds. *Food Engineering Reviews*, *7*(4), 452-490.

Dorni, C., Sharma, P., Saikia, G., & Longvah, T. (2018). Fatty acid profile of edible oils and fats consumed in India. Food chemistry, 238, 9-15.

Du, J., Zhao, G., Shi, Y., Li, Y., Zhu, G., Mao, Y., ... & Wang, W. (2013). A facile method for synthesis of N-doped TiO2 nanooctahedra, nanoparticles, and nanospheres and enhanced photocatalytic activity. *Applied surface science*, *273*, 278-286.

Eastman, J. E., & Moore, C. O. (1984). Cold water soluble granular starch for gelled food composition. US. 4465702.

Edris Sormoli, M., & Langrish, T. A. G. (2016). Spray drying bioactive orange-peel extracts produced by soxhlet extraction: Use of WPI, antioxidant activity and moisture sorption isotherms. *LWT - Food Science and Technology*, *72*, 1–8.

Eggersdorfer, M., & Wyss, A. (2018). Carotenoids in human nutrition and health. Archives of biochemistry and biophysics, 652, 18-26.

El-Reffaei, W. H. M., El-Sebeay, A. S., EL-Ghandour, H., Ragheb, E. M., & Badr, S. E. A. (2016). Effect of deep-fat frying on canola oil, palmolein and sunflower oil blends: B) biologicaland nutritional studies. *Journal of Food and Dairy Sciences*, *7*(2), 81-96.

Elversson, J., & Millqvist-Fureby, A. (2006). In situ coating—An approach for particle modification and encapsulation of proteins during spray-drying. *International journal of pharmaceutics*, *323*(1-2), 52-63.

Endo, Y. (2018). Analytical methods to evaluate the quality of edible fats and oils: the JOCS standard methods for analysis of fats, oils and related materials (2013) and advanced methods. *Journal of oleo science*, *67*(1), 1-10.

Endo, Y. (2018). Analytical methods to evaluate the quality of edible fats and oils: the JOCS standard methods for analysis of fats, oils and related materials (2013) and advanced methods. *Journal of oleo science*, *67*(1), 1-10.

Enríquez-Fernández, B. E., Álvarez de la Cadena y Yañez, L., & Sosa-Morales, M. E. (2011). Comparison of the stability of palm olein and a palm olein/canola oil blend during deep-fat frying of chicken nuggets and French fries. *International journal of food science & technology*, *46*(6), 1231-1237.

Espinosa-Solís, V., García-Tejeda, Y. V., Portilla-Rivera, O. M., & Barrera-Figueroa, V. (2021). Tailoring Olive Oil Microcapsules via Microfluidization of Pickering o/w Emulsions. *Food and Bioprocess Technology*, *14*(10), 1835-1843.

Espinosa-Solis, V., Zamudio-Flores, P. B., Espino-Díaz, M., Vela-Gutiérrez, G., Rendón-Villalobos, J. R., Hernández-González, M., ... & Ortega-Ortega, A. (2021). Physicochemical characterization of resistant starch type-iii (Rs3) obtained by autoclaving malanga (xanthosoma sagittifolium) flour and corn starch. *Molecules*, *26*(13), 4006.

Expert Committee on Food Additives (JECFA). JECFA/61/SC. Rome, Italy.

Ezhilarasi, P. N., Karthik, P., Chhanwal, N., & Anandharamakrishnan, C. (2013). Nanoencapsulation techniques for food bioactive components: a review. *Food and Bioprocess Technology*, *6*(3), 628-647.

Fan, L., & Eskin, N. M. (2015). The use of antioxidants in the preservation of edible oils. *Handbook of antioxidants for food preservation*, 373-388.

Fan, W., Yi, Y., Zhang, Y., & Diao, P. (2015). Effect of an antioxidant from bamboo leaves combined with tea polyphenol on biogenic amine accumulation and lipid oxidation in pork sausages. Food Science and Biotechnology, 24(2), 421-426.

Farag, R. S., El-Agaimy, M. A., & Abd El Hakeem, B. S. (2010). Effects of mixing canola and palm oils with sunflower oil on the formation of trans fatty acids during frying. *Food and Nutrition Sciences*, *1*(01), 24.

Farag, R. S., El-Baroty, G. S., & Basuny, A. M. (2003). Safety evaluation of olive phenolic compounds as natural antioxidants. *International Journal of Food Sciences and Nutrition*, *54*(3), 159-174.

Fennema, O. R. (1996). *Food chemistry* (Vol. 76). CRC Press.

Ference, B. A., Ginsberg, H. N., Graham, I., Ray, K. K., Packard, C. J., Bruckert, E., ... & Catapano, A. L. (2017). Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *European heart journal*, *38*(32), 2459-2472.

Ference, B. A., Ginsberg, H. N., Graham, I., Ray, K. K., Packard, C. J., Bruckert, E., ... & Catapano, A. L. (2017). Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *European heart journal*, *38*(32), 2459-2472.

Fernandes, R. V. D. B., Borges, S. V., & Botrel, D. A. (2013). Influence of spray drying operating conditions on microencapsulated rosemary essential oil properties. *Food Science and Technology*, *33*, 171-178.

Fernandes, R. V. D. B., Borges, S. V., Botrel, D. A., & Oliveira, C. R. D. (2014). Physical and chemical properties of encapsulated rosemary essential oil by spray drying using whey protein–inulin blends as carriers. *International journal of food science & technology*, *49*(6), 1522-1529.

Fernandesa, R. V. B., Marquesa, G. R., Borgesa, S. V., & Botrel, D. A. (2014). Effect of solids content and oil load on the microencapsulation process of rosemary essential oil. *Industrial Crops and Products*, *58*, 173–181.

Fitzpatrick, J. J., Iqbal, T., Delaney, C., Twomey, T., & Keogh, M. K. (2004). Effect of powder properties and storage conditions on the flow ability of milk powders with different fat contents. *Journal of Food Engineering*, *64*, 435–444.

Fitzpatrick, J., Barry, K., Delaney, C., & Keogh, K. (2005). Assessment of the flowability of spray-dried milk powders for chocolate manufacture. *Le Lait*, *85*(4-5), 269-277.

Food Safety and Standard Authority of India, F. No.Stds/ Nutra (DCGI)/ FSSAI--2017(Part file). (2019). Accessed 22 April 2020.

Fortes, I. C. P., & Baugh, P. J. (2004). Pyrolysis–GC/MS studies of vegetable oils from Macauba fruit. *Journal of Analytical and Applied Pyrolysis*, *72*(1), 103-111.

Fritzen-Freire, C. B., Prudêncio, E. S., Amboni, R. D., Pinto, S. S., Negrão-Murakami, A. N., & Murakami, F. S. (2012). Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. *Food Research International*, *45*(1), 306-312.

Froyen, E., & Burns-Whitmore, B. (2020). The effects of linoleic acid consumption on lipid risk markers for cardiovascular disease in healthy individuals: A review of human intervention trials. *Nutrients*, *12*(8), 2329.

Fuchs, M., Turchiuli, C., Bohin, M., Cuvelier, M. E., Ordonnaud, C., Maillard, M. N. P., & Dumoulin, E., (2006). Encapsulation of oil in powder using spray drying and fluidized bed agglomeration. *Journal of Food Engineering*, *75*, 27–35.

Fuchs, M., Turchiuli, C., Bohin, M., Cuvelier, M. E., Ordonnaud, C., Peyrat-Maillard, M. N., & Dumoulin, E. (2006). Encapsulation of oil in powder using spray drying and fluidised bed agglomeration. *Journal of Food Engineering*, *75*(1), 27-35.

Gaca, A., Kludská, E., Hradecký, J., Hajšlová, J., & Jeleń, H. H. (2021). Changes in volatile compound profiles in cold-pressed oils obtained from various seeds during accelerated storage. *Molecules*, *26*(2), 285.

Gad, A. S., Khadrawy, Y. A., Nekeety, A. A. E., Mohamed, S. R., Hassan, N. S., & Wahhab, M. A. A. (2011). Antioxidant activity and hepato protective effects of whey protein and Spirulina in rats. *Nutrition*, *27*, 582–589.

Gallardo, G., Guida, L., Martinez, V., López, M. C., Bernhardt, D., Blasco, R., ... & Hermida, L. G. (2013). Microencapsulation of linseed oil by spray drying for functional food application. *Food Research International*, *52*(2), 473-482.

Gan, P. Y., & Li, Z. D. (2014). Econometric study on Malaysia′ s palm oil position in the world market to 2035. Renewable and Sustainable Energy Reviews, 39, 740-747.

Ganesan, B., Brothersen, C., & McMahon, D. J. (2014). Fortification of foods with omega-3 polyunsaturated fatty acids. *Critical reviews in food science and nutrition*, *54*(1), 98-114.

García-Encina, L. M. (2016). Encapsulation of organic solar cells with polymeric resins, their PV performance and lifetime. *Centro de Investigaciones en Optica, León, Guanajuato, México*.

Gardner, H. W. (1979). Lipid hydroperoxide reactivity with proteins and amino acids: a review. *Journal of Agricultural and Food Chemistry*, *27*(2), 220-229.

Ghaboos, H., & Ardabili, S. (2018). Physico-chemical, textural and sensory evaluation of sponge cake supplemented with pumpkin flour. *International Food Research Journal. 25,* 854–860.

Ghaffari, S., & Roshanravan, N. (2020). The role of nutraceuticals in prevention and treatment of hypertension: An updated review of the literature. *Food Research International, 128,* 108749.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food research international*, *40*(9), 1107-1121.

Ghosh, M., Upadhyay, R., Mahato, D. K., & Mishra, H. N. (2019). Kinetics of lipid oxidation in omega fatty acids rich blends of sunflower and sesame oils using Rancimat. *Food chemistry*, *272*, 471-477.

Gnanasekaran, D., & Chavidi, V. P. (2018). Green fluids from vegetable oil: Power plant. In *Vegetable oil based bio-lubricants and transformer fluids* (pp. 3-26). Springer, Singapore.

Gómez-Mascaraque, L. G., & López-Rubio, A. (2016). Protein-based emulsion electrosprayed micro-and submicroparticles for the encapsulation and stabilization of thermosensitive hydrophobic bioactives. *Journal of colloid and interface science*, *465*, 259-270.

Gómez-Mascaraque, L. G., Perez-Masiá, R., González-Barrio, R., Periago, M. J., & López-Rubio, A. (2017). Potential of microencapsulation through emulsion-electrospraying to improve the bioaccesibility of β-carotene. *Food Hydrocolloids*, *73*, 1-12.

Gomna, A., N'tsoukpoe, K. E., Le Pierrès, N., & Coulibaly, Y. (2019). Review of vegetable oils behaviour at high temperature for solar plants: Stability, properties and current applications. Solar Energy Materials and Solar Cells, 200, 109956.

Gosh, P. (2019). Crisis in Venezuela: A Politico Economic Understanding. International Journal of Political Science, 9(2), 13-20.

Goula, A. M., & Adamopoulos, K. G. (2004). Spray drying of tomato pulp: effect of feed concentration. *Drying technology*, *22*(10), 2309-2330.

Goula, A. M., & Adamopoulos, K. G. (2012). A method for pomegranate seed application in food industries: seed oil encapsulation. *Food and bioproducts processing*, *90*(4), 639-652.

Goula, A. M., Adamopoulos, K. G., & Kazakis, N. A. (2007). Influence of Spray Drying Conditions on Tomato Powder Properties. *Drying Technology : An International Journal.* 37–41. https://doi.org/10.1081/DRT-120038584

Gourichon H. (2013). Analysis of incentives and disincentives for palm oil in Nigeria. Technical notes series, MAFAP, FAO, Rome.

Goyal , R., Khetarpaul,N. & Jood, S. (2018). Effect of blending of crude palm oil with sunflower and groundnut oil on proximate composition, β-carotene and total carotenoids contents of samosa and cutlet. *Annals. Food Science and Technology,* 19(3), 527-534.

Goyal, A., Sharma, V., Sihag, M. K., Tomar, S. K., Arora, S., Sabikhi, L., & Singh, A. K. (2015). Development and physico-chemical characterization of microencapsulated flaxseed oil powder: A functional ingredient for omega-3 fortification. *Powder Technology, 286,* 527-537.

Goyal, A., Sharma, V., Upadhyay, N., Singh, A. K., Arora, S., Lal, D., & Sabikhi, L. (2015). Development of stable flaxseed oil emulsions as a potential delivery system of ω-3 fatty acids. *Journal of food science and technology*, *52*(7), 4256-4265.

Grover, S., Kumari, P., Kumar, A., Soni, A., Sehgal, S., & Sharma, V. (2021). Preparation and Quality Evaluation of Different Oil Blends. *Lett. Appl. NanobBoSci*, *10*, 2126-2137.

Guerra-Rosas, M. I., Morales-Castro, J., Ochoa-Martínez, L. A., Salvia-Trujillo, L., & Martín-Belloso, O. (2016). Long-term stability of food-grade nanoemulsions from high methoxyl pectin containing essential oils. *Food Hydrocolloids*, *52*, 438-446.

Guiotto, E. N., Ixtaina, V. Y., Nolasco, S. M., & Tomás, M. C. (2014). Effect of storage conditions and antioxidants on the oxidative stability of sunflower–chia oil blends. *Journal of the American Oil Chemists' Society*, *91*(5), 767-776.

Gulla, S., & Waghray, K. (2011). Effect of storage on physico-chemical characteristics and fatty acid composition of selected oil blends. *Journal of Life Sciences*, *3*(1), 35-46.

Gunstone, F. (Ed.). (2011). *Vegetable oils in food technology: composition, properties and uses*. John Wiley & Sons.

Haas E, Levin B. Staying healthy with nutrition, rev: the complete guide to diet and nutritional medicine. Celestial Arts; 2012.

Hammond, E. G., & White, P. J. (2011). A brief history of lipid oxidation. *Journal of the American Oil Chemists' Society*, *88*(7), 891-897.

Hardas, N., Danviriyakul, S., Foley, J. L., Nawar, W. W., & Chinachoti, P. (2000). Accelerated stability studies of microencapsulated anhydrous milk fat. *LWT-Food science and technology*, *33*(7), 506-513.

Hashempour-Baltork, F., Torbati, M., Azadmard-Damirchi, S., & Savage, G. P. (2016). Vegetable oil blending: A review of physicochemical, nutritional and health effects. *Trends in Food Science & Technology, 57, 52–58.*

Hebishy, E., Buffa, M., Juan, B., Blasco-Moreno, A., & Trujillo, A. J. (2017). Ultra high-pressure homogenized emulsions stabilized by sodium caseinate: Effects of

protein concentration and pressure on emulsions structure and stability. *LWT-Food Science and Technology*, *76*, 57-66.

Hee, Y. Y., Tan, C. P., Rahman, R. A., Adzahan, N. M., Lai, W. T., & Chong, G. H. (2015). Influence of different wall materials on the microencapsulation of virgin coconut oil by spray drying. *International journal of food engineering*, *11*(1), 61-69.

Hoffmann, M., Swiderski, F., Zalewski, S., & Berger, S. (2002). Frying performance of rapeseed-palm oil blends. *Polish journal of food and nutrition sciences*, *1*(11/52).

Hosseini Ghaboos, S. H., Seyedain Ardabili, S. M., & Kashaninejad, M. (2018). Physico-chemical, textural and sensory evaluation of sponge cake supplemented with pumpkin flour. *International Food Research Journal*, *25*(2).

Huang, L., Zhu, P., Li, G., Lu, D. D., Sun, R., & Wong, C. (2014). Core–shell SiO 2@ RGO hybrids for epoxy composites with low percolation threshold and enhanced thermo-mechanical properties. *Journal of Materials Chemistry A*, *2*(43), 18246-18255.

Huang, X., Yu, H., Tan, H., Zhu, J., Zhang, W., Wang, C., ... & Yan, Q. (2014). Carbon nanotube-encapsulated noble metal nanoparticle hybrid as a cathode material for Li-oxygen batteries. *Advanced Functional Materials*, *24*(41), 6516-6523.

Hundre, S. Y., Karthik, P., & Anandharamakrishnan, C. (2014). Effect of whey protein isolate and beta-cyclodextrin wall systems on stability of microencapsulated vanillin by spray-freeze drying method. *Food Chemistry*.

ICMR Nutrient requirements and RDA for Indians – A report of the Expert Group of the ICMR, 2010.

Iftikhar, H., Zeeshan, M., Iqbal, S., Muneer, B., & Razzaq, M. (2019). Co-pyrolysis of sugarcane bagasse and polystyrene with ex-situ catalytic bed of metal oxides/HZSM-5 with focus on liquid yield. *Bioresource technology*, *289*, 121647.

Ishwarya, S.P., & Anandharamakrishnan, C. (2015). Spray-Freeze-Drying approach for soluble coffee processing and its effect on quality characteristics. *Journal of Food Engineering*, *149*, 171–180.

Ismail, A., Bannenberg, G., Rice, H. B., Schutt, E., & MacKay, D. (2016). Oxidation in EPA-and DHA-rich oils: an overview. *Lipid Technology*, *28*(3-4), 55-59.

J.R. Campos, P. Severino, C.S. Ferreira, A. Zielinska, A. Santini, S.B. Souto and E.B. Souto. Linseed Essential Oil - Source of Lipids as Active Ingredients for Pharmaceuticals and Nutraceuticals. Current Medicinal Chemistry, 2019, 26, 1-22.

Jaarin, K., & Kamisah, Y. (2012). Repeatedly heated vegetable oils and lipid peroxidation. Lipid peroxidation, 2018, 211-28.

Jacobsen, C., Sørensen, A. D. M., Holdt, S. L., Akoh, C. C., & Hermund, D. B. (2019). Source, extraction, characterization, and applications of novel antioxidants from seaweed. *Annual Review of Food Science and Technology*, *10*, 541-568.

Jadhav, N. V., & Vavia, P. R. (2017). Supercritical processed starch nanosponge as a carrier for enhancement of dissolution and pharmacological efficacy of fenofibrate. *International journal of biological macromolecules*, *99*, 713-720.

Jan, K. C., Huang, M. Y., Chang, C. J., & Liu, T. C. (2016). Hypolipidemic Effect of Blended Oil in Hamster: Biochemical Analysis and Gene Expression Profiling. *Journal of Food and Nutrition Research*, *4*(1), 26-32.

Janiszewska-Turak, E. (2017). Carotenoids microencapsulation by spray drying method and supercritical micronization. *Food research international*, *99*, 891-901.

Jarpa-Parra, M., Tian, Z., Temelli, F., Zeng, H., & Chen, L. (2016). Understanding the stability mechanisms of lentil legumin-like protein and polysaccharide foams. *Food Hydrocolloids*, *61*, 903-913.

Jayakumar, Y. A., Sivan, Y. S., Arumughan, C., Sundaresan, A., Jayalekshmi, A., & Kumar, D. R. S. (2001). Consumption profile of preschool children supplemented with different dosages of red palm oil, retinol palmitate and groundnut oil. *Indian J Nutr Diet*, *38*(11), 384-93.

Jayasundera, M., Adhikari, B., Aldred, P., & Ghandi, A. (2009). Surface modification of spray dried food and emulsion powders with surface-active proteins: A review. *Journal of Food Engineering*, *93*(3), 266-277.

Jayme, M. L., Dunstan, D. E., & Gee, M. L. (1999). Zeta potentials of gum arabic stabilised oil in water emulsions. *Food hydrocolloids*, *13*(6), 459-465.

JECFA (2003) Summary and conclusions of the 61st meeting of the Joint FAO/WHO

Jiang, N., Kumar, G. D., Chen, J., Mishra, A., & Solval, K. M. (2020). Comparison of concurrent and mixed-flow spray drying on viability, growth kinetics and biofilm formation of Lactobacillus rhamnosus GG microencapsulated with fish gelatin and maltodextrin. *LWT*, *124*, 109200.

Jimenez, M., Garcia, H. S., & Beristain, C. I. (2004). Spray-drying microencapsulation and oxidative stability of conjugated linoleic acid. *European Food Research and Technology*, *219*(6), 588-592.

Jimenez, M., Garcia, H. S., & Beristain, C. I. (2009). Effect of water activity on physical properties of conjugated linoleic acid (cla) microcapsules. *Journal of Food Process Engineering*, *33*, 434–447.

Kai, N. S., Nee, T. A., Ling, E. L. C., Ping, T. C., Kamariah, L., & Lin, N. K. (2015). Anti–hypercholesterolemic effect of kenaf (Hibiscus cannabinus L.) seed on high–fat diet Sprague dawley rats. *Asian Pacific journal of tropical medicine*, *8*(1), 6-13.

Kamisah, Y., Shamil, S., Nabillah, M. J., Kong, S. Y., Hamizah, N. A. S., Qodriyah, M. S., ... & Jaarin, K. (2012). Deep-fried keropok lekors increase oxidative instability in cooking oils. *The Malaysian Journal of Medical Sciences: MJMS*, *19*(4), 57.

Karaca, A. C., Low, N. H., & Nickerson, M. T. (2015). Potential use of plant proteins in the microencapsulation of lipophilic materials in foods. *Trends in Food Science & Technology*, *42*(1), 5-12.

Karadeniz, M., Sahin, S., & Sumnu, G. (2018). Enhancement of storage stability of wheat germ oil by encapsulation. *Industrial crops and products*, *114*, 14-18.

Karthik, P., & Anandharamakrishnan, C. (2013). Microencapsulation of Docosahexaenoic Acid by Spray-Freeze-Drying Method and Comparison of its Stability with Spray-Drying and Freeze-Drying Methods. *Food and Bioprocess Technology*, *6*(10), 2780–2790.

Kaseke, T., Opara, U. L., & Fawole, O. A. (2021). Blending of sunflower oil with pomegranate seed oil from blanched seeds: Impact on functionality, oxidative stability, and antioxidant properties. *Processes*, *9*(4), 635.

Kaushik, P., Dowling, K., Barrow, C. J., & Adhikari, B. (2015). Microencapsulation of omega-3 fatty acids: A review of microencapsulation and characterization methods. *Journal of functional foods*, *19*, 868-881.

Kenyon, M.M. (1995). Modified starch, maltodextrin and corn syrup solids as wall material for food encapsulation. In: Risch, S.J. and Fineccius, G.A. (Eds), Encapsulation and controlled release of Food Ingredients, pp. 43–50, ACS Symposium Series 590. Washington D.C: American Chemical Society.
Kha, T. C. (2014). *Gac oil extraction and encapsulation by spray drying* (Doctoral dissertation, University of Newcastle).

Kha, T. C., Nguyen, M. H., Roach, P. D., & Stathopoulos, C. E. (2014). Microencapsulation of gac oil by spray drying: optimization of wall material concentration and oil load using response surface methodology. *Drying Technology*, *32*(4), 385-397.

Kher, A., Udabage, P., McKinnon, I., McNaughton, D., & Augustin, M. A. (2007). FTIR investigation of spray-dried milk protein concentrate powders. *Vibrational Spectroscopy*, *44*(2), 375-381.

Kiefer, J., Frank, K., Zehentbauer, F. M., & Schuchmann, H. P. (2016). Infrared spectroscopy of bilberry extract water-in-oil emulsions: sensing the water-oil interface. *Biosensors*, *6*(2), 13.

Koç, M., Güngör, Ö., Zungur, A., Yalçın, B., Selek, İ., Ertekin, F. K., & Ötles, S. (2015). Microencapsulation of Extra Virgin Olive Oil by Spray Drying: Effect of Wall Materials Composition, Process Conditions, and Emulsification Method. *Food and Bioprocess Technology*, *8*(2), 301–318.

Koo, S. Y., Cha, K. H., Song, D. G., Chung, D., & Pan, C. H. (2014). Microencapsulation of peppermint oil in an alginate–pectin matrix using a coaxial electrospray system. *International journal of food science & technology*, *49*(3), 733-739.

Koohikamali, S., & Alam, M. S. (2019). Improvement in nutritional quality and thermal stability of palm olein blended with macadamia oil for deep-fat frying application. *Journal of food science and technology*, *56*(11), 5063-5073.

Koohikamali, S., & Alam, M. S. (2019). Improvement in nutritional quality and thermal stability of palm olein blended with macadamia oil for deep-fat frying application. *Journal of food science and technology*, *56*(11), 5063-5073.

Koohikamali, S., & Alam, M. S. (2019). Improvement in nutritional quality and thermal stability of palm olein blended with macadamia oil for deep-fat frying application. *Journal of food science and technology*, *56*(11), 5063-5073.

Kozłowska, M., & Gruczyńska, E. (2018). Comparison of the oxidative stability of soybean and sunflower oils enriched with herbal plant extracts. Chemical Papers, 72(10), 2607-2615.

Kreivaitis, R., Gumbytė, M., Kazancev, K., Padgurskas, J., & Makarevičienė, V. (2013). A comparison of pure and natural antioxidant modified rapeseed oil storage properties. *Industrial Crops and Products*, *43*, 511-516.

Krokida, M. K., Zogzas, N. P., & Maroulis, Z. B. (1997). Modelling shrinkage and porosity during vacuum dehydration. *International journal of food science & technology*, *32*(6), 445-458.

Kua, Y. L., Gan, S., Ng, H. K., & Morris, A. (2014). The potential of ethyl lactate as a green solvent to extract carotenoids and vitamin e from crude palm oil. *Proceedings of the ISPFVF, University of Nottingham Malaysia Campus, Semenyih, Malaysia*, 11-13.

Kumar, A., Singh, P., Gupta, V., & Prakash, B. (2020). Application of nanotechnology to boost the functional and preservative properties of essential oils. In *Functional and preservative properties of phytochemicals* (pp. 241-267). Academic Press.

Kupongsak, S., & Kansuwan, W. (2012). Effect of vegetable oil blend and frying condition on polar compound formation during deep-fat frying of French fries. *Journal of Applied Sciences Research*, (September), 4777-4782.

Labuschagne, P. (2018). Impact of wall material physicochemical characteristics on the stability of encapsulated phytochemicals: A review. *Food Research International*, *107*, 227–247.

Labuza, T. (1968). Sorption phenomena in foods. *Food Technology*, *22*, 15-24.

Lanigan, R. S., & Yamarik, T. A. (2002). Final report on the safety assessment of BHT (1). *International journal of toxicology*, *21*, 19-94.

Lee, S. H., Heng, D., Ng, W. K., Chan, H. K., & Tan, R. B. (2011). Nano spray drying: a novel method for preparing protein nanoparticles for protein therapy. *International journal of pharmaceutics*, *403*(1-2), 192-200.

Lee, W. J., Tan, C. P., Sulaiman, R., Jr, R. L. S., Chong, G. H. (2018). Microencapsulation of red palm oil as an oil-in-water emulsion with supercritical carbon dioxide solution-enhanced dispersion. *Journal of Food Engineering*, *222*, 100-109.

Lee, Y. I., Jeon, N. J., Kim, B. J., Shim, H., Yang, T. Y., Seok, S. I., ... & Im, S. G. (2018). A low-temperature thin-film encapsulation for enhanced stability of a highly efficient perovskite solar cell. *Advanced Energy Materials*, *8*(9), 1701928.

Lehman, A. J., Fitzhugh, O. G., Nelson, A. A., & Woodard, G. (1951). The pharmacological evaluation of antioxidants. In *Advances in food research* (Vol. 3, pp. 197-208). Academic Press.

Lewandowski, A., Czyżewski, M., & Zbiciński, I. (2012). Morphology and microencapsulation efficiency of foamed spray-dried sunflower oil. *Chemical and Process Engineering*, 95-102.

Leyva, J. D. H., Perez. L. A. B., Acevedo, J. E. A., Ramirez, A., & Echeverry, L. M. J. (2018). Characterization of spray drying microencapsulation of almond oil into taro starch spherical aggregates. *LWT - Food Science and Technology*.

Li, Y., Ma, W. J., Qi, B. K., Rokayya, S., Li, D., Wang, J., ... & Jiang, L. Z. (2014). Blending of soybean oil with selected vegetable oils: impact on oxidative stability and radical scavenging activity. *Asian Pacific Journal of Cancer Prevention*, *15*(6), 2583-2589.

Linke, A., Hinrichs, J., & Kohlus, R. (2020). Impact of the powder particle size on the oxidative stability of microencapsulated oil. *Powder technology*, *364*, 115-122.

Liu, D., Shi, J., Posada, L. R., Kakuda, Y., & Xue, S. J. (2008). Separating tocotrienols from palm oil by molecular distillation. *Food Reviews International*, *24*(4), 376-391.

Liu, F., & Tang, C. H. (2014). Emulsifying properties of soy protein nanoparticles: influence of the protein concentration and/or emulsification process. *Journal of Agricultural and Food Chemistry*, *62*(12), 2644-2654.

Liu, M., Gan, L., Chen, L., Zhu, D., Xu, Z., Hao, Z., & Chen, L. (2012). A novel liposome-encapsulated hemoglobin/silica nanoparticle as an oxygen carrier. *International journal of pharmaceutics*, *427*(2), 354-357.

Liu, Z. Q., Zhou, J. H., Zeng, Y. L., & Ouyang, X. L. (2004). The enhancement and encapsulation of Agaricus bisporus flavor. *Journal of Food Engineering*, *65*(3), 391-396.

Loganathan, R., Subramaniam, K. M., Radhakrishnan, A. K., Choo, Y. M., & Teng, K. T. (2017). Health-promoting effects of red palm oil: evidence from animal and human studies. *Nutrition reviews*, *75*(2), 98-113.

Lozano-Sánchez, J., Giambanelli, E., Quirantes-Piné, R., Cerretani, L., Bendini, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2011). Wastes generated during the

storage of extra virgin olive oil as a natural source of phenolic compounds. *Journal of Agricultural and Food Chemistry*, *59*(21), 11491-11500.

Lubis, A. M. H. S., Ariwahjoedi, B., & Sudin, M. B. (2015). Investigation on oxidation and thermal stability of Jatropha oil. *Jurnal Teknologi*, *77*(21).

Macêdo, R., de Moura, O., de Souza, A., & Macêdo, A. (1997). Comparative studies on some analytical methods: Thermal decomposition of powder milk. *Journal of Thermal Analysis and Calorimetry*, *49*(2), 857-862.

Macêdo, R., de Moura, O., de Souza, A., & Macêdo, A. (1997). Comparative studies on some analytical methods: Thermal decomposition of powder milk. *Journal of Thermal Analysis and Calorimetry*, *49*(2), 857-862.

Maehashi, K., & Huang, L. (2009). Bitter peptides and bitter taste receptors. *Cellular and Molecular Life Sciences, 66, 1661–1671.*

Mahdavi, S. A., Jafari, S. M., Ghorbani, M., & Assadpoor, E. (2014). Spray-drying microencapsulation of anthocyanins by natural biopolymers: A review. *Drying technology*, *32*(5), 509-518.

Mai, K., Andres, J., Bobbert, T., Assmann, A., Biedasek, K., Diederich, S., ... & Spranger, J. (2012). Rosiglitazone increases fatty acid Δ9-desaturation and decreases elongase activity index in human skeletal muscle in vivo. *Metabolism*, *61*(1), 108-116.

Majchrzak, T., Lubinska, M., Różańska, A., Dymerski, T., Gębicki, J., & Namieśnik, J. (2017). Thermal degradation assessment of canola and olive oil using ultra-fast gas chromatography coupled with chemometrics. Monatshefte für Chemie-Chemical Monthly, 148(9), 1625-1630.

Manchanda, S. C., & Passi, S. J. (2016). Selecting healthy edible oil in the Indian context. *Indian heart journal*, *68*(4), 447.

Manorama, R., Chinnasamy, N., & Rukmini, C. (1993). Effect of red palm oil on some hepatic drug-metabolizing enzymes in rats. *Food and chemical toxicology*, *31*(8), 583-588.

Martin-Rubio, A. S., Sopelana, P., Ibargoitia, M. L., & Guillén, M. D. (2018). Prooxidant effect of α-tocopherol on soybean oil. Global monitoring of its oxidation process under accelerated storage conditions by 1H nuclear magnetic resonance. *Food chemistry*, *245*, 312-323.

Masucci, L. C., & Little, C. (2017). Emulsion stability basics. *Processing Solutions for the Food Processing Industries*.

Maszewska, M., Florowska, A., Dłużewska, E., Wroniak, M., Marciniak-Lukasiak, K., & Żbikowska, A. (2018). Oxidative stability of selected edible oils. *Molecules*, *23*(7), 1746.

Matsumura, Y., Satake, C., Egami, M., & Mori, T. (2000). Interaction of Gum Arabic, Maltodextrin and Pullulan with Lipids in Emulsions. *Bioscience, Biotechnology, and Biochemistry*, 64, *1827–1835.*

Mayamol, P. N., Samuel, T., Balachandran, C. P., Babu, R. N., Sundaresan, A., & Arumughan, C. (2009). A novel process for the production of carotene rich red palmolein. India. 195818.

Mba, O. I., Dumont, M. J., & Ngadi, M. (2015). Palm oil: Processing, characterization and utilization in the food industry–A review. *Food bioscience*, *10*, 26-41.

McClements, D. J., & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of food science*, *65*(8), 1270-1282.

McClements, D. J., & Rao, J. (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Critical reviews in food science and nutrition*, *51*(4), 285-330.

McClements, D. J., Decker, E. A., & Weiss, J. (2007). Emulsion-based delivery systems for lipophilic bioactive components. *Journal of food science*, *72*(8), R109-R124.

McClements, D. J., DeLoid, G., Pyrgiotakis, G., Shatkin, J. A., Xiao, H., & Demokritou, P. (2016). The role of the food matrix and gastrointestinal tract in the assessment of biological properties of ingested engineered nanomaterials (iENMs): State of the science and knowledge gaps. *NanoImpact*, *3*, 47-57.

Melton, S. L., Jafar, S., Sykes, D., & Trigiano, M. K. (1994). Review of stability measurements for frying oils and fried food flavor. *Journal of the American Oil Chemists' Society*, *71*(12), 1301-1308.

Menina, A., Zanonia, F., Vakarelovaa, M., Chignolaa, R., Donàa, G., Rizzia, C., Mainentea, F., & Zoccatelli, G. (2018). Effects of microencapsulation by ionic gelation on the oxidative stability of Flaxseed oil. *Food Chemistry*, 269, 293–299.

Michotte, D., Rogez, H., Chirinos, R., Mignolet, E., Campos, D., & Larondelle, Y. (2011). Linseed oil stabilisation with pure natural phenolic compounds. *Food chemistry*, *129*(3), 1228-1231.

Miková, K., Pokorny, J., Yanisshlieva, N., & Gordon, M. (2001). The regulation of antioxidants in food.

Mishra, A. P., Devkota, H. P., Nigam, M., Adetunji, C. O., Srivastava, N., Saklani, S., ... & Khaneghah, A. M. (2020). Combination of essential oils in dairy products: A review of their functions and potential benefits. *Lwt*, *133*, 110116.

Mishra, S., & Manchanda, S. C. (2012). Cooking oils for heart health. *J Prev Cardiol*, *1*(3), 123-131.

Mohammed, A. E., Al-Qahtani, A., Al-Mutairi, A., Al-Shamri, B., & Aabed, K. (2018). Antibacterial and cytotoxic potential of biosynthesized silver nanoparticles by some plant extracts. *Nanomaterials*, *8*(6), 382.

Mohammed, A. N., Ishwarya, S. P., & Nisha, P. (2021). Nanoemulsion versus microemulsion systems for the encapsulation of beetroot extract: comparison of physicochemical characteristics and betalain stability. *Food and Bioprocess Technology*, *14*(1), 133-150.

Mohammed, A., Janakiram, N. B., Brewer, M., Duff, A., Lightfoot, S., Brush, R. S., ... & Rao, C. V. (2012). Endogenous n-3 polyunsaturated fatty acids delay progression of pancreatic ductal adenocarcinoma in Fat-1-p48Cre/+-LSL-KrasG12D/+ mice. *Neoplasia*, *14*(12), 1249-IN46.

Mohammed, N. K., Alhelli, A. M., & Meor Hussin, A. S. (2021). Influence of different combinations of wall materials on encapsulation of Nigella sativa oil by spray dryer. *Journal of Food Process Engineering*, *44*(3), e13639.

Mohammed, N. K., Tan, C. P., Manap, Y. A., Alhelli, A. M., & Hussin, A. S. M. (2017). Process conditions of spray drying microencapsulation of *Nigella sativa* oil. *Powder Technology*.

Mohammed, N. K., Tan, C. P., Manap, Y. A., Muhialdin, B. J., & Hussin, A. S. M. (2020). Spray drying for the encapsulation of oils—A review. *Molecules*, *25*(17), 3873.

Mohanan, A., Nickerson, M. T., & Ghosh, S. (2018). Oxidative stability of flaxseed oil: Effect of hydrophilic, hydrophobic and intermediate polarity antioxidants. *Food Chemistry*, 266, 524–533.

Moigradean, D., Poiana, M. A., & Gogoasa, I. (2012). Quality characteristics and oxidative stability of coconut oil during storage. Journal of Agroalimentary Processes and Technologies, 18(4), 272-276.

Mollet, B., & Rowland, I. (2002). Functional foods: At the frontier between food and pharma. *Current Opinion in Biotechnology*, *5*(13), 483-485.

Montoya, C., Cochard, B., Flori, A., Cros, D., Lopes, R., Cuellar, T., ... & Billotte, N. (2014). Genetic architecture of palm oil fatty acid composition in cultivated oil palm (Elaeis guineensis Jacq.) compared to its wild relative E. oleifera (HBK) Cortés. *PloS one*, *9*(5), e95412.

Mori, C., Kadota, K., Tozuka, Y., Shimosaka, A., Yoshida, M., & Shirakawa, Y. (2019). Application of nozzleless electrostatic atomization to encapsulate soybean oil with solid substances. *Journal of Food Engineering*, *246*, 25-32.

Moriano, M. E., & Alamprese, C. (2020). Whey protein concentrate and egg white powder as structuring agents of double emulsions for food applications. *Food and Bioprocess Technology*, *13*(7), 1154-1165.

Mostafa, R. M., Moustafa, Y. M., Mirghani, Z., AlKusayer, G. M., & Moustafa, K. M. (2013). Antioxidant effect of garlic (Allium sativum) and black seeds (Nigella sativa) in healthy postmenopausal women. *SAGE open medicine*, *1*, 2050312113517501.

Mozaffarian, D., Micha, R., & Wallace, S. (2010). Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. *PLoS medicine*, *7*(3), e1000252.

Mozaffarian, D., Micha, R., & Wallace, S. (2010). Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. *PLoS medicine*, *7*(3), e1000252.

Mujica-Álvarez, J., Gil-Castell, O., Barra, P. A., Ribes-Greus, A., Bustos, R., Faccini, M., & Matiacevich, S. (2020). Encapsulation of vitamins A and E as spray-dried additives for the feed industry. *Molecules*, *25*(6), 1357.

Nadeem, M., Azeem, M. W., & Rahman, F. (2015). Assessment of transesterified palm olein and Moringa oleifera oil blends as vanaspati substitutes. Journal of food science and technology, 52(4), 2408-2414.

Nagendran, B., Unnithan, U. R., Choo, Y. M., & Sundram, K. (2000). Characteristics of Red Palm Oil, a Carotene- and Vitamin E–rich Refined Oil for Food Uses. *Food and Nutrition Bulletin*, *21*(2), 189–194.

Naghshineh, M., Ariffin, A. A., Ghazali, H. M., Mirhosseini, H., & Mohammad, A. S. (2010). Effect of saturated/unsaturated fatty acid ratio on physicochemical properties of palm olein–olive oil blend. Journal of the American Oil Chemists' Society, 87(3), 255-262.

Navarro, R. D., Navarro, F. K. S. P., Ribeiro Filho, O. P., Ferreira, W. M., Pereira, M. M., & Seixas Filho, J. T. (2012). Quality of polyunsaturated fatty acids in Nile tilapias (Oreochromis niloticus) fed with vitamin E supplementation. *Food Chemistry*, *134*(1), 215-218.

Nayana, N., Mary Abraham, L., Padma Ishwarya, S., & Nisha, P. (2021). Spray-dried microcapsules of red palm olein-flaxseed oil blend: Development, physicochemical characterization, and evaluation of its potential applications as a fat replacer and β-carotene fortificant in cupcakes. *Journal of Food Processing and Preservation*, *45*(9), e15663.

Nehdi, I. A., Hadj-Kali, M. K., Sbihi, H. M., Tan, C. P., & Al-Resayes, S. I. (2019). Characterization of ternary blends of vegetable oils with optimal ω-6/ω-3 fatty acid ratios. *Journal of oleo science*, *68*(11), 1041-1049.

Nik, W. W., Ani, F. N., Masjuki, H. H., & Giap, S. E. (2005). Rheology of bio-edible oils according to several rheological models and its potential as hydraulic fluid. *Industrial Crops and Products*, *22*(3), 249-255.

NRC (National Research Council). (1989). Diet and health: implications for reducing chronic disease risk. Report of the committee on diet and health, Food and Nutrition Board. National Academy Press, Washington, DC.

Ogan, I., Mba., Dumontn, M. J., Michael., & Ngadi. (2015). Palm oil: Processing, characterization and utilization in the food industry – A review. *Food Bioscience*, *163*, 1026– 41.

Oil World. (2013). Oil world annual 2013. Retrieved from ⟨http://www.oilworld.biz/app.php⟩ Released June 2013.

Oliveira, P. D., Rodrigues, A. M., Bezerra, C. V., & Silva, L. H. (2017). Chemical interesterification of blends with palm stearin and patawa oil. *Food chemistry*, *215*, 369-376.

Olszowy, M. (2019). What is responsible for antioxidant properties of polyphenolic compounds from plants?. *Plant Physiology and Biochemistry*, *144*, 135-143.

Omar, K. A., Shan, L., Zou, X., Song, Z., & Wang, X. (2009). Effects of two emulsifiers on yield and storage of flaxseed oil powder by response surface methodology. *Pakistan Journal of Nutrition*, *8*(9), 1316-1324.

Orsavova, J., Misurcova, L., Vavra Ambrozova, J., Vicha, R., & Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International journal of molecular sciences*, *16*(6), 12871-12890.

ÖZKAN, O. E., OLGUN, Ç., GÜNEY, B., Mahmut, G. Ü. R., GÜNEY, K., & Saim, A. T. E. Ş. (2018). Chemical composition and antimicrobial activity of Myristica fragrans & Elettaria cardamomum essential oil. *Kastamonu University Journal of Forestry Faculty*, *18*(2), 225-229.

Pan, Y., Wu, Z., Xie, Q. T., Li, X. M., Meng, R., Zhang, B., & Jin, Z. Y. (2020). Insight into the stabilization mechanism of emulsions stabilized by Maillard conjugates: Protein hydrolysates-dextrin with different degree of polymerization. *Food Hydrocolloids*, *99*, 105347.

Pardo, G. D., Roccia, P., Salgado, D., León, A., & Pedroza, R. (2008). Utilization of different wall materials to microencapsulate fish oil. Evaluation of its behavior in bread products. *American Journal of Food Technology*, *3*, 384-393.

Partanen, R., Yoshii, H., Kallio, H., Yang, B., & Forssell, P. (2002). Encapsulation of sea buckthorn kernel oil in modified starches. *Journal of the American Oil Chemists' Society*, *79*(3), 219-223.

Pasukamonset, P., Pumalee, T., Sanguansuk, N., Chumyen, C., Wongvasu, P., Adisakwattana, S., & Ngamukote, S. (2018). Physicochemical, antioxidant and sensory characteristics of sponge cakes fortified with Clitoria ternatea extract. *Journal of food science and technology*, *55*(8), 2881-2889.

Patrick, K. E., Yi, L. V., Muhamyankaka, V., Denis, O., Bouelet, I. S., & Zhang, X. (2013). Development of EPA-DHA microcapsules supplemented probiotic fermented milk. *Akademik Gıda*, *11*(3-4), 6-15.

Pattnaik, M., & Mishra, H. N. (2021). Amelioration of the stability of polyunsaturated fatty acids and bioactive enriched vegetable oil: blending, encapsulation, and its application. *Critical Reviews in Food Science and Nutrition*, 1-23.

Pawlak, A., & Mucha, M. (2003). Thermogravimetric and FTIR studies of chitosan blends. *Thermochimica acta*, *396*(1-2), 153-166.

Paz, E., Martín, A., Estrella, A., Rojo, S. S., Matias, A. A., Duarte, C. M. M., & Cocero, M. J. (2012). Formulation of b-carotene by precipitation from pressurized ethyl acetate-on water emulsions for application as natural colorant. *Food Hydrocolloids*, 26, 17- 27.

Pelegrine, D. H. G., & Gasparetto, C. A. (2005). Whey proteins solubility as function of temperature and pH. *LWT-Food Science and Technology*, *38*(1), 77-80.

Pereira, E., Ferreira, M. C., Sampaio, K. A., Grimaldi, R., de Almeida Meirelles, A. J., & Maximo, G. J. (2019). Physical properties of Amazonian fats and oils and their blends. *Food Chemistry*, *278*, 208-215.

Pham-Hoang, B. N., Romero-Guido, C., Phan-Thi, H., & Waché, Y. (2013). Encapsulation in a natural, preformed, multi-component and complex capsule: yeast cells. *Applied microbiology and biotechnology*, *97*(15), 6635-6645.

Polavarapu, S., Oliver, C. M., Ajlouni, S., & Augustin, M. A. (2011). Physicochemical characterisation and oxidative stability of fish oil and fish oil–extra virgin olive oil microencapsulated by sugar beet pectin. *Food Chemistry*, *127*(4), 1694-1705.

Porter, N. A., Caldwell, S. E., & Mills, K. A. (1995). Mechanisms of free radical oxidation of unsaturated lipids. *Lipids*, *30*(4), 277-290.

Pourashouri, P., Shabanpour, B., Razavi, S. H., Jafari, S. M., Shabani, A., & Aubourg, S. P. (2014). Impact of wall materials on physicochemical properties of microencapsulated fish oil by spray drying. *Food and Bioprocess Technology*, *7*(8), 2354-2365.

Prasad Reddy, M. N., Padma Ishwarya, S., & Anandharamakrishnan, C. (2019). Nanoencapsulation of roasted coffee bean oil in whey protein wall system through nanospray drying. *Journal of Food Processing and Preservation*, *43*(3), e13893.

Premi, M., & Sharma, H. K. (2017). Effect of different combinations of maltodextrin, gum arabic and whey protein concentrate on the encapsulation behavior and oxidative stability of spray dried drumstick (Moringa oleifera) oil. *International journal of biological macromolecules*, *105*, 1232-1240.

Priol, L. L., Dagmey, A., Morandat, S., Saleha, K., Kirat, K. E., & Nesterenko, A. (2019). Comparative study of plant protein extracts as wall materials for the

improvement of the oxidative stability of sunflower oil by microencapsulation. *Food Hydrocolloids*, *95*, 105–115.

Quispe-Condori, S., Saldaña, M. D., & Temelli, F. (2011). Microencapsulation of flax oil with zein using spray and freeze drying. *LWT-Food Science and Technology*, *44*(9), 1880-1887.

Rabail, R., Shabbir, M. A., Sahar, A., Miecznikowski, A., Kieliszek, M., & Aadil, R. M. (2021). An Intricate Review on Nutritional and Analytical Profiling of Coconut, Flaxseed, Olive, and Sunflower Oil Blends. *Molecules*, *26*(23), 7187.

Race, S. (2009). Antioxidants. *The truth about BHA, BHT, TBHQ and otherantioxidants used as food additives. Saltburn by the Sea, UK: Tigmor Books*.

Radhika, M. S., Bhaskaram, P., Balakrishna, N., & Ramalakshmi, B. A. (2003). Red palm oil supplementation: a feasible diet-based approach to improve the vitamin A status of pregnant women and their infants. *Food and Nutrition Bulletin*, *24*(2), 208-217.

Rajam, R., Karthik, P., Parthasarathi, S., Joseph, G. S., & Anandharamakrishnan, C. J. J. O. F. F. (2012). Effect of whey protein–alginate wall systems on survival of microencapsulated Lactobacillus plantarum in simulated gastrointestinal conditions. *Journal of Functional Foods*, *4*(4), 891-898.

Ramadan, M. F., & Wahdan, K. M. M. (2012). Blending of corn oil with black cumin (Nigella sativa) and coriander (Coriandrum sativum) seed oils: Impact on functionality, stability and radical scavenging activity. *Food Chemistry,* 132, 873–879.

Ramakrishnan, S., Ferrando, M., Aceña-Muñoz, L., Mestres, M., De Lamo-Castellví, S., & Güell, C. (2014). Influence of emulsification technique and wall composition on physicochemical properties and oxidative stability of fish oil microcapsules produced by spray drying. *Food and Bioprocess Technology*, 7, 1959–1972.

Ramroudi, F., Yasini Ardakani, S. A., Dehghani-Tafti, A., & Khalili Sadrabad, E. (2022). Investigation of the Physicochemical Properties of Vegetable Oils Blended with Sesame Oil and Their Oxidative Stability during Frying. *International Journal of Food Science*, *2022*.

Ravi, R., Prakash, M., & Bhat, K. K. (2005). Sensory odour profiling and physical characteristics of edible oil blends during frying. Food research international, 38(1), 59-68.

Reddy, K. J., Jayathilakan, K., Pandey, M. C., & Radhakrishna, K. (2013). Evaluation of the physico-chemical stability of rice bran oil and its blends for the development of functional meat products. *International Journal of Food and Nutritional Sciences*, *2*(2), 46.

Reena, M. B., & Lokesh, B. R. (2007). Hypolipidemic effect of oils with balanced amounts of fatty acids obtained by blending and interesterification of coconut oil with

rice bran oil or sesame oil. *Journal of agricultural and food chemistry*, *55*(25), 10461-10469.

Reis, D. R., Ambrosi, A., & Di Luccio, M. (2022). Encapsulated essential oils: A perspective in food preservation. *Future Foods*, 100126.

Ren, C. X., Wang, Y. J., Lin, X. F., Yang, F., Chen, J., Song, H. Q., ... & Guan, W. T. (2020). Palm oil encapsulated by homogenizing and spray drying enhances nutrient digestibility and attenuates diarrhoea of piglets. *Animal Feed Science and Technology*, *266*, 114503.

Restu, W. K., Sampora, Y., Meliana, Y., & Haryono, A. (2015). Effect of accelerated stability test on characteristics of emulsion systems with chitosan as a stabilizer. *Procedia Chemistry*, *16*, 171-176.

Rice, Amy L., and Jennifer B. Burns. "Moving from efficacy to effectiveness: red palm oil's role in preventing vitamin A deficiency." *Journal of the American College of Nutrition* 29, no. sup3 (2010): 302S-313S.

Roberfroid, M. (2007). Prebiotics: the concept revisited. *The Journal of nutrition*, *137*(3), 830S-837S.

Roiaini, M., Ardiannie, T., & Norhayati, H. (2015). Physicochemical properties of canola oil, olive oil and palm olein blends. *International Food Research Journal*, *22*, 1227-1233.

Rosenberg, M., & Sheu, T. Y. (1996). Microencapsulation of volatiles by spray-drying in whey protein-based wall systems. *International Dairy Journal*, *6*(3), 273-284.

Rudzińska, M., Hassanein, M. M., Abdel-Razek, A. G., Kmiecik, D., Siger, A., & Ratusz, K. (2018). Influence of composition on degradation during repeated deep-fat frying of binary and ternary blends of palm, sunflower and soybean oils with health-optimised saturated-to-unsaturated fatty acid ratios. *International Journal of Food Science & Technology*, *53*(4), 1021-1029.

Saénz, C., Tapia, S., Chávez, J., & Robert, P. (2009). Microencapsulation by spray drying of bioactive compounds from cactus pear (Opuntia ficus-indica). *Food chemistry*, *114*(2), 616-622.

Sagiri, S. S., Anis, A., & Pal, K. (2016). Review on encapsulation of vegetable oils: strategies, preparation methods, and applications. *Polymer-plastics technology and engineering*, *55*(3), 291-311.

Saifullah, M., Shishir, M. R. I., Ferdowsi, R., Rahman, M. R. T., & Van Vuong, Q. (2019). Micro and nano encapsulation, retention and controlled release of flavor and aroma compounds: A critical review. *Trends in Food Science & Technology*, *86*, 230-251.

Saini, R. K., Nile, S. H., & Park, S. W. (2015). Carotenoids from fruits and vegetables: Chemistry, analysis, occurrence, bioavailability and biological activities. *Food Research International, 76,* 735–750.

Saito, M., Sakagami, H., & Fujisawa, S. (2003). Cytotoxicity and apoptosis induction by butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). *Anticancer Research, 23*(6C), 4693-4701.

Salar-behzadi, S., Wu, S., Toegel, S., Hofrichter, M., Altenburger, I., Unger, F. M., … Viernstein, H. (2013). Impact of heat treatment and spray drying on cellular properties and culturability of Bifidobacterium bifidum BB-12. *54*, 93–101.

Salimi, A., Maghsoudlou, Y., & Jafari, S. M. (2018). Effect of emulsion stability and spray drying conditions on physicochemical characteristics of encapsulated powders. *Latin American Applied Research-An international journal*, *48*(2), 95-100.

Salminen, H., Aulbach, S., Leuenberger, B. H., Tedeschi, C., & Weiss, J. (2014). Influence of surfactant composition on physical and oxidative stability of Quillaja saponin-stabilized lipid particles with encapsulated ω-3 fish oil. *Colloids and Surfaces B: Biointerfaces*, *122*, 46-55.

Samotyja, U., & Małecka, M. (2010). Antioxidant activity of blackcurrant seeds extract and rosemary extracts in soybean oil. *European Journal of Lipid Science and Technology*, *112*(12), 1331-1336.

Sanchez, M. D. R. H., Cuvelier, M. E., & Turchiuli, C. (2016). Effect of α-tocopherol on oxidative stability of oil during spray drying and storage of dried emulsions. *Food Research International*, *88*, 32-41.

Sánchez-González, L., Pastor, C., Vargas, M., Chiralt, A., González-Martínez, C., & Cháfer, M. (2011). Effect of hydroxypropylmethylcellulose and chitosan coatings with and without bergamot essential oil on quality and safety of cold-stored grapes. *Postharvest biology and technology*, *60*(1), 57-63.

Sanchez-Navarro, M., Arán-Aís, F., Marcilla-Gomis, A., & Orgilés-Barceló, C. (2015). Synthesis and Characterisation of Aminoplast Microcapsules for Controlled Release of Bioactives. Influence of the Resin: Oil Ratio. *Micro and Nanosystems*, *7*(2), 116-123.

Sanguansri, L., Day, L., Shen, Z., Fagan, P., Weerakkody, R., Cheng, L. J., ... & Augustin, M. A. (2013). Encapsulation of mixtures of tuna oil, tributyrin and resveratrol in a spray dried powder formulation. *Food & Function*, *4*(12), 1794-1802.

Santhanam, A. K., Lekshmi, M., Chouksey, M. K., Tripathi, G., & Gudipati, V. (2014). Delivery of omega-3 fatty acids into cake through emulsification of fish oil-in-milk and encapsulation by spray drying with added polymers. *Drying Technology,* 33, 83–91.

Santos, D., Maurício, A. C., Sencadas, V., Santos, J. D., Fernandes, M. H., & Gomes, P. S. (2018). Spray drying: an overview. *Biomaterials-Physics and Chemistry-New Edition*, 9-35.

Santos, J., Santos, I., Conceição, M., Porto, S. L., Trindade, M. F. S. A., Souza, A., ... & Araújo, A. (2004). Thermoanalytical, kinetic and rheological parameters of commercial edible vegetable oils. *Journal of thermal analysis and calorimetry*, *75*(2), 419-428.

Sarafian, T. A., Kouyoumjian, S., Tashkin, D., & Roth, M. D. (2002). Synergistic cytotoxicity of Δ9-tetrahydrocannabinol and butylated hydroxyanisole. *Toxicology letters*, *133*(2-3), 171-179.

Sari, T. P., Mann, B., Kumar, R., Singh, R. R. B., Sharma, R., Bhardwaj, M., & Athira, S. (2015). Preparation and characterization of nanoemulsion encapsulating curcumin. *Food Hydrocolloids*, *43*, 540-546.

Sarma, D., Akhtar, M. S., Das, P., Das, P., Shahi, N., Ciji, A., ... & Debnath, D. (2013). Nutritional quality in terms of amino acid and fatty acid of five coldwater fish species: implications to human health. *National Academy Science Letters*, *36*(4), 385-391.

Sathyan, S., & Nisha, P. (2022). Optimization and Characterization of Porous Starch from Corn Starch and Application Studies in Emulsion Stabilization. *Food and Bioprocess Technology*, 1-16.

Schäfer, J., Höbel, S., Bakowsky, U., & Aigner, A. (2010). Liposome–polyethylenimine complexes for enhanced DNA and siRNA delivery. *Biomaterials*, *31*(26), 6892-6900.

Schramm, L. L. (2014). *Emulsions, foams, suspensions, and aerosols: microscience and applications*. John Wiley & Sons.

Schramm, L. L., Stasiuk, E. N., & MacKinnon, M. (2000). *Surfactants in Athabasca oil sands slurry conditioning, flotation recovery, and tailings processes* (pp. 365-430). Cambridge University Press: Cambridge.

Scrimshaw, N. S. (2000). Nutritional potential of red palm oil for combating vitamin A deficiency. *Food and Nutrition Bulletin*, *21*(2), 195-201.

Shahidi, F. (Ed.). (2005). *Bailey's Industrial Oil and Fat Products, Industrial and Nonedible Products from Oils and Fats* (Vol. 6). John Wiley & Sons.

Sharayei, P., & Farhoosh, R. (2016). Improved frying stability of canola oil blended with palm olein and virgin olive oils as affected by bene kernel oil and its unsaponifiable matter. *European Journal of Lipid Science and Technology*, *118*(10), 1495-1506.

Sharif, H. R., Goff, H. D., Majeed, H., Shamoon, M., Liu, F., Nsor-Atindana, J., ... & Zhong, F. (2017). Physicochemical properties of β-carotene and eugenol co-encapsulated flax seed oil powders using OSA starches as wall material. *Food hydrocolloids*, *73*, 274-283.

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., ... & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in physiology*, *11*, 694.

Sharma, A., Chaurasia, S. P., & Dalai, A. K. (2013). Enzymatic hydrolysis of cod liver oil for the fatty acids production. *Catalysis today*, *207*, 93-100.

Siddeeg, A., & Xia, W. (2015). Oxidative stability, chemical composition and organoleptic properties of seinat (Cucumis melo var. tibish) seed oil blends with peanut oil from China. *Journal of food science and technology*, *52*(12), 8172-8179.

Siddique, B. M., Ahmad, A., Ibrahim, M. H., Hena, S., & Rafatullah, M. (2010). Physico-chemical properties of blends of palm olein with other vegetable oils. *Grasas y aceites*, *61*(4), 423-429.

Siddique, B. M., Muhamad, I. I., Ahmad, A., Ayob, A., Ibrahim, M. H., & Ak, M. O. (2015). Effect of frying on the rheological and chemical properties of palm oil and its blends. *Journal of food science and technology*, *52*(3), 1444-1452.

Silva, J. M., Caridade, S. G., Costa, R. R., Alves, N. M., Groth, T., Picart, C., ... & Mano, J. F. (2015). pH responsiveness of multilayered films and membranes made of polysaccharides. *Langmuir*, *31*(41), 11318-11328.

Simonetto, M., Infante, M., Sacco, R. L., Rundek, T., & Della-Morte, D. (2019). A novel anti-inflammatory role of omega-3 PUFAs in prevention and treatment of atherosclerosis and vascular cognitive impairment and dementia. *Nutrients*, *11*(10), 2279.

Sims, R. J., Fioriti, J. A., & Trumbetas, J. (1979). Effect of sugars and sugar alcohols on autoxidation of safflower oil in emulsions. *Journal of the American Oil Chemists' Society*, *56*(8), 742-745.

Singh, H. (2011). Aspects of milk-protein-stabilised emulsions. *Food Hydrocolloids*, *25*(8), 1938-1944.

Singh, P., Singh, T. P., & Gandhi, N. (2018). Prevention of lipid oxidation in muscle foods by milk proteins and peptides: A review. *Food reviews international*, *34*(3), 226-247.

Sivan, Y. S., Jayakumar, Y. A., Arumughan, C., Sundaresan, A., Balachandran, C., Job, J., ... & Sarma, P. S. (2001). Impact of B-carotene Supplementation Through Red Palm Oil. *Journal of tropical pediatrics*, *47*(2), 67-72.

Sogut, E. (2020). Active whey protein isolate films including bergamot oil emulsion stabilized by nanocellulose. *Food Packaging and Shelf Life*, *23*, 100430

Soliman, E. A., El-Moghazy, A. Y., El-Din, M. M., & Massoud, M. A. (2013). Microencapsulation of essential oils within alginate: formulation and in vitro evaluation of antifungal activity.

Srivastava, Y., Semwal, A. D., & Majumdar, A. (2016). Quantitative and qualitative analysis of bioactive components present in virgin coconut oil. *Cogent Food & Agriculture*, *2*(1), 1164929.

Srivastava, Y., Semwal, A. D., Sajeevkumar, V. A., & Sharma, G. K. (2016). Melting, crystallization and storage stability of virgin coconut oil and its blends by differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR). *Journal of Food Science and Technology, 54, 45–54.*

Sudesh, J., Neelam, K., & Rajni, G. (2009). Blending of crude palm oil with other cooking oils as a source of β-carotene for combating vitamin A deficiency. *Indian Journal of Nutrition and Dietetics*, *46*(9), 378-385.

Sukhotu, R., Guo, S., Xing, J., Hu, Q., Wang, R., Shi, X., ... & Guo, S. (2016). Changes in physiochemical properties and stability of peanut oil body emulsions by applying gum arabic. *LWT-Food Science and Technology*, *68*, 432-438.

Sun, C., & Gunasekaran, S. (2009). Effects of protein concentration and oil-phase volume fraction on the stability and rheology of menhaden oil-in-water emulsions stabilized by whey protein isolate with xanthan gum. *Food Hydrocolloids*, *23*(1), 165-174.

Sun, H., Jiao, R., Xu, H., An, G., & Wang, D. (2019). The influence of particle size and concentration combined with pH on coagulation mechanisms. *Journal of Environmental Sciences*, *82*, 39-46.

Sura, M., Megavath, V. S., Mohammad, A. S., Pendyala, S., Kulkarni, M., Sreeyapureddy, A., & Kuthadi, S. (2020). Studies of the quality parameters of blended oils and sensory evaluation of gram flour products. *Grain & Oil Science and Technology*, *3*(4), 138-145.

Sura, M., Megavath, V. S., Mohammad, A. S., Pendyala, S., Kulkarni, M., Sreeyapureddy, A., & Kuthadi, S. (2020). Studies of the quality parameters of blended oils and sensory evaluation of gram flour products. *Grain & Oil Science and Technology*, *3*(4), 138-145.

Sutaphanit, P., & Chitprasert, P. (2014). Optimisation of microencapsulation of holy basil essential oil in gelatin by response surface methodology. *Food chemistry*, *150*, 313-320.

Tabaszewska, M., & Najgebauer-Lejko, D. (2016). Influence of wpc 80 addition on colour, droplet size and storage stability of w/o emulsions. *Bulgarian Journal of Agricultural Science*, *22*(6), 1021-1026.

Taghvaei, M., & Jafari, S. M. (2015). Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. *Journal of food science and technology*, *52*(3), 1272-1282.

Takeungwongtrakul, S., Benjakul, S., & H-kittikun, A. (2015). Wall materials and the presence of antioxidants influence encapsulation efficiency and oxidative stability of

micro-encapsulated shrimp oil. *European Journal of Lipid Science and Technology*, *117*(4), 450-459.

Tamjidi, F., Nasirpour, A., & Shahedi, M. (2013). Mixture design approach for evaluation of fish oil microencapsulation in gelatin-acacia gum coacervates. *International Journal of Polymeric Materials and Polymeric Biomaterials*, *62*(8), 444-449.

Tamm, F., Herbst, S., Brodkorb, A., & Drusch, S. (2016). Functional properties of pea protein hydrolysates in emulsions and spray-dried microcapsules. *Food Hydrocolloids*, *58*, 204-214.

Tan, J., Li, R., Jiang, Z. T., Tang, S. H., Wang, Y., Shi, M., ... & Wang, H. (2017). Synchronous front-face fluorescence spectroscopy for authentication of the adulteration of edible vegetable oil with refined used frying oil. *Food Chemistry*, *217*, 274-280.

Taneja, A., Ye, A., Jones, J. R., Archer, R., & Singh, H. (2013). Behaviour of oil droplets during spray drying of milk-protein-stabilised oil-in-water emulsions. *International dairy journal*, *28*(1), 15-23.

Tapera, M. (2019). Towards greener preservation of edible oils: A mini-review. *Asian Journal of Applied Chemistry Research*, *4*(1-2), 1-8.

Tarmizi, A. H. A., & Ismail, R. (2014). Use of pilot plant scale continuous fryer to simulate industrial production of potato chips: thermal properties of palm olein blends under continuous frying conditions. *Food science & nutrition*, *2*(1), 28-38.

Tatar, F., Tunç, M. T., Dervisoglu, M., Cekmecelioglu, D., & Kahyaoglu, T. (2014). Evaluation of hemicellulose as a coating material with gum arabic for food microencapsulation. *Food Research International*, *57*, 168-175.

Tavares, L., & Noreña, C. P. Z. (2019). Encapsulation of garlic extract using complex coacervation with whey protein isolate and chitosan as wall materials followed by spray drying. *Food Hydrocolloids*, *89*, 360-369.

Teh, S. S., & Birch, J. (2013). Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. *Journal of Food Composition and Analysis*, 30, 26–31.

Tini, G., Varma, V., Lombardo, R., Nolen, G. T., Lefebvre, G., Descombes, P., ... & Scott-Boyer, M. P. (2020). DNA methylation during human adipogenesis and the impact of fructose. *Genes & Nutrition*, *15*(1), 1-16.

Tiwari, M. R., Tiwari, K. K., & Toliwal, S. D. (2014). Studies on thermal stability of palm-sesame oil blends during deep fat frying.

Tonon, R. V., Grosso, C. R. F. & Hubinger, M. D. (2011). Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. *Food Research International*, 44, 282–289.

Tonon, R. V., Pedro, R. B., Grosso, C. R., & Hubinger, M. D. (2012). Microencapsulation of flaxseed oil by spray drying: Effect of oil load and type of wall material. *Drying Technology*, *30*(13), 1491-1501.

Tontul, I., & Topuz, A. (2013). Mixture design approach in wall material selection and evaluation of ultrasonic emulsification in flaxseed oil microencapsulation. *Drying Technology*, *31*(12), 1362-1373.

Torri, L., Bondioli, P., Folegatti, L., Rovellini, P., Piochi, M., & Morini, G. (2019). Development of Perilla seed oil and extra virgin olive oil blends for nutritional, oxidative stability and consumer acceptance improvements. *Food chemistry*, *286*, 584-591.

Turchiuli, C., Fuchs, M., Bohin, M., Cuvelier, M. E., Ordonnaud, C., Peyrat-Maillard, M. N., & Dumoulin, E. (2005). Oil encapsulation by spray drying and fluidised bed agglomeration. *Innovative Food Science & Emerging Technologies*, *6*(1), 29-35.

Turchiuli, C., Munguia, M. J., Sanchez, M. H., Ferre, H. C., & Dumoulin, E. (2014). Use of different supports for oil encapsulation in powder by spray drying. *Powder Technology*, *255*, 103-108.

Umesha, S. S., Manohar, R. S., Indiramma, A. R., Akshitha, S., & Naidu, K. A. (2015). Enrichment of biscuits with microencapsulated omega-3 fatty acid (Alpha-linolenic acid) rich Garden cress (Lepidium sativum) seed oil: Physical, sensory and storage quality characteristics of biscuits. *LWT-Food Science and Technology*, *62*(1), 654-661.

Upadya, H., Devaraju, C. J., & Joshi, S. R. (2015). Anti-inflammatory properties of blended edible oil with synergistic antioxidants. *Indian Journal of Endocrinology and Metabolism*, *19*(4), 511.

Urbančič, S., Kolar, M. H., Dimitrijević, D., Demšar, L., & Vidrih, R. (2014). Stabilisation of sunflower oil and reduction of acrylamide formation of potato with rosemary extract during deep-fat frying. *LWT-food science and technology*, *57*(2), 671-678.

US EPA (1999) Environmental Protection Agency National Contingency Plan Product Schedule. US Environmental Protection Agency, Feb. 1999, 8 pp.

Vaclavik, V. A., & Christian, E. W. (2014). Fat and oil products. In *Essentials of food science* (pp. 233-261). Springer, New York, NY.

Vaclavik, V., & Christian, E. (2003). Essentials of Food Science. New York.

Valantina, R., Neelamegam, S., & Vignesh, P. (2014). Effect of heating on oxidative stability of palm and rice bran mixture oils. *International Journal of ChemTech Research*, 6, 4671- 4677

Vani, A., Laxmi, R., & Sesikeran, B. (2002). Effects of dietary α-linolenic acid from blended oils on biochemical indices of coronary heart disease in Indians. *Lipids*, *37*(11), 1077-1086.

Vani, A., Laxmi, R., & Sesikeran, B. (2002). Effects of dietary α-linolenic acid from blended oils on biochemical indices of coronary heart disease in Indians. *Lipids*, *37*(11), 1077-1086.

Velez-Erazo, E. M., Bosqui, K., Rabelo, R. S., Kurozawa, L. E., & Hubinger, M. D. (2020). High internal phase emulsions (HIPE) using pea protein and different polysaccharides as stabilizers. *Food Hydrocolloids*, *105*, 105775.

Verhagen, H., Schilderman, P. A., & Kleinjans, J. C. (1991). Butylated hydroxyanisole in perspective. *Chemico-biological interactions*, *80*(2), 109-134.

Vieira, S. A., McClements, D. J., & Decker, E. A. (2015). Challenges of utilizing healthy fats in foods. *Advances in Nutrition*, *6*(3), 309S-317S.

Vitorino, C., Almeida, J., Gonçalves, L. M., Almeida, A. J., Sousa, J. J., & Pais, A. A. C. C. (2013). Co-encapsulating nanostructured lipid carriers for transdermal application: from experimental design to the molecular detail. *Journal of controlled release*, *167*(3), 301-314.

Walallawita, W. K. U. S., Bopitiya, D., Sivakanthan, S., Jayawardana, N. W. I. A., & Madhujith, T. (2016). Comparison of oxidative stability of sesame (Sesamum indicum), soybean (Glycine max) and mahua (mee)(Madhuca longifolia) oils against photo-oxidation and autoxidation. *Procedia food science*, *6*, 204-207.

Wang, J. Y., Snoeck, D., Van Vlierberghe, S., Verstraete, W., & De Belie, N. (2014). Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete. *Construction and building materials*, *68*, 110-119.

Wang, S. N., Sui, X. N., Wang, Z. J., Qi, B. K., Jiang, L. Z., Li, Y., ... & Wei, X. (2016). Improvement in thermal stability of soybean oil by blending with camellia oil during deep fat frying. European Journal of Lipid Science and Technology, 118(4), 524-531.

Wei, B., Hu, X., Li, H., Wu, C., Xu, X., Jin, Z., & Tian, Y. (2014). Effect of pHs on dispersity of maize starch nanocrystals in aqueous medium. *Food Hydrocolloids*, *36*, 369-373.

Wei, B., Xu, X., Jin, Z., & Tian, Y. (2014). Surface chemical compositions and dispersity of starch nanocrystals formed by sulfuric and hydrochloric acid hydrolysis. *PloS one*, *9*(2), e86024.

Wen, P., Zong, M. H., Linhardt, R. J., Feng, K., & Wu, H. (2017). Electrospinning: A novel nano-encapsulation approach for bioactive compounds. *Trends in Food Science and Technology*, 70, 56–68.

World Health Organization. (2008). *WHO European action plan for food and nutrition policy 2007–2012* (No. WHO/EURO: 2008-4245-44004-62047). World Health Organization. Regional Office for Europe.

World Health Organization. (2017). *WHO report on the global tobacco epidemic, 2017: monitoring tobacco use and prevention policies*. World Health Organization.

Xu, Y. Y., Howes, T., Adhikari, B., & Bhandari, B. (2012). Investigation of relationship between surface tension of feed solution containing various proteins and surface composition and morphology of powder particles. *Drying Technology*, *30*(14), 1548-1562.

Xu, Y. Y., Howes, T., Adhikari, B., & Bhandari, B. (2013). Effects of emulsification of fat on the surface tension of protein solutions and surface properties of the resultant spray-dried particles. *Drying Technology,* 31, 1939–1950.

Yamauchi, K., Shimizu, M., & Kamiya, T. (1980). Emulsifying properties of whey protein. *Journal of food science*, *45*(5), 1237-1242.

Yang, Y., Song, X., Sui, X., Qi, B., Wang, Z., Li, Y., & Jiang, L. (2016). Rosemary extract can be used as a synthetic antioxidant to improve vegetable oil oxidative stability. *Industrial Crops and Products*, *80*, 141-147.

Yang, Z., Peng, Z., Li, J., Li, S., Kong, L., Li, P., & Wang, Q. (2014). Development and evaluation of novel flavour microcapsules containing vanilla oil using complex coacervation approach. *Food chemistry*, *145*, 272-277.

Ye, A., Cui, J., Taneja, A., Zhu, X., & Singh, H. (2009). Evaluation of processed cheese fortified with fish oil emulsion. *Food research international*, *42*(8), 1093-1098.

Zbicinski, I., Strumillo, C., & Delag, A. (2002). Drying kinetics and particle residence time in spray drying. *Drying Technology*, *20*(9), 1751-1768.

Zhang, C., Zhang, X., Dai, J., & Bai, C. (2008). Synthesis and properties of PDMS modified waterborne polyurethane–acrylic hybrid emulsion by solvent-free method. *Progress in Organic Coatings*, *63*(2), 238-244.

Zhang, K., Zhang, H., Hu, X., Bao, S., & Huang, H. (2012). Synthesis and release studies of microalgal oil-containing microcapsules prepared by complex coacervation. *Colloids and Surfaces B: Biointerfaces*, *89*, 61-66.

Zribi, A., Jabeur, H., Matthäus, B., & Bouaziz, M. (2016). Quality control of refined oils mixed with palm oil during repeated deep-frying using FT-NIRS, GC, HPLC, and multivariate analysis. *European Journal of Lipid Science and Technology*, *118*(4), 512-523.

Zuidam, N. J., & Heinrich, E. (2010). Encapsulation of aroma. In *Encapsulation technologies for active food ingredients and food processing* (pp. 127-160). Springer, New York, NY.

Appendix

Appendix

Sl.no	Instruments	Manufacturer
1	Overhead stirrer	Remi, RQ126/D, with 40V, Mumbai, India
2	Spectrophotometer	Shimadzu UV-2600, Japan
3	Hot air oven	Globe Tex, Digital Laboratory Hot Air Oven, Ghaziabad, India
4	GC-MS/MS	Thermo scientific TRACE 1310/TSQ 8000
5	Homogenizer	T25 ULTRA-TURRAX digital; dispersing tool: S 25 N - 8G; IKA, Private Limited, China.
6	Mini spray dryer	LABULTIMA, Process technologies Pvt Ltd., Mumbai, India, Model LU 228, ADVANCED
7	Water activity meter	Rotronic HygroPalm23-AW-A, Switzerland
8	Moisture analyzer	Mettler Toledo, India
9	Hunter lab, ColorFlex EZ	Hunter Associate Laboratory Inc., Reston
10	Scanning Electron Microscope	Carl Zeiss EVO-18, Germany
11	Oven	Bajaj OTG, 4500 TMCSS, Mumbai, India
12	Texture analyser	Lloyd instrument, AMTECK, TA1,
13	Malvern Zeta sizer	Zeta Nano-ZS; Malvern Instruments, UK
14	Conductivity meter	Labman- LMCM20, Scientific Instruments Pvt. Ltd. Chennai, India
15	Oakton pH 700	Benchtop Meter, Oakton Instruments, USA
16	FTIR-ATR spectrometer	Perkin Elmer, Spectrum Two, US
17	TG-DTA	Perkin Elmer, ST6000
18	Rheometer	Anton Paar GmbH, Ostfildern-Scharnhausen, Germany
19	Drop shape analyser	KRÜSS GmBH, Hamburg, Germany
20	Fluorescence microscope	Olympus IX83 , Olympus Corporation of the Americas, Center Vally, PA, USA
21	BET	Micromeritics Instrument Corporation, GA, USA)

ABSTRACT

Name of the student: Nayana.N	**Registration No.: 10BB18A39031**
Faculty of study: Biological Science	**Year of submission: 2022**
ACSIR academic centre/CSIR Lab: CSIR-NIIST	**Name of supervisor: Dr P. Nisha**
Title of the thesis: 'Encapsulation of Edible Lipids for Functional Food Applications: Process Development and Characterization'.	

The consumer preference for healthier, safe, shelf stable and easy to handle lipids offers opportunities for the researchers to develop innovative lipids. Blending and encapsulation are two methods which could be investigated for developing tailored fatty acid profile with improved stability, for nutritional/ functional food products. Convenience is one of the leading trends in the market and conversion of edible lipids in to encapsulated powder can offer more stable, easy to handle, transportant and convenient product. In the first experimental chapter, red palm olein which is an excellent natural source of β-carotene was blended with coconut oil (CNO), sunflower oil (SFO), rice bran oil (RBO), and olive oil (OL), in the ration of in the ratio 1:4, and the thermal, oxidative and storage stability and β-carotene has been evaluated. The blended oils demonstrated improved shelf and thermal stability. Application of oil in the form of microencapsulated powders has been less studied. Therefore, in the second chapter, encapsulation of vegetable oil blends with improved fatty acid composition as a carrier of oil soluble vitamins is attained by blending RPO and flax seed oil (FSO) in 70:30 ratios. The resulting encapsulates were characterized for water activity, bulk density, particle morphology, carotene content and encapsulation efficiency. The peroxide value (PV) and free fatty acids (FFA) were analyzed during shelf life studies. The carotene content of the blend was found to be 270 ppm. The microencapsulated oil was further used in the preparation of muffins to replace butter and it was found that while replacing 40 % of butter with encapsulated oil blend, 36.87 % of the recommended dietary allowance (RDA) for β- carotene was met from 100 g product. Further to this, in the final chapter, to address the convenience factor, powdered ghee for food applications were fabricated with whey protein as wall material. The key point of this study is that we could achieve an oil pay load up to 70 % (dry basis of encapsulates) which was not attained before. Encapsulation parameters were optimised based on the emulsion and spray drying trials. Process efficiency, product quality, yield, powder properties, morphology, thermal analysis, in vitro release studies, shelf life, product formulation and characterisation were done and the ghee powder showed better stability against oxidation and heat. The simulated treatments confirmed the optimal release behaviour, thus encapsulates could be used to deliver thermally stable bioactive compounds.

LIST OF PUBLICATIONS EMANATING FROM THE THESIS

Related to thesis published

- **Nayana N**, Mary Abraham L, Padma Ishwarya S, Nisha P. Spray-dried microcapsules of red palm olein-flaxseed oil blend: Development, physicochemical characterization, and evaluation of its potential applications as a fat replacer and β-carotene fortificant in cupcakes. *J Food Process Preserv*. 2021;00:e15663. https://doi.org/10.1111/jfpp.15663

Manuscript under preparation

- **Nayana N**, Nisha P. Development and characterisation of microencapsulated emulsion for food and health care application. *Food research international.* (Manuscript under preparation)

- **Nayana N**, Nisha P. Blending and encapsulation by spray drying as sustainable approaches to improve the oxidative stability of edible oils. *Food reviews international*. (Manuscript under preparation)

Published - Not related to thesis

- Chandran, J., **Nayana, N**., Roshini, N., & **Nisha, P**. (2017). Oxidative stability, thermal stability and acceptability of coconut oil flavoured with essential oils from black pepper and ginger. *Journal of food science and technology*, *54*(1), 144-152.

- Chandran, J., **Nayana, N**., & Nisha, P. (2019). Phenolics in Vegetable Oils. *Phenolic Compounds in Food*, 407–414. https://doi.org/10.1201/9781315120157-21

- Shakeela, Heeba, Mini, Navami M., Abraham, Billu, **N Nayana** and Nisha, Prakasan. "Influence of coating material and processing parameters on acrylamide formation in potato patties" International Journal of Food Engineering, vol. 18, no. 5, 2022, pp. 399-409. https://doi.org/10.1515/ijfe-2021-0337

LIST OF CONFERENCES

- Micro encapsulation of Red Palm Olein (RPO) and flax seed oil (FSO) blends for food enrichment applications: optimisation studies, **Nayana.N**, Litty Mary Abraham, Hari Krishnan R, and Nisha.P, International Conference on "Holistic Approaches for Startup, Food Innovation and Human Resource Training for Agriculture and Food Industry Gemmation" (2018), organized by AFSTI at **CSIR-CFTRI, Mysore**. (Poster presentation).

- Development of functional soup by replacing corn starch with that from indigenous sources: Optimization studies and functional properties, **Nayana .N**, Sharon Mariam Jacob, P. Nisha, International Conference on "Food and Nutrition Challenges: Role of Food Science and Technology", 26th ICFoST, (2017), organized by AFSTI at IICT, Hyderabad. (Poster presentation).

- "Ancient grains as source of prebiotic dietary fibre", **Nayana .N**, Sithara Thomas, P. Nisha, "International Conference on Food Value Chain: Innovations and Challenges", (2016), NIFTEM. (Poster presentation).

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Spray-dried microcapsules of red palm olein-flaxseed oil blend: Development, physicochemical characterization, and evaluation of its potential applications as a fat replacer and β-carotene fortificant in cupcakes

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Abstract

There is increasing interest in the blending of edible vegetable oils containing essential fatty acids and natural vitamins. The present study aims to develop spray-dried microencapsulates of a blend of red palm oil and flaxseed oil. The wall material composition and inlet temperature of spray drying were varied to maximize the encapsulation efficiency. Among the different trials, maximum encapsulation efficiency was achieved at a core-to-wall ratio of 1:2, an oil payload of 34%, and an inlet temperature of 180°C. The encapsulates were characterized for water activity, bulk density, particle morphology, carotene content, and encapsulation efficiency. To evaluate the potential of spray-dried oil encapsulate as a fat replacer, it was incorporated in cupcake formulation at 40% replacement of butter. The spray-dried oil encapsulates retained more than 70% of β-carotene. Even at a higher oil payload, the spray-dried microcapsules showed good flowability and low moisture content (2.60%). The test cupcake met 36.87% of the recommended dietary allowance for β-carotene. Thus, this study established the potential of spray-dried oil microcapsules as a fat replacer and β-carotene fortificant in cupcakes.

Practical applications

The blending of edible oils is considered the least expensive approach for attaining the balanced fatty acid composition. Encapsulation increases the shelf life and protects the core component. Red palm oil (RPO) is a minimally processed oil that retains most natural carotenoids, and flaxseed oil (FSO) is rich in its unsaturated fatty acid composition. Thus, the present study aims to use the RPO-FSO encapsulate as a β-carotene fortificant.

1 | INTRODUCTION

Tailoring the balance of fatty acids in edible oils and transforming them into various household and industrial applications are gaining more interest. Deficiency due to the lack of balanced fatty acids in the diet, especially polyunsaturated fatty acids (PUFA), is one of the significant nutritional problems globally (Karthik & Anandharamakrishnan, 2013; Menina et al., 2018). According to the Food and Nutrition Board's Committee on diet and health, the recommended ratio of SFA (saturated fatty acid)/MUFA (monounsaturated fatty acid)/PUFA (PUFA) in edible oils is 1/2/1 and that of omega-3/omega-6 is 0.05 (National Research Council, 1989). The blending of edible oils is considered the least expensive approach that results in desired fatty acid composition (Guiotto et al., 2014; Srivastava et al., 2016). For instance, palm oil blending with canola and olive oil has been found to enhance the essential fatty acid

composition (Roiaini et al., 2015). Palm oil is known for its saturated fatty acid composition, which gives good stability during frying and storage (Hashempour-Baltork et al., 2016).

Red palm oil (RPO) is a minimally processed palm oil that naturally contains tocopherols and tocotrienols (500–1,000 ppm) and carotenoids (500–700 ppm) (Lee et al., 2018; Mba et al., 2015). Its primary constituent fatty acids are palmitic acid (42%) and oleic acid (42%). Despite its various benefits, RPO is deficient in PUFA. Flaxseed oil (FSO) has gained wide acceptability as a vegan source of essential fatty acids, alpha-linolenic acid (50%–60%), vitamin E (tocopherols ranging from 20 to 70 mg/100 g), and vitamin A (carotenoids: ~57 ppm) (Goyal et al., 2015; Mohanan et al., 2018). Therefore, the blending of RPO and FSO in appropriate proportions could result in a product with improved fatty acid composition and enriched β-carotene content. However, PUFA is susceptible to oxidative degradation due to its high degree of unsaturation (Ramadan & Wahdan, 2012).

Using the microencapsulation approach, edible oils can be powdered using appropriate techniques to improve handling. Moreover, encapsulation promotes stability of oils and fats through long period of storage, besides facilitating easy transport. Therefore, converting oil to powder can be an innovative and consumer-friendly approach to improve the nutritional and oxidative stability of oils and to widen their applications in the culinary/health/functional food and nutraceutical sectors (Campos et al., 2019). Microencapsulation of RPO has been reported to improve nutrient availability in piglets (Ren et al., 2020). FSO has been successfully microencapsulated by spray drying (SD) using different wall materials. The resultant oil encapsulates exhibited good dissolution and reconstitution behavior and good storage stability at room temperature (Goyal et al., 2015; Sharif et al., 2017). However, there are no reports on the microencapsulation of RPO and FSO blends until now.

Various encapsulation techniques have been employed for converting oils into powder form by utilizing a wide range of carrier or wall materials. The commonly used microencapsulation techniques include emulsification, SD, and freeze drying (Liu et al., 2004). Among the above, SD is considered as an industry-friendly drying-cum-encapsulation technique. SD is a practical approach to improve the oxidative stability of oils (Carneiro et al., 2013; Priol et al., 2019). Choosing an ideal wall material is the key to achieve optimal encapsulation efficiency; the general traits expected of a wall material include, but are not limited to, bland flavor, high solubility, emulsification ability, film-forming, and drying properties. Diverse wall materials have been used for encapsulating oils, including gums (gum Arabic, xanthan), proteins (whey protein, soy protein), polysaccharides, and modified starches (Leyva et al., 2018). Among the hydrolyzed starch-based wall materials, maltodextrin was used extensively by different authors for the encapsulation process. However, maltodextrin lacks emulsifying capacity and thereby leads to poor emulsion stability and lower retention of volatiles and oils (Kenyon, 1995). Consequently, use of maltodextrin as wall material for the encapsulation of lipids often demands an additional emulsifying agent.

In the above context, whey protein is a type of wall material which exhibits good encapsulation properties due to its gelling behavior and ability to entrap both volatile and nonvolatile core substances (Edrisi Sormoli & Langrish, 2016; Gad et al., 2011). Gum Arabic exhibits heat-resistant properties (Bucurescu et al., 2018; Salar-Behzadi et al., 2013). The protective effect of gum Arabic has been attributed to the stabilization of the phospholipid membrane by hydrogen bonding (Anandharamakrishnan & Padma Ishwarya, 2015). Thus, the aim of present work is to microencapsulate the RPO-FSO blend by SD using different combinations of whey protein and gum Arabic as wall materials. The resultant product is expected to have enhanced shelf-stability, in addition to an improved fatty acid profile and enriched β-carotene content. The physical and structural properties of the developed RPO-FSO microencapsulate were evaluated besides its encapsulation efficiency and β-carotene content. Further, the potential applications of the resultant oil encapsulate as a healthy fat substitute and β-carotene fortificant were assessed, using cupcakes as the model food product.

2 | MATERIALS AND METHODS

2.1 | Materials

Crude palm oil was procured from M/s Godrej Industries, Tamil Nadu. The RPO used in this study was produced from crude palm oil, using the minimal processing technology developed by CSIR-NIIST, Thiruvananthapuram, India (Mayamol et al., 2009). FSO was purchased from the local market in Thiruvananthapuram. Whey protein isolate (WPI, >95% purity and having a molecular weight of 18.3 kDa) was obtained from ACE International LLP, New Delhi, India. Gum Arabic, xanthan gum, and guar gum were bought from Premia Food Additives, Mumbai, India. Sodium bicarbonate, sodium hydroxide, anhydrous sodium sulfate, potassium iodide (>99.5% purity), and solvents including hexane, methanol, glacial acetic acid, and diethyl ether (>99.9% purity) were procured from Merck Limited, Mumbai, India. All the chemicals were of analytical grade. The ingredients for the preparation of cupcakes, including self-rising flour, sugar, butter, and eggs, were procured from the local market of Thiruvananthapuram.

2.2 | Methods

2.2.1 | Preparation and characterization of blends

The RPO and FSO were blended in two different ratios, 70:30 and 60:40, to obtain blended oil with an improved fatty acid profile. The RPO and FSO were mixed thoroughly using an overhead stirrer (Remi, RQ126/D, with 40V, Mumbai, India), based on the procedure of Guiotto et al. (2014), with slight modifications. GC-MS analysis of the blended oil was performed to confirm the fatty acid composition.

2.2.2 | Fatty acid profiling

For the characterization, fatty acids were subjected to esterification to form fatty acid methyl esters (FAMEs) (AOCS, 2017; Method Ce 2-66). Briefly, 1 ml of 2% methanolic sulphuric acid was added to 0.5 g of oil and refluxed at 55°C for 3 hr. Thinlayer chromatography of the sample was done using hexane, diethyl esters, and glacial acetic acid in the ratio 80:20:1, ensuring the completion of methylation. Proper methylation is indicated by the formation of a single band of methyl ester below the solvent front. After refluxing, the cooled oil sample was washed with 2-ml hexane two to three times, then with 2% sodium bicarbonate for two to three times, and finally with water. The aqueous layer was then drained off, and the top layer was filtered through cotton, which contained anhydrous sodium sulfite. The FAMEs were then analyzed using GC-MS/MS (GC-MS/MS, Model: Thermo scientific TRACE 1310/TSQ 8000) equipped with TR-FAME (Thermoscientific) 30 m × 0.25 mm × 0.25 μm capillary column. The carrier gas was helium at a flow rate of 1.5 ml/min. The initial column temperature was set at 140°C with a holding time of 4 min. Subsequently, the temperature was increased up to 230°C at the rate of 4°C/min, followed by a holding time of 20 min. MS (TSQ 8000) with triple quadrupole was used for the identification of FAME. Qualitative analysis of each sample was performed using the NIST library by comparing with standard curves derived for each of the major fatty acids under investigation.

2.3 | Emulsion preparation

Oil-in-water emulsions of the RPO-FSO blends (core) were prepared before encapsulation by SD. WPI and gum Arabic (GA) at different proportions were used as the wall material (Table 1). Both WPI and GA play a dual role as drying aid as well as surfactant/emulsifier (Chuyen et al., 2019; Kha et al., 2014; Madene et al., 2006). Preliminary trials were carried out to find out the most efficient combination of WPI and GA that resulted in maximum product yield (Table 2). The wall materials in the optimized proportions were entirely dispersed in the required amount of water, followed by oil addition. The core-to-wall ratio was fixed at 1:2. The mixture was homogenized using a rotor-stator homogenizer (T25 ULTRA-TURRAX digital; dispersing tool: S 25N - 8G; IKA India Private Limited, Bengaluru), at 5,000 rpm for 10 min at room temperature (28 ± 2°C). The emulsion thus obtained was immediately taken for SD.

TABLE 1 Effect of different compositions of gum arabic and whey protein isolate on the yield

Trails	Wall material	Composition (w/w)	Yield (%)
S1	GA:WPI	2:1	40.90 ± 0.12
S2	GA:WPI	1:1	55 ± 0.04
S3	GA:WPI	1:2	62.07 ± 0.10

Note: Significant at p value = 2.23×10^{-7}; $p \leq .05$.
Abbreviations: GA, gum Arabic; WPI, whey protein isolate.

2.4 | Microencapsulation by SD

Immediately after the emulsion preparation, conversion of emulsion to encapsulated powder was performed in a mini spray dryer (LABULTIMA, Process technologies Pvt Ltd., Mumbai, India, Model LU 228 ADVANCED), operating in a co-current configuration. A two-fluid nozzle having an orifice diameter of 1 mm was used for atomization. SD was carried out at various inlet and outlet air temperatures, at a constant feed flow rate and aspirator rate. The air pressure was held steady at 1.5 kg/cm^2. The emulsion was fed at 60°C into the main drying chamber at a feed flow rate of 1.2 ml/s, controlled by a peristaltic pump. And the airflow rate was set at 73 m^3/h (1.2 m^3/min). The samples of encapsulated oil encapsulate were stored in Duran bottles until further analysis.

2.5 | Characterization of spray-dried encapsulates

2.5.1 | Water activity (a_w)

A digital water activity meter (Rotronic HygroPalm23-AW-A, Switzerland) was used to measure the water activity of the spray-dried oil encapsulate. The sample was placed in a sample cup of 14-mm depth, completely covering the bottom of the cup. A sealed container was formed by placing the probe above the sample cup, and the value of water activity was recorded from the digital display of the water activity meter.

2.5.2 | Moisture content

The moisture content of the oil encapsulate was determined using a Moisture analyzer (HC 103 Mettler Toledo, India). About 0.5 g of oil encapsulate was placed in the analyzer, set at a temperature of 105°C until a constant weight was reached. The value of moisture content (in percentage, %) was recorded from the digital display of the moisture analyzer.

2.5.3 | Color analysis

The oil encapsulate color was determined using Hunter lab, ColorFlex EZ (Hunter Associate Laboratory Inc., Reston) Port up, or Port forward dual-beam spectrophotometer. The results were expressed as Hunter color values of L^*, a^*, b^*, where L denotes lightness/ darkness, a^* denotes redness and greenness, and b^* denotes yellowness and blueness. The total color difference or change in color between the two samples was calculated using the below formula.

$$\Delta E = \sqrt{\left(L_o^* - L^*\right)^2 + \left(a_o^* - a^*\right)^2 + \left(b_o^* - b^*\right)^2} \quad (1)$$

TABLE 2 Influence of wall material composition on the yield and encapsulation efficiency of spray-dried oil encapsulates

Trail	Wall material	The proportion of wall materials (w/w)	Core-to-wall ratio	Temperature of spray-drying (inlet/outlet) (°C)	[a]Encapsulation efficiency (%)	[b]Yield %
1	MD+WPI	1:2	1:2	181/75 ± 5	12.5 ± 0.21	20.0 ± 0.14
2	MD+GA	1:2	1:2	181/80 ± 5	14.2 ± 0.10	15.0 ± 0.07
3	MD+GA+WPI	1:2:1	1:2	180/75 ± 5	18.4 ± 0.04	32.1 ± 0.14
4	GA+WPI	2:1	1:2	181/80 ± 5	6.8 ± 0.50	40.9 ± 0.12
5	GA+WPI	1:1	1:2	181/80 ± 5	20.0 ± 0.021	55.0 ± 0.04
6	GA+WPI	1:2	1:2	181/85 ± 5	40.0 ± 0.04	62.1 ± 0.10

Abbreviations: GA, gum Arabic; MD, maltodextrin; WPI, whey protein isolate.

[a]Significant at p value = 1.52×10^{-10}; $p \leq .05$.

[b]Significant at p value = 2.23×10^{-7}; $p \leq .05$.

TABLE 3 Comparison of results obtained by changing the proportion of GA: WPI

Sample	GA: WPI (wt/wt)	[a]Bulk density (g/ml)	[b]Tapped density (g/ml)	Flowability	[c]Total colour change	[d]Moisture %	[e]Water activity
S2	1:1	0.33 ± 0.21	0.38 ± 0.14	Good	73.23 ± 1.10	1.90 ± 0.04	0.35 ± 0.00
S3	1:2	0.33 ± 0.12	0.38 ± 0.32	Good	73.96 ± 0.40	2.60 ± 0.16	0.46 ± 0.01

Abbreviations: GA, gum Arabic; WPI, whey protein isolate.

[a]Significant at p value = .97; $p \geq .05$.

[b]Significant at p value = 1.00; $p \geq .05$.

[c]Significant at p value = 1.2×10^{-7}; $p \leq .05$.

[d]Significant at p value = .02; $p \leq .05$.

[e]Significant at p value = .01; $p \leq .05$.

where L_o^*, a_o^*, and b_o^* are the color values of the standard reference type and L^*, a^*, and b^* are the color values of the test sample (oil encapsulate) (Table 3).

2.5.4 | Bulk density

Bulk density (g/ml) was determined by the method proposed by Goula et al. (2007), with slight modifications. About 2 g of oil encapsulate was gradually added into an empty 10-ml graduated cylinder. The value of Bulk density was calculated as the volume occupied by the mass of the powder added to the cylinder (Equation 2).

$$\text{Bulk density} = \frac{\text{Mass of the powder}}{\text{volume of the powder}} \quad (2)$$

2.5.5 | Tapped density

The tapped density of the sample was determined by Chinta et al. (2009) with some modifications. About 2 g of oil encapsulate was gradually added into an empty 10-ml graduated cylinder. After tapping the cylinder for ten times, the volume occupied by the sample was noted. The tapped density was calculated using the below equation.

$$\text{Tapped density} = \frac{\text{Mass of the powder}}{\text{volume of the powder after tapping}} \quad (3)$$

2.5.6 | Flowability

Flowability was evaluated in terms of the Carr compressibility index (CI) and Hausner ratio (HR) (Fitzpatrick et al., 2004), which were calculated from the bulk and tapped densities of the powders using the following equations:

$$\text{HR} = \frac{\rho_T}{\rho_B} \quad (4)$$

$$\text{CI} = \left(\frac{\rho_T - \rho_B}{\rho_T}\right) \times 10 \quad (5)$$

where ρ_T and ρ_B are tapped and bulk density, respectively. Table 4 presents the correlation between the values of the Carr index, Hausner ratio, and powder flowability.

2.5.7 | Particle (true) density

For the determination of particle density, approximately 1 g (m_0) of the sample was filled in a burette containing toluene. The rise in toluene level (V_1) was measured and calculated as true particle density. Toluene was used because of its ability to penetrate the finest external pores connected to the surface without dissolving the material (Premi & Sharma, 2017).

$$\text{Particle density} = \left(\frac{m_0}{V_1}\right) \quad (6)$$

TABLE 4 Correlation between powder flowability, Hausner ratio and Carr index (Turchiuli et al., 2005)

Carr's index	Flowability	Hausner ratio
≤10	Excellent	1.00–1.11
11.0–15.0	Good	1.12–1.18
16–20	Fair	1.19–1.25
21–25	Passable	1.26–1.34
26–31	Poor	1.35–1.45
32–37	Very poor	1.46–1.59
>38	Awful	>1.60

2.5.8 | Product yield

The product yield was calculated as the ratio of the amount of powder collected after every SD experiment to the initial amount of solids in the feed solution. The dry weight of the oil encapsulate was obtained by deducting the weight of residual moisture present in it, which was estimated using the moisture analyzer. Then, the product yield was calculated using the below expression.

$$Y = \frac{(W_2 - W_1) - X_{wb}(W_2 - W_1)}{F_v T_s} \times 100 \quad (7)$$

where Y is the powder yield (%), X_{wb} is the moisture content (w.b), F_v is the feed volume (ml), T_s is the total solid content (mg/L), and W_1 and W_2 are the weight of the powder bottle before and after SD (g), respectively.

2.5.9 | Solubility

Solubility is expressed as the percentage of dried supernatant with the amount of powder originally added (Chew et al., 2018). Solubility was determined according to the methods of Eastman and Moore (1984) and Cano-Chauca et al. (2005), with some modifications. About 100 ml of distilled water was transferred into a blender jar. The powder sample (1 g, dry basis) was carefully added into the blender operating at high velocity for 5 min. The solution was placed in a tube and centrifuged at 3,000× *g* for 5 min. An aliquot of 25 ml of the supernatant was transferred to preweighed Petri dishes and immediately oven-dried at 105°C for 5 hr. Then the solubility (%) was calculated by weight difference.

2.5.10 | Wettability

Wettability is expressed as time in seconds, necessary for a given amount of powder to penetrate the quiet surface of the water, that is, the ability of a powder to absorb water on the surface and become wet. The wettability test was carried out according to the procedure proposed by Fuchs et al. (2006). A powder sample of 0.1 g was sprinkled over 100-ml distilled water at 20°C without agitation. The time (in seconds) taken for all the powder particles to submerge was recorded as wettability.

2.5.11 | Determination of carotenoid content of microencapsulated oil encapsulate

About 0.5–1 g of oil was weighed into a 25 ml volumetric flask. The oil was dissolved in hexane and made up to the mark. The absorbance of the oil solution was recorded at 446 nm in a spectrophotometer (BS 684, section 2.2:1977; Dian et al., 1996).

$$\text{Carotene} = \frac{V \times 383 \times (A_s - A_b)}{1,000 \times W} \quad (8)$$

where V is the volume of oil made up with hexane (ml), W is the weight of the sample (g), A_s is the sample absorbance, and A_b is the blank absorbance.

2.5.12 | Microencapsulation efficiency

Total oil content in microcapsules was quantified using the AOAC Official Method 925.32 (2012). Briefly, 1 g of powder was transferred to a fat-extraction tube, and 10 ml HCl was added slowly. The tubes were kept in a boiling water bath. After cooling to room temperature (25°C), 25 ml of ethyl ether and 25 ml of petroleum ether were added, and the tubes were vigorously shaken for a minute. The ether solution (supernatant) was separated and filtered through packed cotton. The remaining aqueous phase was further extracted twice with 15 ml of ethyl ether and 15 ml of petroleum ether. The solvent was evaporated in a rotary evaporator (Hei-VAP-Value Digital (G3), Heidolph Instruments, Schwabach, Germany), and the oil was dried in a vacuum oven at 100°C to constant weight. Extractable oil, usually referred to as surface oil (EO), was determined according to the methodology of Davidov-Pardo et al. (2008). This nonencapsulated oil is defined as the fraction that can be easily extracted with organic solvents without disrupting the solid matrix. Briefly, 4 g microcapsule powder was drip washed with 75 ml of ethyl ether for 15 min at 25°C. The suspension was filtered through a Whatman No. 1 filter paper, and the powder on the filter was rinsed three times with ethyl ether. The solvent was dried and rota-evaporated to obtain the surface oil mass. Encapsulation efficiency (EE %) was calculated from the following equation:

$$\text{EE}\,\% = \left\{\frac{\text{TO} - \text{EO}}{\text{TO}}\right\} 100 \quad (9)$$

where TO is the total oil content in microcapsules and EO is the extractable oil content determined as previously described.

2.5.13 | Scanning electron microscopy (SEM)

Morphology and particle size of spray-dried oil encapsulate were determined using a Scanning Electron Microscope (Carl Zeiss EVO-18, Germany) and using a software Digimizer Version 4.6.1 (Digimizer Version 4.6.1, Copyright © 2005–2016, MedCalc Software, Belgium; pixel per mm of the image was 9.7 units). For SEM, the samples were transferred to a cryo-preparation chamber, which was maintained at a constant temperature of −10°C using a "Peltier-cooling" stage. The prepared samples were mounted on an aluminum stub using carbon tape. The samples were examined under vacuum using an accelerating beam at a voltage of 10 kV. The micrographs were recorded at a magnification of 1,000–3,000×.

2.5.14 | Chemical quality analysis of encapsulated and individual oils

The free fatty acid (FFA) content of the RPO and FSO samples, blended oils, and spray-dried encapsulates were estimated using AOCS Ca5a-40 (1989). Briefly, the sample (2 g) was dissolved in methanol (30 ml). The contents were boiled until the first bubble's appearance and titrated against 0.1 N standardized alkali using phenolphthalein as an indicator.

Peroxide value (PV) was estimated by following AOCS Cd8-53 (1998). Sample (2–5 g) was mixed with 30-ml acetic acid–chloroform reagent in a 250-ml glass stoppered flask and shaken until the sample was dissolved. Then, 0.5 ml of saturated potassium iodide solution was added to the above mixture and kept under dark conditions for a few minutes. After mixing with 30-ml distilled water, the mixture was titrated against standardized 0.1-N sodium thiosulphate using freshly prepared starch solution as an indicator. The endpoint is the disappearance of blue color. The PV of oils was expressed in units of milliequivalents of peroxide per kilogram oil. A blank sample was set up as reagent control.

The stability of oil in the microencapsulated powders was evaluated by extracting the oil from the encapsulated powders using the procedure described by Lee et al. (2018). The oil thus obtained was used for further analysis.

β-carotene estimation by spectrophotometric method

The β-carotene content of the RPO, FSO, and the spray-dried oil encapsulate was estimated using a spectrophotometer according to the method (BS 684, section 2.2:1977). The oil (0.5–1 g) was dissolved in hexane and made up to known volume. The absorbance was read at 446 nm using the spectrophotometer (Shimadzu UV-2600, Japan). The carotene content was calculated using Equation (9) and expressed in ppm. The encapsulate oil was extracted using the procedure described by Lee at al. (2018). About 20 ml of aqueous ethanol (85%) was added to 2 g of the sample, followed by the addition of 50 ml of petroleum ether. The samples were then stirred using a magnetic stirrer at 1,200 rpm (IKA, RCT basic, 220 V) for 30 min. After 30 min of stirring, the top layer that separated was carefully decanted into a tared round bottom flask. Petroleum ether (5 ml) was used to re-extract the remaining ethanol solution, and the procedure was repeated until the yellowish RPO microcapsules turned white. The oil content was calculated after solvent evaporation. And this oil was further used for the determination of β-carotene content, using the methodology described in Section 2.5.12.

2.5.15 | Product formulation and characterization

To evaluate the potential of the spray-dried oil encapsulate as a replacer of hard stock fat (e.g., butter), product development studies were carried out by replacing 40% of butter in the formulation of cupcakes with the oil encapsulate. The control and test cupcakes were prepared using the formulations given in Table 5. The ingredients were then mixed and blended using an electric whisk until a creamy consistency was attained. The batter was transferred into the cupcake mold and baked in a preheated oven (Bajaj OTG, 4500 TMCSS, Mumbai, India) for 12 min at 180°C until the crumb turned golden brown. The product was cooled at room temperature for 3 hr. The sensory and texture characteristics of the control and test cupcakes were compared.

2.5.16 | Cupcake characterization

Sensory evaluation

A hedonic test was performed to determine the acceptability and any significant differences in the sensory attributes between the control and test cupcakes. Care was taken to avoid interference from other sources. The samples were presented to ten semi-trained panelists familiar with the techniques of sensory analysis. They were asked to score the product for appearance, color, texture, taste, and overall acceptability with a scale representing quality grade description given in Table 6.

Color and texture profile analysis

The color of the control and test cupcakes was determined using the methodology mentioned in Section 2.5.3. A texture analyzer

TABLE 5 Ingredient composition of cupcakes

Ingredients	T1	T2 (40% replacement with oil powder)
Flour	100 g	100 g
Sugar	75 g	75 g
Butter	64 g	39 g
Oil powder	Nil	25.6 g
Vanilla essence	2.5 g	2.5 g
Egg	2 no.s	2 no.s
Salt	To taste	To taste
Baking powder	5 g	5 g

TABLE 6 Hedonic scale grade description

Preference	Grade
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

equipped with a 50 N load cell (TA1, AMTECK, Lloyd instrument) was used to determine the texture profile of cupcakes. The force required to compress cupcakes by 50% was measured with a rounded bottom stainless steel probe at a speed of 10 mm/s. Texture measurements were performed in triplicates for each sample, and the mean values were reported. Before the test, the sample was placed centrally under the probe to avoid irregular areas of the crust regions. The Nexygen MT software program was used to quantify the parameters of interest in this work: hardness (N), cohesiveness (TPA), springiness, chewiness (N), adhesiveness (TPA), and gumminess (N).

2.6 | Statistical analysis

All the measurements were performed in triplicates, and the results are expressed as mean ± standard deviation. The significance of the difference between the means of all the parameters was examined by the one-way analysis of variance (ANOVA) at a confidence level of 95%, using the EXCEL 2010 (Microsoft, USA).

3 | RESULTS AND DISCUSSION

3.1 | Quality analysis of red palm olein, FSO, and their blends

Initially, the quality of RPO and FSO was assessed in terms of their FFA content, PV, and β-carotene content. The FFA content of RPO and FSO was found to be 0.647 ± 0.04% and 0.740 ± 0.18%, respectively, and the corresponding PVs were 2.75 ± 0.636 and 1.432 ± 1.04 millieq/kg. These values are in alignment with the values reported by Teh and Birch (2013) (2.04 millieq/g) and Domian et al. (2017) (1.45 millieq/g). PV of oil is an indication of the amount of hydroperoxides present in it. Hydroperoxides are compounds that arise from lipid oxidation. Hence, PV is a measure of oil quality. Generally, PVs of fresh oils are less than 10 millieq/kg, and the rancid taste is noticeable in oils with PV ranging between 30 and 40 millieq/kg (Choe & Min, 2006; Kamsiah et al., 2012). Thus, in this study, the freshness of both RPO and FSO is confirmed as their PVs was less than 10 millieq/kg.

β-carotene content of the RPO and FSO were estimated to be 390 ± 0.08 and 34.4 ± 0.02 ppm, respectively. Earlier studies have also reported a β-carotene content of 370–700 ppm in RPO (Lau et al., 2008; Manorama & Rukmini, 1992; Mba et al., 2015). Further, the carotene content of the blend was found to be 270.029 ± 0.12 ppm.

Based on the published literature, the fatty acid profile of individual oils and the value obtained with GC-MS/MS (Figure 1) for blended oils are provided in Table 7. Notably, there was a considerable improvement in the α-linolenic acid content of blended oil. Moreover, the blended oil had an omega-3 to omega-6 ratio of 0.61. This is relevant as the RPO is inherently devoid of PUFA. Thus, blending of RPO and FSO in the ratio of 70:30 showed a balanced fatty acid profile with enhanced content of PUFA, compared to pure oils.

3.2 | Optimization of wall material composition and SD conditions for the microencapsulation of RPO-FSO blend

The inlet temperature of SD is reported to play a major role in determining the end product's quality (Anandharamakrishnan & Padma Ishwarya, 2015; Koç et al., 2015). In this study, an inlet/outlet temperature combination of 180 ± 5/80 ± 5°C was selected for the microencapsulation of the RPO-FSO blend. This inlet temperature is within the range of 160–200°C reported in earlier investigations on the SD encapsulation of highly unsaturated oils (Carneiro et al., 2013; Davidov-Pardo et al., 2008; Tonon et al., 2011; Wen et al., 2017).

To determine the best effective combination of wall materials, the spray-dried RPO-FSO encapsulates were prepared using different proportions of GA, whey protein, and maltodextrin (Table 2), at a constant oil payload of 34%. Product yield and encapsulation efficiency were determined for each trial. Based on the results, WPI and GA in the ratio of 1:2 were selected as the wall material for further studies, as this combination resulted in maximum product yield (62.07%) (Table 1) and highest encapsulation efficiency (40%) among all the other wall material compositions (Table 2). The superiority of WPI and GA as encapsulating agents for oils has been demonstrated in several studies. For instance, Kha et al. (2014) encapsulated Gac oil using a combination of GA and WPI as wall materials. The efficiency of WPI has been attributed to its skin-forming behavior that results in a particle surface without any pores and cracks after SD. The antioxidant activity of WPI (Gad et al., 2011) is an added advantage of using it as a wall material for the SD encapsulation of unsaturated oils (Ramakrishnan et al., 2014).

Similarly, GA's effectiveness for oil encapsulation is due to its excellent emulsifying property, viscoelastic film-forming ability, high solubility, and low viscosity in aqueous systems (Matsumura et al., 2000). Generally, proteins are not easily soluble in water; therefore, a common approach is to combine the proteins and gums to utilize their emulsifying, film-forming/matrix-forming ability (Labuschagne, 2018). Sunflower oil protected by WPI and

Library Search Report

Data File:	70one	Original Data Path:	C:\Xcalibur\Data
Current Data Path:	C:\Xcalibur\Data	Sample Type:	Unknown
Sample ID:	1	Sample Name:	
Operator:	Admin	Acquisition Date:	03/12/18 12:47:11 PM
Run Time(min):	45.61	Comments:	
Vial:	43	Scans:	13411
Low Mass(m/z):	50.00000	High Mass(m/z):	750.02199
Sample Weight:	0.00	ISTD Amount:	0.000
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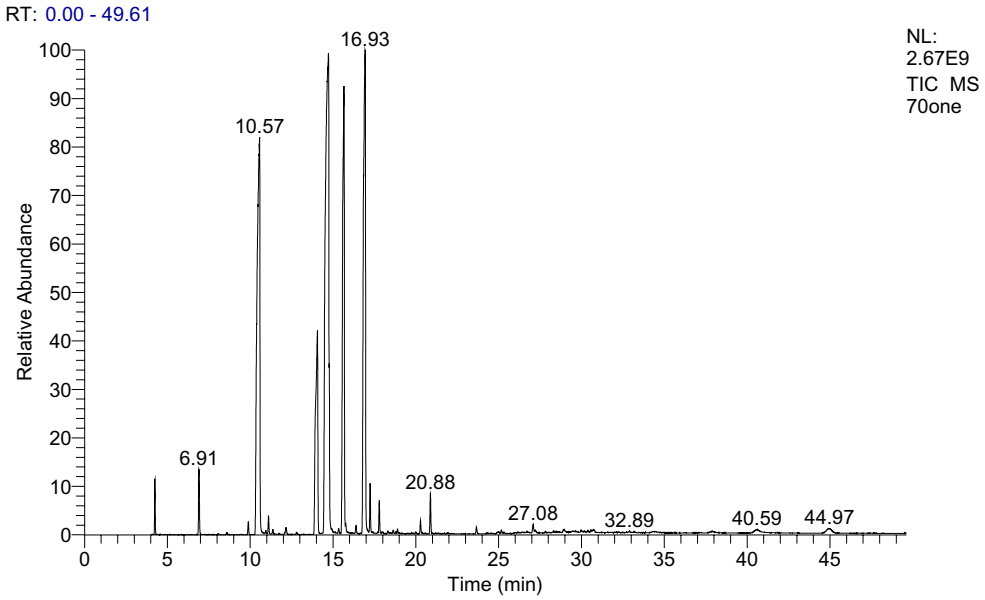

FIGURE 1 GC MS/MS profile of blended oil (red palm oil [RPO] + flaxseed oil [FSO])

TABLE 7 Fatty acid composition of individual and blended oils

Sl. no	Fatty acid composition	[a]Percentage of fatty acids in RPO (red palm oil)	[b]Percentage of fatty acids in FSO (flaxseed oil)	Percentage of fatty acids in RPO-FSO blends by GC-MS/MS
1	Palmitic acid	42	6	21.57
2	Stearic acid	5	2.5	8.61
3	Oleic acid	42	19	30.95
4	Linoleic acid	10	24.1	14.05
5	Alpha-linolenic acid	–	47.4	8.61

[a]Montoya (2014).

[b]Bayrak et al. (2010).

maltodextrin depicted similar characteristics (Xu et al., 2013). In the present study, incorporation of a combination of GA with whey protein as wall material improved the barrier properties of the wall and this is because incorporation of polysaccharides in minor quantities will improve the encapsulation efficacy of whey protein (Anandharamakrishnan & Padma Ishwarya, 2015). However, WPI and GA, in combination with maltodextrin (MD), resulted in lower product yield and encapsulation efficiency. This may be because of maltodextrin's poor emulsification capacity, which led to low oil retention (Fernandesa et al., 2014).

From Table 1, it could also be noted that the yield increased with an increase in the proportion of WPI at a constant inlet temperature. When the ratio of GA: WPI was increased from 1:1 to 1:2, the product yield and encapsulation efficiency increased from 55% to 62% and 20 to 40%, respectively. However, the encapsulation efficiency was lower than that reported in previous studies. This may be due to the high atomization pressure (147.1 kPa) and high airflow rate (1.2 m^3/min) used in this study. High atomization pressure leads to smaller droplet size and thereby low moisture content (moisture in small droplets under rapid evaporation than in big ones, due to the

enhanced surface area of smaller droplets). Low moisture results in cracks on the surface of particles that can lower the encapsulation efficiency. Further, the drying air flow rate bears an inverse relationship with the encapsulation efficiency. Increasing aspiration rate offers more mass flow rate of drying air, which leads to higher moisture evaporation from feed droplets due to higher heat and mass transfer values (Aghbashlo et al., 2013; Carmona et al., 2018). Huang et al. (2014) also reported a decrease in the encapsulation efficiency of tilapia oil at atomization pressure greater than 100 kPa and airflow rate beyond 0.67 m^3/min.

The carotene content of the oil encapsulates from the S2 and S3 trials were 30.0 ± 0.12 and 84 ± 0.03 ppm, respectively. This corresponds to carotene retention of 74.5% and 77.8% after SD, relative to the carotene content of the oil blend before encapsulation. Therefore, the thermal stability of encapsulated β-carotene and the protective effect of the wall materials on the core is evident. The carotene retention thus obtained is in line with the studies of De Paz et al. (2012), who reported 70% of carotene retention. SD with carbohydrate and protein-based wall materials such as those used in this study has been reported to improve the stability of β-carotene (De Paz et al., 2012; Donhowe & Kong, 2014; Kha et al., 2014).

3.3 | Morphology of encapsulates

The SEM images of the oil encapsulate revealed spherical-shaped particles with surface dents (Figure 2). Based on digital image processing, the mean radius of S3 oil encapsulate produced at the inlet temperature of 180/80 ± 5°C was found to be 8.741 ± 3.551 μm and that of S2 oil encapsulate was 11.658 ± 0.553 μm. Encapsulate S3 showed more uniform and smooth surface with no fissures. As discussed in Section 3.2 on encapsulation efficiency, the aspirator flow rate and the feed flow rate play a major role in determining the particle morphology. An increase in concentration of whey protein and gum might have helped in the film formation and lowering the permeability of encapsulates to gases. Further, smooth surface morphology of S3 may be responsible for its low surface oil content and, thereby, high encapsulation efficiency (Hundre et al., 2015). As S3 showed an optimal morphology, and a higher encapsulation efficiency and yield, further physicochemical properties of the encapsulate were carried out only with S3.

3.4 | Powder properties

3.4.1 | Moisture content and water activity

Low moisture content and water activity of microcapsules are preferable as high moisture content accelerates the oxidation of fat and decreases the powder flow (Carneiro et al., 2013; Turchiuli et al., 2013). The moisture content and water activity of the oil encapsulate obtained in this study are given in Table 3. Water activity is a measure of the available water present in the microorganisms for their growth. Restriction of water content in foods will always lead to the possibility of a reduction in microbial spoilage and, consequently, to an increase in the shelf life of foods. Effect of water activity on conjugated linoleic acid's physical properties microencapsulated using different matrices showed that the ideal storage condition prevails at a water activity of 0.5 or below (Jimenez et al., 2009). Lipid oxidation is delayed in dried products having water activity in the range of 0.2–0.4 (Labuza, 1968). The value of moisture content (2.6%) and water activity (0.46) of encapsulate obtained in the study falls within the above range.

3.4.2 | Bulk density and tapped density

Density is an important parameter in powders when packed or stacked in bulk. By definition, density decreases as volume increases for a constant mass. Therefore, similar relationship between the bulk density of the powder and the diameter of the particles is expected. The bulk density of the oil encapsulate was 0.335 ± 0.12 g/ml (Table 3). The bulk density of spray-dried FSO encapsulate produced using MD/GA and MD/WP as wall materials were 0.28 and 0.40 g/ml, respectively (Carniero et al., 2013). At 20% oil payload, Tonon et al. (2011) obtained FSO microcapsules with a bulk density of 0.303 and 0.473 g/ml, using whey protein concentrate and GA as wall material, respectively. In another study that used a combination of GA and WPI for Gac oil's encapsulation, the bulk density ranged from 0.24 to 0.33 g/ml (Kha et al., 2014). Thus, the value of bulk density obtained in this study is within the range reported by similar studies. The complementing film-forming property of GA and WP might have resulted in rapid crust formation around the oil core during the constant rate period of SD. As a result, air occlusion within

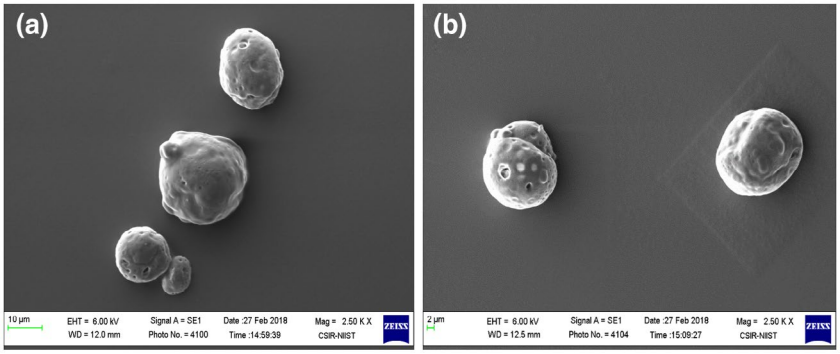

FIGURE 2 Scanning electron microscope (SEM) images of encapsulated powders S3 (a) and S2 (b)

the particles may have been restricted, leading to higher bulk density. A low amount of occluded air prevents lipid oxidation. Further, powders with higher bulk density are advantageous as they can be stored in large quantities in smaller packages, relative to products with lower densities (Carniero et al., 2013).

Tapped density indicates the weight and amount of powder that can fit in a container. The value of the tapped density of a powdered product is always higher than its bulk density (Chew et al., 2018). The above findings hold good in this study, as well. The oil encapsulates exhibited higher tapped density (0.385 ± 0.32 g/ml) than its bulk density (0.335 ± 0.12 g/ml). FSO encapsulate was prepared with whey protein as wall material showed tapped density in the range of 0.454–0.498 g/ml (Goyal et al., 2015). The reason for the low tapped density of encapsulate in this study can be attributed to its high bulk density due to the reasons explained above. When subjected to tapping, small particles roll between the particle voids and reach the densest packing condition (Ishwarya & Anandharamakrishnan, 2015). High bulk density of particles implies a low amount of occluded air (voids) between the particles (Carniero et al., 2013). This justifies the low tapped density of oil encapsulates observed in this study.

3.4.3 | Flowability

The flowability of powder was evaluated based on the Hausner ratio (HR) and Carr's compressibility index (Carr, 1965), which was calculated from the loose and tapped bulk densities of the encapsulate. In this study, the oil encapsulate showed an HR and CI of 1.149 and 12.987, respectively. The FSO encapsulated in whey protein concentrate at 35% oil load was reported to have an HR and CI of 1.55 and 33.82, respectively (Goyal et al., 2015). From Tables 3 and 4, it is apparent that the oil encapsulate obtained in this study exhibits superior flowability than that reported in the above study. The surface composition of the powder would affect the flowability so as to overcome the surface interactions among the particles (Chew et al., 2018). Thus, the low Hausner ratio of the oil encapsulates obtained in this study is indicative of its less cohesive nature and superior flowability (Table 4). From the results, it is evident that the low moisture content (discussed in Section 3.3.1), high inlet temperature, and the wall material composition played a major role to attain a good flowability of the encapsulate even at a high proportion of oil in the feed (34% on a dry basis with respect to the wall materials).

3.4.4 | Solubility

Microencapsulation enhances the solubility of oil in water (Mohammed et al., 2017). The solubility of the RPO-FSO oil encapsulate was found to be 60%. This value is either comparable to or higher than the solubility of spray-dried encapsulates reported in other studies. Fernandes et al. (2013) reported solubility in the range of 55.75% to 67.75% for rosemary essential oil encapsulated within GA. The solubility of encapsulates is strongly influenced by the wall material composition (Fernandes et al., 2013). Whey protein, which is present in a higher proportion in the wall material composition used in this study, is reported to impart better solubility (Goyal et al., 2015) but depending on whether it is in its native or denatured state. (Anandharamakrishnan et al., 2008; Pelegrine & Gasparetto, 2005). Anandharamakrishnan et al. (2008) reported that at inlet and outlet temperatures in the range of 160–190°C and 65–90°C, respectively, there is less chance of whey protein denaturation. As the inlet/outlet temperature used in this study falls within the range mentioned above, the high solubility of oil encapsulate is justified.

3.4.5 | Wettability

The wettability of microcapsules indicates their ability to absorb water related to the powder's reconstitution. Shorter the dissolution time in water, the better the physical attributes in food processing (Chew et al., 2018). The wettability varied from 543 to 550 s for the oil encapsulates (S3). The moisture content of particles (2.6%) at 180°C may be the reason for good wettability. Microencapsulated flax seed oil formulations prepared using WP as wall material showed similar dissolution behavior and were completely dissolved in less than 15 min (Goyal et al., 2015). GA is highly soluble in water due to its high number of hydrophilic hydroxyl groups. GA helps to increase the adherence capacity of water molecules on the surface of microcapsules, thus shortening the instantizing time through greater interaction with water (Edrisi Sormoli & Langrish, 2016; Fernandesa et al., 2014).

3.4.6 | Color

The color of any food product is affected by the ingredients used in its formulation. The color of encapsulated powder was measured using HUNTERLAB colorimeter and reported as L^*, a^*, and b^* values. The L^*, a^*, and b^* values of the encapsulates were 82.95 ± 0.08, 2.95 ± 0.008, and 33.37 ± 0.008, respectively. In this work, core material was a combination of two vegetable oil fractions, which contain FSO and RPO. Thus, it could be inferred from the data that the wall material significantly affects the encapsulated powder's color. The results of present work agree with the values reported in earlier studies: L^*, a^*, and b^* values for microencapsulated flax seed oil powder with whey protein were 88.60, 0.06, and 13.56 (Goyal et al., 2015) and fish oil encapsulate showed color values varying from $L^* = 78.01$ to 82.76, $a^* = 1.37$ to 2.54 and $b^* = 18.97$ to 24.23, respectively, when encapsulated with GA (Binsi et al., 2017).

3.5 | Spray-dried RPO-FSO microcapsules as a functional ingredient in cupcakes

Since the encapsulates are enriched with essential fatty acids and micronutrients, it was used as a fat replacer and β-carotene fortificant

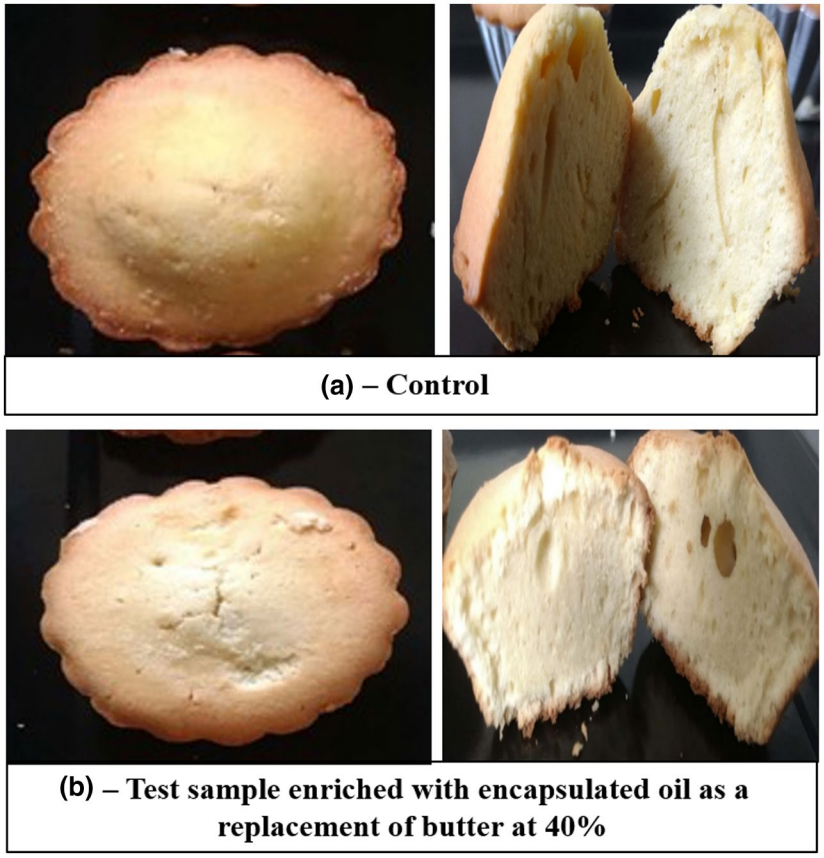

FIGURE 3 Photographs of cupcakes: (a) control and (b) test sample enriched with encapsulated oil as a replacement of butter at 40%

in cupcakes. The control cupcakes were prepared with butter as the source of fat, and the test cupcakes were formulated by replacing 40% of butter with the oil encapsulate shown in Figure 3a,b. The control cake's external appearance differed from that of the test cupcakes with a slightly shrunken structure. This observation may be attributed to the replacement of butter with oil encapsulate, reducing the moisture in the batter and releasings during baking. This may have suppressed the rise in dough volume, which may also occur from reduced batter stability.

The sensory evaluation results showed (Table 8) that there was no significant difference in the overall acceptability values between the control and test cupcakes. Although the oil encapsulate contained FSO known for its fishy smell due to its high PUFA content, the test cupcake containing the oil encapsulate was liked by panelists. This may be due to whey protein, which interacts with the compounds responsible for off-flavor compounds and masks the off-taste (Maehashi & Huang, 2009). Moreover, encapsulation by SD using gums and whey protein concentrate as wall materials has been demonstrated to attenuate off-taste. Indeed, enhanced palatability of bioactive compounds with unfavorable sensory characteristics is an important functional property of spray-dried encapsulates that permits their inclusion in different food products (Anandharamakrishnan & Padma Ishwarya, 2015).

The texture profile analysis results showed that the test cupcake samples exhibited similar textural quality as that of control, except for chewiness and springiness, which were significantly lower for the test sample (Table 9). The significantly reduced springiness and chewiness of the oil encapsulate-fortified cakes may be attributed to the weaker and less elastic structure of the cakes caused by the polyphenols present in the oil core (RPO and FSO). A similar effect of polyphenols on cake springiness and gumminess has been reported by Pasukamonset et al. (2018). Springiness is a measure of the elasticity, which is observed as the degree to which the sample recovers between the first and second compression during the texture profile analysis (Santhanam et al., 2014). Chewiness and springiness are directly related as the former are obtained by multiplying gumminess and springiness. And chewiness is the amount of energy required to disintegrate food for swallowing (Ghaboos &

TABLE 8 Sensory evaluation

Parameters	Control	Test cake
Appearance	8.5 ± 0.5	7.5 ± 0.5
Colour	8.1 ± 0.7	8.7 ± 0.4
Texture	8.5 ± 0.7	7.3 ± 0.4
Taste	8.8 ± 0.3	8.5 ± 0.5
Flavour	7.3 ± 0.4	8.7 ± 0.4
Softness	8.5 ± 0.5	8.3 ± 0.4
Overall acceptability	8.2 ± 0.4	8 ± 0.6

Note: Significant at p value = .01; $p \leq .05$.

TABLE 9 Texture profile analysis of cupcake samples

Texture parameters	Control cake	Test cake
Adhesiveness (TPA)	1.34 ± 0.02	1.13 ± 0.15
Cohesiveness (TPA)	0.51 ± 0.05	0.53 ± 0.003
Springiness (TPA) (mm)	17.71 ± 0.26	28.42 ± 0.29
Chewiness (N)	4.98 ± 0.51	0.55 ± 0.05
Hardness (N)	2.49 ± 0.05	2.37 ± 0.01
Springiness	3.29 ± 0.30	0.48 ± 0.01
Firmness (N)	2.58 ± 0.05	2.37 ± 0.01
Gumminess (N)	1.51 ± 0.146	1.15 ± 0.06

Note: Significant at p value = .01; $p \leq .05$.

Ardabili, 2018). Thus, the reduction of springiness and chewiness of test cakes is desirable.

Further, the results of TPA exhibited a good correlation with the inferences derived from sensory analysis. A study done by Santhanam et al. (2014) also revealed that fish oil encapsulates could be successfully used as a fortificant in cakes without much alteration in the textural and sensory qualities. The present study yet again confirmed that oil encapsulates can be easily incorporated in the formulation of baked foods without much alteration in the textural and sensory qualities.

The β-carotene content of the test cake was 208.32 ± 0.02 ppm. Therefore, consumption of one test cupcake (90 g) enriched with the RPO-FSO oil encapsulate at 40% replacement of butter (fat) will meet 36.87% of recommended dietary allowances (RDA) requirement of β-carotene proposed by the regulating agencies (FSS Act, 2019). According to this regulation, a person should gain 4,800 μg of β-carotene per day (ICMR, 2010). Thus, encapsulation of blended edible oil containing RPO: FSO as core ingredient can be opted as a good enrichment method of Vitamin A, especially β-carotene.

4 | CONCLUSIONS

Thus, this work demonstrated the possibility of offing a blend of red palm olein and FSO as core material for encapsulation by SD. The proportion of wall materials was found to exert a substantial influence on the spray-dried oil microcapsules' yield and entrapment efficiency. After microencapsulation, the oil encapsulates retained >70% of the carotene content of its blended-oil core. The use of GA and whey protein as wall materials for the encapsulation of oil enabled the incorporation of oil encapsulates in the cupcake formulation at 40% replacement of conventional fat (butter) with desirable sensory appeal and textural characteristics of the final product. Moreover, one-third of the RDA of β-carotene was met by replacing butter in the cupcake formulation with the oil encapsulate. Also, encapsulation mitigated the off-flavor typical of FSO and thereby retained the sensory quality of the cupcakes fortified with the oil encapsulate, on par with the control cakes. Hence, the potential application of the spray-dried encapsulate of RPO- FSO blend developed in this study is in formulating functional foods with nutraceutical benefits.

5 | ETHICAL REVIEW

This study does not involve any human or animal testing.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

N. Nayana: Formal analysis; Investigation; Methodology; Validation; Writing-original draft. **Litty Mary Abraham:** Formal analysis; Methodology. **S. Padma Ishwarya:** Methodology; Writing-review & editing. **P. Nisha:** Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Writing-review & editing.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

Aghbashlo, M., Mobli, H., Madadlou, A., & Rafiee, S. (2013). Influence of wall material and inlet drying air temperature on the microencapsulation of fish oil by spray drying. *Food and Bioprocess Technology*, *6*(6), 1561–1569. https://doi.org/10.1007/s11947-012-0796-7

Anandharamakrishnan, C., & Padma Ishwarya, S. (2015). Selection of wall material for encapsulation by spray drying. In C. Anandharamakrishnan & S. Padma Ishwarya (Eds.), *Spray drying techniques for food ingredient encapsulation* (pp. 77–100). John Willey & Sons Ltd.

Anandharamakrishnan, C., Rielly, C. D., & Stapley, A. G. F. Ã. (2008). Loss of solubility of a -lactalbumin and b -lactoglobulin during the spray drying of whey proteins. *LWT - Food Science and Technology.*, *41*, 270–277. https://doi.org/10.1016/j.lwt.2007.03.004

AOAC. (2012). *Approved methods of the Association of Official Analytical Chemists, 925.32 (fat in eggs. Acid hydrolysis method)* (19th ed.). Author.

AOCS. (1989). *Approved methods of the American Oil Chemists Society, Ca 5a-40* (4th ed.). Author.

AOCS. (1998). *Approved methods of the American Oil Chemists Society, Cd 8–53 (peroxide value)* (5th ed.). Author.

AOCS. (2017). *Approved methods of the American Oil Chemists Society, Ce 2–66 (preparation of methyl esters of fatty acids)* (7th ed.). Author.

Bayrak, A., Kiralan, M., Ipek, A., Arslan, N., Cosge, B., & Khawar, K. M. (2010). Fatty acid compositions of linseed (Linum Usitatissimum L.) genotypes of different origin cultivated in Turkey. *Biotechnology*

and Biotechnological Equipment, *24*(2), 1836–1842. https://doi.org/10.2478/V10133-010-0034-2

Binsi, P. K., Nayak, N., Sarkar, P. C., Jeyakumari, A., Muhamed Ashraf, P., Ninan, G., & Ravishankar, C. N. (2017). Structural and oxidative stabilization of spray dried fish oil microencapsulates with gum arabic and sage polyphenols: Characterization and release kinetics. *Food Chemistry*, *219*, 158–168. https://doi.org/10.1016/j.foodchem.2016.09.126

Bucurescu, A., Blaga, A. C., Estevinho, B. N., & Rocha, F. (2018). Microencapsulation of curcumin by a spray-drying technique using gum arabic as encapsulating agent and release studies. *Food and Bioprocess Technology*, *11*(10), 1795–1806. https://doi.org/10.1007/s11947-018-2140-3

Campos, J. R., Severino, P., Ferreira, C. S., Zielinska, A., Santini, A., Souto, S. B., & Souto, E. B. (2019). Linseed essential oil—source of lipids as active ingredients for pharmaceuticals and nutraceuticals. *Current Medicinal Chemistry*, *26*, 1–22. https://doi.org/10.2174/0929867325666181031105603

Cano-Chauca, M., Stringheta, P. C., Ramos, A. M., & Cal-Vidal, J. (2005). Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. *Innovative Food Science & Emerging Technologies*, *6*(4), 420–428. https://doi.org/10.1016/j.ifset.2005.05.003

Carmona, P. A. O., Garcia, L. C., Ribeiro, J. A. D. A., Valadares, L. F., Marçal, A. D. F., de França, L. F., & Mendonça, S. (2018). Effect of solids content and spray-drying operating conditions on the carotenoids microencapsulation from pressed palm fiber oil extracted with supercritical CO_2. *Food and Bioprocess Technology*, *11*(9), 1703–1718. https://doi.org/10.1007/s11947-018-2132-3

Carneiro, H. C. F., Tonon, R. V., Grosso, C. R. F., & Hubinger, M. D. (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *Journal of Food Engineering*, *115*, 443–451. https://doi.org/10.1016/j.jfoodeng.2012.03.033

Carr, R. L. (1965). Evaluating flow properties of solids. *Chemical Engineering Journal*, *72*, 163–168.

Chew, S. C., Tan, C. P., & Nyam, K. L. (2018). Microencapsulation of refined kenaf (*Hibiscus cannabinus* L.) seed oil by spray drying using β-cyclodextrin/gum Arabic/sodium caseinate. *Journal of Food Engineering*, *237*, 78–85. https://doi.org/10.1016/j.jfoodeng.2018.05.016

Chinta, D. D., Graves, R. A., Pamujula, S., Praetorius, N., Bostanian, L. A., & Mandal, T. K. (2009). Spray-dried chitosan as a direct compression tableting excipient. *Drug Development and Industrial Pharmacy*, *35*, 43–48. https://doi.org/10.1080/03639040802149053

Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive reviews in food science and food safety*, *5*(4), 169–186.

Chuyen, H. V., Roach, P. D., Golding, J. B., Parks, S. E., & Nguyen, M. H. (2019). Encapsulation of carotenoid-rich oil from Gac peel: Optimisation of the encapsulating process using a spray drier and the storage stability of encapsulated powder. *Powder Technology*, *344*, 373–379. https://doi.org/10.1016/j.powtec.2018.12.012

Davidov-Pardo, G., Roccia, P., Salgado, D., León, A. E., & Pedroza-Islas, R. (2008). Utilization of different wall materials to microencapsulate fish oil evaluation of its behavior in bread products. *American Journal of Food Technology*, *3*, 384–393. https://doi.org/10.3923/ajft.2008.384.393

De Paz, E., Martin, A., Estrella, A., Rodriguez-Rojo, S., Matias, A. A., Duarte, C. M. M., & Cocero, M. J. (2012). Formulation of β- carotene by precipitation from pressurized ethyl acetate-on water emulsions for application as natural colorant. *Food Hydrocolloids*, *26*(1), 17–27. https://doi.org/10.1016/j.foodhyd.2011.02.031

Determination of carotene in vegetable oils. (1977). *British standard Methods of Analysis – BS*, *684*, Section 2.20.

Dian, N. L. H. M., Sudin, N., & Yusoff, M. S. A. (1996). Characteristics of microencapsulated palm-based oil as affected by type of wall material. *Journal of the Science of Food and Agriculture*, *70*(4), 422–426. https://doi.org/10.1002/(SICI)1097-0010(199604)70:4<422:AID-JSFA514>3.0.CO;2-5

Domian, E., Cenkier, J., Górska, A., & Kopytowska, A. B. (2017). Effect of oil content and drying method on bulk properties and stability of powdered emulsions with OSA starch and linseed oil. *LWT- Food Science and Technology*, *88*, 95–102. https://doi.org/10.1016/j.lwt.2017.09.043

Donhowe, E. G., & Kong, F. (2014). Beta-carotene: Digestion, microencapsulation, and in vitro bioavailability. *Food and Bioprocess Technology*, *7*(2), 338–354. https://doi.org/10.1007/s11947-013-1244-z

Eastman, J. E., & Moore, C. O. (1984). *Cold water soluble granular starch for gelled food composition*. US. 4465702.

Edrisi Sormoli, M., & Langrish, T. A. G. (2016). Spray drying bioactive orange-peel extracts produced by soxhlet extraction: Use of WPI, antioxidant activity and moisture sorption isotherms. *LWT - Food Science and Technology*, *72*, 1–8. https://doi.org/10.1016/j.lwt.2016.04.033

Fernandes, R. V. D. B., Borges, S. V., & Botrel, D. A. (2013). Influence of spray drying operating conditions on microencapsulated rosemary essential oil properties. *Food Science and Technology*, *33*, 171–178.

Fernandesa, R. V. B., Marquesa, G. R., Borgesa, S. V., & Botrel, D. A. (2014). Effect of solids content and oil load on the microencapsulation process of rosemary essential oil. *Industrial Crops and Products*, *58*, 173–181. https://doi.org/10.1016/j.indcrop.2014.04.025

Fitzpatrick, J. J., Iqbal, T., Delaney, C., Twomey, T., & Keogh, M. K. (2004). Effect of powder properties and storage conditions on the flow ability of milk powders with different fat contents. *Journal of Food Engineering*, *64*, 435–444. https://doi.org/10.1016/j.jfoodeng.2003.11.011

Fuchs, M., Turchiuli, C., Bohin, M., Cuvelier, M. E., Ordonnaud, C., Maillard, M. N. P., & Dumoulin, E. (2006). Encapsulation of oil in powder using spray drying and fluidized bed agglomeration. *Journal of Food Engineering*, *75*, 27–35. https://doi.org/10.1016/j.jfoodeng.2005.03.047

Gad, A. S., Khadrawy, Y. A., Nekeety, A. A. E., Mohamed, S. R., Hassan, N. S., & Wahhab, M. A. A. (2011). Antioxidant activity and hepato protective effects of whey protein and Spirulina in rats. *Nutrition*, *27*, 582–589. https://doi.org/10.1016/j.nut.2010.04.002

Ghaboos, H., & Ardabili, S. (2018). Physico-chemical, textural and sensory evaluation of sponge cake supplemented with pumpkin flour. *International Food Research Journal*., *25*, 854–860.

Goula, A. M., Adamopoulos, K. G., & Kazakis, N. A. (2007). Influence of spray drying conditions on tomato powder properties. *Drying Technology: An International Journal*, *22*(5), 1129–1151. https://doi.org/10.1081/DRT-120038584

Goyal, A., Sharma, V., Sihag, M. K., Tomar, S. K., Arora, S., Sabikhi, L., & Singh, A. K. (2015). Development and physico-chemical characterization of microencapsulated flaxseed oil powder: A functional ingredient for omega-3 fortification. *Powder Technology*, *286*, 527–537. https://doi.org/10.1016/j.powtec.2015.08.050

Guiotto, N. E., Ixtaina, V. Y., Nolasco, S. M., & Thomas, M. C. (2014). Effect of storage conditions and antioxidants on the oxidative stability of sunflower–chia oil blends. *Journal of the American Oil Chemical Society*, *91*, 767–776. https://doi.org/10.1007/s11746-014-2410-9

Hashempour-Baltork, F., Torbati, M., Azadmard-Damirchi, S., & Savage, G. P. (2016). Vegetable oil blending: A review of physicochemical, nutritional and health effects. *Trends in Food Science & Technology*, *57*, 52–58. https://doi.org/10.1016/j.tifs.2016.09.007

Huang, H., Hao, S., Li, L., Yang, X., Cen, J., Lin, W., & Wei, Y. (2014). Influence of emulsion composition and spray-drying conditions on microencapsulation of tilapia oil. *Journal of Food Science and Technology*, *51*, 2148–2154.

Hundre, S. Y., Karthik, P., & Anandharamakrishnan, C. (2015). Effect of whey protein isolate and β-cyclodextrin wall systems on stability of microencapsulated vanillin by spray–freeze drying method. *Food Chemistry*. *174*, 16–24. https://doi.org/10.1016/j.foodchem.2014.11.016

ICMR nutrient requirements and RDA for Indians—A report of the Expert Group of the ICMR. (2010). https://www.nin.res.in/nutrition2020/RDA_short_report.pdf

Ishwarya, S. P., & Anandharamakrishnan, C. (2015). Spray-freeze-drying approach for soluble coffee processing and its effect on quality characteristics. *Journal of Food Engineering*, *149*, 171–180. https://doi.org/10.1016/j.jfoodeng.2014.10.011

Jimenez, M., Garcia, H. S., & Beristain, C. I. (2009). Effect of water activity on physical properties of conjugated linoleic acid (cla) microcapsules. *Journal of Food Process Engineering*, *33*, 434–447. https://doi.org/10.1111/j.1745-4530.2008.00283.x

Kamisah, Y., Shamil, S., Nabillah, M. J., Kong, S. Y., Hamizah, N. A. S., Qodriyah, M. S., Azlina, M. F. N., Azman, A., & Jaarin, K. (2012). Deep-fried keropok lekors increase oxidative instability in cooking oils. *Malaysian journal of medical sciences*, *19*, 57–62.

Karthik, P., & Anandharamakrishnan, C. (2013). Microencapsulation of docosahexaenoic acid by spray-freeze-drying method and comparison of its stability with spray-drying and freeze-drying methods. *Food and Bioprocess Technology*, *6*(10), 2780–2790. https://doi.org/10.1007/s11947-012-1024-1

Kenyon, M. M. (1995). Modified starch, maltodextrin and corn syrup solids as wall material for food encapsulation. In S. J. Risch & G. A. Fineccius (Eds.), *Encapsulation and controlled release of food ingredients* (pp. 43–50), ACS Symposium Series 590. American Chemical Society.

Kha, T. C., Nguyen, M. H., Roach, P. D., & Stathopoulos, C. E. (2014). Microencapsulation of gac oil by spray drying: Optimization of wall material concentration and oil load using response surface methodology. *Drying Technology*, *32*, 385–397. https://doi.org/10.1080/07373937.2013.829854

Koç, M., Güngör, Ö., Zungur, A., Yalçın, B., Selek, İ., Ertekin, F. K., & Ötles, S. (2015). Microencapsulation of extra virgin olive oil by spray drying: Effect of wall materials composition, process conditions, and emulsification method. *Food and Bioprocess Technology*, *8*(2), 301–318. https://doi.org/10.1007/s11947-014-1404-9

Labuschagne, P. (2018). Impact of wall material physicochemical characteristics on the stability of encapsulated phytochemicals: A review. *Food Research International*, *107*, 227–247. https://doi.org/10.1016/j.foodres.2018.02.026

Labuza, T. (1968). Sorption phenomena in foods. *Food Technology*, *22*, 15–24.

Lau, H. L. N., Choo, Y. M., Ma, A. N., & Chuah, C. H. (2008). Selective extraction of palm carotene and vitamin E from fresh palm-pressed mesocarp fiber (Elaeis guineensis) using supercritical CO2. *Journal of Food Engineering*, *84*(2), 289–296.

Lee, W. J., Tan, C. P., Sulaiman, R., Smith, R. L., & Chong, G. H. (2018). Microencapsulation of red palm oil as an oil-in-water emulsion with supercritical carbon dioxide solution-enhanced dispersion. *Journal of Food Engineering*, *222*, 100–109. https://doi.org/10.1016/j.jfoodeng.2017.11.011

Leyva, J. D. H., Perez, L. A. B., Acevedo, J. E. A., Ramirez, A., & Echeverry, L. M. J. (2018). Characterization of spray drying microencapsulation of almond oil into taro starch spherical aggregates. *LWT - Food Science and Technology*, *101*, 526–533. https://doi.org/10.1016/j.lwt.2018.11.079

Liu, Z. Q., Zhou, J. H., Zeng, Y. L., & Ouyang, X. I. (2004). The enhancement and encapsulation of *Agaricus bisporus* flavor. *Journal of Food Engineering.*, *65*, 391–396. https://doi.org/10.1016/j.jfoodeng.2004.01.038

Madene, A., Jacquot, M., Scher, J., & Desobry, S. (2006). Flavour encapsulation and controlled release - A review. *International Journal of Food Science and Technology*, *41*(1), 1–21. https://doi.org/10.1111/j.1365-2621.2005.00980.x

Maehashi, K., & Huang, L. (2009). Bitter peptides and bitter taste receptors. *Cellular and Molecular Life Sciences*, *66*, 1661–1671. https://doi.org/10.1007/s00018-009-8755-9

Manorama, R., & Rukmini, C. (1992). Crude palm oil as a source of beta-carotene. *Nutrition Research*, *12*, S223–S232. https://doi.org/10.1016/s0271-5317(05)80466-x

Matsumura, Y., Satake, C., Egami, M., & Mori, T. (2000). Interaction of gum arabic, maltodextrin and pullulan with lipids in emulsions. *Bioscience, Biotechnology, and Biochemistry*, *64*, 1827–1835. https://doi.org/10.1271/bbb.64.1827

Mayamol, P. N., Samuel, T., Balachandran, C. P., Babu, R. N., Sundaresan, A., & Arumughan, C. (2009). *A novel process for the production of carotene rich red palmolein*. India. Patent No:195818.

Mba, O. I., Dumont, M.-J., & Ngadi, M. (2015). Palm oil: Processing, characterization and utilization in the food industry—A review. *Food Bioscience*, *163*, 1026–1041. https://doi.org/10.1016/j.fbio.2015.01.003

Menina, A., Zanonia, F., Vakarelovaa, M., Chignolaa, R., Donàa, G., Rizzia, C., Mainentea, F., & Zoccatelli, G. (2018). Effects of microencapsulation by ionic gelation on the oxidative stability of Flaxseed oil. *Food Chemistry*, *269*, 293–299. https://doi.org/10.1016/j.foodchem.2018.06.144

Mohammed, N. K., Tan, C. P., Manap, Y. A., Alhelli, A. M., & Hussin, A. S. M. (2017). Process conditions of spray drying microencapsulation of *Nigella sativa* oil. *Powder Technology*, *315*, 1–14. https://doi.org/10.1016/j.powtec.2017.03.045

Mohanan, A., Nickerson, M. T., & Ghosh, S. (2018). Oxidative stability of flaxseed oil: Effect of hydrophilic, hydrophobic and intermediate polarity antioxidants. *Food Chemistry*, *266*, 524–533. https://doi.org/10.1016/j.foodchem.2018.05.117

Montoya, C., Cochard, B., Flori, A., Cros, D., Lopes, R., Cuellar, T., Espeout, S., Syaputra, I., Villeneuve, P., Pina, M., Ritter, E., Leroy, T., & Billotte, N. (2014). Genetic architecture of palm oil fatty acid composition in cultivated oil palm (Elaeis guineensis Jacq.) compared to its wild relative E. oleifera (H.B.K) Corte. *PLoS One*, *9*(5). https://doi.org/10.1371/journal.pone.0095412

National Research Council. (1989). *Diet and health: Implications for reducing chronic disease risk. Report of the committee on diet and health, Food and Nutrition Board*. National Academy Press https://doi.org/10.17226/1222.

Pasukamonset, P., Pumalee, T., Sanguansuk, N., Chumyen, C., Wongvasu, P., Adisakwattana, S., & Ngamukote, S. (2018). Physicochemical, antioxidant and sensory characteristics of sponge cakes fortified with *Clitoria ternatea* extract. *Journal of Food Science and Technology*, *55*(8), 2881–2889. https://doi.org/10.1007/s13197-018-3204-0

Pelegrine, D. H. G., & Gasparetto, C. A. (2005). Whey proteins solubility as function of temperature and pH. *LWT - Food Science and Technology*, *38*(1), 77–80. https://doi.org/10.1016/j.lwt.2004.03.013

Premi, M., & Sharma, H. K. (2017). Effect of different combinations of maltodextrin, gum arabic and whey protein concentrate on the encapsulation behavior and oxidative stability of spray dried drumstick (*Moringa oleifera*) oil. *International Journal of Biological Macromolecules*, *105*, 1232–1240. https://doi.org/10.1016/j.ijbiomac.2017.07.160

Priol, L. L., Dagmey, A., Morandat, S., Saleha, K., Kirat, K. E., & Nesterenko, A. (2019). Comparative study of plant protein extracts as wall materials for the improvement of the oxidative stability of sunflower oil by microencapsulation. *Food Hydrocolloids*, *95*, 105–115. https://doi.org/10.1016/j.foodhyd.2019.04.026

Ramadan, M. F., & Wahdan, K. M. M. (2012). Blending of corn oil with black cumin (*Nigella sativa*) and coriander (*Coriandrum sativum*) seed oils: Impact on functionality, stability and radical scavenging

activity. *Food Chemistry*, *132*, 873–879. https://doi.org/10.1016/j.foodchem.2011.11.054

Ramakrishnan, S., Ferrando, M., Aceña-Muñoz, L., Mestres, M., De Lamo-Castellví, S., & Güell, C. (2014). Influence of emulsification technique and wall composition on physicochemical properties and oxidative stability of fish oil microcapsules produced by spray drying. *Food and Bioprocess Technology*, *7*, 1959–1972. https://doi.org/10.1007/s11947-013-1187-4

Ren, C. X., Wang, Y. J., Lin, X. F., Yang, F., Chen, J., Song, H. Q., & Guan, W. T. (2020). Palm oil encapsulated by homogenizing and spray drying enhances nutrient digestibility and attenuates diarrhoea of piglets. *Animal Feed Science and Technology*, *266*, 114503. https://doi.org/10.1016/j.anifeedsci.2020.114503

Roiaini, M., Ardiannie, T., & Norhayati, H. (2015). Physicochemical properties of canola oil, olive oil and palm olein blends. *International Food Research Journal*, *22*, 1227–1233.

Salar-Behzadi, S., Wu, S., Toegel, S., Hofrichter, M., Altenburger, I., Unger, F. M., Wirth, M., & Viernstein, H. (2013). Impact of heat treatment and spray drying on cellular properties and culturability of *Bifidobacterium bifidum* BB-12. *Food Research International*, *54*(1), 93–101. https://doi.org/10.1016/j.foodres.2013.05.024

Santhanam, A. K., Lekshmi, M., Chouksey, M. K., Tripathi, G., & Gudipati, V. (2014). Delivery of omega-3 fatty acids into cake through emulsification of fish oil-in-milk and encapsulation by spray drying with added polymers. *Drying Technology*, *33*, 83–91. https://doi.org/10.1080/07373937.2014.934832

Sharif, H. R., Goff, H. D., Majeed, H., Shamoon, M., Liu, F., Atindana, J. N., Haider, J., Liang, R., & Zhong, F. (2017). Physicochemical properties of β-carotene and eugenol co-encapsulated flax seed oil powders using OSA starches as wall material. *Food Hydrocolloids*, *73*, 274–283. https://doi.org/10.1016/j.foodhyd.2017.07.002

Srivastava, Y., Semwal, A. D., Sajeevkumar, V. A., & Sharma, G. K. (2016). Melting, crystallization and storage stability of virgin coconut oil and its blends by differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR). *Journal of Food Science and Technology*, *54*, 45–54. https://doi.org/10.1007/s13197-016-2427-1

Teh, S. S., & Birch, J. (2013). Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. *Journal of Food Composition and Analysis*, *30*, 26–31. https://doi.org/10.1016/j.jfca.2013.01.004

Tonon, R. V., Grosso, C. R. F., & Hubinger, M. D. (2011). Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. *Food Research International*, *44*, 282–289. https://doi.org/10.1016/j.foodres.2010.10.018

Turchiuli, C., Fuchs, M., Bohin, M., Cuvelier, M. E., Ordonnaud, C., Peyrat-Maillard, M. N., & Dumoulin, E. (2005). Oil encapsulation by spray drying and fluidised bed agglomeration. *Innovative Food Science & Emerging Technologies*, *6*(1), 29–35.

Turchiuli, C., Lemarié, N., Cuvelier, M. E., & Dumoulin, E. (2013). Production of fine emulsions at pilot scale for oil compounds encapsulation. *Journal of Food Engineering*, *115*, 452–458. https://doi.org/10.1016/j.jfoodeng.2012.02.039

Wen, P., Zong, M. H., Linhardt, R. J., Feng, K., & Wu, H. (2017). Electrospinning: A novel nano-encapsulation approach for bioactive compounds. *Trends in Food Science and Technology*, *70*, 56–68. https://doi.org/10.1016/j.tifs.2017.10.009

Xu, Y. Y., Howes, T., Adhikari, B., & Bhandari, B. (2013). Effects of emulsification of fat on the surface tension of protein solutions and surface properties of the resultant spray-dried particles. *Drying Technology*, *31*, 1939–1950. https://doi.org/10.1080/07373937.2013.802331

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