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Extraction and Microemulsion of Lupin Protein

Prepared by: Manar Thameen Hamad Al Badareen

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Prepared by: Manar Thameen Hamad Al Badareen

B.Sc. Chemistry and Chemical Technology
Al-Quds University - Palestine

Supervisor: Prof. Ibrahim Kayali
Co-supervisor: Dr. Michel Hanania

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Extraction and Microemulsion of Lupin Protein

Prepared by: Manar Thameen Hamad Al Badareen
Registration No.: 21710907

Supervisor: Prof. Ibrahim Kayali
Co-supervisor: Dr. Michel Hanania

Master Thesis submitted and accepted, Date: 21/12/2021
The name and signature of examining committee member are as follows:

1- Head of Committee: Prof. Ibrahim Kayali Signature:
2- Co-Supervisor: Dr. Michel Hanania Signature:
3- Internal Examiner: Dr. Fuad Rimawi Signature:
4- External Examiner: Dr. Hatem Maraqah Signature:

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Dedication

This thesis is dedicated to:
I dedicate this thesis to my beloved great parents, who never stop giving of themselves in countless ways, for nursing me with affection, love and for their dedicated support in the success of my work.
My beloved brothers and sisters;
My beloved kid: Ameer, whom I can't force myself to stop loving.
To all my family, the symbol of love and giving,
My friends who encourage and support me,
All the people in my life who touch my heart,
I dedicate this research.

Manar Thameen Hamad Al Badareen

Declaration

I certify that thesis submitted for Master degree, is a result of my own research, except where otherwise acknowledged, and that this study has not been submitted for a higher degree to any other university or institution.

Signed: 

Name: Manar Thameen Hamad Al Badareen

Date: 21/12/2021

Acknowledgement

Praise be to Allah the cherisher and sustainer of the worlds. Praise be to Allah that is worthy of this Majesty, Bounty and Honour. Praise be to Allah who expanded my breast, made things easy, and guided me to succeed in bringing this modest study into being.

Prayer and Peace be upon the most honorable of all creatures and the leader of all prophet, the prophet Mohammad bin Abdullah, peace be upon him-who was sent as a guidance and mercy to all humanity.

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Abstract

Lupin seeds extracted proteins were incorporated in microemulsion to provide useful information for the rational design of transparent microemulsions as delivery systems potentially for bioactive compounds for applications in food, beverage and non-food areas.

In this study, protein and oil were extracted from sweet and bitter lupin by using Soxhlet method, that were used to define the phase behavior of a three–component system (surfactant/oil/water) and construct the ternary phase diagrams and identify the optimum area of ternary phase diagrams corresponding to the formation of microemulsions.

The formation of microemulsions with droplet size smaller than 100 nm in diameter and stabilised by non-ionic surfactants was investigated by using emulsion titration methods. A series of ternary systems consisting of three components (lupin oil, Tween 80/ Propylene glycol and water/lupin protein) were prepared at different ratios via gentle agitation by the phase inversion composition method involving the spontaneous formation of microemulsion.

The phase behavior and microemulsion formation of the ternary mixtures prepared were characterised by visual observation for their phase separation and optical clarity (e.g. transparency and opacity).

As a consequence, phase diagrams based on small molecule surfactants (Tween 80) were constructed which define the ratios of three components in the composition of the ternary mixtures that allow the formation of oil-in-water (o/w) or water-in-oil (w/o) microemulsions.

Overall, the o/w microemulsions were found to form at a small region of the ternary phase diagrams with a relatively large ratio of water/lupin protein.

Between the two ternary phase diagrams based on Tween 80/PG, there were some differences in their composition regions responsible for the formation of microemulsions as well as for other types of phases formed, including bi- and multiphase, liquid crystals, gel and coarse emulsions. In this study, microemulsions were produced by a method called ‘titration method’.

Type and concentration of surfactant (Tween 80) and oils (Lupin oil) were investigated for their influence on the solubilisation of oil molecules from emulsion droplets into surfactant micelles, thus the formation of microemulsion. The results showed that Tween 80/ PG had better capacity of oil droplet solubilisation when added to water/lupin protein, because the protein has emulsifying properties.

The results indicated that the physical characteristics of the developed microemulsions did not change under different storage temperatures.

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Abbreviations, Symbols and Terminology:

O/W: Oil dispersed in Water

W/O: Water dispersed in Oil

MEs: Microemulsions

PG: Propylene Glycol

HLB: Hydrophile Lipophile Balance

SLO: Sweet Lupin Oil

BLO: Bitter Lupin Oil

SLP: Sweet Lupin Protein

BLP: Bitter Lupin Protein

MM: Molecular Masses

ANFs: Anti-Nutritive Substances

SC: Stratum Corneum

ACE inhibitors: Angiotensin-converting-enzyme inhibitors

Chapter 1: Introduction

Microemulsion

The term "Microemulsion" refers to a thermodynamically stable, fluid, optically clear dispersions of two immiscible liquids such as oil and water. Microemulsions form when a surfactant, or more commonly a mixture of surfactants and co-surfactants, lowers the oil/water interfacial tension to ultra-low values, allowing thermal motions to spontaneous disperse the two immiscible phases (Klier et al., 2000; Kale et al., 2016; Mishra et al., 2014).

According to the International Union of Pure and Applied Chemistry, a microemulsion is an isotropic and thermodynamically stable dispersion comprised of water, oil, and surfactant(s) with dispersed domain diameters ranging from 1 to 100 nm, most often 10 to 50 nm (Kale et al., 2016).

Depending on the composition, three types of microemulsions are most likely to develop (Mishra et al., 2014), as it can be seen in Figure 1:

- Oil in water microemulsions wherein oil droplets are dispersed in the continuous aqueous phase
- Water in oil microemulsions wherein water droplets are dispersed in the continuous oil phase;
- Bi-continuous microemulsions wherein microdomains of oil and water are interdispersed within the system.

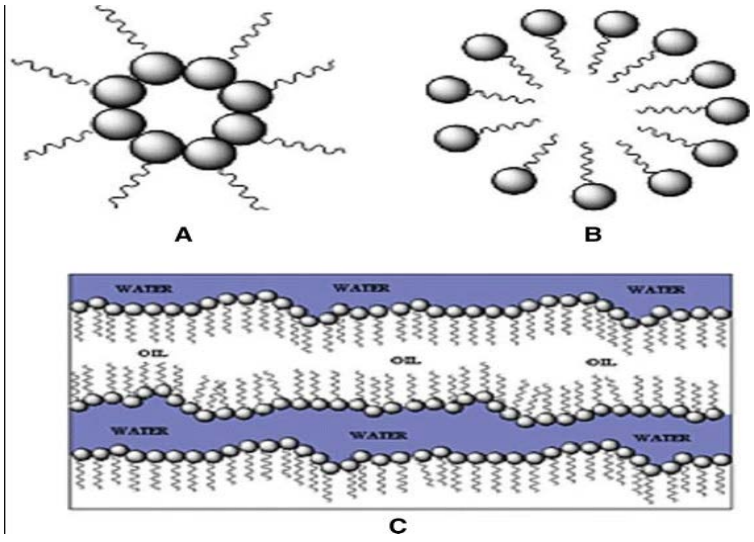


Figure 1: Microemulsion structures: **A):** reverse microemulsion, **B):** direct microemulsion and **C):** bicontinuous microemulsion **(**adapted from Bera & Mandal, 2015).

Microemulsions are thermodynamically stable and therefore do not require high inputs of energy or shear conditions for their formation. They are also clear, as compared to emulsions which are cloudy (Lee, 2011).

The physicochemical properties of microemulsions—thermodynamic stability, spontaneous formation, ease of manufacturing, and other properties (Klier et al., 2000; Lee, 2011; Madhav

et al., 2011) in cosmetic and pharmaceutical applications are linked to their growing interest in them as drug carrier systems for oral, topical, and parenteral administration, in food applications, in the petrochemical industry, enhanced oil recovery, nanoparticle synthesis, chemical reaction media (Madhav et al., 2011; Mishra et al., 2014).

Microemulsions have also found broad applicability in formulated products that include oily and aqueous components. These include consumer and industrial cleaning formulations, pharmaceutical formulations for improved drug solubilization, coating formulations and many others (Klier et al., 2000).

In theory, microemulsions can be utilized in a variety of ways to deliver drugs, but topical application of microemulsions is gaining favor. The three important variables that determine drug transdermal penetration are the drug's mobility in the vehicle, its release from the vehicle, and the drug's absorption into the skin. These variables affect the drug's permeability in the skin, notably the stratum corneum, as well as the thermodynamic activity that propels the medication into the skin (Madhavet al., 2011).

As a topical drug delivery system, microemulsions outperform standard creams, emulsions, gels, and solutions. The current formulations have a low solubilizing capacity with lipophilic drugs, which is their principal drawback. Microemulsions have the benefit over gels in that they can be utilized to solubilize drugs and improve topical drug availability. Microemulsions have an advantage over creams in that they improve stratum corneum hydration, which increases drug dermal permeability and skin flux (Madhav et al., 2011; Sabale & Vora, 2012).

These drug delivery systems are currently of primary interest to pharmacists due to their embryonic potential to operate as therapeutic enzymes and peptide-based drug delivery vehicles with a wide spectrum of active therapeutic protein and peptide molecules (Anil & Kannan, 2018).

Legumes are an important part of many traditional diets around the world. The increased need for low-cost, non-genetically modified vegetable proteins has encouraged food scientists to look into a variety of protein options. When compared to other legumes like beans and peas, lupin seeds, for example, have a low carbohydrate content and a high protein content (Torres et al., 2005; Martínez-Villaluenga et al., 2006).

Lupin seeds have become an integral part of human nutrition due to their high protein content and healthy fatty acid composition. Anti-nutritive chemicals like as isoflavones, on the other hand, are substantially lower in lupin than in soy, and lupin protein isolates are almost isoflavone-free (Sirtori et al., 2004).

Lupin proteins have so-called technofunctional properties in food products, in addition to their nutritional benefits. Hydration capacity, foaming and emulsifying properties, protein solubility, and gelation are all important technofunctional qualities of the proteins that make this vegetable protein interesting for the food industry (Lampart-Szczapa et al., 2006).

Lupin seeds are well-known for providing a variety of necessary nutrients, such as carbohydrates, dietary fiber, protein, minerals, and vitamins, all of which have biologically important properties, such as lowering the glycaemic index and lowering blood pressure and

cholesterol. In addition, (Çakir et al., 2019) found that a protein extract from lupin can inhibit cell migration in colon cancer cells.

In the disciplines of functional foods and pharmaceutics, the use of food-grade delivery methods for the encapsulation, preservation, and controlled release of bioactive substances has recently gotten a lot of attention. Plant proteins (for example, lupin protein) are abundantly available and may be converted into a variety of delivery platforms, including micro- and nanoencapsulations.

Chapter 2: Literature Review

2.1 Introduction to Lupin

Legumes represent, together with cereals, the main plant source of proteins in human diet (Duranti et al., 2008; Kohajdová et al., 2011). Human consumption of lupine seeds has expanded in recent years due to their high nutritional value, which includes not only proteins but also lipids, dietary fiber, minerals, and vitamins (Bartkiene et al., 2016).

Lupin or lupine are trivial names for plants of the genus Lupinus belonging to the Leguminosae family, subfamily Papilonoideae (Trugo et al., 2003). Lupine agriculture dates back at least 2,000 years, and it is most likely to have started in Egypt or the Mediterranean region (Putnam et al., 1989). This genus is very diverse and contains several known species (Trugo et al., 2003).

In nature, there are approximately 400 species of lupin (genus Lupinus). Only a few species, including white lupin (Lupinus albus), blue lupin (Lupinus angustifolius), yellow lupin (Lupinus luteus), and pearl or Tarrwilupin (Lupinus mutabilis), have been extensively studied for their agronomical characteristics and nutritional values (Putnam et al., 1989; Philippe et al., 2003; Khan et al., 2015; Vogelsang-O'Dwyer et al., 2020).

Lupin seeds have been used as food and feed from ancient times (Trugo et al., 2003). As people have become more aware of the unique nutritional properties and health benefits of lupin, interest in it as a meal has grown. Lupin, a non-starchy grain legume, has a lower fat content (6%), a higher quantity of essential amino acids, vital dietary minerals, higher protein (40%) and dietary fiber (28%) than other legume crops, making it a good food ingredient and a low-cost alternative (Khan et al., 2015).

The lupine plant, like other grain legumes such as beans, peas, and lentils, fixes atmospheric nitrogen and produces high-protein seed (Putnam et al., 1989). Protein concentration varies according to species and cultivars, as well as growing conditions and soil types (Bartkiene et al., 2016; Sobotka et al., 2016).

To reduce the amount of alkaloids in edible lupins, they are frequently boiled and preserved in salts. Because lupin seeds are gluten-free and safe for celiac disease sufferers, they are used to replace grains or soy in baked products or pasta (Calvanoet al., 2020). Lupins can be found in a wide range of products, including cakes, snacks, hamburgers, biscuits, infant foods, soups, salads, and milk, meat, and soy bean substitutes (Carvajal-Larenas et al., 2015).

In the food and chemical industries, lupin protein isolate is highly regarded for its water and oil absorption, emulsifying capacity, activity, and stability, foaming capacity and stability, and gelation capacity (Carvajal-Larenas et al., 2015), (Janusz, 2017).

The more general use of lupin products as food ingredients for situations requiring added foaming capability and foam stabilization, such as the replacement of egg albumin (Pollard et al., 2002).

Lupin alkaloids may provide medical benefits due to their immunosuppressive, antiarrhythmic, and hypocholesterolemic capacity, in addition to being toxic in human diet.

Lupins also contain phenolic antioxidant compounds and prebiotic oligosaccharides, both of which may help bifidobacteria proliferate (Carvajal-Larenas et al., 2015).

Lupin seeds appear to have therapeutic effects and can be classified as a functional or nutraceutical food that helps lower the glycemic index and aid in the prevention of obesity, diabetes, and cardiovascular disease (Thambiraj et al., 2019; Calvanoet al., 2020), hypotensive, anti-carcinogenic and anti-obesity activities (Duranti et al., 2008, Prusinski, 2017).

It's a good nerve tonic, heart stimulant, diuretic, anti-Kalokzima for some skin illnesses including psoriasis, sugar reducer, and dewormer (Hanania et al., 2018).

Has been used for various therapeutic purposes since ancient time. It has been used as a potent drug in hyperpigmentary skin disorders such as Kalaf (melasma), Barañ (freckles), Til (moles), etc (Qaiyyum & Mohammad, 2020).

2.2 Chemical Composition of Lupin

Legume seeds are a high source of protein, with lupin being one of the richest (Kohajdova et al., 2011). Protein concentration varies according to species and cultivars, as well as growing conditions and soil types (Bartkiene et al., 2016).

Whole lupin seeds have a protein level that is comparable to or greater than soy beans. On a dry weight basis, Lupinus albus contains around 35% protein (Uauy et al., 1995).

Thus, L. luteus (yellow lupin) seed contains about 30% of raw protein composed from globulins (legumin and vicillin) and albumins (Lásztity et al., 2001) are principal proteins in the cotyledons of legume seeds (Konopska-Waliszkiewicz, 1988).

Sobotka and coworkers reported that lupin seeds had high protein content, ranging from 28% to 48%, lipids, dietary fiber, has found with a rich source of phytochemicals importantly bioactive peptides, alkaloids, polyphenols, phytosterols, tocopherols, etc. (Sobotka et al., 2016, Khan et al., 2015), minerals, vitamins, macro- (Na, Mg, K, Ca) and microelements, including essential (Cr, Mn, Co, Ni, Fe, Zn, Se and Cu) and nonessential (Al, As, Sr, Cd, Pb and Ag) elements depending on species (Bartkiene et al., 2016).

Lupine contains many important components such as: carbohydrates 32 % and oils 18-21 %. The seeds are also rich with vitamin K, vitamin C, and omega 3 and 6 fatty acids (Hanania et al., 2018).

Lupin meal is a promising crop for both protein and oil production since it provides a high concentration of essential amino acids. Unsaturated (78%) and polyunsaturated (25-30%) fatty acids are abundant in lupin oil (Alamanou & Doxastakis, 1995; Doxastakis, 2000). Oleic and linoleic acid are the most abundant fatty acids (Klupšaitė & Juodeikienė, 2015).

Non-starch polysaccharide lupin components are common. The oligosaccharides of the raffinose family are abundant in lupin seeds (Doxastakis, 2000).

2.3 Anti-nutritional compounds and toxicity of lupin grain

The nutritional content of lupin grains is determined not only by the nutrients they contain, but also by the anti-nutritive substances (ANFs) they contain (Reinhard et al., 2006). Protease inhibitors, lectins, tannins, saponins, and phytic acid (phytates) are antinutritive components found in lupins, as well as flavonoids in various amounts and naturally occurring bitter principles. The bitter compounds in the seed make it unpleasant and even toxic at times. (Sujak et al., 2006; Doxastakis, 2000).

Bitter (high alkaloid content) and sweet (low alkaloid content) lupin seeds are distinguished by their alkaloid content (Lack of alkaloids or very low concentration of alkaloids). Lupin seeds are characterized as "sweet" or "bitter" based on the amount of alkaloids present, which can range from 0.01 percent to 4% (Allen, 1998).

White lupin seeds, in contrast to other legumes (peas, soybeans, and beans), have a low or extremely low amount of anti-nutritive chemicals (Muzquiz et al., 1998; Enneking & Wink, 2000).

Lupin seeds, which are otherwise a promising source of protein, are especially high in alkaloids. Heating and processing during food preparation can remove or inactivate several ANFs to a substantial extent. The detoxification of seed materials is known to be successful using wet milling and processing procedures used during protein concentration and isolation (Alamanou & Doxastakis, 1995, Doxastakis, 2000).

In the process of domestication and breeding, the total alkaloid content of sweet lupin cultivars has been greatly lowered, and it now stands at less than 0.2 percent (Janusz, 2017).

At concentrations of up to 6%, more than 150 alkaloids from the quinolizidine, piperidine, and indole groups have been identified, conferring plant resistance to diseases and herbivores (Sujak et al., 2006; Reinhard et al., 2006).

Oligosaccharides may benefit the immune system by having antioxidative and anticancer properties, as well as decreasing cholesterol levels (Rocheort & Pannozo, 2007). Alkaloids found in low concentrations in lupin seeds have been shown to have no negative impact on human and animal health. However, their presence continues to limit the widespread usage of lupine seeds (Sobotka et al., 2016).

For instance, oligosaccharides found in lupin seeds are poorly absorbed in the small intestine and fermented by bacteria in the cecum, causing digestive problems in humans and animals (Sobotka et al., 2016).

2.4 Composition of Lupin Proteins

Globulins and albumins are the most abundant protein classes in legume seeds; prolamin and glutelin fractions are also found, albeit in very small amounts (Doxastakis, 2000; Carvajal-Larenas et al., 2015). The water-soluble albumin fraction contains enzyme proteins, protease inhibitors, amylase inhibitors, and lectins with molecular masses (MM) ranging from 5000 to 80,000 Da (Klupait & Juodeikien, 2015), whereas globulins are isolated in salt solutions (Doxastakis, 2000).

Globulins are proteins extracted at high ionic strength, and represent about 80% of the protein in L. albus comprising the storage protein of the seed, and quantitatively representing the major protein component (Carvajal-Larenas et al., 2015). Consist primarily of the (α-conglutin or 11S-like protein, β-conglutin or 7S-like protein, and γ-conglutin). These proteins have molecular weights ranging from 8000 to 600,000 Da. These proteins exhibit a minimum solubility at pH values between four and five (isoelectric point) (Klupšaitė & Juodeikienė, 2015).

The group's complexity was revealed as the analytical methodologies utilized were refined, based not only on the presence of essentially distinct protein families, but also on different oligomeric component relationships and protomer size and composition variability within each family (Doxastakis, 2000).

The albumin fraction contains molecules that are part of the seed's functional proteins. Glycosidases and proteases are two examples of enzymes. Others, such as trypsin inhibitors and lectins, play a significant part in plant defense. Albumin has a high amount of lysine and sulfur amino acids, particularly methionine (Doxastakis, 2000; Carvajal-Larenas et al., 2015).

2.5 Lupin Seed Oil

The lupin oil presents lipid profile similar to other sources of oils, as olive oil, and can be of great potential use for human consumption (Fontanari et al., 2017).

Lupin features around 11–18% oil in its composition (Fontanari et al., 2017), while the L. albus contain 13 % oil (Yáñez et al., 1983).

Bitter and sweet lupins are the two most common types of lupins. The alkaloid content of these two types of plant species varies (Sbihi et al., 2013).

Lupin seeds contain 8% and 12% oil, respectively, in their bitter and sweet varieties. Oleic acid (46.28 and 48.72 percent) was found in high concentrations in BLO and SLO, followed by linoleic acid (21.55 and 20.90 percent), linolenic acid (7.69 and 8.95 percent), and palmitic acid (7.69 and 8.95 percent) (7.39 and 7.5 percent). BLO and SLO contained 184.70 mg and 317.01 mg of total tocopherol per 100 g oil, respectively (Sbihi et al., 2013).

Overall the amount of saturated fatty acids was lower than unsaturated fatty acids in both types of oils (Khalid & Elhardallou, 2019). Table 1 shows the composition of lupin seed oil for both sweet and bitter types.

Table 1: Bitter and sweet lupine seed oils have different fatty acid compositions (Khalid & Elhardallou, 2019).

Saturated and unsaturated Fatty acids %	Lupine seed flours oil	
	Bitter lupine	Sweet lupine
Myristic $C_{14:0}$	0.140±0.03 [a]	0.199±0.03 [a]
Palmitic $C_{16:0}$	8.990±0.69 [a]	7.612±0.69 [a]
Stearic $C_{18:0}$	2.001±0.14 [a]	1.711±0.14 [b]
Arachidinic $C_{20:0}$	2.123±0.27 [a]	2.657±0.27 [a]
Oleic $C_{18:1}$	50.234±0.62 [a]	45.00±0.62 [b]
*Lino*leic $C_{18:2}$	22.546±1.18 [b]	24.900±1.18 [a]
Linolenic $C_{18:3}$	13.564±0.67 [a]	14.897±0.67 [a]
Palmitoleic $C_{16:1}$	0.440±0.11 [a]	0.660±0.11 [a]
Arachidic $C_{20:1}$	1.551±0.12 [a]	1.789±0.12 [a]
Total saturated fatty acids (TSFA)	13.251±0.54 [a]	12.179±0.54 [a]
Total unsaturated fatty acids (TUFA)	88.330±0.54 [a]	87.246±0.54 [a]
(TUFA)/ (TSFA)	6.67±0.23 [b]	7.14±0.23 [a]
Total essential fatty acid	36.114±0.84 [b]	39.797±0.84 [a]

2.6 Lupin Protein Isolation & Ultrafiltration

The food industry is growing increasingly interested in the creation of legume protein concentrates or isolates due to its functional characteristics and potential to increase the nutritional value of food. Protein concentrates/isolates with diverse properties are extracted using a variety of techniques (Klupait & Juodeikien, 2015).

The majority of methods for producing protein concentrates and isolates from leguminous seeds employ an alkaline medium with a moderate NaOH solution at a pH of around 9. The proteinaceous material is then separated from the alkaline extract through isoelectric point precipitation or ultrafiltration (Lampart-Szczapa, 1996).

Using water and NaCl solution to extract protein from undefatted and defatted lupin flour in an alkaline and neutral environment. As a result, we were able to acquire the highest yield and protein content. The highest level of sum of Sulphur amino acids was also obtained using this method (Lampart-Szczapa, 1996).

Additionally, Sussman and coworkers isolated protein from full-fat flakes of lupin by salt-induced extraction followed by precipitation with cold demineralized water (Sussman et al., 2011). Hexane-defatted flakes of lupin were extracted under various conditions with 80 percent ethanol, 80 percent methanol, or their aqueous solutions in order to eliminate the poisonous quinolizidine alkaloids and other nonprotein components (Blaicher et al., 1981).

Hanania and coworkers described the extraction processes for bitter Lupinus albus seeds that were either soaked in water or not, and then exposed to Soxhlet extraction with commercial ethanol (~95%), 80 percent, 70 percent, 60 percent, and 50 percent ethanol. Soaked samples yielded 12.5 percent, 23.9 percent, 12.6 percent, 16.1 percent, and 16.6 percent extraction

yields, while unsoaked samples yielded 11.6 percent, 20.9 percent, 19.3 percent, 20.5 percent, and 25 percent extraction yields (Hanania et al., 2018). Lupin protein concentrates with a protein content of greater than 70% and alkaloids of 0.1-0.2 percent (Blaicher et al., 1981).

Lupin seeds were extracted with diluted NaOH and then precipitated with HCl to produce protein isolates. The isolates were examined for their protein concentration, amino acid composition, biological value, and functional properties. Good solubility and moderate emulsifying, foaming, and gel-forming properties were found in the isolates (Lásztity et al., 2001).

A concentrated form of lupin protein with a protein content of 90% was prepared by alkaline extraction at pH 9.0 followed by acidic precipitation at a higher pH 5.0, resulting in reduced acid usage. This is one of the most commonly used methods for protein isolates (Jayasena et al., 2011) (defined as having protein contents of 90% to 95%) (Klupšaitė & Juodeikienė, 2015), thus giving a more cost-effective approach to protein isolate synthesis.

The emulsifying and foaming properties of lupin seed protein isolates prepared by wet extraction methods, such as isoelectric precipitation, dialysis and polyacrylamide gel, were investigated. The isoelectric precipitation isolate mostly comprises the globulin fraction but not the albumin fraction, whereas the dialysis and polyacrylamide gel isolates contain all of the protein fractions as well as a significant quantity of polysaccharides. Because of steric repulsion effects, protein polysaccharide complexes improve emulsion stability (Alamanou & Doxastakis, 1997).

Various isolation procedures were used to investigate the emulsifying and water binding capacities of lupin protein (isolates and concentrates), including protein extraction in a single or double step in alkaline or acid conditions, followed by precipitation and drying at isoelectric pH to promote water binding capacity, or dry milling and alkaline extraction to improve emulsifying capacity (Manrique and Thomas, 1976).

2.7 Bioactive Proteins and Peptides

Food proteins are a source of nutraceutical and bioactive peptides that promote health and prevent diseases. Legumes are a good source of bioactive compounds as proteins (Marcela, 2017). Plants, animals, fungi, bacteria, and their products all contain diverse proteins, making nature the most abundant source of bioactive peptides (Daliri et al., 2017).

The most abundant sources of bioactive proteins and peptides generated from diet appear to be bovine milk, cheese, and dairy products. This could be linked to the specific goals for which milk is employed beyond nourishment in the first few months of life: "On a substrate basis, bioactive molecules in milk and colostrum are mother language." Bioactive peptides and proteins, on the other hand, can be found in a wide range of animals and plants. Bovine blood, gelatin, meat, eggs, and a range of fish, including tuna, sardine, herring, and salmon, as well as wheat, maize, soy, rice, mushrooms, pumpkin, and sorghum (Möller et al., 2008), bioactive proteins have been detected directly or after release by hydrolysis or fermentation (Möller et al., 2008).

Peptide sequences encoded in the parent protein, which become active when cleaved intact, perform the majority of a protein's physiological functions. Bioactive peptides are released

during enzymatic proteolysis (gastrointestinal digestion, in vitro hydrolysis using proteolytic enzymes) of proteins and food processing (cooking, fermentation, ripening) (Daliri et al., 2017; Chakrabarti et al., 2018).

Recently, there has been an increase in interest in the use of bioactive peptides for therapeutic purposes, particularly in the treatment of neoplasms, viral inflammations (infections), immune system disorders, neurological and cardiologic disorders (Dziuba & Darewicz, 2007), as well as in treatment with antibiotics, antioxidative, antimicrobial and antimycotic agents (Danquah & Agyei 2012).

Peptides derived from dietary proteins by enzymatic proteolysis have been found to have opiate, antithrombotic, antihypertensive, immunomodulating, antilipemic, osteoprotective, antioxidative, antibacterial, ileum contracting, anticariogenic, and growth-promoting properties. Certain peptides can have a variety of biological effects (Möller et al., 2008; Danquah & Agyei, 2012).

Lupin seeds are interesting as food ingredient because of their high protein content, which is at least similar to that of soybeans (Day, 2013). White lupin seeds contain two classes of proteins: albumin and globulin fractions (Duranti et al., 2008).The most abundant storage proteins in lupin seeds are the globulins, which comprise two major protein types, β-conglutin (7S globulin, vicilin-like protein) and α-conglutin (11S globulin, legumin-like protein) and minor components, γ-conglutin and δ-conglutin (Schlegel et al., 2019; Arnoldi, A., Boschin et al., 2015).

Lupin protein exhibits similar properties to that of soy protein and displays several health benefits. It is demonstrated that lupin protein hydrolysates exhibit significant cholesterol lowering activity, anti-inflammatory activity and ACE inhibitory activity that was comparable to soy protein hydrolysates and superior to lentil, common bean and chickpea protein hydrolysates. About 18 peptides from β-conglutin component of Lupinusalbus are identified as active hypocholesterolemic agents (Kamran & Fozia, 2018).

Legume proteins: such as soy, lupin, chickpea, yellow pea, common bean and lentil proteins display direct health promoting properties and/or their consumption leads to the production of bioactive peptides by digestive enzymes (Kamran & Fozia, 2018) which governed by the the type, number, sequence and properties of amino acids present in the peptide, as they would interact with other proteins in the body and modulate natural processes (Chakrabarti et al., 2018; Danquah & Agyei, 2012).

2.8 Lupin Extract Microemulsion

Protein and peptide delivery technologies have advanced significantly in recent decades, with the majority of research efforts focused on the administration of a wide range of important proteins and peptides with enormous molecular weight. These macromolecules have a low rate of absorption into the bloodstream when taken orally, as well as a short half-life, necessitating frequent administration in optimal amounts to achieve desired therapeutic efficiency (Anil & Kannan, 2018).

Parenteral (intravenous/subcutaneous) dosing is the most common mode of delivery for protein medicines. However, because the majority of proteins have short half-lives, this method has the drawback of requiring several medication administrations and poor patient

compliance. Other routes, such as the oral, pulmonary, and nasal, have been investigated as alternatives, and some products are already on the market. However, issues such as GI breakdown, limited bioavailability, and local discomfort continue to be issues (Bivek et al., 2018).

When compared to other routes, skin could be a viable option for administering protein medicines over the skin since it avoids first-pass metabolism, provides longer drug release, and has low proteolytic activity (Bivek et al., 2018). The biggest organ of the body is the human skin. It acts as a barrier to prevent the body from losing water and vital components, as well as hazardous molecules from penetrating the body (Anil & Kannan, 2018).

The stratum corneum, dermis, epidermis, and subcutaneous tissue are the four separate layers that make up the skin (Anil & Kannan, 2018). The stratum corneum (SC), the skin's outermost layer, is made up of keratin-filled, non-viable cells encased in a crystalline intercellular lipid domain that is impervious to practically all chemicals and medications with a molecular weight greater than 500 Da (Gautam et al., 2016; Anil & Kannan, 2018; Bivek et al., 2018). All intradermal, subcutaneous and transdermal drug delivery must pass through this epidermal barrier without interfering with the skin's normal function.

Water, oil, and an amphiphile make up microemulsions, which are optically isotropic systems. Thermodynamic stability, optical clarity, ease of preparation, and greater diffusion and absorption rates are all advantages of these systems. Furthermore, it has been claimed that microemulsion substances can efficiently cross the diffusion barrier and infiltrate the skin's stratum corneum. As a result, it has the potential to be used for both transdermal and cutaneous drug administration (Vadlamudi et al., 2014).

Direct (o/w, oil dispersed in water) and reversed (w/o, water dispersed in oil) microemulsions are the most prevalent. In ternary systems like microemulsions, two immiscible phases (water and oil) are present with a surfactant, the surfactant molecules may form a monolayer at the oil-water interface, with the surfactant molecules' hydrophobic tails dissolved in the oil phase and the hydrophilic head groups in the aqueous phase (Madhav & Gupta, 2011).

The presence of surfactant and cosurfactant in the system makes the interfacial tension very low. Therefore microemulsions form spontaneously and have the ability to deliver larger amounts of water and topically applied agents into the skin than water alone or other traditional vehicles such as lotions or creams (Ravikumar et al., 2011).

Surfactants with a low HLB (about 3-6) are preferred for water dispersed in oil (w/o) microemulsion formulation, whereas surfactants with a higher HLB (around 8-18) are preferred for oil dispersed in water (o/w) microemulsion formulation. Surfactants having an HLB value greater than 20 are frequently used in conjunction with co-surfactants to reduce the total amount of surfactant utilized (Anil & Kannan, 2018).

The main challenges in the application of protein therapeutics are their high molecular weight, delicate nature and complexity, and inconsistent structure. Furthermore, several physiochemical and enzymatic activities can easily denature, degrade, and ultimately inactivate such macromolecules during their formulation, storage, and delivery. Their biologic qualities have also been attributed for their lack of stability (Anil & Kannan, 2018).

Protein bioavailability and mucosal permeability are largely attributed to the many proteolytic enzymes found in the gut, lungs, and skin. Due to quick removal from the body due to immediate phagocytosis, endocytosis, glomerular filtration, proteolysis due to certain enzymatic processes, and numerous immunological aspects of these active protein molecules, therapeutic bioavailability is also inadequate. Various investigations have been conducted to improve protein bioavailability and alter the physicochemical features of these therapeutic macromolecules. Functional additives have been incorporated into innovative drug delivery systems adapted specifically for these purposes (Anil & Kannan, 2018).

In light of these facts, colloidal drug delivery systems, primarily microemulsions (MEs), targeting intradermal and transdermal sites have become the primary focus of this research in order to improve the therapeutic indices of protein biomolecules by allowing for localized and extended release at the target site without causing undesirable side effects.

Chapter 3: Materials and Methods

3.1 Materials

Bitter and Sweet lupin seeds were obtained from the local market which were used to extract oil and protein. Non-ionic small molecule surfactants polysorbate 80 (Tween 80), were purchased from Eigenmanne and used as emulsifiers, while propylene glycol was purchased from Daw and used as co-surfactant and purified water was obtained from Beit Jala Pharmaceutical Company (BJP). All components were used as supplied while all other chemicals used were of reagent grade.

3.2 Preparation of Lupine seed flour

Lupin seeds (Sweet and Bitter) were ground using a household mill and then sieved. Protein isolates were then made from the fine flour of each seed variety.

3.3 Protein isolates are prepared via alkaline water extraction and isoelectric precipitation (Isolate-PI).

The flour samples were defatted using hexane as a solvent in a Soxhlet apparatus to a final fat content of less than 0.5%. The extraction of protein in lupin seeds was carried out according to a known procedure (Jayasena et al., 2011) at room temperature (20±2°C). The details of the procedure are shown in Fig. 2.

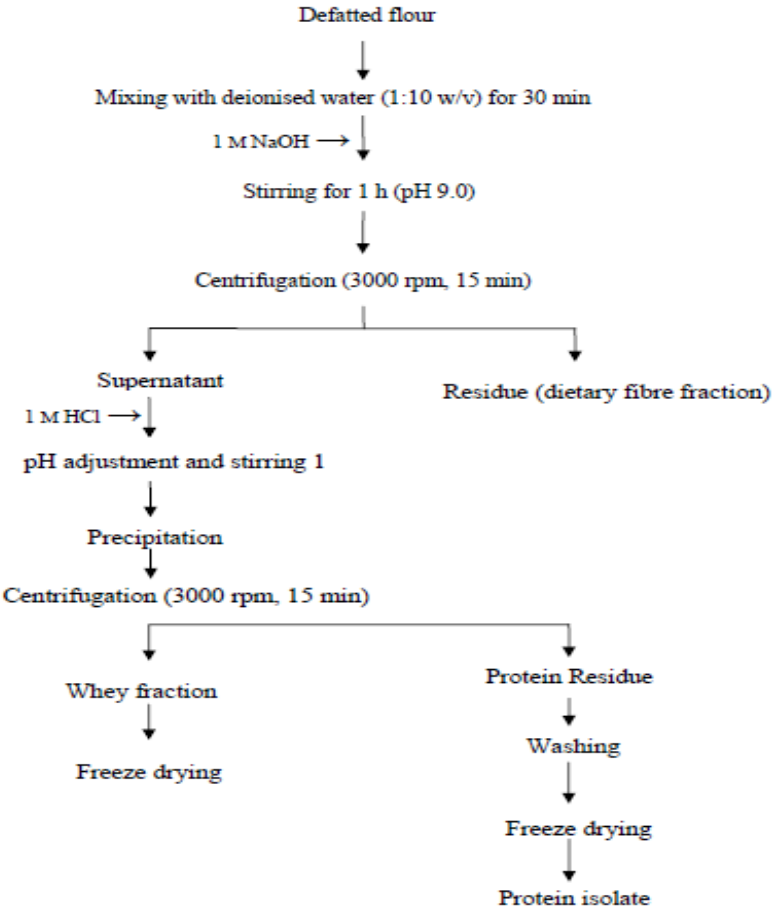


Figure 2: Protein Isolate Preparation Flow diagram (Jayasena et al., 2011)

3.4 Preparation of oil from Bitter and sweet lupin

The oil was extracted from the flour samples using hexane as solvent. The oil fractions were processed separately (Wäsche, et al., 2001).

Hexane extraction was carried out for 8 hours at the boiling point of the solvent (68–70°C). Using a rotary evaporator, the solvent was distilled out at 45°C under vacuum (Sbihi, et al., 2013). 0il samples were stored in dark in a tightly fitting glass bottles and kept in refrigerator for analysis (Khalid & Elhardallou, 2019).

3.5 Microemulsion Preparation

Lupin oil, Tween 80, Propylen glycol, and water/lupin protein were used to make the oil phase, surfactant, co-surfactant, and aqueous phase of the microemulsions, respectively. To establish the composition of polar, nonpolar, and surfactant phases that would form a microemulsion, a pseudoternary phase diagram was created.

Titrations of water/Lupin protein into a mixture of oil, surfactant, and co-surfactant were used to create phase diagrams. The effect of temperature on the construction of pseudoternary phase diagrams is also significant. The studies were carried out at 25 ± 0.5°C, 30 ± 0.5°C and 40 ± 0.5°C because to the direct relationship between the microemulsion region and the ambient temperature. The greatest microemulsion area was found by determining the surfactant/cosurfactant (s/co-s) ratio.

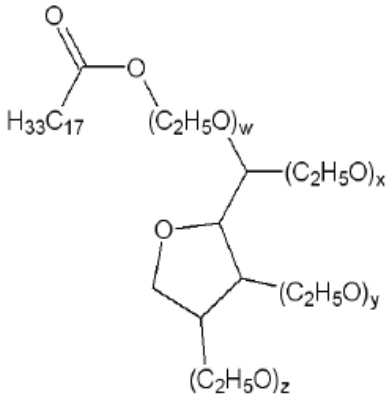
Different ratios were utilized in this study, as stated in table 2-3. The oil phase was mixed with the surfactant and co-surfactant mixtures in the prescribed ratios, Water/lupin protein was gradually titrated into the resultant combination. The microemulsion zone was characterized as the transparent fluid formulation.

3.6 Preparation and construction of phase diagrams

3.6.1 Content preparation for phase diagrams

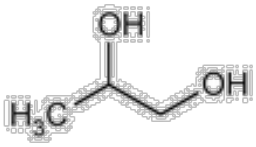
Preparation of Tween 80: Propylene glycol (1:1):

100 ml of Tween 80 and 100 ml of Propylene glycol were mixed in a glass bottle. The mixture was stirred for 1 min using magnetic stirrer until a clear, homogenized solution was obtained, figure 3 shows the molecular structure and HLB values of the Tween 80 (polysorbate 80), (Karjiban, et al., 2012) and figure 4 shows the chemical structure and HLB values of the Propylene glycol (Syed, et al., 2014).



, HLB = 15

Figure 3: Molecular structure and HLB values of the Tween 80 (polysorbate 80). x, y, z, and w were selected as 5 (Karjiban, et al., 2012).



, HLB = 4.45

Figure 4: Chemical structure and HLB values of the Propylene glycol (Syed, et al., 2014).

Preparation of the Lupin Protein: Purified water (1:1) (the titrant used in titration):
10 mg of the protein isolates were mixed with 10 ml of purified water. The pH was adjusted in a range of 2.0-10.0 using NaOH (0.1 N) or HCl (0.1 N). The suspension was stirred at room temperature from 30 min to 1 hr using magnetic stirrer until a clear homogenized solution appeared.

3.6.2 Phase diagram construction

The microemulsion area was defined using ternary phase diagrams, which specify the appropriate concentration ranges of three components (oil, water, and surfactant) that result in microemulsion production.

The preparation of samples (ternary systems) was carried out at 20°C, 30°C and 40°C through the water dilution method (titrated with water). Water was added dropwise to the mixture of lupin oil and surfactant/co-surfactant (Tween 80/propylene glycol) in different weight ratios until its solubilization limit was reached. Samples were prepared in culture tubes sealed with Viton lined screw caps and mixing gently using vortex mixer.

In Table 2, the titration percentage required amounts are shown.
Table 2 (A): Percentage of titration vs. weights

%	Total added weight (g)	Net weight Each interval (g)
4	0.0417	0.0417
8	0.0870	0.0453
12	0.1364	0.0494
16	0.1905	0.0541
20	0.2500	0.0595
24	0.3158	0.0658
28	0.3889	0.0732
32	0.4706	0.0817
36	0.5625	0.0919
40	0.6667	0.1042
44	0.7857	0.1191
48	0.9231	0.1374
52	1.0833	0.1603
56	1.2727	0.1894
60	1.5000	0.2273

Table 2 (B): Percentage of titration vs. weights

64	1.7778	0.2278
68	2.125	0.3472
72	2.5714	0.4464
76	3.1667	0.5953
80	4.0000	0.8333
84	5.2500	1.2070
88	7.3330	2.0833
92	11.500	4.1667

Each phase diagram contains varied amounts of Sweet lupin oil and (Tween 80/ PG (1:1)) for phase diagram (1), Bitter lupin oil and (Tween 80/ PG (1:1)) for phase diagram (2), as shown in Tables 3 and 4.

Table 3: Components of the phase diagram for sweet lupin.

Tube No.	**Tween 80/ PG (g) (1:1) –(B)**	**Sweet Lupin Oil (g) --(C)**
100% (C)	-	0.9936
0.5:9.5	0.0509	0.9491
1:9	0.1007	0.9015
2:8	0.2011	0.7968
3:7	0.3016	0.6972
4:6	0.4093	0.5931
5:5	0.5099	0.4857
6:4	0.5923	0.4131
7:3	0.7010	0.2955
8:2	0.8036	0.1987
9:1	0.9099	0.0971
9.5:0.5	0.9643	0.0356
100% (B)	1.0047	-

Table 4: Components of the phase diagram for Bitter lupin.

Tube No.	Tween 80/ PG (g) (1:1) –(B)	Bitter Lupin Oil (g) --(C)
100% (C)	-	1.0066
0.5:9.5	0.0560	0.9471
1:9	0.1008	0.9002
2:8	0.1999	0.7994
3:7	0.3075	0.6936
4:6	0.4175	0.5921
5:5	0.5060	0.4825
6:4	0.6064	0.3903
7:3	0.7084	0.3172
8:2	0.8240	0.1760
9:1	0.9093	0.0996
9.5:0.5	0.9507	0.0493
100% (B)	0.9999	-

These samples were titrated with (Water/sweet lupin protein (1:1)), while other samples were titrated with (Water/bitter lupin protein (1:1)), respectively, by adding dropwise until its solubilization limit was reached. (See table 2 for each titration percentage required amount). Then the samples were mixed gently using vortex mixer. After achieving equilibrium by storing the created ternary systems at 20°C, 30°C and 40°C for an overnight period, the samples were examined visually and the number of phases and anisotropy were determined using a cross polarizer.

Samples, which exhibited a single phase (i.e. monophasic) with no phase separation and optical transparency, two phase diagram were placed, and phase was examined at three temperatures. Finally, the phase diagram was drawn by using (Origin Pro 2018), as demonstrated in figure 5 and 6.

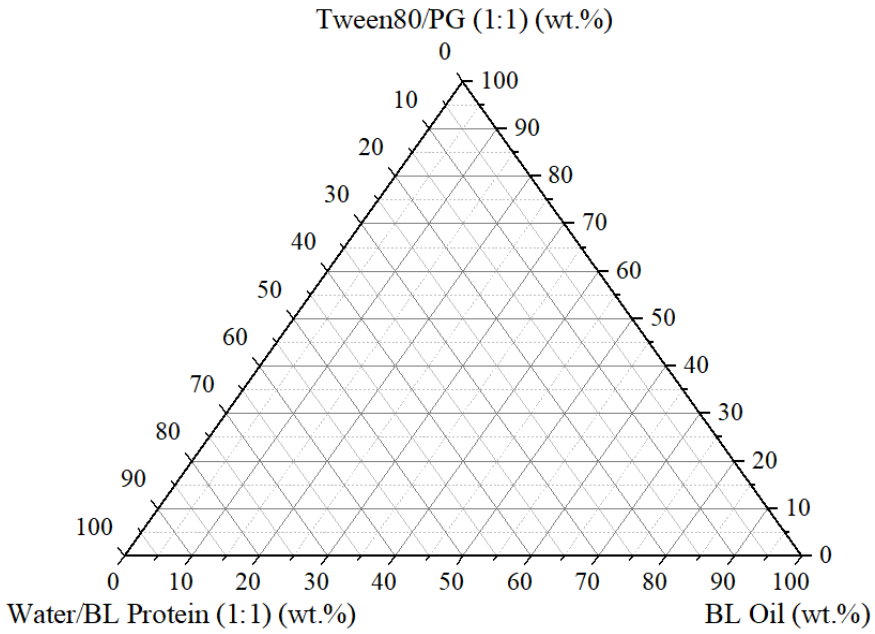


Figure 5: Ternary phase diagram composed of Sweet Lupin Oil, s/co-s (Tween 80/PG) and (Water/SL Protein)

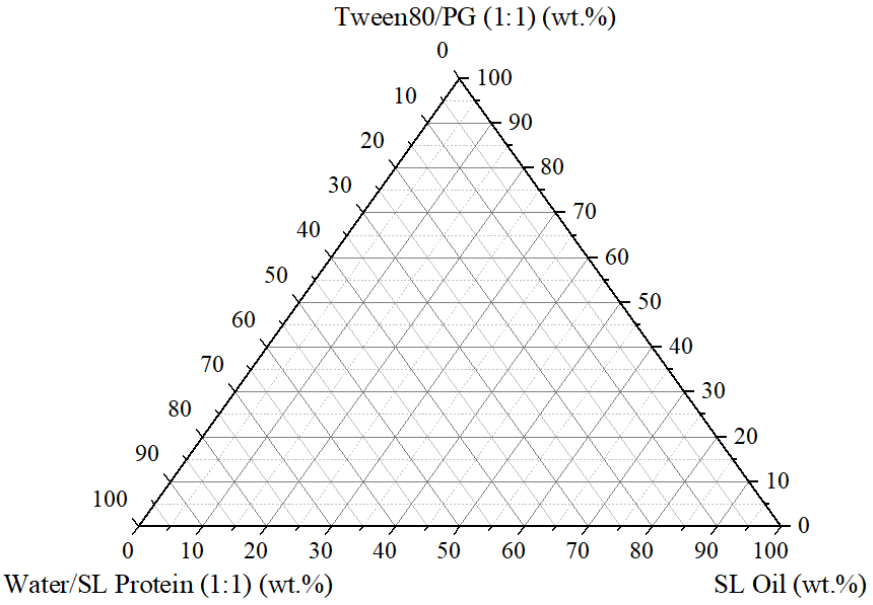


Figure 6: Ternary phase diagram composed of Bitter Lupin Oil, s/co-s (Tween 80/PG) and (Water/BL Protein)

Chapter 4: Results and Discussion

4.1 Microemulsion phase diagram results:

Our findings suggest that successful microemulsion formulations of lupin protein and lupin oil from both sweet and bitter lupins can be developed with optimal properties.

Phase diagrams

The aim of construction of ternary phase diagrams was to find out the existence region of microemulsions. The ternary phase diagrams of lupin oil, (Tween 80: Propylene glycol) (1:1) and (Water: lupin protein) (1:1) for each of the sweet and bitter lupin are presented in figure 5 and 6.

Two varieties of lupin protein were employed in this work to explore their impact on the phase behavior of a three-component system (surfactant/oil/water) and to determine the optimum area of ternary phase diagrams related to microemulsion production.

After one day of sample preparation, the samples were visually assessed for optical properties and phase separation. The findings of ternary systems made utilizing a two-step procedure in which lupin oil and a surfactant/co-surfactant (Tween 80: PG) were mixed at various ratios initially and then diluted with water were used to create the ternary phase diagrams (water: lupin protein).

4.1.1 Pseudo phase diagram # 1 at 25°C, 30°C and 40°C:

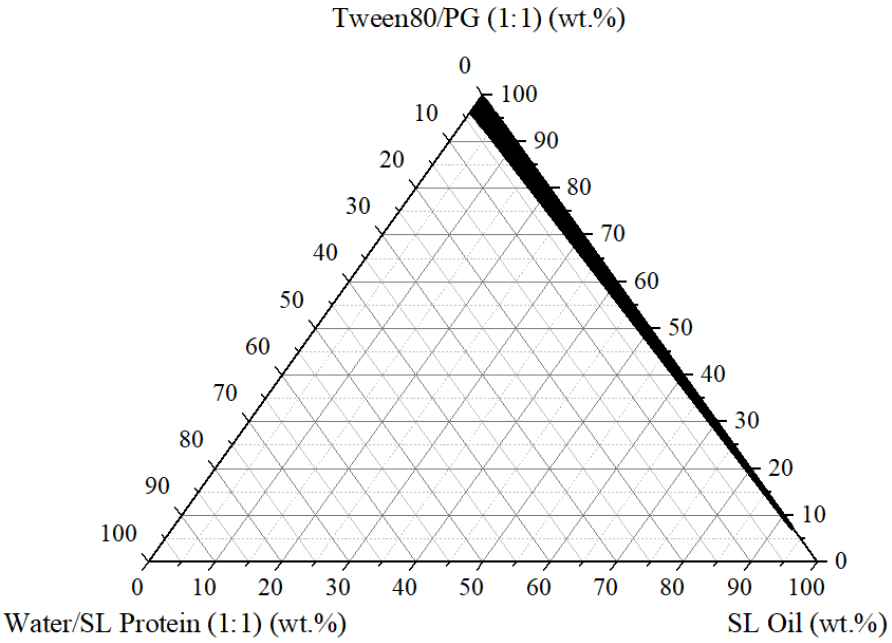
Tween 80 with PG in the ratio (1:1) in the corner (B) and SLO in the corner (C) demonstrates that microemulsion was obtained that started as a single clear, isotropic and not shiny solution by the crossed polarizers upon the addition of 4% (Water : SLP) in the ratio (1:1) in the tube No. (9.5:0.5) which contains 0.9643g of Tween 80 with PG in the ratio (1:1) and 0.0356g of SLO, and in the tube No. (100% B) which contains 1.0047g Tween 80 with PG in the ratio (1:1).

The microemulsion maintains its qualities as the temperature rises, owing to the fact that the nonionic surfactant utilized is soluble in (Water: SLP) and that protein has emulsifying properties. Since, SLO is insoluble in the (Water: SLP).

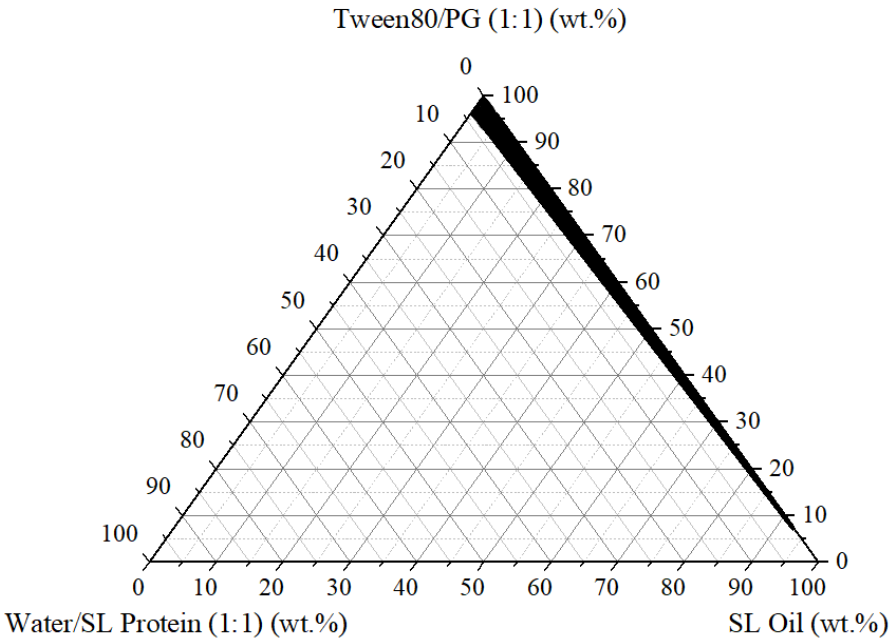
As a result, Tween 80 with PG (1:1) is employed as a tuning parameter for all ingredients, and it clearly contributes to the formation of the microemulsion for tube No. (9.5:0.5) and tube No. (100% B) up to 96% of (Water: SLP).

The physical features of the generated microemulsions did not alter under varied storage temperatures, according to the findings. Figure 7 shows a pseudo-ternary phase diagram for all of the temperature conditions investigated.

Phase diagram # 1 at 25°C:



Phase diagram # 1 at 30°C:



Phase diagram # 1 at 40°C:

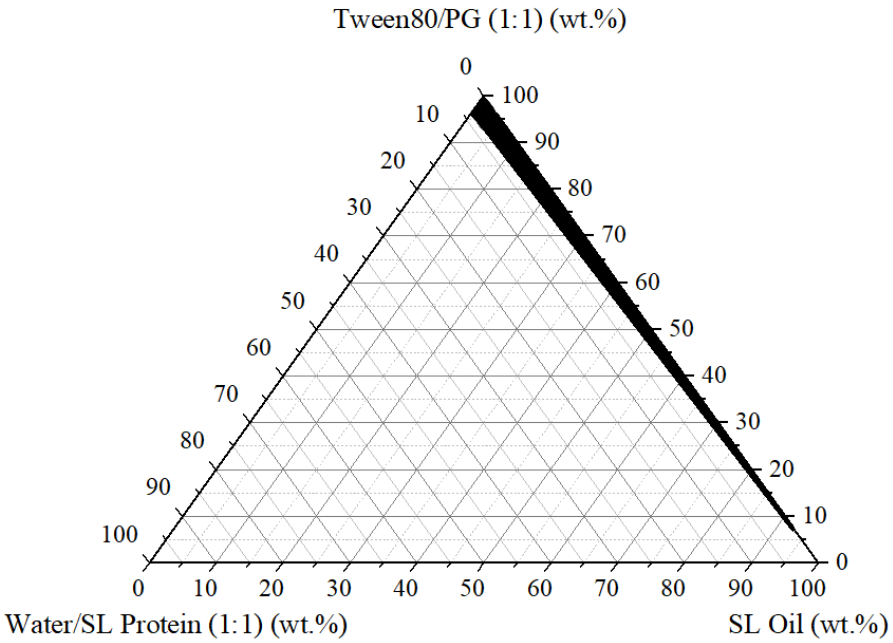


Figure 7: Pseudo-ternary phase diagram for all temperature conditions investigated (the phase diagram constructed from: Sweet lupin oil, (Tween 80 / PG) (1:1) and Water: SL Protein (1:1).

4.1.2 Pseudo phase diagram # 2 at 25°C, 30°C and 40°C:

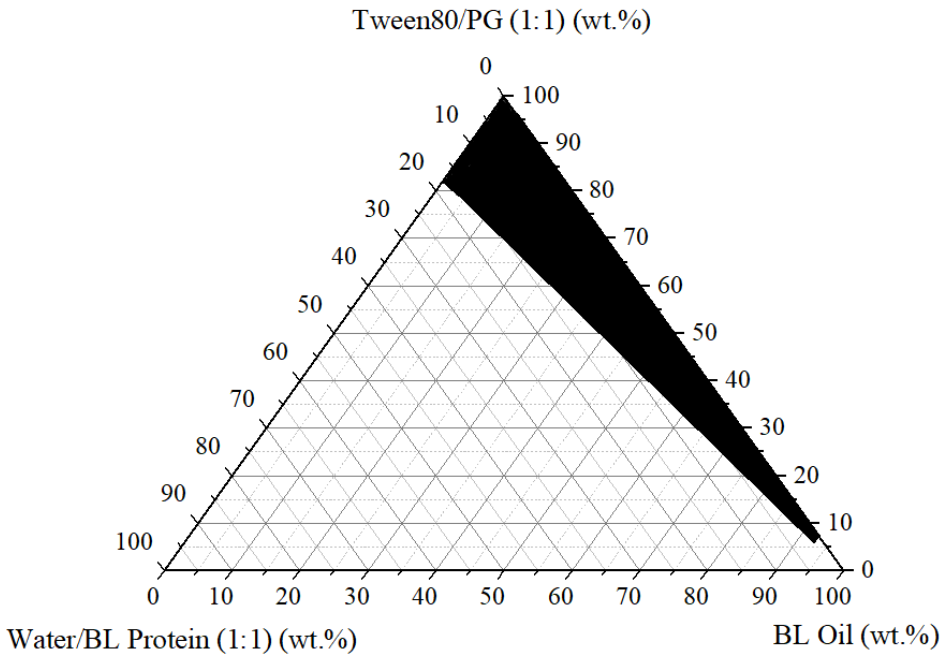
Tween 80 with PG in the ratio (1:1) in the corner (B) and BLO in the corner (C) demonstrates that microemulsion was obtained that started as a single clear, isotropic and not shiny solution by the crossed polarizers upon the addition of 4% (Water : BL P) in the ratio (1:1) in the tube no. (8:2) which contains 0.8240 g of Tween 80 with PG in the ratio (1: 1) and 0.1760g of BLO, in the tube no. (9:1) which contains 0.9093g of PG in the ratio (1: 1) and 0.0996g of BLO, in the tube no. (9.5:0.5) which contains 0.9507g of PG in the ratio (1: 1) and 0.0493g of BLO and in the tube no. (100% B) which contains 0.9999g of PG in the ratio (1: 1).

The microemulsion maintains its qualities as the temperature rises, owing to the fact that the nonionic surfactant utilized is soluble in (Water: BLP) and that protein has emulsifying properties. Since, BLO is insoluble in the (Water: BLP).

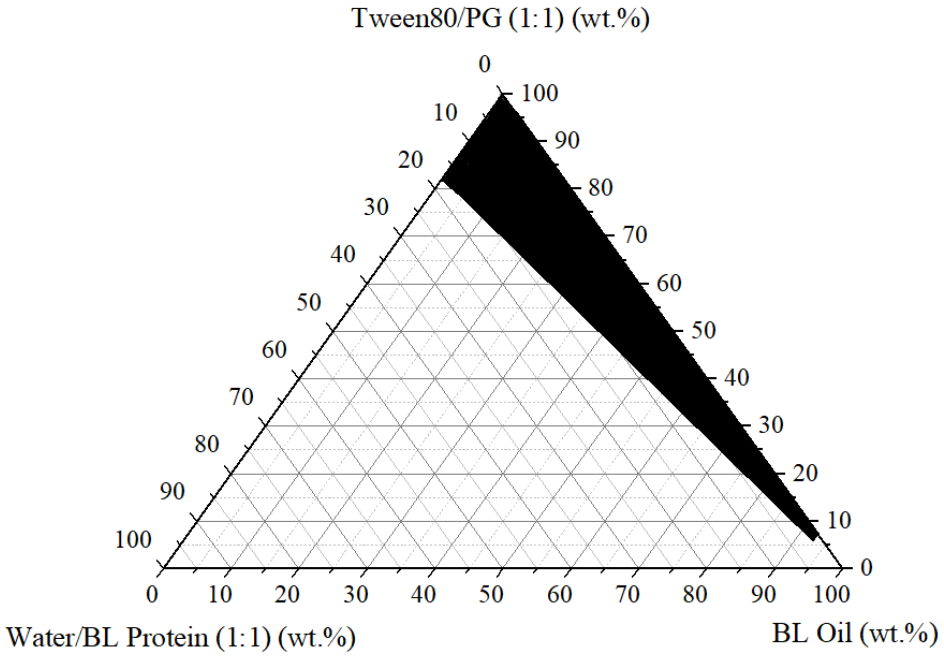
As a result, Tween 80 with PG (1:1) is employed as a tuning parameter for all ingredients, and it clearly contributes to the formation of the microemulsion for tube no. (8:2), (9:1), (9.5: 0.5) and (100% B) up to 96% of (Water: BLP).

The physical features of the generated microemulsions did not alter under varied storage temperatures, according to the findings. Figure 8 shows a pseudo-ternary phase diagram for all of the temperature conditions investigated.

Phase diagram # 2 at 25°C:



Phase diagram # 2 at 30°C:



Phase diagram # 2 at 40°C:

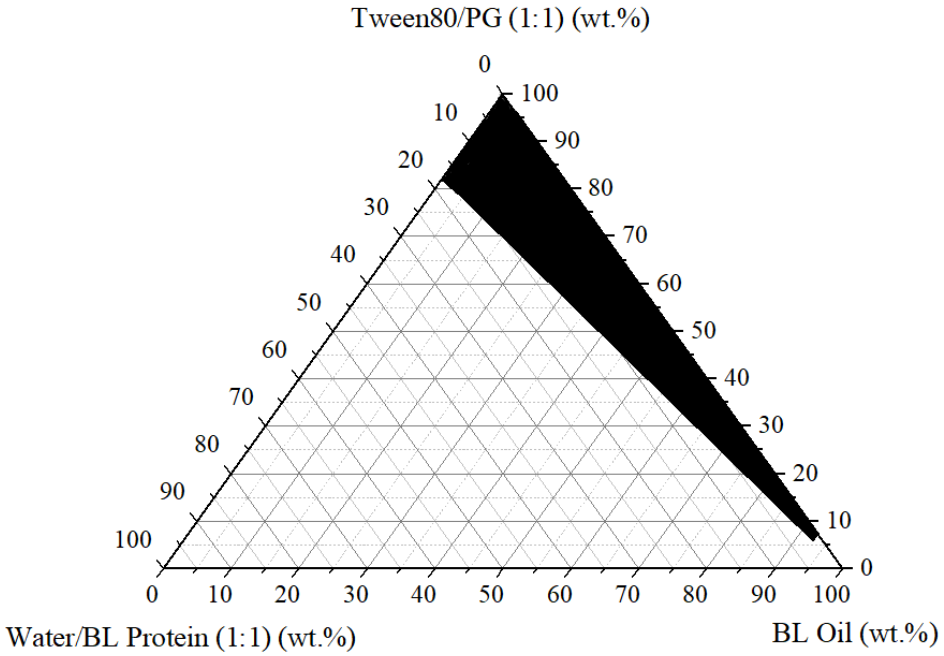


Figure 8: Pseudo ternary phase diagram at all studied temperature conditions (the phase diagram constructed from: Bitter lupin oil, (Tween 80 / PG) (1:1) and Water: BL Protein (1:1).

The results showed that the area of regions indicating microemulsions differed between the two phase diagrams.

The visual inspection trial lasted four months, with ME samples being drawn weekly during the first month and monthly for the following months. There was no evidence of phase separation, precipitation, or flocculation during the visual inspection, which indicates good physical stability of both preparations.

4.1.3 Visual appearance:

When a small amount of water is added to an oil and non-ionic surfactant mixture, a w/o microemulsion is formed. The volume proportion of water grows as the dilution advances, and as a result, water droplets join. When the emulsion inversion point is reached, further dilution with water promotes the production of bicontinuous structures, also known as lamellar phase, which is then converted into an o/w microemulsion. The formation of microemulsion is dependent on the solubilisation of the oil employed. Because oil is completely dissolved around the phase inversion threshold, microemulsions are easier to form at high surfactant concentrations. At low or medium surfactant concentrations, however, incomplete oil solubilization occurs in bigger droplets, resulting in a coarse emulsion and even multiphase systems.

The developed microemulsion containing lupin oil, tween 80, propylene glycol and water/lupin protein was found to be transparent at increased tween 80, propylene glycol and water/lupin protein content and decreased oil content. At first, the microemulsion showed

turbid appearance but at increased water/lupin protein content and S/Cos ratio 1:1 it showed transparent flowable microemulsion.

Visual appearance of of Sweet lupin oil, (Tween 80:PG), (Water:Sweet lupin protein) at 25°C, 30°C and 40°C:

As shown in figure 9 visual appearance of samples that formed by titrating gradually for (Water: Sweet lupin protein) to the (100% (C), 0.5:9.5, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 9.5:0.5 and 100% (B), ratio of oil (B) to surfactant (C)) of the phase diagram for Sweet lupin oil, (Tween 80: PG), (Water: SLP) ternary systems.

For the SLO/ (Tween 20/PG) (1:1)/ (Water/SLP) (1:1) system, the samples in dilution tube no. (9.5:0.5) and tube no. (100% B) of the ternary phase diagram were used to make microemulsions with optical transparency, as shown in Figure 9.

The type of microemulsion formed when a low weight fraction of (water:SLP) was added in tube no. (9.5:0.5) and tube no. (100% B) was an o/w microemulsion; however, further dilution with (water:SLP) causes the formation of bicontinuous structures, also known as lamellar phase, followed by conversion to an o/w microemulsion. These emulsion samples were not opaque and had a translucent appearance in terms of transparency and opacity.

The rest of the samples in the phase diagram (100% (C), 0.5:9.5, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:3, 8:2, 9:1) had a higher ratio of oil to surfactant. The sample was discovered to have a thick gel-like texture at low water dilution levels. The material became a turbid dispersion with some phase separation and/or creaming as the dilution progressed.

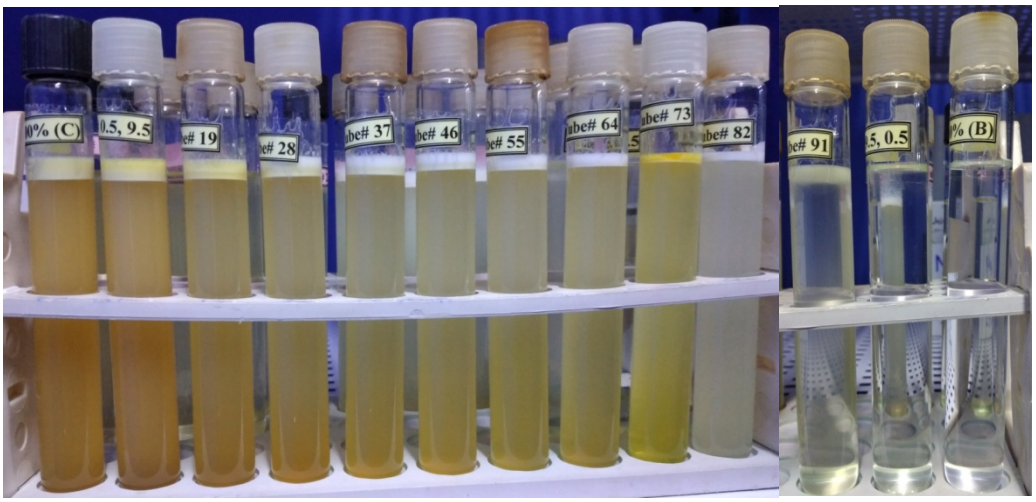


Figure 9: Visual appearance of Sweet lupin oil, (Tween 80: PG), (Water: Sweet lupin protein) at 25°C, 30°C and 40°C.

Visual appearance of of Bitter lupin oil, (Tween 80:PG), (Water:Bitter lupin protein) at 25°C, 30°C and 40°C:

As shown in Figure 10, the visual appearance of the samples formed by titrating gradually for (Water: BLP) to (100% (C), 0.5: 9.5, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 9.5:0.5 and 100% (B), ratio of oil (C) to surfactant (B)) of the phase diagram of bitter lupin oil , (Tween 80:PG), (Water:BLP) ternary systems.

For the BLO / (Tween 20/PG) (1:1)/ (Water/ BLP) (1:1) system, the samples in dilution tube no. (8:2), (9:1), (9.5: 0.5) and (100% B) of the ternary phase diagram were used to make microemulsions with optical transparency, as shown in Figure 10.

The type of microemulsion formed when a low weight fraction of (water: BLP) was added in tube no. (8:2), (9:1), (9.5: 0.5) and (100% B) was an o/w microemulsion; however, further dilution with (water: BLP) causes the formation of bicontinuous structures, also known as lamellar phase, followed by conversion to an o/w microemulsion. These emulsion samples were not opaque and had a translucent appearance in terms of transparency and opacity.

The rest of the samples in the phase diagram (100% (C), 0.5:9.5, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3) had a higher ratio of oil to surfactant. The sample was discovered to have a thick gel-like texture at low water dilution levels. The material became a turbid dispersion with some phase separation and/or creaming as the dilution progressed.

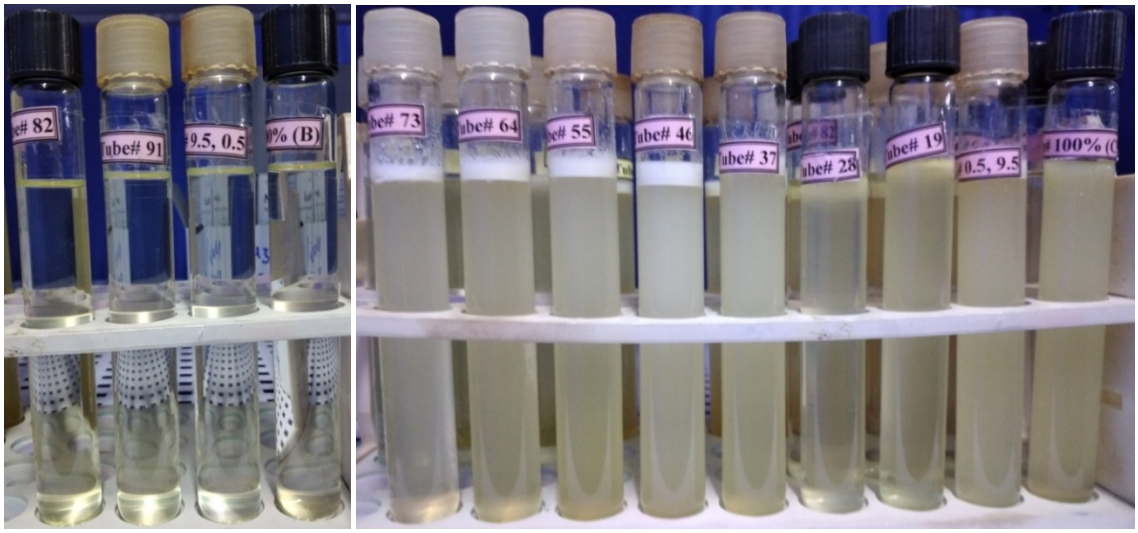


Figure 10: Visual appearance of Bitter lupin oil, (Tween 80: PG), (Water: Bitter lupin protein) at 25°C, 30°C and 40°C.

Microemulsion was found to be transparent at increased tween 80, propylene glycol and water/lupin protein content and decreased oil content. At first, the microemulsion showed turbid appearance but at increased water/lupin protein content and S/Cos ratio 1:1 it showed transparent flowable microemulsion.

Our results were similer those reported by El-Adawy (2001) who found that the both bitter and sweet lupin isolates (Isolate-PI, Protein isolate generated by alkaline water extraction/isoelectric precipitation) reduce fat absorption.

According to El-Adawy (2001), the emulsification capacities of lupin protein isolate are equivalent to those of other well-known vegetable proteins such as soybean. As a result, its incorporation into meat products, such as minced meat analogs, will be highly anticipated. This broadens the spectrum of uses for lupin protein isolates in the food industry.

The alkaloid content had little effect on the seed's oil content, but it did have a substantial impact on the protein content. Bitter seeds have a higher protein level, according to the findings (Staples ET AL., 2017).

Overall, we noted that the area of the microemulsion that formed from bitter lupin more than that formed from sweet lupin.

Chapter 5: Conclusion

Lupin is a dietary source with a variety of health benefits. Lupin has many beneficial features, and they are the greatest choice for manufacturing protein isolates due to their high protein concentration, low cost, and popular appeal. Protein isolates are increasingly used in food items due to their functional characteristics, and they have been used for numerous therapeutic purposes since ancient times.

The study's main goal was to create and compare ternary phase diagrams with two types of Lupin oil, a surfactant/co-surfactant (Tween 80: PG), and water with two types of lupin protein (Sweet and Bitter lupin protein), with the purpose of determining whether regions of ternary phase diagrams allow for the development of o/w or o/w microemulsions.

Two types of lupin proteins (BLP&SLP) were employed in this phase diagram investigation to see how they affected the creation of the optimum region of ternary phase diagrams correlating to the development of microemulsions. Bitter lupin protein formed a larger micro-emulsion area than sweet lupin protein.

The secondary emulsions were transparent at low oil content, but as the oil content increased, they turned muddy. These surfactants, particularly Tween 80, showed a higher capacity for oil droplet solubilization, which can be attributed to their longer hydrocarbon tail. Because of the oil molecules integrated between the non-polar tails, as well as the emulsifying characteristics of the lupin protein.

In all oils, the interesting findings achieved with a surfactant mixture of Tween 80 and PG, which encouraged the production of ME and turbid emulsion phases but no gel phase.

Visual inspection of samples and birefringence measurements using a polarized microscope were used to accomplish this. O/w microemulsions were found to form primarily at the corner of the surfactant-rich area based on the ternary phase diagrams generated.

The physical features of the created microemulsion did not change over the course of three months at various storage temperatures, according to the findings. Furthermore, we established that lupin protein-based microemulsions have the best features for oral or topical administration. As a result, we were able to create a stable microemulsion for oral and topical application of lupin seed protein.

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استخلاص و عمل مستحلب دقيق من بروتين الترمس

إعداد الطالبة: منار ثمين حماد البدارين

إشراف: البروفيسور إبراهيم كيالي

المشرف الثاني: الدكتور ميشيل حنانيا

الملخص:

تم دمج البروتينات المستخرجة من بذور الترمس في المستحلب الدقيق لتوفير معلومات مفيدة حول التصميم العقلاني للمستحلبات الدقيقة الشفافة كنظم توصيل محتملة للمركبات النشطة بيولوجيًا للتطبيقات في مجالات الأغذية والمشروبات والمجالات غير الغذائية.

في هذه الدراسة، تم استخلاص البروتين والزيت من الترمس الحلو والمر باستخدام طريقة Soxhlet، والتي تم استخدامها لتحديد سلوك الطور لنظام ثلاثي المكونات (خافض للتوتر السطحي / زيت / ماء) وإنشاء مخططات الطور الثلاثية وتحديد مستوى المنطقة الأمثل من مخططات الطور الثلاثية المقابلة لتشكيل المستحلبات الدقيقة.

تم فحص تكون المستحلبات الدقيقة بحجم قطيرة أصغر من 100 نانومتر ومثبتة بواسطة مواد خافضة للتوتر السطحي غير الأيونية باستخدام طرق معايرة المستحلب. تم تحضير سلسلة من الأنظمة الثلاثية؛ ثلاثية المكونات (زيت الترمس، توين 80 / البروبيلين جليكول والماء / بروتين الترمس) بنسب مختلفة عن طريق التحريض اللطيف بواسطة طريقة تكوين انعكاس الطور التي تتضمن التكوين التلقائي للمستحلب الدقيق.

تم تمييز سلوك الطور وتشكيل المستحلب الدقيق للمخاليط الثلاثية المحضرة بالملاحظة البصرية لفصل الطور والوضوح البصري (مثل الشفافية والتعتيم).

نتيجة لذلك، تم إنشاء مخططات الطور بناءً على المواد الخافضة للتوتر السطحي الجزيئية الصغيرة (توين 80) والتي تحدد نسب المكونات الثلاثة في تكوين المخاليط الثلاثية التي تسمح بتكوين مستحلبات دقيقة من نوع الزيت في الماء (o/w) أو من نوع الماء في الزيت (w/o).

بشكل عام، وجد أن المستحلبات الدقيقة من نوع الزيت في الماء (o/w) تتشكل في منطقة صغيرة من مخططات الطور الثلاثية مع نسبة كبيرة نسبيًا من الماء / بروتين الترمس.

بين مخططي الطور الثلاثي على أساس Tween 80 / PG، كانت هناك بعض الاختلافات في مناطق تكوينها المسؤولة عن تكوين المستحلبات الدقيقة وكذلك أنواع أخرى من المراحل المتكونة، بما في ذلك ثنائية ومتعددة الأطوار، والبلورات السائلة، والهلام والمستحلبات الخشنة. في هذه الدراسة، تم إنتاج المستحلبات الدقيقة بطريقة تسمى "طريقة المعايرة."

تم فحص نوع وتركيز المادة الخافضة للتوتر السطحي (توين 80) والزيوت (زيت الترمس) لتأثيرها على إذابة جزيئات الزيت من قطرات المستحلب إلى مذيلات الفاعل بالسطح، وبالتالي تكوين المستحلب الدقيق.
أظهرت النتائج أن Tween 80/PG لديها قدرة أفضل على إذابة قطرات الزيت عند إضافتها إلى الماء / بروتين الترمس، لأن البروتين له خصائص استحلاب.

أشارت النتائج إلى أن الخصائص الفيزيائية للمستحلبات الدقيقة المطورة لم تتغير تحت درجات حرارة التخزين المختلفة.