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Extraction and Microemulsion of Lupin Protein

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In present study, protein and oil were extracted from sweet and bitter lupin that were used to define the phase behaviour of a three-component system (lupin oil, Tween 80/propylene glycol and water/lupin protein). Ternary phase diagrams were constructed and the optimum area of ternary phase diagrams corresponding to the formation of microemulsions were identified using emulsion titration method. Phase inversion composition method was applied, which involved the spontaneous formation of microemulsion, characterized by visual observation for their phase separation and optical clarity (*e.g.* transparency and opacity). Generally, o/w microemulsions were formed at a small region of the ternary phase diagrams with a relatively large ratio of water/lupin protein. Some differences between the sweet and bitter lupin diagrams were observed in regions, including bi- and multiphase, liquid crystals, gel and coarse emulsions. The physical characteristics of the microemulsions did not change with different storage temperatures.

Keywords: Sweet lupin, Bitter lupin, Protein extraction, Microemuslion, Phase diagram.

INTRODUCTION

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. Lupin or lupine are trivial names for plants of the genus *Lupinus* belonging to the Leguminosae family, subfamily Papilonoideae [1]. Lupine agriculture dates back at least 2000 years and it is most likely to have started in Egypt or the Mediterranean region [2]. This genus have very diversity and contains several known species [1].

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 [2-5]. Lupin seeds are characterized as "sweet" or "bitter" based on the amount of alkaloids present [6], which can range from 0.01 to 4%. Lupin seeds are well-known for providing a variety of necessary nutrients, such as carbohydrates, dietary fibers, 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, it was found that protein extract from lupin could inhibit cell migration in colon cancer cells [7].

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 [8]. 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 [9]. Depending on the composition, three types of microemulsions are most likely to develop; oil in water (o/w) microemulsions, water in oil microemulsions (w/o) and bi-continuous microemulsions [10].

Microemulsions have found broad applicability in the 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 [11]. In theory, micro-

emulsions can be utilized in a variety of ways to deliver drugs, but topical application of microemulsions is gaining favour [12].

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 [13,14]. Globulins and albumins are the most abundant protein classes in legume seeds; prolamin and glutelin fractions are also found, albeit in very small amounts [15,16]. 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 [17], whereas globulins are isolated in salt solutions [16].

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 [18]. In literature, various isolation procedures were used to investigate the emulsifying and water binding capacities of lupin protein either isolates or concentrates [19].

In this study, protein and oil were extracted from the sweet and bitter lupin using Soxhlet method, which were used to define the phase behaviour 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. According to our best knowledge and upon searching the literature, no similar work has been done.

EXPERIMENTAL

Bitter lupin (*Lupinus albus*) and sweet lupin (*Lupinus angustifolius*) seeds were obtained from the local market. Non-ionic small molecule surfactant polysorbate 80 (Tween 80) emulsifier was purchased from Eigenmann & Veronelli, propylene glycol, a cosurfactant, was purchased from Daw Chemicals, while purified water was obtained from Beit Jala Pharmaceutical Company, Palestine. All compounds were used as supplied while all other used chemicals were of reagent grade.

Preparation of lupin seed flour and oil extraction (defatting): Lupin seeds (sweet and bitter) were ground using a household mill and then sieved to form a fine powder. The flour samples were defatted using hexane as a solvent in a Soxhlet apparatus for 8 h to a final fat content of less than 0.5%. The solvent was evaporated under vacuum using rotary evaporator [20]. Oil samples were stored in dark in a tightly fitting glass bottles and kept in refrigerator for analysis [21].

Extraction of protein: The extraction of protein in lupin seeds was carried out according to a known procedure [14] at room temperature. Briefly, the defatted flour was stirred with deionized water (1:10) for 30 min. The pH of the solution was adjusted to 9.0 using 1.0 M NaOH solution and further stirred for 1 h. The resultant mixture was centrifuged at 3000 rpm for 15 min, where 1 M HCl was added to the supernatant to a pH 4.5 and the mixture was stirred for 1 h. The solvent was centrifuged at 3000 rpm for 15 min, decanted and then the residue was washed several times with water and finally stored in refrigerator.

Microemulsion preparation: Lupin oil, propylene glycol, Tween 80 and water/lupin protein were used to make the oil phase, surfactant, cosurfactant and aqueous phase of the microemulsions, respectively. To establish the composition of polar, non-polar and surfactant phases that would form a microemulsion, a pseudo-ternary phase diagram was constructed.

Titrations of water/lupin protein into a mixture of oil, surfactant and cosurfactant were used to construct 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, 30 ± 0.5 and 40 ± 0.5 ºC. The largest microemulsion area was found by determining the surfactant/cosurfactant (s/co-s) ratio.

Preparation of Tween 80:propylene glycol (1:1): Tween 80 (100 mL) and propylene glycol (100 mL) were mixed in a glass bottle. The mixture was stirred for 1 min using magnetic stirrer until a clear, homogenized solution was obtained.

Preparation of lupin protein:purified water (1:1): The protein isolates (100 mg), both sweet and bitter, were mixed separately with 100 mL of purified water. The pH was adjusted to 10.0 using 0.1 M NaOH solution. The suspension was stirred at room temperature from 30 min to 1 h using magnetic stirrer until a clear homogenized solution appeared.

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 through the water dilution method (titration with water). Water was added dropwise to the mixture of lupin oil and surfactant/cosurfactant (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.

After the dropwise addition of water, samples were mixed gently using a Vortex mixer. After achieving equilibrium by storing the formed ternary systems at 25, 30 and 40 ºC overnight, the samples were examined visually and the number of phases and anisotropy were determined using a cross polarizer.

Phase diagrams were constructed for both sweet and bitter lupin samples that exhibited a single phase (*i.e.* monophasic) with no phase separation and optical transparency, by using Origin Pro 2018 Program.

RESULTS AND DISCUSSION

Microemulsion phase diagram: In pseudo-phase diagram (Fig. 1a-c), where Tween 80 with propylene glycol (PG) in the ratio (1:1) is in the corner (B) and sweet lupin oil (SLO) is in the corner (C), a microemulsion was clearly obtained. It started as a single clear, isotropic and not shiny solution, examined

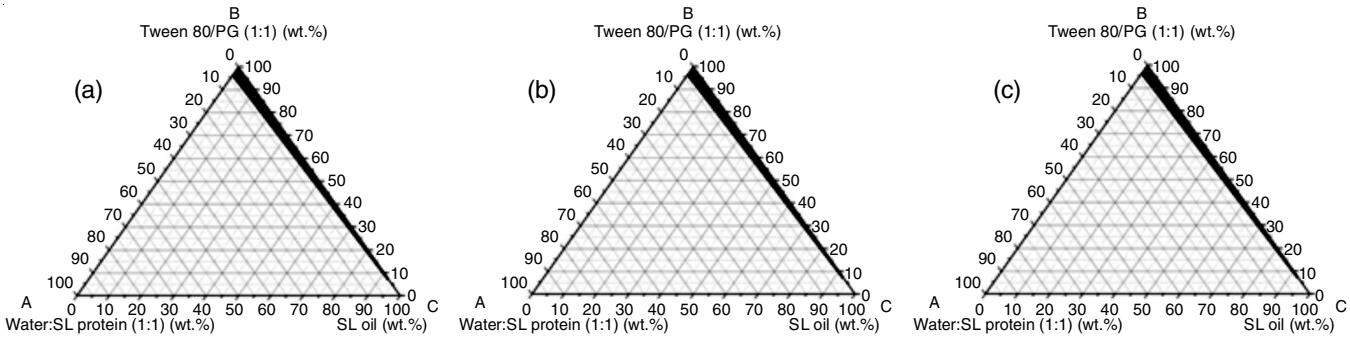

Fig. 1. Pseudo phase diagram of sweet lupin oil that is constructed from sweet lupin oil, (Tween 80/PG) (1:1) and water:SL protein (1:1). (a) 25 °C, (b) 30 °C and (c) 40 °C

by the cross polarizer. As shown in Fig. 2, two samples only, tube No. (9.5:0.5) and tube No. (100% B) showed clear one phase microemulsion. It is worth to note that the first number of each tube represents the amount of oil, while the second number indicates the amount of Tween/PG 1:1.

According to the findings, the microemulsion maintained its quality and physical properties as the temperature was raised (Fig. 1b-c), owing to the fact that the non-ionic surfactant utilized is soluble in water:sweet lupin protein and that the protein has emulsifying properties. 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). Consequently, successful microemulsion formulations of lupin protein and lupin oil from sweet lupins can be developed with optimal properties.

Pseudo-phase diagram for bitter lupin oil (BLO): Tween 80 with PG in the ratio (1:1) in the corner (B) and BLO in the corner (C) demonstrates that microemulsion was obtained. It started as a single clear, isotropic and not shiny solution, examined by the cross polarizer. Four samples only (tubes No. 8:2, 9:1, 9.5:0.5 and 100% B) showed clear one phase microemulsion (Fig. 3). It is worth to mention that the first number of each tube represents the amount of oil, while the second number indicates the amount of Tween/PG 1:1.

Similar to the sweet lupin characteristics, the microemulsion maintains its qualities as the temperature rises, owing to the fact that the non-ionic surfactant utilized is soluble in water:BLP and that protein has emulsifying properties. Since, BLO is insoluble in the (sater:BLP). The physical features of the generated microemulsions did not alter under varied storage temperatures, according to the findings. Fig. 3 shows a pseudo-ternary phase diagram for all of the temperature conditions investigated.

Visual appearance: The visual inspection trial lasted for four months, with microemulsion 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.

When a small amount of water is added to an oil and non-ionic surfactant mixture, a w/o microemulsion is formed. The

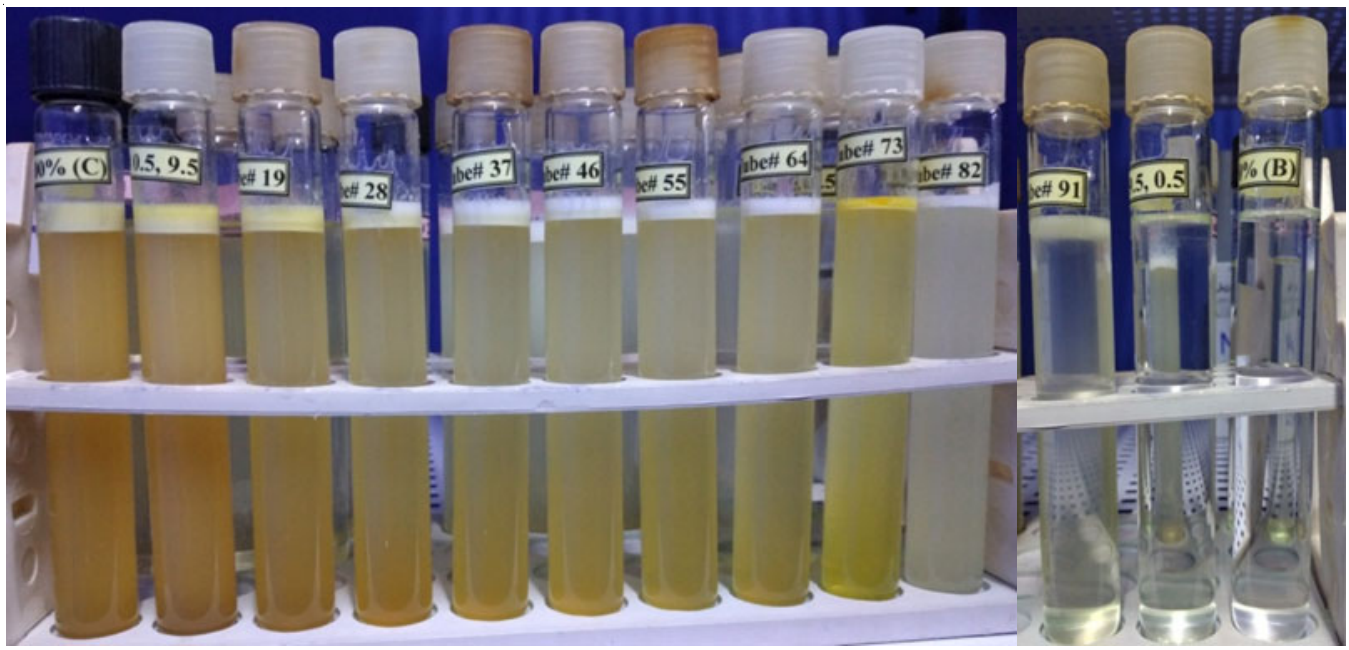

Fig. 2. Visual appearance of sweet lupin oil, (Tween 80: PG), (water:sweet lupin protein (SLP)) at 25, 30 and 40 °C

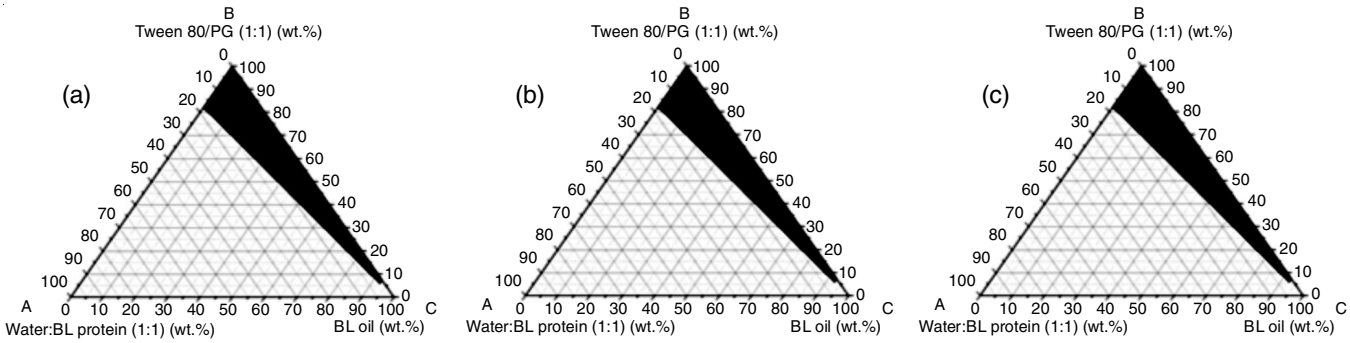

Fig. 3. Pseudo phase diagram of bitter lupin oil, that is constructed from bitter lupin oil, (Tween 80/PG) (1:1) and water:BL protein (1:1). (a) 25 °C, (b) 30 °C and (c) 40 °C

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 [22]. The formation of microemulsion is dependent on the solubilization 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 [23].

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 sweet lupin oil, (Tween 80:PG), (water:sweet lupin protein): For the SLO/(Tween 20/PG) (1:1)/(water/SLP) (1:1) system, the samples in dilution tubes No. (9.5:0.5 and 100% B, Fig. 2) of the ternary phase diagram were used to make microemulsions with optical transparency. The type of microemulsion formed when a low weight fraction of (water:SLP) was added in tube 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 and 9:1) had a higher ratio of oil to surfactant. These samples were 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.

Visual appearance of bitter lupin oil, (Tween 80:PG), (water:bitter lupin protein): 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, (Fig. 4). The type of microemulsion formed when a low weight fraction of (water:BLP) was an o/w microemulsion. On the

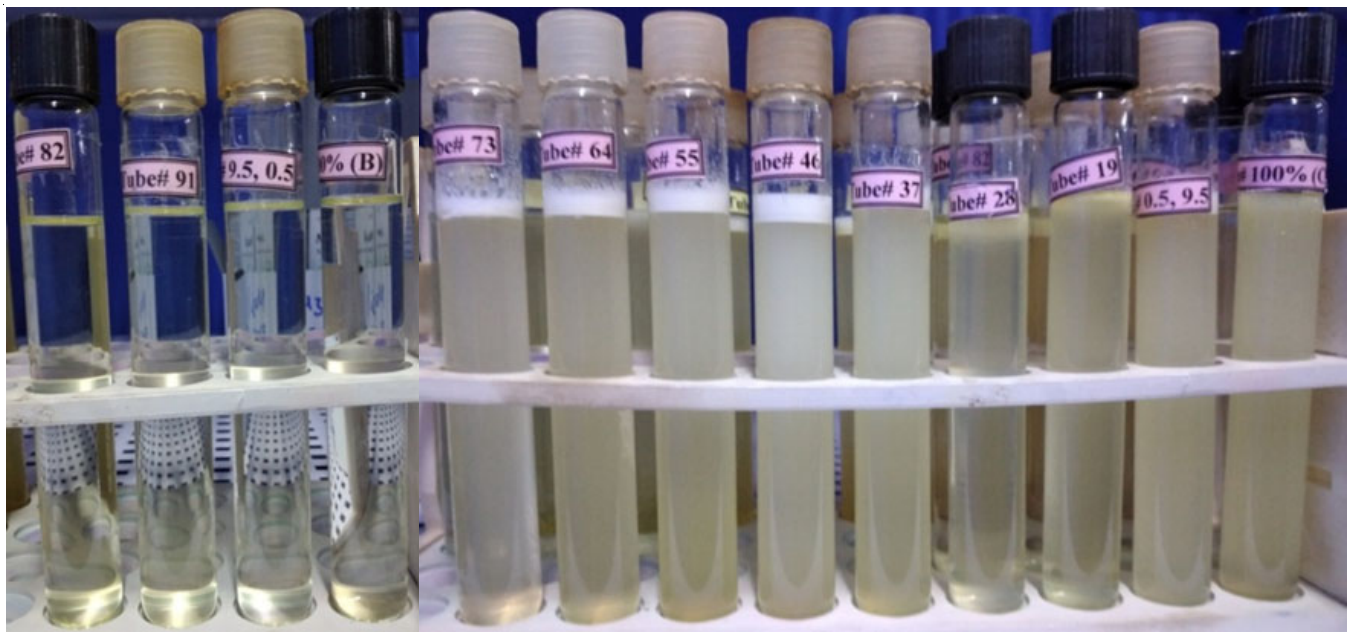

Fig. 4. Visual appearance of bitter lupin oil, (Tween 80: PG), (water:bitter lupin protein (SLP)) at 25, 30 and 40 °C

other hand, 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 and 7:3) had a higher ratio of oil to surfactant. The samples had 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. 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.

Present results were similar to reports [24], which showed that both bitter and sweet lupin isolates (isolate-PI, protein isolate generated by alkaline water extraction/isoelectric precipitation) reduce the fat absorption. The emulsification capacities of lupin protein isolate are equivalent to those of other well-known vegetable proteins such as soybean [24]. 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. Moreover, the alkaloid content had little effect on the seeds oil content, but it did have a substantial impact on the protein content. Bitter seeds have a higher protein level, according to the findings [25]. Overall, it is noted that the microemulsion area formed from bitter lupin were more than sweet lupin.

Conclusion

In this study phase diagrams with two types of lupin oil, a surfactant/cosurfactant (Tween 80:PG) and water with two types of lupin protein (sweet and bitter lupin protein) were constructed. It was found that bitter lupin protein formed a larger microemulsion area than that of sweet lupin protein. The secondary emulsions were transparent at low oil content, but as the oil content increased, they turned opaque. These surfactants, particularly Tween 80, showed a higher capacity for oil droplet solubilization, which can be attributed to their longer hydrocarbon tail. This is because oil molecules were integrated between the non-polar tails, as well as the emulsifying characteristics of the lupin protein. The physical features of the formed microemulsion did not change over the course of four months at various storage temperatures (25, 30 and 40 ºC). Furthermore, it is established that lupin protein-based microemulsions have the best features for the oral or topical administration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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