Investigation of emulsification and application of 2 distinct emulsifiers: Soybean Lecithin and Phospholipase A-1 Hydrolyzed Lecithin

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Abstract: Liquid soybean lecithin oil (LSLO) and PLA1 hydrolyzed soybean lysolecithin oil (LPSLO) have been recognized as potential emulsifiers. This study aims to investigate the the variation in addition of soybean lecithin and soybean lysolecithin influences the Emulsification Ability and Stability in emulsions. An experimental setup entailing five concentrations (1%, 1.5%, 2%, 2.5%, and 3% of total mixture mass with LLO and LPSLO with water was studied where the continuous phase and soybean oil as the dispersed phase. These were prepared by homogenizer and studied at various time intervals to 30 mins. LPSLO performed significantly better in EA and ES than the control at lower concentrations, which was attributed to its higher hydrophilicity so it could integrate more efficiently with water without being differentiated. However, the difference was less pronounced at higher concentrations, with LSLO being equivalent to or better than LPSLO in some cases. Despite being limited due to experimental setup drawbacks, the method is reproducible increasing its credibility.

1. Introduction

Emulsification entails the process of stabilizing mixtures of immiscible liquids such as oil and water. It is a crucial process utilized in various industries such as cosmetics, food production and pharmaceuticals (Hasenhuettl)[1]. For instance, emulsifiers are essential in creating stable products such as mayonnaise, ice cream, and dressings to ensure a consistent mixture of oil and water. Currently, there is a demand for natural emulsifiers consisting of polysaccharides, polysaccharides, saponins and proteins (Ozturk and McClements)[2]. One of the most frequently used emulsifiers is Liquid soybean lecithin oil (LSLO) due to lower interfacial tension between oil and water (Tabaniag et al., “Stabilization of Oil/Water Emulsions Using Soybean Lecithin as a Biobased Surfactant for Enhanced Oil Recovery”)[3]. On the other hand, liquid PLA1 hydrolyzed soybean lysolecithin oil (LPSLO) is capable of potentially enhancing effectiveness in particular emulsification environments. This is brought about by a modified form of lecithin hydrolyzed by enzymes. Therefore, this current study aimed to investigate the difference between lecithin and lysolecithin emulsification ability through an experiment. Subsequently, it aimed to study how does the variation in addition of soybean lecithin and soybean lysolecithin influence the Emulsification Ability and Stability in emulsions. Thereby, it would aim to investigate the difference between lecithin and lysolecithin emulsification ability through an experiment, to use the experiment’s results to evaluate the effectiveness and to deduce the importance of applying lecithin and lysolecithin in the idea of “pre-digestion.

Furthermore, it was hypothesized that:

H1 hypothesis: For all samples, the emulsifiers' Emulsification Ability (or EA) and Emulsification Stability (or ES) will vary according to different emulsifiers proportion addition. The higher the addition of emulsifiers, the better EA and ES at and over a certain period of time, and vice versa.

H0 hypothesis (null hypothesis): For all samples, there is no significant relationship between a change in the proportion addition of emulsifiers and their EA and ES.

The findings of the research would contribute to the industrial application to a great extent. Additionally, it can also contribute to highly efficient and low-cost production processes, by studying

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the optimal concentrations of these emulsifiers in different applications like the food industry, cosmetics field or pharmaceutical area. Thereby, the results would be instrumental in developing new sustainable industrial products.

2. Review

2.1. Overview of Emulsification

Emulsification is the process of lowering the interfacial tension, which is the force that separates and maintains a boundary between two liquid phases. The presence of surfactants which are amphiphilic molecules is the key determinant of their stability (Chen and Tao)[4]. Thereby, these detergents lower the surface tension among immiscible liquids, thus improving their dispersion and cohesion over time. The determination of the appropriate surfactant for different types of emulsions can be measured by the degree to which a surfactant is hydrophilic or lipophilic known as hydrophilic-lipophilic balance (HLB).(See Supplementary materials available in Zenodo at 10.5281/zenodo.14287842)[5].

2.1.1. Lecithin

Lecithin is a type of phospholipid with an additional organic compound choline in the phosphate group (Rincón-León). It is a natural emulsifier that is found in eggs, soybean (oil), sunflower, and other food sources that are consumed daily (Joe). Phospholipids function as a component of phospholipid bilayer for the universal basis for cell-membrane structure functioning as a selectively semi-permeate certain substances and increase the efficiency of exchanging substances (Cooper; Alberts)[6-7]. Additionally, the amphipathic property of lecithin allows it to act as a surfactant that lowers the interfacial tension between two immiscible liquids (Yamamoto and Araki)[8]. It allowed them to mix and form a stabilised emulsion, and the process of creating an emulsion is called emulsification.

2.1.2. Phospholipase A_1

PLA_1 is an enzyme that hydrolyzes phospholipids to produce a lysophospholipid and a fatty acid chain (Richmond and Smith). By using PLA_1 to hydrolyze lecithin at the S_n1 position, the lysolecithin loses a saturated palmitic fatty acid chain, which increases the hydrophilic properties and thus performs a higher emulsification ability than the original lecithin (Estiasih et al.)[9].

2.1.3. Chemistry of lecithin and lysolecithin

LSLO is a liquid emulsifier that has a translucent, yellowish-brown, viscous appearance and a high concentration of lecithin ("Soy Lecithin Liquid (Organic) - Get Natural Essential Oils"). The chemical structure of lecithin in LSLO contains one saturated palmitic acid (CH_3-$(CH_2)_{14}$-COOH) and an 8-9 cis monounsaturated oleic fatty acid chain (CH3-(CH2)7-CH=CH-(CH2)7-COOH) (see Figure 1). Additionally, the cis double bond that existed on the latter fatty acid chain disrupts the regular linear structure of the fatty acid chain creating a 'kink' that provides lower hydrophobic properties than the former one (Joe).

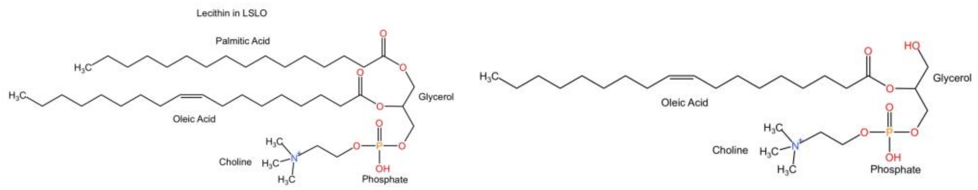

Figure 1. Lewis's structure of Lecithin (above) and PLA1 in LSLO and LPSLO, respectively.

Furthermore, the liquid PLA_1 hydrolyzed LPSLO is a manufactured liquid emulsifier that contains a high concentration of lysolecithin. In this case, the hydrogen in the hydroxyl group in the S_n1 position of glycerol and the hydroxyl group in the saturated palmitic acid chain is lost after esterification or condensation reaction (see Figure 1)

2.1.4. Structural Comparison

In the structure of PLA1 digested lecithin in LPSLO, the removal of the 8-9 cis monounsaturated oleic acid chain leads to increased hydrophilic properties. Consequently, the formation of the hydroxyl group with methine group in glycerol forms a hydrogen bond further increasing the hydrophilic property in LPSLO.

The structural composition can also be studied through oil phase percentage (OP%) as the above layer is more purely oil than emulsion following disposal.

$$Oil\ Phase\ Percentage\ (OP\%) = \frac{H_{Oil}}{H_{Total}} \times 100$$

2.2. Existing Knowledge of Lecithin and Lysolecithin

Soybean lecithin (LSLO) is a natural emulsifier for different applications due to its amphiphilic nature. Numerous studies have detailed the ability of LSLO to modify food emulsions, leading to amazing characteristics related to texture and stability (Tabaniag et al.)[3]. Furthermore, enzymatic production improved emulsification characteristics for instances, Estiasih et al. LPSLO emulsions with their higher hydrophilicity were more stable in both food as well as pharmaceutical formulations than the less-foldable LSLO [9]. Similarly, another study by Jala et al. found that the modified substances acted to be the best emulsifier for medium acidic conditions.

2.3. Gaps in Knowledge

Despite studies targeting lecithin and lysolecithin, there is a lack of comparative studies on emulsifiers in different concentrations and conditions. Moreover, most of the studies encompassed the effectiveness of LSLO and LPSLO in forming emulsions rather than examining different concentrations. Therefore, this study would look into concentration where performance differences between LSLO and LPSLO become significant for industrial applications. Additionally, it investigated how this can provide cost and sustainability for processes that demand high emulsion stability by using LPSLO-based applications.

3. Methodology

3.1. Materials

The study utilised LSLO and LPSLO as the primary emulsifiers. The materials used in this study are included below in Table 1.

Table 1. Materials utilized in the study

Item	Quantity	Method of use
LSLO	100 mL	One of the emulsifiers
LPSLO	100 mL	One of the emulsifiers
Tap water	3200 mL	The continuous phase of the emulsion
Soybean oil	3200 mL	Dispersed phase of the emulsion

Here, the water was in the continuous phase and soybean oil was dispersed phase (oil dispersed in the water) in o/w emulsion preparation.

3.2. Apparatus

The list of apparatus utilized in this study included beaker, graduated cylinder, balance, stopwatch, homogenizer and glass rod.

Variables

3.2.1. Independent variables

The independent variables are the amount of addition of emulsifiers which are regular and consecutive in proportion to the total mass of the mixture, tap water and soybean oil. According to the reference, the addition of an emulsifier is preferred around 2.5% to the total mass of mixtures (Tabaniag et al.)[3], Subsequently, this study utilised the number of emulsifiers to be 1%, 1.5%, 2%, 2.5%, and 3%. Since the mass of water and oil were fixed at 300 g each, therefore, the mass of LSLO and LPSLO added are respectively 6 g, 9 g, 12 g, 15 g, and 18 g.

3.2.2. Dependent variables

The dependent variables in this experiment were the results of Emulsification Ability (or EA) and Emulsification Stability (or ES) of emulsions. This was measured by observing the stratification layer ratio (±1%), in this case, the water (stratification) layer ratio (or *war*), in the emulsions at and over a certain time.

3.2.3. Controlled variables

The controlled variables included mass of water, mass of soyabean oil, temperature, type of cylinder and pH. The details are included in the Supplementary materials available in Zenodo at 10.5281/zenodo.14287842[5].

3.2.4. Ethical considerations

The ethical considerations entailed organism samples, waste disposal, chemical spillage and glass breakage. During the entire experiment precautionary steps were undertaken to thoroughly prevent and maintain bioethics.

3.3. Procedure

Before starting the experiment, the adoption of safety was ensured. The oil-in-water (o/w) emulsions were prepared by mixing a certain mass of LSLO or LPSLO with fixed volumes of distilled water and soybean oil. the tests used five different concentrations: 1%, 1.5%, 2 %, and up to, the total mass of the mix(chp- water and oil) (3%). The experiment was meant to determine the emulsification ability (EA) and emulsification stability (ES) of the obtained systems by changing the concentration of the applied surfactants. The water and oil weight was kept constant at 300 g, also the emulsifier was randomly added from 6 up to 18 grams.

Furthermore, after quantifying the desired amount, the two phases were combined in a 1000 mL beaker and an emulsifier (either LSLO or LPSJL) was added. For the preparation of each emulsion, water and oil were weighed on an electronic balance with a precision defined as ±0.01. This way, the mixing would be homogeneous through a homogenizer at 3000 revolutions per minute (rpm) for two minutes and all samples were homogenized (Estiasih et al.)[9]. Following this, reading and recording the stratified height at time intervals of 5min,10min, 15 mins and 30min respectively were conducted. If the water layer was less than 10 ml, its height was measured with a ruler to determine war allowing an accurate data collection (See Supplementary materials available in Zenodo at 10.5281/zenodo.14287842[5].).

4. Results and Discussion

4.1. Results

4.1.1. Qualitative Observation

Each observation on the qualitative approach is included in the See Supplementary materials available in Zenodo at 10.5281/zenodo.14287842[5]. Every LSLO and LPSLO added emulsions have “breaking”, additionally, through the increase in LSLO addition, there is a significant decrease in water volume that stratified from LSLO added emulsions in 24 hours. On the other hand, LSLO added emulsions have “coalescence” or “flocculation”. There is a change in water in 30 min (See Supplementary materials available in Zenodo at 10.5281/zenodo.14287842[5]).

4.1.2. Quantitative data

The quantitative data provided detailed insight. “Emulsification Ability” entails an extent of different types but the same addition of emulsifier to produce the stratification after the same time, ceteris paribus. The stratification production could be calculated as the stratification ratio to the total emulsion. By comparing the value of average wlr in 1% g LSLO and LPSLO added emulsions at a 30-minute break, the former emulsions have a value of 26.33% but the latter emulsions present a lower value of 7.48%. Therefore, it shows that the LPSLO has better EA than LSLO in 1% g addition at a 30-minute break. However, the EA could vary through adding different addition of emulsifiers. In tables presented in the supplementary materials[5], by comparing the value of average wlr in 2.5% g LSLO and LPSLO added emulsions at 30-minute break, the former emulsions present a value of 4.79% but the latter emulsions present higher value of 5.41%. Thus, it shows that the LSLO has better EA than LPSLO in 2.5% g addition at 30-minute break (see Fig. 2).

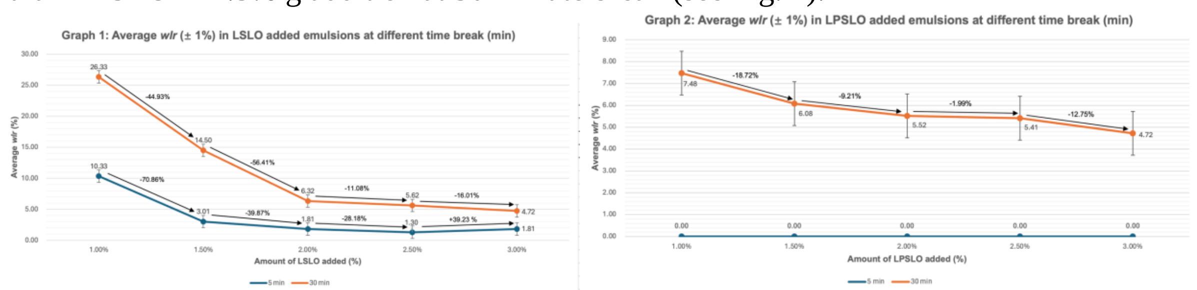

Figure 2. Average *wlr* for LPSLO and LSLO at different time

Emulsification Stability (ES) included the effectiveness of the same type but different amounts of emulsifiers in sustaining its stratification over a certain time period, ceteris paribus. The value of ES is measured by calculating the average Δwlr of certain emulsions over a certain time. The smaller the difference means the better emulsions hold their stratification, which means the better ES of certain emulsifiers on certain emulsion. For instance, in supplementary materials[5], the average Δwlr for 1.00% g and 3.00% g LSLO added emulsions are 16.00% and 2.91%. Because the latter emulsions present a lower value of average Δwlr than the former one, the higher addition of LSLO shows a better ES than the lower addition. For instance, the average Δwlr for 1.00% g and 3.00% g LPSLO added emulsions are 7.48% and 4.72%. The smaller value of average Δwlr for the latter emulsions means the better ES of these emulsions compared to the former emulsions.

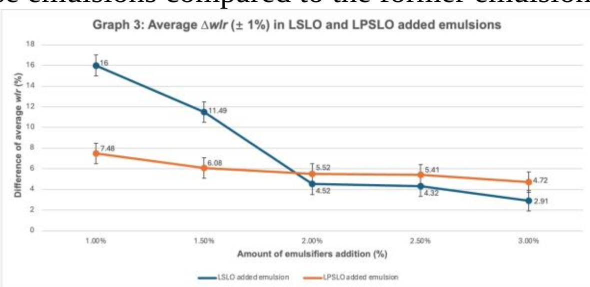

Figure 3. The graph of average Δwlr in LSLO and LPSLO added emulsions at a different time break

4.2. Discussion

4.2.1. Analysis of EA

It was found that the LPSLO emulsions have less *wlr*. It could present the effectiveness of this change on reducing the value of wlr and indicates to what extent the LSLO and LPSLO added to reach the most optimum amount of emulsifiers addition (or the so-called “critical point”). It also included the extent of cost effectiveness in terms of economic considerations. Here, the value of %Δ average wlr itself was not compared, but the degree of the decrease was. Additionally, there is a downward trend when increasing the amount of LSLO addition (see Figure 3). Based on the graph, the biggest difference in the blue curve appears between the average Δwlr from 1.50% g to 2.00% g addition is 6.97% (11.49%-4.52%). Therefore, taking the economic consideration, at 2.00% g of LSLO addition, the emulsions would offer the emulsions with the best ES. Moreover, the drop from 2.50% g to 3.00% g addition implies the average may further decrease thus 3.00% g addition has not yet reached the "critical point" of LSLO addition if the former assumption is right.

Similarly, the LPSLO-added emulsions curve (or the orange curve) also shows a general downward trend. the drop from 2.50% g to 3.00% g addition may also show that the LPSLO addition may not yet reach its "critical point" or other reasons, such as the operation error, etc. • However, unlike the blue curve, the little deviations in between each average Δwlr in the orange curve shows implies general ES than the LSLO added emulsions. Thus, it will be hard to observe the best addition point for ES.

It is apparent that the changes in average Δwar in the blue curve are much more significant than in the orange curve. Interestingly, at 2.00% g and beyond, the increase of LSLO or LPSLO addition has a smaller change in average Δwlr compared to the former addition, which is a truly useful discovery throughout this experiment. Furthermore, the drops in both curves from 2.50% g to 3.00% g LSLO or LPSLO addition suggest that a certain amount of addition may not reach their "critical point" or operation error, which is also valuable for the discussion and improvements for future experiments. Thus, the general downward in both blue and orange curves show that the higher LSLO or LPSLO added into the emulsions, the increase of ES of the emulsions. More importantly, before 2.00% g LSLO or LPSLO addition, the LPSLO-added emulsions present better ES; but for the rest of the addition, the LSLO-added emulsions present better ES. Additionally, by considering the economic costs, the certain LSLO or LPSLO additions should be taken into consideration carefully by reading the graph.

4.2.2. Discussion on analysis

Phospholipase A1-hydrolyzed lecithin is more hydrophilic compared to the commonly used lecithin sources such as liquid soybean lecithin oil. The identification was made based on the HLB value. These findings align with statements from studies that suggested that emulsifiers having higher HLB values were ideal for strengthening oil-in-water emulsions because of their ability to have a higher affinity for the water phase (Gupta et al.)[10]. Thus, the models developed previously regarding emulsification highlighted surfactant properties, hydrophilicity, and molecular structure as the most important characteristics that determined the emulsification ability and stability. Thus, the findings also align with the hypothesis whereby, it presents different levels of results. Emulsifier content significantly affected HMD performance and stability (Li et al.)[11]. This is consistent with the results of this study because LPSLO had better performance than LSLO at lower concentrations, while higher concentrations improved the effectiveness of LSLO. Thus, emulsifier type and amount need to be optimized based on application requirements for predigestion.

5. Conclusion

5.1. Key conclusion

The concentration of soybean phospholipids in soybean oil is approximately 2 to 3 per cent of various phospholipids, and more than 60 to 80 per cent of soybean phospholipids are soybean lecithins (Rincón-León). However, First Grade Soybean Oil removes many nutritional accompaniments in order to have better looks and smells, which directly reduces the nutritional accompaniment from the original soybean oil. Therefore the current finding can be instrumental in the food industry.

5.2. Strength and limitations

Firstly, the methodology of this experiment is very easy to imitate due to the ratio and materials utilized. This reciprocability increases the credibility of the study. Additionally, all the samples of emulsions were repeated with 6 trials to reduce the effect of uncertainties and increase the reliability of the experiment's results. Thirdly, the use of precise measurement tools such as the specific graduated cylinder, the electronic balance and the standard ruler ensured that the quantitative measurements in this investigation were relatively accurate. However, the study could be limited due to stability, dispersion, and temperature control. Stringent steps such as utilizing credible source and adherence to methodological rigour were followed to overcome the limitations.

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