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Enzymatic preparation and characterization of soybean lecithin-based emulsifiers

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SUMMARY: Simple enzymatic methods were developed for the synthesis of lysolecithin, glycerolyzed lecithin and hydrolyzed lecithin. The products were characterized in terms of their acetone insoluble matter, hexane insoluble matter, moisture, phospholipid distribution and fatty acid composition. The HLB value ranges of different products with different acid values were detected. The efficiency of optimally hydrolyzed lecithin was examined at high calcium ion, low pH, and aqueous solutions and compared with commercially available standard lecithin-based emulsifiers. Overall, lysolecithin powder was proven to be the best emulsifier even at strong and medium acidic conditions.

***KEYWORDS**: Concentrated lecithin; Glycerolyzed lecithin; Hydrolyzed lecithin; Lysolecithin; Powdered lecithin*

RESUMEN: ***Preparación enzimática y caracterización de emulsionantes a base de lecitina de soja.*** Se han desarrollado métodos enzimáticos simples para la síntesis de lisolecitina, lecitina esterificada a glicerol y lecitina hidrolizada. Los productos se caracterizaron en términos de su composición en materia insoluble en acetona, materia insoluble en hexano, humedad, distribución de fosfolípidos y ácidos grasos. Además, se detectaron rangos de los valores de HLB de diferentes productos con valores de ácido diferentes. La eficiencia de la lecitina hidrolizada de forma óptima fue estudiada en función de una alta concentración de ion calcio, pH bajo, y soluciones acuosas y se compara con emulsionantes basados en lecitina estándar disponibles en el mercado. En general, el polvo de lisolecitina mostró ser el mejor emulsionante incluso en condiciones ácidas fuertes y medias.

***PALABRAS CLAVE:** Lecitina concentrada; Lecitina en polvo; Lecitina esterificada a glicerol; Lecitina hidrolizada; Lisolecitina*

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1. INTRODUCTION

Lecithins and their partially hydrolyzed/modified products have found numerous applications in food, pharmaceutical and personal care industries due to their superior emulsification properties (Nieuwenhuyzen, 1981; Nieuwenhuyzen and Tomas, 2008; Aoi, 1990; Fujita and Suzuki, 1990). The most widely used lyso phospholipids (LPLs) is lysolecithin, which is obtained through hydrolysis of one fatty acyl residue from lecithin (Nakai *et al*., 1988; Kudo and Nishi, 1990; Yesair, 1997; Kim *et al*., 1997;

Haas *et al*., 1994; Sarney *et al*., 1994; Mustranta *et al*., 1995). Conventional methods of fat hydrolysis were reported to be inappropriate for phospholipids (PLs) as the high temperature and pressure reaction caused fouling of reactors (Haas *et al*., 1993).

Structural modification of PLs had been made consistently to achieve beneficial nutritional and functional properties. It can be achieved enzymatically by using phospholipases and lipases in reactions such as hydrolysis, alcoholysis, esterification, transesterification, and transphosphatidylation. Enzymatic reactions offer a non-destructive and energy efficient route for PL hydrolysis. Therefore, numerous phospholipases A_1 (PLA_1) and A_2 (PLA_2) have been identified, and their abilities to hydrolyze the fatty acyl ester bonds of PL have been characterized (de Maria *et al*., 2007).

The addition of mono- and diglycerides to lecithins or partially hydrolyzed lecithins has been found to improve functional properties such as baking performance, anti-spattering and anti-staling. Modified lecithins have been used in food and feed products such as frozen dough, bakery products, emulsified meat products, ice cream, dressings and other emulsion systems (Schmitt *et al*., 2005). Presently lecithin-based products enriched with mono- and diglycerides are prepared by the addition of mono- and diglycerides to lecithins. However, the direct preparation of mono and diglyceride-rich lecithin products in a controlled manner through enzymatic hydrolysis and glycerolysis would be useful. Therefore, the present study attempts to prepare such products and the products were characterized for their composition. Further, the optimally hydrolyzed product was evaluated for its emulsifying efficiency and compared with commercially available standard lecithin-based emulsifiers.

2. MATERIALS AND METHODS

2.1. Materials

Concentrated soybean lecithins, and crude gum solution were donated by Qinhuangdao Golden Sea Industry, Beijing, China. Powdered lecithins were obtained from the ADM (Shanghai, China). They were stored at –20 °C until the de-oiling or modification process. Organic solvents (Analytical and HPLC grade) and chemicals (glycerol and calcium chloride) were purchased from Sinopharm Chemical Reagent Co., Ltd., (Shanghai, China). For column chromatography, silica gel (60–120, 100–200 mesh particle size) was purchased from Qingdao Haiyang Chemical Co., Ltd., (Qingdao, China). Certified standard materials phosphatidylcholine (L-α-PC), phosphatidylethanolamine (L-α-PE), phosphatidylinositol (L-α-PI) from soybean and phosphatidic acid (L-α-PA) with purities greater than 98% were obtained from Sigma Chemical Co. (Shanghai, China). Lecitase Ultra [Phospholipase A_1 (PLA_1, E.C.3.1.1.32)] from Aspergillus oryzae (10, 000 U/mL) and *Candida antarctica* lipase (CAL) B were purchased from Novozymes A/S (Tianjin, China). The term CALB-PLA_1 means the combination of CAL B and PLA_1 was used in the reaction. The PL compositions of concentrated lecithin, aqueous-hydrolyzed lecithins (lysolecithin-1), solvent-hydrolyzed lecithins (lysolecithin-2) and glycerolyzed lecithin were different from each other (Table1). Concentrated lecithin contains approximately equal amounts of PC, PE and PI (~30%) and a small amount of PS (<10%). After the enzymatic hydrolysis and glycerolysis the amount of Lyso PLs had increased (>50%) in lysolecithin 1, lysolecithin 2 and glycerolyzed lecithins.

2.2. Methods

2.2.1. Characterization of the concentrated soybean lecithin

Concentrated soybean lecithin was characterized in terms of acetone-insoluble matter, hexane-insoluble matter, humidity (moisture content) and acid value according to the AOCS official methods (Ja 4–46, Ja 3–87, Ja 2b–87, Ja 6–55) (AOCS, 2001). Phospholipid composition was determined using HPLC-ELSD (Model: Agilent 1100, Agilent, Beijing, China) according to Becart *et al*., (1990). HPLC (Agilent 1100) equipped with a silica gel column (Lichrospher Si 60, 5 μm, 12 cm x 4 mm, Merck) and evaporative light scattering detector (ELSD, Alltech 3300) was used. Eluent A was chloroform/methanol/ammonium hydroxide, 80/19.5/0.5 (v/v/v) and eluent B was chloroform/methanol/water/ammonium hydroxide, 60/34/5.5/0.5 (v/v/v/v). The gradient was: 0–14 min: linear from A/B, 50/50 to 100% B, 14–25 min: hold 100% B, 25–30 min: 100% B to A/B, 50/50 and 15 min at A/B, 50/50 for column regeneration. The flow rate of the eluent was 1 mL/min. The pressure of the nebulizer gas (air) at ELSD was maintained at 3.2 bars and the drift tube temperature was set at 40 °C. The column temperature was also set at 40 °C. Samples (10 mg) were dissolved in 10 mL chloroform/methanol/water (70/25/5). 20 μL of the sample were injected into the HPLC. The sample (10 mg) was dissolved in (10 mL) chloroform-methanol (2:1) solution. 20 μL of the sample were injected into the HPLC. Analyses were performed in triplicate. The retention times of phosphatidylethanolamine (PE), phosphatidylinositol (PI), lysophosphatidylethanolamine (Lyso PE), phosphatidylcholine (PC), lysophosphatidylcholine (Lyso PC) and phosphatidic acid (PA) were approximately 5.0, 7.0, 8.0, 8.8, 9.5 and 14.5 min, respectively. The percentages of individual constituents were also confirmed by quantitative column chromatography. Lecithin fatty acid composition was

Table 1. Characterization of concentrated soybean lecithin and modified lecithin

	Concentrated soybean lecithin	Lysolecithin 1*	Lysolecithin 2*	Glycerolyzed lecithin
Acetone insoluble matter[a] (%)	63.2±0.4	49.7±0.4	52.3±0.4	54.1±0.4
Hexane insoluble matter[a] (%)	1.9±0.01	2.0±0.01	2.0±0.01	2.0±0.01
Moisture (Air oven method)[a] (%)	3.1±0.2	4.5±0.2	4.3±0.3	4.2±0.3
Acid value[a] (mg KOH/g)	27.3±2.1	60±2.3	55±2.1	54±2.0
Phospholipid distribution[a] (%)				
PC	30.8±2.1	11±1.1	15.9±1.9	8.8±1.2
PE	32.5±0.2	15.2±1.6	20.7±0.8	12.8±1.7
PI	27.9±0.3	16.7±0.7	18.7±1.5	15.7±0.8
PA	8.8±0.5	8.0±0.9	8.7±0.7	6.9±0.9
Lyso PC	-	19.7±1.9	14.8±1.2	22.2±2.1
Lyso PE	-	17.4±1.2	11.8±1.1	19.9±1.1
Lyso PI	-	11.2±0.9	9.2±0.9	12.0±1.1
Lyso PA	-	0.8±0.3	0.2±0.2	1.7±0.2
Fatty-acid profile[b] (%)				
C16:0	20.4±1.5	20.3±1.6	20.3±1.5	20.2±1.4
C18:0	4.6±0.1	4.5±0.1	4.6±0.1	4.4±0.1
C18:1	9.5±0.2	9.6±0.2	9.5±0.2	9.7±0.2
C18:2	57.6±2.5	57.7±2.4	57.6±2.3	57.6±2.3
C18:3	7.9±0.1	7.9±0.1	8.0±0.1	8.1±0.1
Unsaturated/saturated ratio	3.0	3.0	3.0	3.0

[a]Arithmetic means of triplicate determinations with their confidence interval at 95%.
[b]Arithmetic means of duplicate determinations.
*Lysolecithin 1: aqueous-hydrolyzed lyso lecithin.
*Lysolecithin 2: solvent-hydrolyzed lyso lecithin.

determined by gas chromatography (Model: Agilent HP6890, Agilent, Beijing, China) according to the AOCS Ce 2–66 method (AOCS, 2001). Analyses were performed in duplicate.

2.2.2. De-oiling of the concentrated soybean lecithin

De-oiled lecithin was prepared from concentrated soybean lecithin through acetone- fractionation. Concentrated soybean lecithin was repeatedly extracted with cold acetone at 0 °C (acetone/gum ratio of 5:1 v/w; stirred for 30 min at 400 rpm for 3 times). The acetone solution fractions were subjected to centrifugation. After decantation of the acetone soluble part, the insoluble matter (PLs) located at the bottom of the centrifugation tube was collected and solvent traces were evaporated under vacuum conditions.

2.2.3. Preparation of lysolecithin (lysolecithin 1) through PLA1-catalyzed hydrolysis in aqueous medium

Concentrated soybean lecithin (50 g) and water (100 mL) were heated to 30 and 50 °C, respectively. They were then mixed and magnetically stirred for a period of 15 mins (with 400 rpm speed). The reaction was initiated by the addition of PLA_1 enzyme solution (0.5%; w/w of lecithin). The reaction was conducted at 50 °C for 25 min. All the reactions were carried out in duplicate. Aliquots (4 mL) were withdrawn from the reaction mixture at regular intervals for analysis of acid value according to the AOCS official method (Ja 6–55) (AOCS, 2001). At the end of the reaction, the reaction mixtures were heated at 110 °C for 30 min to inactivate the enzyme. Subsequently, the water was evaporated from the reaction mixture at 80 °C for 1 h.

2.2.4. Preparation of lysolecithin (lysolecithin 2) through PLA1-catalyzed hydrolysis in solvent medium

Concentrated soybean lecithin (50 g) and solvents (100 mL tert-butanol/hexane) were heated at 50 and 60 °C, respectively. They were then mixed with stirring at 400 rpm with a magnetic bar. The reaction was initiated by the addition of PLA_1enzyme solution (0.5 %; w/w of lecithin). The reaction was conducted at 60 °C for about 6 h. All the reactions were carried out in duplicate. Aliquots (4 mL) were withdrawn from the stirred reaction mixture at regular intervals for analysis of acid value according to

the AOCS official method (Ja 6–55) (AOCS, 2001). At the end of the reaction, the reaction mixtures were heated at 110 °C for 30 min to inactivate the enzyme. After that, the solvent was evaporated from the reaction mixture at 60 °C for 1 h.

2.2.5. CAL B-PLA1 catalyzed glycerolysis of lecithin

Concentrated soybean lecithin (50 g) and glycerol (25 g) at a molar ratio of 1:4.5, tert-butanol (75g, 100 wt % of total substrates) and water (7.5 g, 10 wt % of total substrates) were mixed and mechanically stirred (500 rpm) at 50 °C. The reaction was initiated by adding a CAL B solution (5 wt% of lecithin) and PLA_1solution (5 wt% of lecithin). The reaction mixture was incubated at 50 °C for 8 h. At the end of the reaction, the reaction mixture was heated at 110 °C for 30 min to deactivate the enzymes. Solvent was evaporated at 60 °C for 30 min to recover the product. The composition of the reaction product was quantified using HPLC analysis.

2.2.6. CAL B-PLA1 catalyzed hydrolysis of lecithin

Concentrated soybean lecithin (50 g) and glycerol (25 g) at a molar ratio of 1:4.5, and water (300 wt% of total substrates) were mixed and mechanically stirred (500 rpm) at 50 °C. The reaction was initiated by adding a CAL B solution (10 wt% of lecithin) and PLA_1solution (10 wt% of lecithin). The reaction mixture was incubated for 1 h at 50 °C. At the end of the reaction, the reaction mixture was heated at 110 °C for 30 min to deactivate the enzymes. Water was removed through rotary evaporation at 80 °C for 1 h. Composition of the reaction product was quantified using HPLC analysis.

2.2.7. Quantification of the glycerolyzed and hydrolyzed lecithins

Quantitative analyses of the glycerolyzed and hydrolyzed products were carried out by HPLC (Agilent 1100) equipped with a silica gel column (Lichrospher Si 60, 5 µm, 12 cm x 4 mm, Merck) and evaporative light scattering detector (ELSD, Alltech 3300). Eluent A was chloroform/methanol/ammonium hydroxide, 80/19.5/0.5 (v/v/v) and eluent B was chloroform/methanol/water/ammonium hydroxide, 60/34/5.5/0.5 (v/v/v/v). The gradient was: 0–14 min: linear from A/B, 50/50 to 100% B, 14–25 min: hold 100% B, 25–30 min: 100% B to A/B, 50/50 and 15 min at A/B, 50/50 for column regeneration. The flow rate of the eluent was 1 mL/min. The pressure of the nebulizer gas (air) in ELSD was maintained at 3.2 bars and the drift tube temperature was set at 40 °C. The column temperature was also set at 40 °C. The samples (10 mg) were dissolved in 10 mL chloroform/methanol/water (70/25/5). 20 µL of the sample were injected into the HPLC.

2.2.8. HLB value of lyso lecithins with different acid values

The HLB value was determined according to a method by Gupta *et al*., (1983) with slight modification. HLB standards (5.0, 5.2, 5.4, 5.6, 5.8, and 6.0) were prepared using cottonseed oil and turpentine. To measure the HLB values of lysolecithins, sample solutions were prepared (lysolecithins: standards: water [1:3:16] w/v/v). These sample solutions were mixed for 5 min at 200 rpm/min. 20 mL of the mixed solution were transferred to a graduated cylinder. Water separation from the mixtures was monitored for a period of 12 h. The equation used for the determination of HLB value was:

$$X = \frac{HLB_0 - HLB_1}{HLB_2 - HLB_1} X\ M_0$$

$$Y = M_0 - X$$

Where X=weight (mg) of high HLB value containing surfactant (Tween 80, turpentine), M_0=weight (mg) of standard oil required for the experiment, Y=low HLB value containing surfactant (span 80, cotton seed oil), HLB_0=HLB of sample (concentrated lecithin or related product), HLB_1=required HLB of cotton seed oil, HLB_2=required HLB of turpentine.

2.2.9. Emulsifying properties of concentrated and de-oiled lecithins

2.2.9.1. Effects of calcium ion. Calcium ion tolerance of the emulsions was determined according to the method reported by Ye and Singh (2001) with slight modifications. Refined soybean oil (50 mL), calcium chloride solution (0.1 wt%, 50 mL) and varying amounts of lecithin were mixed and homogenized for 60 min at room temperature to ensure complete dispersion. The homogenized solution was kept in a measuring cylinder (100 mL) for a period of time. The amount of water separated from the emulsion at different storage times was recorded.

2.2.9.2. Effects of pH. The effects of pH on emulsifying properties were determined according to the method reported by Seung *et al*., (2011) with minor modifications. Soybean oil (50 mL), aqueous solutions of different pHs (adjusted by HCl and KOH) and lecithin/or modified lecithin products (0.5 wt % of total solution) were mixed. A coarse emulsion premix was prepared by homogenizing oil and aqueous phases using a high-speed blender for 2 min at room temperature. The premixed emulsions were further homogenized by five passes through a high pressure homogenizer. All the emulsions

were then stored in amber glass bottles at 20 °C for up to 15 days. The amount of water separated from the emulsion at different storage times was recorded.

2.2.9.3 Aqueous solution. The emulsifying properties of concentrated and de-oiled lecithins in an aqueous solution was conducted according to the method by Aura *et al*., (1994) with slight modifications. Concentrated/de-oiled/modified lecithin (1 g, 1 wt % of total solution) was added to water (100 mL) and homogenized for 1 min. The solution was kept in a measuring cylinder (100 mL) for a period of time. The amount of water separated from the emulsion at different storage time was recorded.

3. RESULTS AND DISCUSSION

3.1. Characterization of the concentrated and modified soybean lecithin

Table 1 shows the characterization of concentrated and modified soybean lecithin in terms of acetone insoluble matter, hexane insoluble matter, moisture content, acid value, PL distribution and fatty acid composition. Concentrated lecithin contains 40% neutral lipids (36.1% TAG and 3.9% partial glycerides) and 60% of PLs (53% PL and 7% Lyso PL). After the hydrolysis, the contents of neutral lipids and PLs were 40 (35.3% TAG; 4.7% partial glycerides) and 60% (41.7% Lyso PL 18.3% PL), respectively. Glycerolyzed lecithin contains higher amounts of partial glycerides (33.5%) and minor amount of TAG (2.7%). Lyso PLs and PL contenst in glycerolyzed lecithins were 52 and 11.8%, respectively.

The PL compositions of concentrated lecithin, aqueous-hydrolyzed lecithins (lysolecithin-1), solvent-hydrolyzed lecithins (lysolecithin-2) and glycerolyzed lecithin were different from each other (Table1). Concentrated lecithin contains approximately equal amounts of PC, PE and PI (~30%) and a small amount of PS (<10%). After the enzymatic hydrolysis and glycerolysis the amount of Lyso PLs increased (>50%) in lysolecithin 1, lysolecithin 2 and glycerolyzed lecithins. All the enzymes have higher affinity for Zwitterionic PLs (PC and PE) resulting in a significant ($P<0.05$) increment in their lyso-counterparts (Lyso PC and Lyso PE). In contrast, anionic PLs such as PA are almost unmodified. The reason for enzymes exhibiting high selectivity towards Zwitterionic PLs could be their tertiary structure. That means the tertiary structure of enzymes is more favorable to react with these PLs. The fatty acid profiles were similar for concentrated and modified lecithins. The major fatty acids were linoleic (>50%) and palmitic acids (>20%). The remaining fatty acids were comprised of stearic, oleic and linolenic acids.

3.2. Determination of HLB values

The HLB values of the lysolecithin products are shown in Table 2. Lysolecithin 1 (acid value approximately 60) was highly hydrophilic with a HLB of approximately 7.6 to 8.0. Meanwhile, lysolecithin 2 (acid value of approximately 55) was more hydrophobic than lysolecithin 1 with HLB of approximately 6.5 to 7.0. The degree of hydrolysis of lysolecithins (indirectly acid value) leads to the differences in HLB values which means the higher hydrolyzed lecithins will have a high lyso PL content and HLB and vice-versa (Estiasih 2013; www.solae.com). In general, based on the requirement of hydrophilicity the degree of hydrolysis will be varied starting from 20% to 60%. If the hydrolysis is between 20–30% the hydrolysis can be considered minor hydrolysis.

3.3. Emulsifying properties of concentrated and de-oiled lecithins

3.3.1. Effect of calcium ion concentration on emulsifying properties of lecithins

The calcium ion tolerance of PL and Lyso PL was in the following ascending order: concentrated lecithin < powder lecithin < lysolecithin from crude gum solution ≈ lysolecithin from concentrated

TABLE 2. HLB values of modified lecithins with different acid values

HLB values of modified lecithins with different acid values		
Types of lecithins	**Acid value (mg KOH/g)**	**HLB value range**
Lysolecithins 1[a]	60.6	7.6–8.0
	60.3	7.6–8.0
	54.8	6.5–7.0
	53.6	6.5–7.0
Lysolecithins 2[a]	52.8	6.0–6.5
	48.6	5.5–5.9
	45.7	5.2–5.6
Lecithin with minor hydrolysis[a]	44.0	5.0–5.5
	43.0	5.2–5.5
	41.8	5.1–5.4
	40.7	5.0–5.4
	39.0	5.0–5.3
Glycerolyzed Lecithin	54.0	6.5–7.0
Concentrated Lecithin	31.0	4.0–4.5

[a]Arithmetic means of triplicate determinations with their confidence interval at 95%. Minor hydrolysis using both solvent and aqueous media. These are the values of different experiments (batches) with different acid values.

lecithin < lysolecithin powder from lysolecithin (Figure 1A). The concentrated lecithin and its powder exhibited poor performance (at all the studied concentrations) in high calcium ion solution due to their higher sensitivity towards calcium ions. Lysolecithin has better emulsifying properties than concentrated lecithin-based emulsion indicating better calcium tolerance towards calcium ions. This is in agreement with previous findings that showed Lyso PE-stabilized emulsion did not flocculate in the presence of calcium ions and milk protein (Duin *et al.*, 1963; Hoof *et al.*, 2005).

3.3.2. Effects of pH on emulsifying properties of concentrated and de-oiled lecithin

At highly acidic conditions (pH=2), the ascending order of emulsion stability was as follows: concentrated lecithin < powder lecithin < lysolecithin from crude gum solution ≈ lysolecithin from concentrated lecithin < lysolecithin powder from lysolecithin (Figure 1B). Apart from lysolecithins prepared from crude gum solution and the concentrated lecithin, the powdered lysolecithin also had good emulsifying properties at pH 2 (strongly acidic condition). Whereas, when the concentrated lecithin, powdered lecithins were used as emulsifiers the oil phase in the emulsion was separated at a 24 h time period which led to complete demulsification of the emulsion. This finding is in agreement with previous observations that lysolecithin improves the stability of emulsion with high salt and over a wide range of pH. Therefore, it is used as an ingredient in foods requiring longer shelf life (Aoi, 1990).

At moderate acidity condition (pH=4) (Fig 1C), the ascending order of emulsion stability observed was as follows: concentrated lecithin ≈ powder lecithin < lysolecithin from crude gum solution ≈ lysolecithin from concentrated lecithin < lysolecithin powder from lysolecithin. At the moderate to highly acidic conditions (pH < 5) the lysolecithin and its

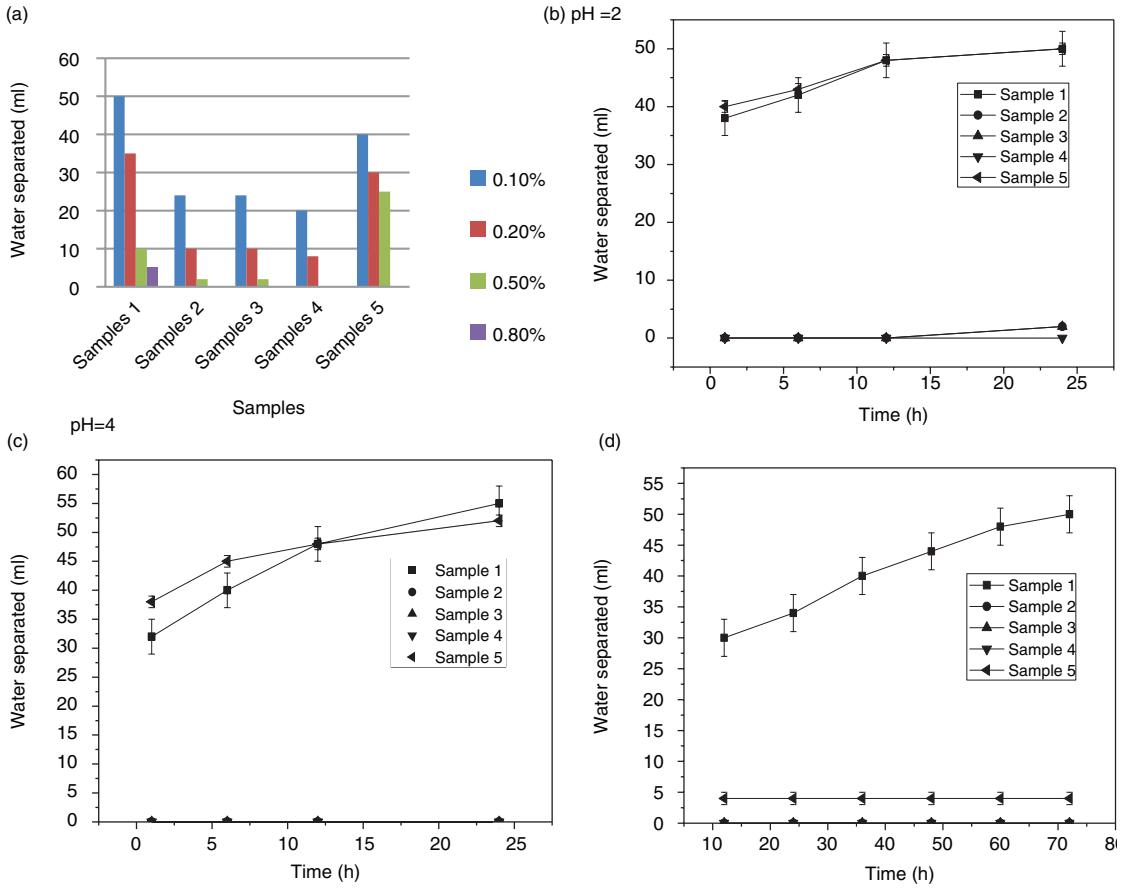

FIGURE 1. **A**) Effect of calcium ion on the stability of emulsions at different lecithin concentrations (at 10 min time periods); (B and C) Effect of pH on stability of emulsion; sample 1, concentrated lecithin (0.5%); sample 2, Lysolecithin from crude gum solution (0.5%); sample 3, lysolecithin from con lecithin (0.5%); sample 4, lysolecithin powder (0.5%); sample 5, powdered lecithin (0.5%); D) Stability of aqueous based emulsion system. Each value is the average of two determinations. Lysolecithin samples were stable for at least 12 days. All values are arithmetic means of duplicate determinations.

powder exhibited good performance in stabilizing the emulsification system and the performance was much better than concentrated lecithin and its powder.

3.3.3. Aqueous solution

The emulsifying properties of concentrated and de-oiled lecithins are shown in Figure 1D. Powdered lysolecithin exhibited superior emulsifying properties. This is followed by lysolecithin from concentrated lecithin, lysolecithin from crude gum solution > powder lecithin > concentrated lecithin. It was observed that lysolecithin solutions were stable at least for 12 days. As lysolecithin exhibits high moisture retention, its solubilizing property, its lipophilic and hydrophilic moiety's holding nature and emulsifying power are excellent. Especially, enzymatic hydrolyzed lecithin possesses technological and commercial advantages over native lecithins such as enhanced O/W emulsifying property, increased emulsion stability under acidic conditions and in the coexistence with salts, improved capability to bind proteins and starch and excellent mold- or pan-releasing property (Hirai *et al*., 1998; Erickson, 2008). Consequently, the demand for lysolecithins has increased in recent years.

The less hydrolyzed lecithins (lower AV and HLB) were ignored for the evaluation in the present study as their properties are expected to be more or less similar to normal concentrated lecithin. In general, lecithins that exhibit HLB values between 4–6, 7–9 and 8–10 are considered as water in oil (w/o) emulsifiers, wetting agents and oil in water (o/w) emulsifiers, respectively. In our case standard concentrated lecithins, lecithins with minor hydrolysis, lysolecithin 2 falls in the range of 4–6 HLB values and therefore, they are expected to have applications in w/o emulsions such as margarine, spreads, icings, frostings and petroleum emulsions. On the other hand, the optimally hydrolyzed lecithin such as lysolecithin 1 falls in the range of 8–10 HLB values and therefore, they will have the application in o/w emulsions such as mayonnaise, infant formulas, and hand and body lotions. Since the glycerolyzed lecithin also exhibited HLB near to 7.0, this product can be suitable in o/w emulsions and it will not be suitable for w/o emulsions.

4. CONCLUSIONS

The food grade emulsifiers such as lysolecithins, hydrolyzed lecithin and glycerolyzed lecithin were prepared using simple enzymatic methods. The optimally hydrolyzed lecithin was examined at high calcium ion, low pH, and aqueous solutions and compared with commercially available standard lecithin-based emulsifiers. Overall, lysolecithin powder was proven to be the best emulsifier due to its high moisture retention, solubilizing property and enhanced o/w emulsifying properties. Further, it is the best due to its high emulsifying efficiency under acidic conditions and also in high calcium ion concentrations.

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