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MODIFICATION OF CANOLA LECITHIN TO ENHANCE EMULSIFYING PROPERTIES FOR FOOD USES

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ABSTRACT

To improve the emulsifying properties of lecithin extracted from crude canola oil, a lecithin-protein complex was prepared by sonicating of canola lecithin suspension with soluble canola protein isolate (CPI) at pH 7.0. Also, the prepared lecithin-protein complex was treated by 99 % ethyl alcohol or heat at 95°C for one minute to improve its emulsifying properties. The emulsifying activity (EA) of canola lecithin was much improved by complex formation with CPI. Moreover, the output data of contour plot of emulsion stability (ES) as observed at different time (20, 40 and 60 min.) and emulsifier concentrations (5, 10 and 15 mg/ml water) clearly indicated that the lecithin-protein complex treated by heat or ethanol markedly improved emulsion stability and retarded coalescence and creaming. Mathematical models of quadratic type were proposed to predict the EA values of lecithin types at different concentrations. The obtained data are useful for evaluation the relationship between the amount of lecithin and the EA for different applications in food formulations. The microstructure studies of salad dressing samples prepared using modified canola lecithin appeared the smallest oil droplets irregular in both size and shape.

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1. INTRODUCTION

During processing of raw vegetable oils, lipid residues are obtained as by-products. These residues, commonly called lecithins, are complex mixtures mainly of different phospholipids. Crude lecithin contains of about 60 % acetone insoluble phospholipids and 40% triglyceride oil. The mixture of phospholipids in crude lecithin [phosphatidyl choline (PC): phosphatidyl ethanolamine (PE): phosphatidyl inositol (PI)] gives weak water-oil and oil-water emulsifying properties. Improved emulsifying properties could be obtained by modification of crude lecithin (**Temelli and Dunford, 1995**). Commercial canola lecithin contains 80 % acetone insoluble phospholipids. The phospholipids composition of canola lecithin is 46.3% phosphatidyl choline, 36.2% Phosphatidyl ethanolamine and 17.5% phosphatidyl inositol (**Neidleman, 1993**). The vegetable phospholipids are of the greatest economic importance at present (**Gober *et al* 1993**).

Emulsions are thermodynamically unstable systems. Sufficient long-term physical stability is crucial, and kinetic stability is clearly an important goal in the development of a new emulsion formulation. Common requirements of a stable emulsion over the time-scale of observation are no discernible changes size distribution of the droplets or their state of aggregation, nor in the spatial arrangement within the vessel (**Dickinson, 2003**). This can only be achieved by adequate control of the instability processes, which often is challeng-

ing since emulsion instability is a complex process and may involve a combination of different mechanisms such as creaming or sedimentation, flocculation and coalescence (**Claesson *et al* 2004**). Since the different destabilizing processes may occur simultaneously, a complete mechanistic understanding is normally not achievable with reasonable efforts, but substantial improvements may be obtained by a pragmatic approach. Flocculation and coalescence can be restricted by increasing the magnitude of the energy barrier that prevents the droplets to come in close contact. The two main ways to achieve this is electrostatic repulsion as a result from electrical double layers (e.g when using ionic surfactants) and steric repulsion due to adsorbed non-ionic surfactants or polymers (**Tadros, 2004**). Emulsifying agents promote emulsion formation and long-term stabilization by interfacial action. Emulsifying agents are typically rather small molecules such as monoglycerides, polysorbates, sucrose esters, lecithin, etc., but can also be larger, as exemplified by milk and egg proteins. The small molecules that are good emulsifying agents are often not particularly well suited for providing long-term stability (**Dalgleish, 1995**). Emulsifiers are important since they affect many of the emulsion properties. An emulsifier is surface active and reduces the interfacial tension between oil and water and therefore, facilitates the disruption of emulsion droplets during homogenization. The emulsifier adsorbs to the surfaces of emulsion droplets to form a protective coating that prevents the droplets from aggregating with each other (**McClements and Demetriades, 1998**). Proteins are among the most widely used emulsifiers (**Turgeon *et al* 1996**). Furthermore, technological modification of plant lecithins opens the opportunity to alter lecithin properties towards a better suitability for use by increasing their dispersibility in water.

Salad dressings have grown in popularity during recent years. In Egypt for example, many consumers have turned to salads as a healthy eating option, which means that also, the dressings have to be healthy. It has been shown that most consumers are not prepared to sacrifice taste, flavor or any other quality of foods for any perceived health benefit (**Mcilveen and Armstrong, 1995**). This implies that food industry is facing a challenge to produce a wide variety of dressings, including dressings with low cholesterol content, in order to meet the consumer demands. An important part of the flavor perception derived from eating a food product is determined by the nature and quantity of the flavor components and the availability of these components to the sensory system as a function of time (**Overbosch *et al* 1991**). This means that the food matrix plays an important role in controlling flavor release at each step of food product preparation and consumption (**Druaux and Voilley, 1997**).

Dressing is an oil-water emulsion, in which the total flavor has been shown to be a combination of aroma, taste and mouthfeel (**McClements and Demetriades, 1998**). Differences in perceived flavor intensities in different products can often be explained by the physicochemical properties of the flavorants eliciting these sensations and especially by their oil-water distribution (**De Roos, 1997**). The fat content is of great importance not only for the perceived intensity but also for the temporal profile of the flavors (**Druaux and Voilley, 1997**). Besides, fat is important for many other properties such as texture, lubricity, emulsification and color (**Vafiadis, 1996**).

Because of high quantities of soybean grown and processed, and owing to the relatively high percentage of phosphatides in soybean oil practically all over the world, soybean oil is the principal commercial source of natural and modified lecithins. Canola seed must be also considered as a major and potential source of oil and lecithin. While a lot of data have been published on soybean lecithin, canola lecithin has not received serious attention. Basing on it, the task of this study was to extract lecithin from canola oil and to improve its emulsification activity by complex-formation with canola protein isolate. Heat and ethanol were suggested as a treatment to enhance the emulsifying properties of lecithin-protein complex. Also, the objective of this study was to characterize the influences of modified canola lecithin for different applications in food formulations.

2. MATERIALS AND METHODS

2.1. Materials

The crude canola oil used for extraction lecithin and canola meal were obtained from Cairo Oil Processing Company, El-Badrashin, Giza, Egypt. The corn germ oil was purchased from Arma Food Industry Company, 10th of Ramadan City, Egypt. Xanthan gum, guar gum and beta-carotene were purchased from Sigma chemical and Gumix International Companies. Sucrose, mustard, acetic acid, lemon and NaCl were purchased from the local market, Cairo, Egypt.

2.2. Methods

2.2.1. Degumming

Degumming process was carried out according to **Smiles *et al* (1988)** using 85 % phosphoric acid (1.7 g / Kg oil) and 2 % water. Crude canola oil (50 g) was placed in 200 ml centrifuge cups and heated to 60 °C in water bath. The degumming agent was added with stirring for 5 min. After degumming, the oil was cooled to 40 °C and centrifuged at 4000 xg for 30 min. Then, degummed oil was separated from the gummy lecithin residue by decantation.

2.2.2. Extraction of lecithin

The extraction of lecithin was carried out according to **Sosada *et al* (1994)**. The wet gum precipitate after degumming was collected. Wet gum was diluted with an appropriate volume of acetone and blended in warring blendor (Model 32 BL 80) at high speed for 5 min. The mixture was centrifuged at 2000 xg for 10 min. The extraction with acetone was repeated three times, and the precipitate was dried under vacuum at 25 °C for 12 hrs.

2.2.3. Preparation of canola protein isolate (CPI)

Canola protein isolate was prepared from defatted canola meal according to the method described by **Klockeman *et al* (1997)**.

2.2.4. Preparation of lecithin-protein complex

Canola lecithin-CPI complex was prepared according to the method described by **Hirotsuka *et al* (1984)**. A suspension of lecithin in water was added to 4 % CPI and sonicated using an Insonator (IKA labortechnik, Type U50) for 10 min at maximum output to form the complex. The dry weight ratio of protein/lecithin was usually adjusted to 4:1.

2.2.5. Treatment of lecithin-protein complex by ethanol

The lecithin-protein complex was treated by ethanol according to the procedure of **Hirotsuka *et al* (1984)**. An equal volume of 99 % ethanol was added to a lecithin-protein complex solution during stirring. After standing for 30 min., the pH of the mixture was adjusted to 4.5 with HCl 0.1 N and the mixture was centrifuged at 2000 g for 10 min. The precipitate was washed twice with 20–fold distilled water to remove the excess of the residual ethanol, then dried under vacuum at 25 °C for 12 hrs.

2.2.6. Thermal treatment of lecithin-protein complex

Thermal treatment of the lecithin-protein complex was carried out by the procedure of **Hirotsuka *et al* (1984)**. Suspension of lecithin-protein complex (2 %) was heated in boiling water and the temperature achieved in the suspension was 95 °C for 1 min. The suspension was then cooled immediately in ice water at 4 °C.

2.2.7. Emulsification activity and emulsion stability

The method of **Pearce and Kinsella (1978)** was used to determine the emulsification activity and emulsion stability (EA and ES) of canola lecithin, CPI and modified canola lecithin. Ten ml corn oil was added to 30 ml aqueous lecithin solutions (adjusted to pH 7.0 with diluted NaOH 0.1 N) then, homogenized by Virtis homogenizer (Model 6-105 AF) at 10,000 rpm for 60 sec. A 0.1 ml of sample was immediately taken from the bottom of the container and diluted to 50 ml with 0.1 % sodium dodecyl sulfate solution. The absorbance of the diluted emulsion was measured at 500 nm. The initial A_{500} measurement was taken as the EA, while ES was measured after 20, 40 and 60 min. The concentration of CPI, canola lecithin and its modified forms were 5, 10 and 15 mg/ml water.

2.2.8. Preparation of salad dressing

Dressing samples (1 kg of each dressing) were produced according to the formula presented by **Wendin and Hall (2001)**. The dressing formula of sample contained 300 g corn germ oil, 85.0 g sucrose, 9.0 g beta carotene, 8.3 g mustard, 25.0 g acetic acid, 25.0 g lemon juice, 14.7 g NaCl, 5 g thickener (1:1 of xanthan gum and guar gum), 525 g water. Emulsifiers were also added at the equilibrium concentration of emulsification activity of modified canola lecithin (3.01 g/kg).

Sucrose, beta carotene, mustard, acetic acid, lemon juice, NaCl, thickener, water and emulsifiers were first mixed using electric mixer on liquefy velocity for 5 sec. The corn germ oil was then slowly added to the system on puree velocity and more rapidly after the mass begins to thicken, with raising gradually the velocity from puree to liq-

uefy during 50 sec. All the ingredients were then mixed on liquefy velocity for 20 sec.

2.2.9. Microstructure and oil droplet size of salad dressing

The microstructure of prepared salad dressing samples was studied according to the procedure introduced by **Langton *et al* (1999)** using Carl zis light microscope. The salad dressing samples were placed in the cavity of the object slide. The whole preparation procedure was performed above ice in order to keep the temperature low. The temperature microscope stage was set to keep a temperature of 10 °C, the samples had a slightly higher temperature, around 15 °C. The size of oil droplet measurements was recorded as diameter mean.

2.2.10. Statistical analysis

Duncan multiple range at 5% level of significance was used to compare between means. Results followed by different alphabetical letters significantly differed. Regression and ANOVA analysis were carried out using the procedure of Statistical Analysis System (**SAS, 1996**).

Predicting of emulsification activity (EA) was assumed by quadratic polynomial regression model for the independent variable of emulsifier concentration (C). The model proposed for response of EA is:

$$EA=EA_0+aC+bC^2$$

EA_0 is a constant value of the EA; C is the concentration (mg/ml water); a and b are constant coefficients. Regression analysis was carried out using the quadratic polynomial equation of **Sigma Plot (2002)**.

The contour plot was used as a method to study the response surface of emulsion stability as dependent variable with emulsifier concentrations, and times as independent variables. The response surface method was applied using Harvard ChartXl software version 2.0.

3. RESULTS AND DISCUSSION

3.1. Effect of concentration on emulsification activity

The emulsification activity of extracted canola lecithin and canola protein isolate (CPI) at pH 7.0 and concentrations of 5, 10 and 15 mg/ml water is shown in **Figure (1)**. The data indicated that the emulsification activity of canola lecithin or CPI increased with increasing their concentration. It was clearly noticed that the extracted lecithin had higher emulsification activity than that of CPI.

3.2. Effect of modification treatments on emulsification activity

To improve the emulsifying properties of canola lecithin or CPI, the complex-formation was prepared by sonicating a water suspension of canola lecithin with CPI. The lecithin-CPI complex was then treated by ethanol or heat. **De Kruif and Tuinier, (2001)** reported that the interaction of biopolymers is of direct importance for the macroscopic properties of food products such as: flow, stability and texture.

The obtained data presented in **Table (1)** indicated that as the concentration of modified lecithin increased, the emulsification activity significantly increased ($p<0.05$). The emulsification activity of lecithin-CPI complexes treated by heat registered the highest one, while lecithin-CPI complex treated by ethanol and lecithin-CPI complex were in the second and third orders, respectively. The rate of increase in emulsification activity of modified canola lecithin at concentration of 15 mg/ml water was higher than that at concentration of 5 mg/ml water compared to crude canola lecithin. The increasing rate of emulsification activity of lecithin-CPI complex treated by heat or ethanol compared to extracted crude canola lecithin was 64.5 and 54.4 % at concentration of 5 mg/ml water, while the emulsification activity was improved to 99.5 and 90.4 % at concentration of 15 mg/ ml water, respectively. **Aynié *et al* (1992)** reported that the interaction occurred through lipid polar heads and protein polar side chains due to hydrogen bonds and/or electrostatic interactions. Proteins with hydrophobic regions or lecithin are examples of such molecules, as they contain segments that prefer solution into an aqueous environment and segments that prefer solution into a nonpolar environment (**Aynié *et al* 1992; Tomas *et al* 1994**). During the homogenization of a fat into a solution in the presence of amphiphilic molecules, a membrane quickly forms around the fat globule. This membrane acts to lower the interfacial tension (surface free energy) between oil and water depending on the amount of surfactant adsorbed and the density of the fat globule can increase (**Chen *et al* 1993**). Both mechanisms have a stabilizing effect, slowing the rate of creaming and coalescence that may have otherwise occurred.

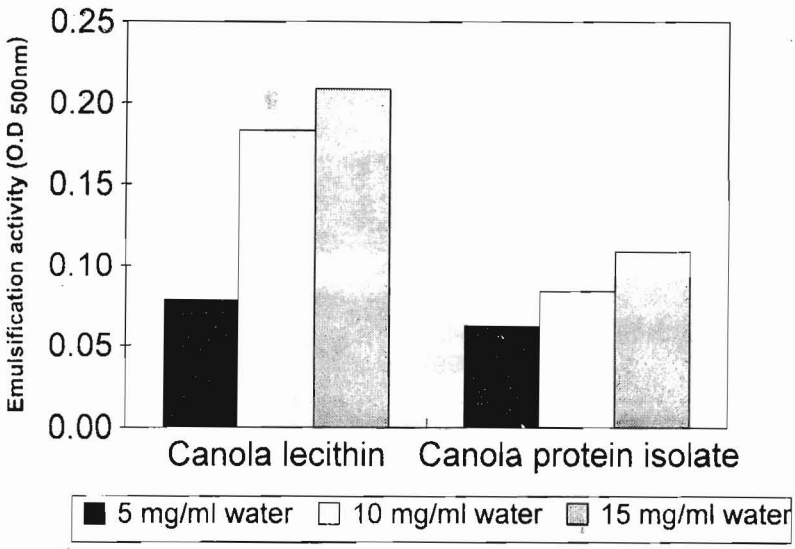

Figure 1. Emulsification activity (O.D $_{500\ nm}$) of crude canola lecithin and canola protein isolate at different concentrations

Table 1. Emulsification activity ($O.D_{500\ nm}$) of modified canola lecithin at different concentrations

Lecithin type	Lecithin concentration (mg/ml water)		
	5	10	15
SCLP	0.093^{Cc}	0.289^{Bb}	0.368^{Ca}
SCLP-E	0.122^{Bc}	0.291^{Bb}	0.398^{Ba}
SCLP-H	0.130^{Ac}	0.304^{Ab}	0.417^{Aa}

SCLP, sonicated canola lecithin with canola protein isolate; **SCLP-E**, sonicated canola lecithin with canola protein isolate treated by ethanol; **SCLP-H**, sonicated canola lecithin with canola protein isolate treated by heat

Capital letters compared between the means in the same column.

Small letters compared between the means in the same raw.

Different alphabets are significantly ($P<0.05$).

3.3. Mathematical models of relationship between emulsifier concentrations and emulsification activity

From the mathematical models for prediction of EA values of prepared emulsions, it could be mentioned that the EA of CPI, canola lecithin and their modified forms was dependent on emulsifier concentration. Therefore, a trial was carried out to find suitable equation for predication of EA at different concentrations. The most suitable model found to adequately represent this relationship was a quadratic polynomial equation. R^2-values for this mathematical model were found to vary between 0.9424 and 0.9998. **Figure (2)** gives the constants of the proposed mathematical models for each type of the tested lecithin. With the help of these constants the EA values could be predicted for identify the optimum concentration required to produce a high stable emulsion when applied in quadratic polynomial equations.

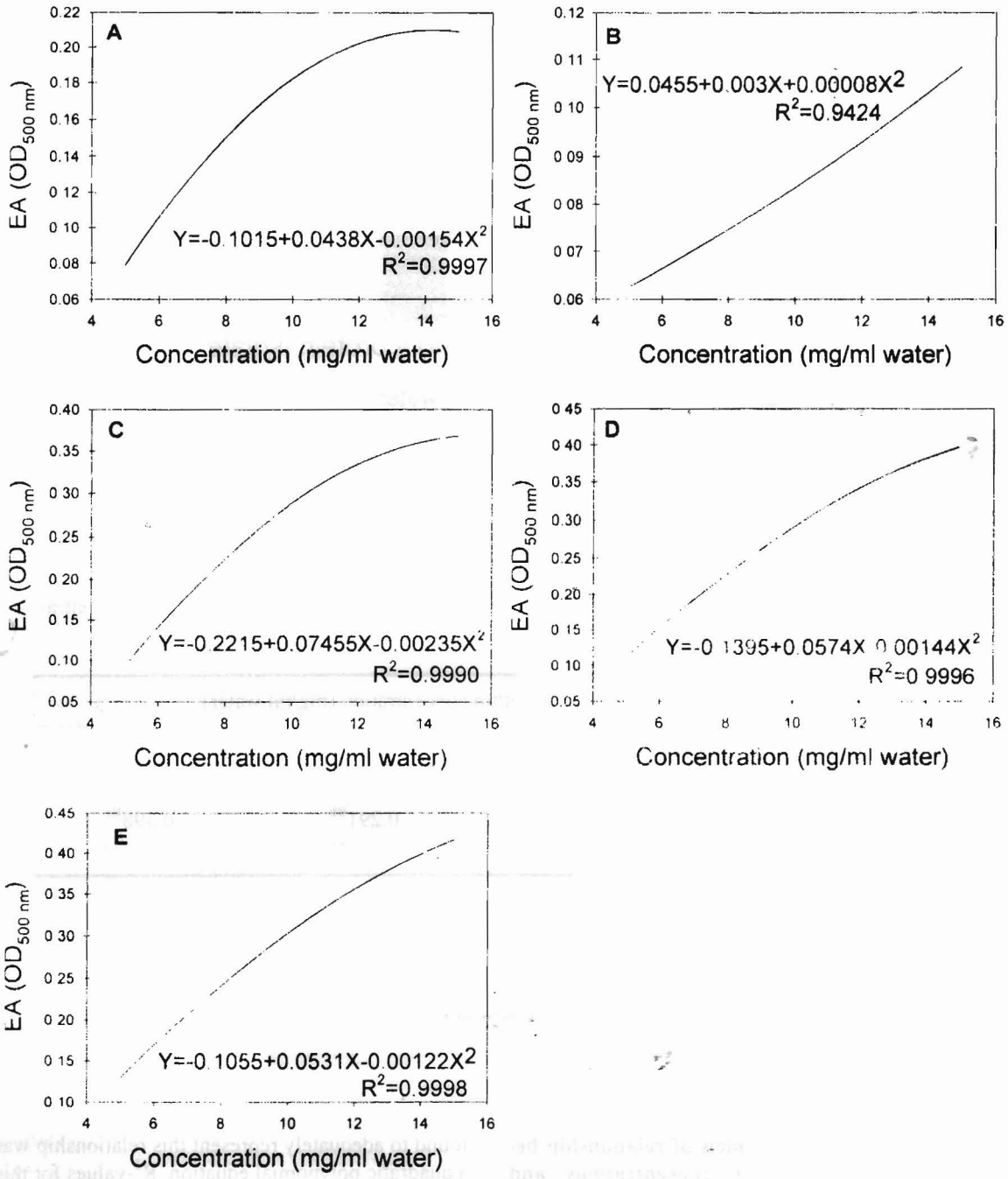

Figure 2. Mathematical models for prediction EA values of **A**, canola lecithin; **B**, canola protein isolate; **C**, canola lecithin-CPI complex; **D**, canola lecithin-CPI complex treated by ethanol; **E**, canola lecithin-CPI complex treated by heat at different concentrations

3.4. Emulsion stability

Figure (3) shows the contour plot of ES as observed at different times and concentrations. It was clearly noticed that, the ES followed the same trend of EA, i.e., the improver the EA, the greater in its stability. ES increased with increasing concentration from 5 mg/ml water to 15 mg/ml water. On the other hand, ES decreased with increasing holding time from zero to 60 min. After 60 min at concentration values ranging between 5 and 15 mg/ml water, the prepared emulsion using lecithin-CPI complex treated by heat demonstrated significantly ($P<0.05$) the strongest stability, followed by the emulsion prepared using lecithin-CPI complex treated by ethanol, whereas, the emulsions prepared using canola lecithin and CPI showed the lowest emulsion stability. The observed coalescence and creaming in emulsions prepared using canola lecithin or CPI was due to increasing of oil droplet diameter. However, addition of lecithin-protein complex treated by heat or ethanol markedly improved emulsion stability and retarded coalescence and creaming. It appears that the most important factor affecting creaming stability is particle diameter, in accordance with Stoke's low. While, according to **Agboola *et al* (1998)**, the mechanism by which creaming stability is preserved in system containing modified lecithin is unclear. The increasing of emulsifying stability of modified lecithin may also due to the improvement of their hydrophilic/lypophilic balance that lowered more effectively the interfacial tension of the film between oil droplets and water in the emulsion. These observations are agreed with those of **Yamamoto and Araki, (1997)**.

3.5. Response surface study of emulsion stability at different concentrations and times

Table (2) shows the optimum values of emulsion stability at different emulsifier concentrations and times; the data were obtained from the response surface study by contour plot of concentrations, times and emulsion stability. It can be seen that, canola lecithin-CPI complex treated by heat or ethanol were effective in enhancement of emulsion at concentrations less than other modified canola lecithins. These findings are in accordance with **Mizutani and Nakamura, (1988)**. They showed that the emulsifying activity of soy lecithin-protein complex was much higher than that of soy lecithin vesicles having no protein or soy protein and increased further with ethanol treatment. **Hirotsuka *et al* (1984)** mentioned that the enhancement of EA of lecithin-soy protein complex treated by heat or ethanol due to the conformation of soy proteins was changed by this treatment, and their aggregation occurred. In this process of aggregation, lecithin was firmly associated with the protein, and the final products of partially denatured lecithin-protein complex may contain polymerized proteins with amphipathic structure where hydrophobic surface may have increased. **Fang and Dalgleish, (1993)** found that the casein-oil-lecithin interaction enhance the stability of the oil-in-water emulsions because, the hydrodynamic thickness of the adsorbed protein layer on the hydrophobic oil surface was modified by the presence of lecithin.

Table 2. Optimum values of emulsion stability for CPI, canola lecithin and their modified forms at different times and concentrations.

Lecithin type	Time (min)	Lecithin concentration (mg/ml water)	ES
CPI	20	14.1	0.095
	40	15.0	0.090
	60	13.2	0.080
CL	20	14.4	0.120
	40	12.4	0.100
	60	12.4	0.100
SCLP	20	13.2	0.200
	40	13.9	0.200
	60	12.4	0.150
SCLP-E	20	15.0	0.250
	40	13.5	0.200
	60	14.6	0.200
SCLP-H	20	14.7	0.250
	40	12.8	0.200
	60	13.9	0.200

CPI, canola protein isolate; **CL**, canola lecithin; **SCLP**, sonicated canola lecithin with canola protein isolate; **SCLP-E**, sonicated canola lecithin with canola protein isolate treated by ethanol; **SCLP-H**, sonicated canola lecithin with canola protein isolate treated by heat

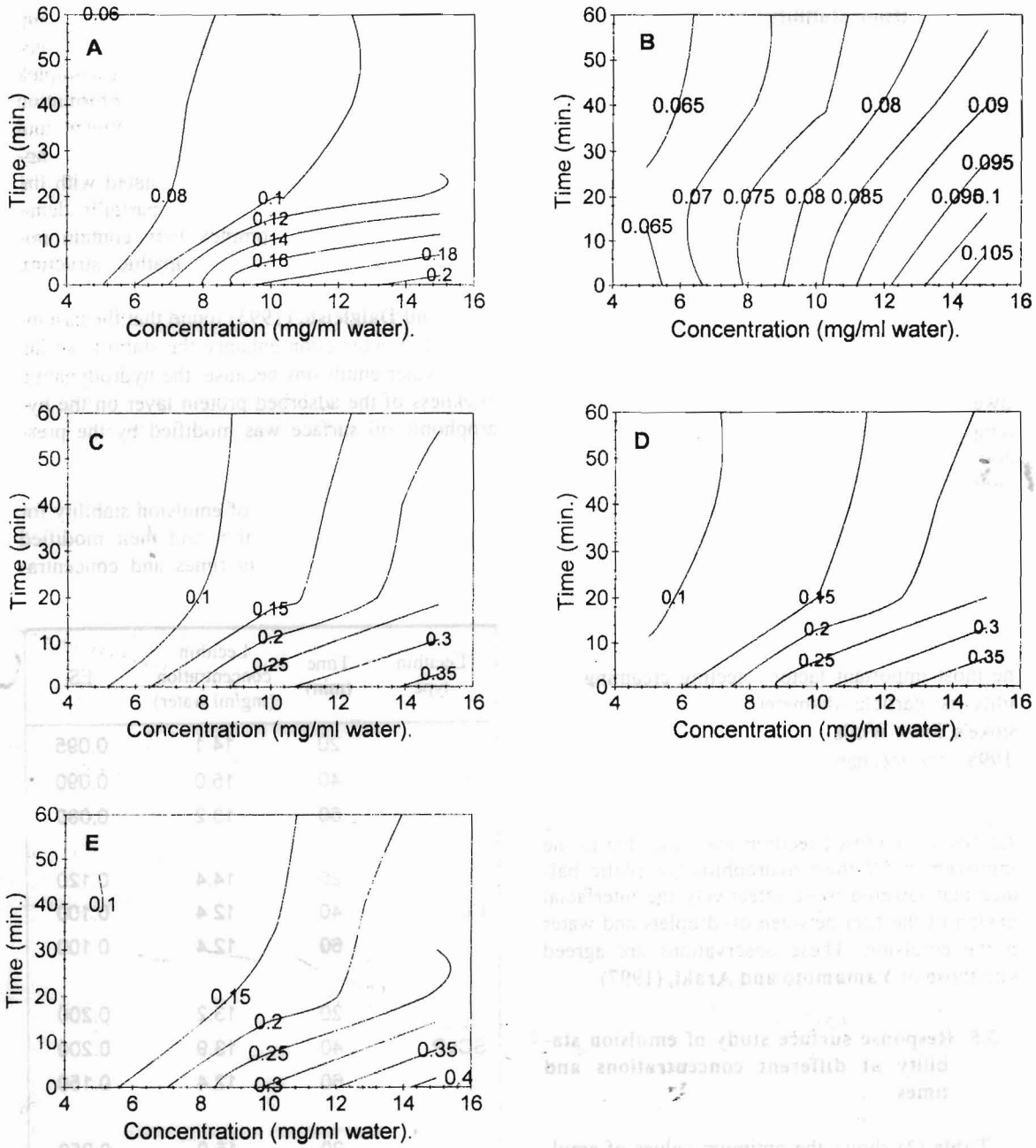

Figure 3. Contour plot of emulsion stability for **A**, canola lecithin; **B**, canola protein isolate; **C**, canola lecithin-CPI complex; **D**, canola lecithin-CPI complex treated by ethanol; **E**, canola lecithin-CPI complex treated by heat at different concentrations and times

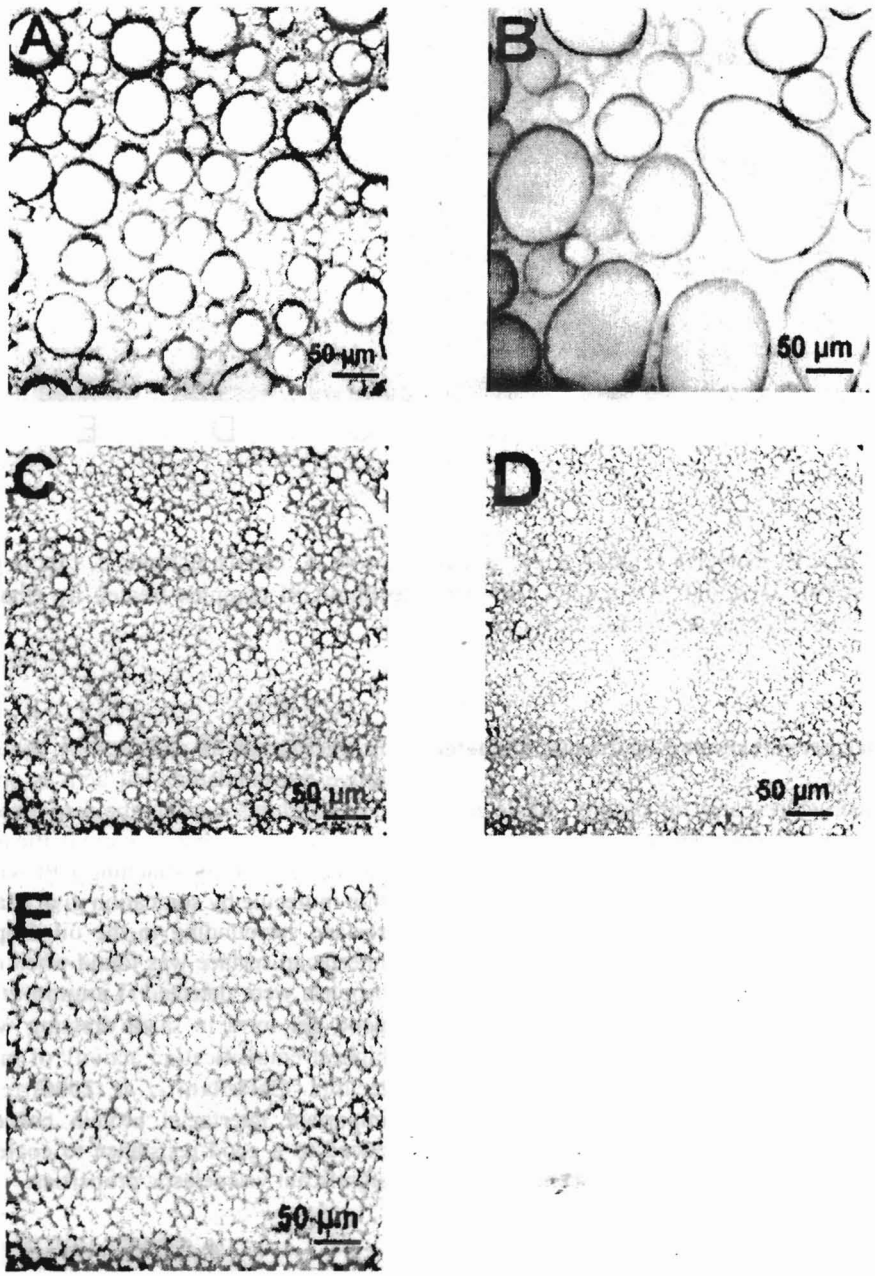

Fig. 4. Microstructure of salad dressing prepared with **A**, canola lecithin; **B**, canola protein isolate; **C**, canola lecithin-CPI complex; **D**, canola lecithin-CPI complex treated by ethanol; **E**, canola lecithin-CPI complex treated by heat

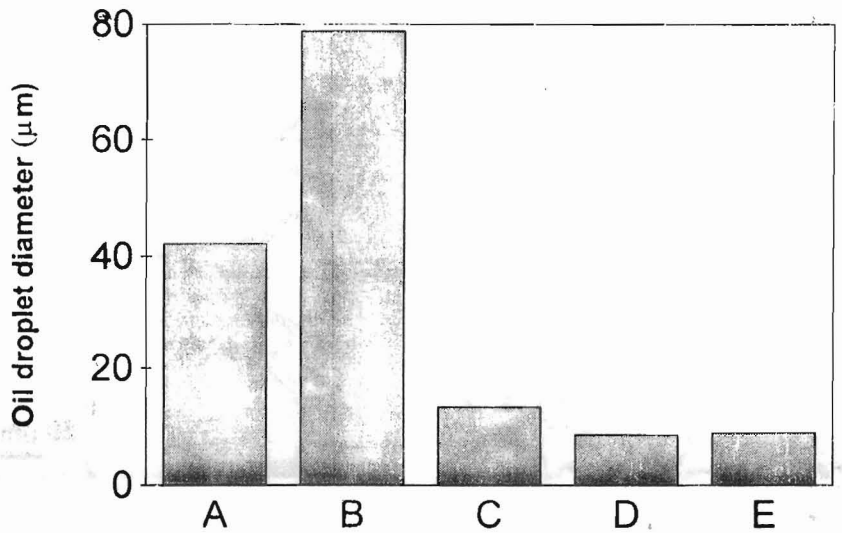

Fig. 5. Oil droplet diameter of salad dressing prepared with **A**, canola lecithin; **B**, canola protein isolate; **C**, canola lecithin-CPI complex; **D**, canola lecithin-CPI complex treated by ethanol; **E**, canola lecithin-CPI complex treated by heat

3.6. Microstructures and oil droplet diameter

The microstructures and oil droplet diameter of salad dressing samples prepared using canola lecithin, CPI, canola lecithin-CPI complex, canola lecithin-CPI complex treated by heat or canola lecithin-CPI complex treated by ethanol are shown in **Figures (4 and 5)**. Micrographs A and B appeared that the salad dressing prepared using CPI or canola lecithin contained larger oil droplets and some droplets joined together. This result due to the flocculation and coalescence mechanisms that occurred in salad dressing emulsion prepared using CPI or canola lecithin. Increase in droplet size possibly due to oil droplet coalescence, which occurred after the droplets, had been in prolonged contact (**Abu-Jdayil, 2003**). On the other hand, the modified canola lecithin salad dressing appears smallest oil droplets than those in salad dressing prepared using CPI or canola lecithin alone. The micrograph C for salad dressing prepared using sonicated canola lecithin-CPI complex contained oil droplets differed in size. However, micrographs D and E for salad dressing prepared using sonicated canola lecithin-CPI complex treated by heat or ethanol appear the smallest oil droplets irregular in both size and shape. The small size of the oil droplets, in salad dressing emulsions shown in micrograph D and E, was contributed to improve the emulsification activity in modified canola lecithin according the data presented in **Table (1)**. The improvement in emulsification activity of canola lecithin by attaching CPI with canola lecithin molecule by sonication give a large interfacial surface surrounding to the oil droplets. A larger interfacial surface was found when many small oil droplets were detected (**Langton *et al* 1999**) The good gel form in salad dressing was due to the highest emulsification activity of modified canola lecithin. **Castellani *et al* (2006)** reported that an important interfacial protein concentration conduced to a good resistance to coalescence due to significant viscoelastic properties.

4. CONCLUSION

From the previous data it could be noticed that ethanol or thermal treatment of sonicated canola lecithin-SPI complex improved the emulsification activity and emulsion stability indices. The modified canola lecithin inhibit strongly the coalescence and flocculation of oil in emulsion systems. The micrograph of prepared salad dressing using modified canola lecithin exhibited smallest oil droplets irregular in both size and shape.

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تعديل ليسيثين الكانولا لتحسين الخواص الاستحلابية للاستخدامات الغذائية

[١١]

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تم فى هذه الدراسة تحسين الخواص الاستحلابية لليسيثين المستخلص من زيت الكانولا الخام وذلك عن طريق تحضير معقد الليسيثين مع البروتين، وقد اجرى معاملة معلق ليسيثين الكانولا مع معزول بروتين الكانولا الذائب عند رقم الأس الهيدروجينى ٧,٠ بالموجات الفوق صوتية، كما اجرى معاملة هذا المعقد الناتج بكحول الميثانول تركيزة ٩٩%، أو تعريضة للحرارة عند ٩٥°م لمدة دقيقة واحدة لتحسين خواصه الاستحلابية. ودلت النتائج على ان النشاط الاستحلابى لليسيثين الكانولا قد حدث فية تحسن كبير نتيجة تكوينة المعقد مع البروتين المعزول من الكانولا. كما اتضح من تحليل النتائج المتحصل عليها لثبات المستحلب عند ازمنة مختلفة (٢٠، ٤٠، ٦٠ دقيقة) والمحضر بإستعمال تركيزات مختلفة (٥، ١٠، ١٥ ملليجرام/مل ماء) من الليسيثين أن معاملة معقد الليسيثين مع البروتين بالكحول أو بالحرارة قد أدى الى تحسين كبير فى ثبات المستحلب ومنع التصاق حبيبات الدهن ببعضها وانفصالها.

وقد تم اجراء تحليل انحدار لإستنباط نموذج رياضى بغرض التنبؤ بقيم النشاط الاستحلابى لانواع الليسيثين المستعملة ونشاطها الاستحلابى لمختلف التطبيقات فى الخلطات الغذائية. وقد أظهرت الدراسات الميكروسكوبية للتركيب الدقيق لعينات من سلطات التغطية المحضرة بإستعمال ليسيثين الكانولا المعدل مدى صغر وتماثل قطيرات الزيت من حيث الشكل والحجم.

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