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OPTIMIZATION OF EMULSIFICATION AND MICROENCAPSULATION OF EVENING PRIMROSE OIL AND ITS OXIDATIVE STABILITY DURING STORAGE BY RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Microencapsulation is a technique by which small droplets of liquid or solid particles are coated with a thin film of wall materials to protect susceptible ingredients in food products to assure their quality or effectiveness. Microencapsulation of liquid lipid into powdery matrixes of wall materials includes two unit operations: emulsification of the lipid with an aqueous solution of wall material and drying of the emulsion. The effects of hydrophile-lipophile balance (HLB) value, emulsifier content, and oil content on the evening primrose oil-in-water emulsion stability were studied by response surface methodology (RSM). The HLB value, emulsifier content, and oil content all had significant effects on the emulsion stability ($p<0.05$). Of them, the HLB value and emulsifier content contributed more effects than the oil content. The optimized HLB value, emulsifier content, and oil content were used to mix with wall materials: gum arabic (GA), maltodextrin (MD), and/or sodium caseinate (NaC). The oil was encapsulated with these materials individually or in combination by spray-drying and their oxidative stability during storage was compared. The microcapsules with a single wall material were relatively susceptible to oxidation than those with multiple wall materials. The most desirable composition of the mixture of GA, MD, and NaC by RSM was 17.24, 75 and 7.76%, respectively.

PRACTICAL APPLICATIONS

RSM provided a valuable means to help us understand the relative or interactive effects of three important parameters, HLB value, emulsifier content, and oil content on the emulsion stability of oil-in-water system. The information obtained would be useful for the preparation of similar o/w emulsion system as needed in some product development for foods. In addition, the effects of gum arabic, maltodextrin, and sodium caseinate, on the oxidative stability of

microencapsulated oil were also studied by RSM. The results revealed the relative or interactive effects of these materials and gave the optimal conditions in minimizing the oxidative instability in this study. Since these wall materials are readily available and widely used in a variety products, the information provided by this study would be useful for product-developing professionals to use these materials more efficiently in terms of obtaining optimized microencapsulated products against lipid oxidation and cost effectiveness.

Key Words: evening primrose oil, oil-in-water (o/w) emulsion, response surface methodology, γ-linolenic acid, spray-drying, emulsifier, microencapsulation

INTRODUCTION

Evening primrose oil (*Oenothera biennis*) is becoming used in increasing amounts in nutritional and pharmaceutical preparations (Christie, 1999). Gamma-linolenic acid is a main active component of the oil responsible for many physiological effects, such as hypocholesterolemic effect (Sugano *et al.*, 1986), therapeutic effects in atopic eczema, diabetic neuropathy, rheumatoid arthritis and premenstrual pains, etc.(Bugnariu, 1996). A high content of polyunsaturated fatty acids in evening primrose oil (>80%) makes it susceptible to oxidation. Microencapsulation is a technique by which small droplets of liquid or solid particles are coated with a thin film of protective biomaterials (Sheu and Rosenberg 1995), or wall materials as often used in many articles (Hogan *et al.* 2001; Kagami *et al.* 2003). The encapsulated materials can be protected against evaporation, oxidation, and

chemical reactions (Rosenberg *et al.* 1990).

Microencapsulation of liquid lipid into powdery matrixes of wall materials includes two unit operations: emulsification of the lipid with an aqueous solution of wall material and drying of the emulsion (Minemoto *et al.* 1997). A well-prepared and stable emulsion is essential to the success of the later drying process in terms of the uniformity of droplet distribution and covering effects of wall materials. Emulsifiers are often used to facilitate formation of emulsions and enhance their stability. Hydrophile-lipophile balance (HLB) is an important parameter to optimize the stability and the size of the dispersed droplets in a two-phase system of immiscible liquids (Davis 1994). Stable emulsions, especially where synthetic surfactants are used, are best formulated with emulsifiers or combination of emulsifiers having HLB values close to that required of the oil phase (Aulton 1995). Emulsifiers, Tween 80 and Span 80, have been used for forming a range of HLB values to investigate the effect of HLB on the essential oil-water emulsion stability (Orafidiya and Oladimeji 2002). In their study, the oil and emulsifier amounts used were fixed over the HLB range. Theoretically, HLB is affected by the types and the amount of emulsifiers, and oil content would affect HLB due to the increase of lipophilic portion and the usage level of emulsifiers due to more lipophilic portion needed to be covered. These three factors are important to form a stable emulsion; however, the interactive effects of HLB value, emulsifier content, and oil content on the emulsion stability of oil-in-water system (o/w) have not been reported. Response surface methodology (RSM) is a very effective tool when many factors and interactions between factors affect the desirable responses (Wanasundara and Shahidi 1998). Therefore, the interactive effects of HLB value, low molecular weight emulsifier content, and oil content on the emulsion stability of oil-in-water system (o/w) were studied by RSM.

Spray-drying is the microencapsulation technique most commonly used in the food

industry (Gibb *et al.*, 1999). Spray-drying may enhance oxidation due to a very large surface area being produced on the microcapsules, if the wall materials can not provide a good oxygen barrier (Desorby *et al.*, 1997). Therefore, selection of wall materials becomes important for spray-drying microencapsulation. Regarding wall materials used, many have been reported. For instance, Sodium caseinate for microencapsulation of orange peer oil (Kim and Morr, 1996), chitosan for that of krill oil (Butos *et al.*, 2003), whey protein concentrate for that of conjugated linoleic acid (Jimenez *et al.*, 2006), n-octenylsuccinate-derivatized starch for fish oil (Drush and Schwarz 2006) starched, and modified celluloses for that of fish oil (Kolanowski *et al.* 2006). Blending different encapsulating substances as a wall material is a useful practice, which may take advantage of the merits of respective substances or reduce the cost via the replacement of the expensive ones. Gum arabic has traditionally been the choice material for lipid encapsulation due to its efficient emulsification characteristics (Kenyon 1995), but cost and limited supply have restricted its use for encapsulation. Hence, maltodextrins and modified starches were used as alternative carrier materials to partially replace it (Krishnan *et al.* 2004). Blends of sodium caseinate and con syrup with various DE have been studied for microencapsulation of soybean oil and the result showed good encapsulating properties at certain weight ratio and DE value (Hogan *et al.* 2001). In these blending studies, they used one-factor-at-a-time method, which can not show the interactive effect among the factors (Wanasundara and Shahidi 1998). Furthermore, the characteristics and interactive effects of the blend of gum arabic, maltodextrins, and sodium caseinate as an encapsulating material have not been reported.

Gum arabic and sodium caseinate are natural substances, and maltodextrins are a GRAS ingredient; therefore, they would be favorable for use in health-appealing functional products, such as evening primrose oil, in terms of consumer preference for natural products. Maltodextrins are a good compromise between cost and effectiveness, are bland in flavor, and

have low viscosity at high solid ratio (Desorby *et al.*, 1997); however, it is hygroscopic. Sodium caseinate would appear to offer the physical and functional characteristics required to encapsulate lipid core materials (Kinsella 1984). However, it becomes hardly dissolvable in water at high content. Therefore, it would be advisable to make a blend of these materials, which may take the advantages of them and minimize the disadvantages when they are used alone. To achieve this purpose, it is useful to know the interactive effects among gum arabic, maltodextrin, and sodium caseinate in terms of obtaining optimal microencapsulation efficacy and cost reduction.

As to the research of oxidative stability of evening primrose oil, there have been few reports on this subject. Khan and Shahidi (2002) studied the photooxidative stability of stripped and non-stripped evening primrose oil; Ouyang *et al.* (2005) studied the stability of β-cyclodextrin inclusion complex of evening primrose oil. However, the effect of mixture of gum arabic, maltodextrin, and sodium caseinate as wall materials on the oxidative stability of microencapsulated evening primrose oil by RSM has not been reported.

The objectives of this research were to study the effects of HLB value, low molecular weight emulsifier content, and oil content on the emulsion stability of evening primrose oil-in-water system (o/w) by response surface methodology in order to find the optimal conditions for preparing a stable emulsion for the spray-dying microencapsulation of evening primrose oil, and to study the effects of mixture of gum arabic, maltodextrin, and sodium caseinate as wall materials on the oxidative stability of the microencapsulated oil.

MATERIALS AND METHODS

Materials

Evening primrose oil was provided by Goerlich Pharma Int. GmbH (Edling, Germany).

Tween 80, Span 80 and propylene glycol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Maltodextrin (DE:11-14), sodium caseinate and gum arabic were obtained from Roquette Frères (Lestrem, France), Fonterra Brands Ltd. (New Zealand) and Taiwan Gum Arabic Co. (Taipei, Taiwan), respectively.

Methods

Preparation of Emulsion

Two emulsifiers, Tween 80 (HLB:15), Span 80 (HLB: 4.3) were blended in different weight ratios to make a range of HLB values. Three variables (HLB value, emulsifier content, and oil content) were arranged by the central composite design as in Table 1. The required amount of Span 80 was dissolved in the oil phase and that of Tween 80 in the aqueous phase; then, both were mixed together according to the designed ratio and homogenized by a homogenizer (IKA-Werke T20 S1, Staufen, Germany) at 9500 rpm for 3 min.

Droplet Size Analysis

A gentle rotation of the emulsion container was done before sampling to obtain even dispersion of the oil droplets. After sampling, the emulsion was diluted (1: 100) using propylene glycol and the droplet size of the emulsions was measured with an optical microscope (Nikon OPTIPHOT-2) fitted with X40 objective and a standardized X10 eyepiece micrometer scale (Orafidiya and Oladimeji, 2002). To ensure uniformity of distribution of the droplets, 20 replicates of the emulsion slide (about 1200 droplets) were observed and the size distribution of the droplets was recorded by Nikon Digital Camera COOLPIX 950 (Nikon Instech Co., Kanagawa, Japan). The geometric mean droplet diameter (M) was calculated using the following formula: $\log M = (\Sigma_i n_i \log x_i)/ \Sigma_i n_i$, where n_i is the number of droplets whose diameter lies in an interval of which the mid-point is x_i (Smith and Jordan, 1964).

Effect of Centrifugation on Emulsion Stability

Five milliliters of each of the emulsion preparations were taken into glass tubes and subjected to centrifugation at 15ºC and10,000 x g for 10 min. This condition was tested not to cause creaming. The mean droplet diameter was determined as the above method. The percentage increase in the mean droplet diameter of each emulsion formulation was calculated as follows: $x\% = (x_2-x_1)100/x_1$, where x is the difference in the mean droplet diameter of the centrifuged emulsion x_2, and the uncentrifuged emulsion x_1 (Orafidiya and Oladimeji, 2002).

Microencapsulation

The emulsion was prepared as described above using the following condition: HLB value 11, 1.4 wt % emulsifiers (Tween 80 and Span 80) and 11 wt % oil. Gum arabic, maltodextrin and sodium caseinate were used as wall materials. The quantity of wall materials used in the emulsion was based on a ratio of 1.5 times the oil weight. The individual wall material was well hydrated separately and then homogenized with the emulsifiers and oil at 9500 rpm for 3 min to prepare a mixture solution. To conduct the experiment more effectively and economically in the presence of the three wall materials as independent variables and various usage levels in each variable, a statistical mixture design was applied and these wall materials were formulated as in Table 2. The amount of sodium caseinate was constrained to a lower usage level as compared with the other two substances, because of its poor solubility at high concentration in this system. The mixture solutions with a total solid content of 23% were dried by a spray-dryer (GB 22, Yamato, Japan). They were pumped into the spray-dryer at a flow rate of 2.4ml/min and dried at an inlet temperature of 140ºC and outlet temperature of 80±5ºC. The head pressure of atomizer was 0.1 MPa and the dry air flow rate was 0.4 m^3/min.

Structure of Microcapsules

The microcapsules were deposited on double-coated carbon conductive tapes adhered to copper stubs; the samples were then sputter-coated with a thin gold layer and then analyzed by a scanning electron microscope (JSM 5300, JEOL Co., Tokyo, Japan).

Accelerated Oxidation and Headspace Oxygen Analysis

The microcapsules at an amount of 1.5 g were weighted in a 14 ml glass serum bottle. The bottle was sealed air-tightly with a septum secured with an aluminum cap. The prepared sample bottles were stored in an oven at 60ºC for 4 days. The headspace oxygen was determined by injecting 250 ul headspace air of the sample bottle into a gas chromatograph (GC 14B, Shimaz Co., Japan) equipped with a thermal conductivity detector. A stainless steel column (2 m x 3.2 mm) packed with 60/80 molecular sieve (Supelco, Bellefonte, PA) was used. Helium was used as a carrier gas with a column flow rate of 30 ml/min. The temperatures of injector, oven, and detector were 120, 110 and 160ºC, respectively. The computer software (SISC Chromatography Data System, Davis, CA) was used for integration and calculation of the peak areas in the gas chromatograms.

Statistical Analysis

The statistical mixture design and analyses were conducted using Statistica for Windows (StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

Effects of HLB Value, Emulsifier Content, and Oil Content on Emulsion Stability

Several methods have been used to evaluate emulsion stability in literature, such as turbidity, creaming, droplet size analysis after centrifugation (Orafidiya and Oladimeji 2002), and conductivity (Azzam and Omari 2002). Turbidity, creaming and conductivity methods were tested and found not suitable for comparison of the emulsion stability in this study due to the simultaneous change of HLB value, emulsifier content, and oil content (data not shown). Therefore, the increase of mean oil droplet diameter in the emulsion after centrifugation was used to evaluate the emulsion stability. The analysis of variance (ANOVA) in a second-order regression model was conducted to compare the influence of the HLB value, emulsifier content, and oil content on the emulsion stability. The degree of the influence exerted by the three variables is represented by the statistical term of "effect", which means the value of difference of the dependent variable between the highest level and the lowest level of the corresponding independent variable used within the experimental range. The higher the value, the more influential the independent variable is. In this case, the dependent variable is the increase of the mean oil droplet diameter and the independent variables are the HLB value, emulsifier content, and oil content. In addition, the leading sign of the value shows the direction of the effect. Namely, the positive sign denotes "increase" while the negative sign means "decrease". Briefly, the higher the positive value, the lower the emulsion stability is in this study. The effects of the HLB value, emulsifier content, and oil content on the mean oil droplet diameter in the emulsion after centrifugation are given in Table 3. The results show they all have significant effects on the change of mean oil droplet diameter at $p<0.05$.

As far as the HLB value is concerned, the linear term, HLB (L) and the quadratic term, HLB (Q), both show significant effects on the change of the mean oil droplet diameter. Because both values are positive, their effects are unfavorable to the emulsion stability. That is, when the HLB value increases, the difference of the mean oil droplet diameter (DMD) before and after centrifugation also increases. Fig. 1c shows a trend that when the amount of

oil and emulsifier is fixed, the increase of the HLB value results in the increase of DMD, which corresponds to the above "effect" analysis. The emulsifier content also gives a significant effect. The linear term, emulsifier (L), has a negative effect, while the quadratic term, emulsifier (Q), shows a positive effect. This result suggests that the amount of emulsifier must have some limitation in usage; namely, an appropriate amount of the emulsifier could decrease the DMD, but overdose would cause the increase of the DMD. As for the oil content, it shows a significant effect as well. The linear term, oil (L), shows a significant effect, but the quadratic term, oil (Q), does not at $p<0.05$. This means the linear effect, or main effect, is dominant and also indicates that more oil used would bring about the increase of DMD in the emulsion.

Through the interaction analysis, it is found that the HLB value and emulsifier content have an interactive effect [HLB (L) x emulsifier (L)], $p=0.0086 < 0.05$] and the effect is relatively large judging from the negative value. It indicates that the HLB value and emulsifier content must be manipulated simultaneously in looking for optimal conditions of minimizing the DMD to form a stable emulsion. In contrast, the HLB value and oil content [HLB (L) x oil (L)], $p=0.1568 > 0.05$] or the emulsifier content and oil content [emulsifier (L) x oil (L)], $p=0.6825 > 0.05$] do not have interactive effects. The above effect analysis can be summarized as follows: the three variables all have significant effects on the change of mean oil droplet diameter in the emulsion after centrifugation, of which the HLB value and emulsifier content give a higher effect than that of the oil content. This result gives us useful information that at appropriate condition of fixed HLB value and emulsifier content, the oil content can be reasonably increased without markedly affecting the emulsion stability. This characteristic may be desirable when incorporating more oil in o/w type products is preferred.

In addition to giving the information of interactive effect, the RSM can facilitate us in selecting suitable conditions to obtain desirable experimental results. In this work, at the fixed

emulsifier content or HLB value, either the contour plot of emulsifier content *vs*. oil content (Figure 1a) or that of oil content *vs*. HLB value (Fig. 1b) is a centric graph, which can be used to predict the optimal values of independent variables. In contrast, at the fixed oil content, the contour plot of HLB value *vs*. emulsifier content (Fig. 1c) is ridge-like. As a result, there is no commonly converged point available from the surface plots of the three variables probably due to the marked interactive effect between the HLB value and the emulsifier content. The centric graphs can be used to predict the optimal values of independent variables. In the mean time, the ridge graph also shows a trend that is useful for selection of the most suitable conditions. In the present work, the optimal combination of the HLB value, emulsifier and oil contents, is obtained from Figure 1(a) and their values are 11, 1.4%, and 11%, respectively. Subsequently, this condition was used for preparation of emulsion for spray drying. The reasons for using emulsifiers (Tween 80 and Span 80) to form an o/w emulsion prior to mixing with the wall materials are some materials may have no or reduced emulsifying ability to the oil when used individually or in combination such as maltodextrin. In addition, if wall materials have some emulsifying activity, a two-step emulsification, namely using low molecular weight emulsifiers to form an emulsion first then adding the second high molecular weight emulsifier and homogenizing the mixture again, can enhance the emulsifying activity of the latter. Miyaguchi *et al.* (2003) investigated the effect of low molecular weight emulsifiers (SE-16, Tween 80, and sodium cholate) on the emulsifying activity of porcine sarcoplasmic proteins (SP). They found that the emulsifying activity of SP alone was the same as that of simultaneous addition of SP and either of these low molecular weight emulsifiers (one-step emulsification). However, the emulsifying activity of SP was markedly enhanced by the two-step emulsification. They also used other proteins, such as sodium caseinate, for comparison; the result also showed the same tendency. Therefore, we used the two-step method to prepare the emulsion mixture for spray drying.

Effect of Wall Materials on the Oxidative Stability of Microencapsulated Evening Primrose Oil

Peroxide value (PV), and conjugated diene content (CD), and headspace oxygen uptake (HOU) methods have been commonly used to evaluate the oxidative stability of oils. The disadvantages of peroxide value and conjugated diene content are as follows. PV fails to adequately measure low peroxide value because of the difficulties in determining the titration end point; the magnitude of changes in absorption at 233 nm for CD determination is not related the degree of oxidation because the effects on the various unsaturated fatty acids vary in quality and magnitude (Gray 1985). When the rates of decomposition of hydroperoxides or conjugated dienes exceeds the rate of formation of hydroperoxides or conjugated dienes, the PV, and CD could be decreased even though the oxidation increases during storage; in contrast, the HOU continuously increases to form hydroperoxides in a gas-tight bottle as the lipid oxidation progresses (Chung *et al.* 2004). Chung *et al.* (2004) investigated the relationships among HOU, PV, and CD methods in measuring lipid oxidation. They used HOU to calculate the theoretical peroxide value (TPV), which is based on the assumption that all headspace oxygen consumed is used to form peroxides. The average coefficient of determination (r^2) between TPV, PV, and CD and storage time for the 7 oil samples containing antioxidants and pro-oxidants under light or dark were 0.99, 0.96, and 0.95, respectively. Hence, they suggested that the headspace oxygen method is simple and reproducible and may be the best analytical method to evaluate the oxidative stability of oils. In this study, the potential candidates for headspace oxygen consumption would be the not encapsulated surface oil, encapsulated oil, and wall materials. But, from the headspace oxygen analysis of the wall materials alone (data not shown), there is no significant difference in the headspace oxygen depletion from the control (the air only bottle) was found during the

storage period. This indicated the wall materials themselves did not interfere with the headspace oxygen analysis. Therefore, the HOU can truthfully reflect the state of lipid oxidation of the microencapsulated oil. Hence, this method was used in this study to evaluate the oxidative stability of microcapsules during accelerated storage. Evening primrose oil (EPO) was encapsulated with various wall materials as in Table 2. The encapsulated EPO and the wall materials alone were stored at 60ºC for 4 days. For encapsulated oil, at low temperature lipid oxidation seems rate-limiting while at higher temperature oxygen permeation through the matrix due to lower activation energy becomes rate-determining (Orlien *et al*. 2004). In order to compare the relative protective effect of the wall materials against oxygen permeation especially in severe conditions within a short storage period, the higher temperature (60ºC) was used to accelerate the experiment. All samples remained in a powder state without melting phenomenon observed after the heating treatment. Although the accelerated experiment may not fully reflect the same situation as the normal room temperature storage; however, it can be rationally inferred that those that show better oxidative stability in severe conditions should perform as well as in normal conditions. Fig. 2 illustrates that as the storage time increased, the headspace oxygen depletion increased in all samples. EPO encapsulated with different wall materials individually or in combination varied in their oxidative stability. Headspace oxygen depletion in the microcapsules with maltodextrin (MD) alone rapidly increased at day 2 as compared with that of the microcapsules with the other wall materials. Because the oily surface was apparently observed from the microcapsules after spray-drying, it showed that MD could not entrap the oil effectively. As expected, the microcapsules with MD were rather susceptible to oxidation. Similarly, gum arabic (GA) neither exhibited a good protective effect against oxidation at day 4. The unfavorable effects of using MD or GA alone could be improved by adding another component to either of both materials. For example, MD mixed with GA gave a marked

improvement in the oxidative stability at day 2 and day 4. The poor entrapping effect on lipids by MD has been reported in other study (Imagi *et al.* 1992). It was suggested that MD required the addition of both lecithin and a stabilizer, xanthan gum, to entrap lipids effectively. In our study GA might give a similar effect as the xatham gum in the above case. It can be reasoned that GA increased the viscosity of MD/GA emulsion system as compared with that of MD alone system, and this might help disperse and stabilize the oil droplets more efficiently until they were spray dried. The viscosity of an emulsion is important because it can affect the behavior of the emulsion on subsequent drying and the rate of formation of a semi-permeable membrane at the surface of drying particles, movement of core materials to the surface of powder particles during the spray-drying process, the size of dried particles, and the thickness of capsule walls (Hogan *et al.* 2001). GA was found a good entrapping agent for methyl linoleate, but it did not have a great ability to retard the oxidation of the entrapped methyl linoleate (Imagi *et al.* 1992). This result is similar to our study in GA encapsulated EPO against oxidation.

MD and/or GA mixed with a low amount of sodium caseinate (NaC) showed a great improvement on oxidative stability as compared with using MD and/or GA alone. It is speculated that NaC has an emulsifying ability which may cover the exposed interfacial area oil droplet not or loosely covered by the low molecular weight emulsifiers (Tween 80 and Span 80) and thereby enhance the stability of the emulsion system via a two-step emulsification (Miyaguchi *et al*. 2003). Though NaC can enhance the stability of oil droplets in the emulsion mixture, a detrimental effect on oxidative stability would occur if the amount of NaC increased too much. This can be reasoned that the excess NaC might destabilize the emulsion system due to depletion flocculation or interfere with the structure formation of the wall, which is largely made of GA and MD, because of competing with them for water thus affecting their solubility and molecular arrangement in the emulsion system. Phase separation

has been reported in the polysaccharide-protein emulsion system such as NaC/xanthan mixture, when the protein concentration was high (Hemar 2001).

The structure of the microcapsules was observed by scanning electron microscopy. The figures show that the microcapsules with GA, MD or GA/MD (Fig. 3a, b, c) seem to have more hollow-structure particles than those with GA/MD/NaC (Fig. 3d). The poor integrity of the microstructure is one of the detrimental factors to oxidative stability. Consequently, this may partly support the results in the headspace oxygen depletion analyses. Other factors such as permeability of the wall, surface oil, particle sizes and shapes may also contribute to the oxidative stability of microcapsules and their effects can also be reflected in the uptake of headspace oxygen. The effect of above mentioned factors may mainly be attributed to the performance of wall materials in a microencapsulating process if the other operating conditions are the same. Therefore, wall materials can be regarded as primary factors affecting the formation and oxidative stability of microcapsules.

To compare the relative effects of the wall materials individually or in mixture on the oxidative stability, a statistical analysis was performed and the results are showed in Fig. 4. To eliminate the effect resulting from different measuring scales used by the components, the standardized pseudo-component mode was used for the relative comparison of the effects. The pseudo-component transformation is defined as $x'_i = (x_i - L_i)/(Total - L)$. Where, x'_i stands for the i'th pseudo-component, x_i stands for the original component value, L_i stands for the lower constraint (limit) for the i'th component, L stands for the sum of all lower constraints (limits) for all components in the design, and *Total* is the mixture total. Pareto chart is a histogram used in a statistical analysis to show the effects by absolute value in descending order, which can make the comparison of the impact of the factor effects on the dependent variable of interest more easily and clearly. The chart shows the wall materials, whenever alone or in combination, all give significant effects on the oxidative stability of the

microcapsules at $p< 0.05$ (Fig. 4). The positive sign of the value shows an unfavorable effect on the oxidative stability of microcapsules and the value gives the intensity of the effect while the negative sign shows an enhancing effect on the oxidative stability. Therefore, from the Fig. 4, GA, MD or NaC individually all give unfavorable effects on the oxidative stability and their influence in a descending order is GA followed by MD and NaC. In contrast, MD with NaC, MD with GA or GA with NaC all has the interactive effects to one another which can enhance the oxidative stability. Moreover, the oxidative stability of the EPO encapsulated with MD and GA mixture can be further improved by the addition of NaC at a low quantity.

To obtain the most desirable response in this study, a desirability function approach is used. It is one of the most widely used methods in industry for the optimization of multiple response processes. It is based on the idea that the quality of a product or process that has multiple quality characteristics, with one of them outside of some desired limits, is completely unacceptable. The method finds operating conditions x that provide the most desirable response values. For each response $Y_i(x)$, a desirability function $d_i(Y_i)$ assigns numbers between 0 and 1 to the possible values of Y_i, with $d_i(Y_i) = 0$ representing a completely undesirable value of Y_i and $d_i(Y_i) = 1$ representing a completely desirable or ideal response value. The individual desirabilities are then combined using the geometric mean, which gives the overall desirability D, namely $D = (d_1(Y_1) \times d_2(Y_2) \times \ldots \times d_k(Y_k))^{1/k}$, with k denoting the number of responses (Derringer and Suich 1980). In this study we used the headspace oxygen depletion of microcapsules as a quality control criterion for the oxidative-stability evaluation with the notion that the lower the headspace oxygen depletion, the higher the desirability. By using the "response desirability profiling" function provided by the statistical software (Statistica), the predicted quantity values for the mixture of GA, MD, and NaC were 17.24, 75 and 7.76%, respectively.

In conclusion, the RSM provided a valuable means to help us understand the relative or

interactive effects of three important parameters, HLB value, emulsifier content, and oil content on the emulsion stability of oil-in-water system. The information obtained would be useful for the preparation of similar o/w emulsion system as needed in some product development for foods. In addition, the effects of gum arabic, maltodextrin, and sodium caseinate, on the oxidative stability of microencapsulated oil were also studied by RSM. The results revealed the relative or interactive effects of these materials and gave the optimal conditions in minimizing the oxidative instability in this study. Since these wall materials are readily available and widely used in a variety products, the information provided by this study would be useful for product-developing professionals to use these materials more efficiently in terms of obtaining optimized microencapsulated products against lipid oxidation and cost effectiveness.

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TABLE 1. ROTATABLE CENTRAL COMPOSITE DESIGN OF HLB VALUE, EMULSIFIER CONTENT, AND EVENING PRIMROSE OIL CONTENT AS WELL AS CHANGES OF DROPLET DIAMETER AFTER CENTRIFUGATION.

Run	HLB value	Emulsifier[1] (%, w/w)	Oil(%, w/w)	MB[2] (μm)	MA[3] (μm)	Diameter increase (%)
1	9	0.6	9	1.47	1.69	14.7
2	9	0.6	21	1.49	2.50	67.8
3	9	1.8	9	1.35	2.09	54.8
4	9	1.8	21	1.35	2.75	103.9
5	13	0.6	9	1.37	4.52	230.8
6	13	0.6	21	1.40	3.46	247.1
7	13	1.8	9	1.33	2.33	75.2
8	13	1.8	21	1.49	2.65	77.9
9	7.6	1.2	15	1.39	2.40	72.7
10	14.3	1.2	15	1.54	3.62	135.1
11	11	0.2	15	1.57	5.30	237.3
12	11	2.2	15	1.40	3.35	152.1
13	11	1.2	4.9	1.37	2.12	54.7
14	11	1.2	25.1	1.39	3.38	143.2
15	11	1.2	15	1.45	1.85	27.6
16	11	1.2	15	1.49	2.29	53.4
17	11	1.2	15	1.46	1.97	34.9

[1] Amount of a blend of Tween 80 and Span 80 in ratios corresponding to each designed HLB value.

[2] Mean droplet diameter before centrifugation.

[3] Mean droplet diameter after centrifugation.

TABLE 2. STATISTICAL MIXTURE DESIGN OF GUM ARABIC, MALTODEXTRIN, AND SODIUM CASEINATE FOR THE ENCAPSULATION OF EVENING PRIMROSE OIL.

Run	Gum arabic (g)	Maltodextrin (g)	Sodium caseinate (g)
1[1]	24.75	0	0
2	0	24.75	0
3	21.04	0	3.71
4	0	21.04	3.71
5	0	22.895	1.855
6	22.895	0	1.855
7[1]	12.375	12.375	0
8[1]	10.52	10.52	3.71
9	11.4475	11.4475	1.855

[1] The run was duplicated.

TABLE 3. EFFECTS OF HLB VALUE, EMULSIFIER CONTENT, AND OIL CONTENT ON THE INCREASE OF MEAN DROPLET DIAMETER IN THE EVENING PRIMROSE OIL EMULSION SYSTEM AFTER CENTRIFUGATION

Factor	Effect	*p* value
HLB (L)[1]	73.7*[2]	0.0094
HLB (Q)[1]	34.9*	0.0496
emulsifier (L)	-57.4*	0.0152
emulsifier (Q)	97.2*	0.0065
oil (L)	39.5*	0.0314
oil (Q)	-29.4	0.0648
HLB (L) x emulsifier (L)	-100.3*	0.0086
HLB (L) x oil (L)	-20.8	0.1568
emulsifier (L) x oil (L)	-4.4	0.6825

[1] L, linear term; Q, quadratic term.

[2] significant effects at $p<0.05$.

FIGURE CAPTIONS

FIGURE 1. EFFECTS OF THE HLB VALUE, EMULSIFIER AND OIL CONTENTS ON THE CHANGE OF MEAN DIAMETER OF THE OIL DROPLETS IN THE EMULSION AT

THE (A) HLB VALUE OF 11, (B) EMULSIFIER CONTENT OF 1.4%, AND (C) OIL CONTENT OF 11%.

FIGURE 2. HEADSPACE OXGEN DEOLETION OF ENCAPSULATED EVENING PRIMROSE OIL DURING STORAGE AT 60 °C (NAC-H: HIGH NAC, NAC-L: LOW NAC)

FIGURE 3. OUTER STRUCTURES OF MICROCAPSULES CONSISTING OF (A) GA (B) MD, (C) GA/MD, AND (D) GA/MD/NAC.

FIGURE 4. PARETO CHART OF THE EFFECTS OF GUM ARABIC, MALTODEXTRIN, AND SODIUM CASEINATE IN THE STANDARDIZED PSEUDO-COMPONENT FORM ON THE HEADSPACE OXYGEN DEPLETION OF THE MICROENCAPSULATED PRIMROSE OIL.

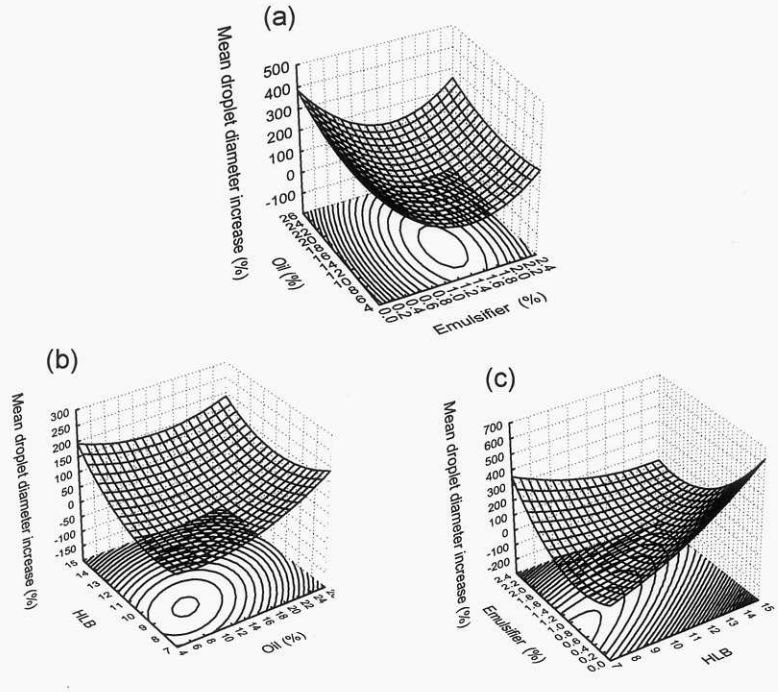

Fig. 1. Effects of the HLB value, emulsifier and oil contents on the change of mean diameter of the oil droplets in the emulsion at the (a) HLB value of 11, (b) emulsifier content of 1.4%, and (c) oil content of 11%.

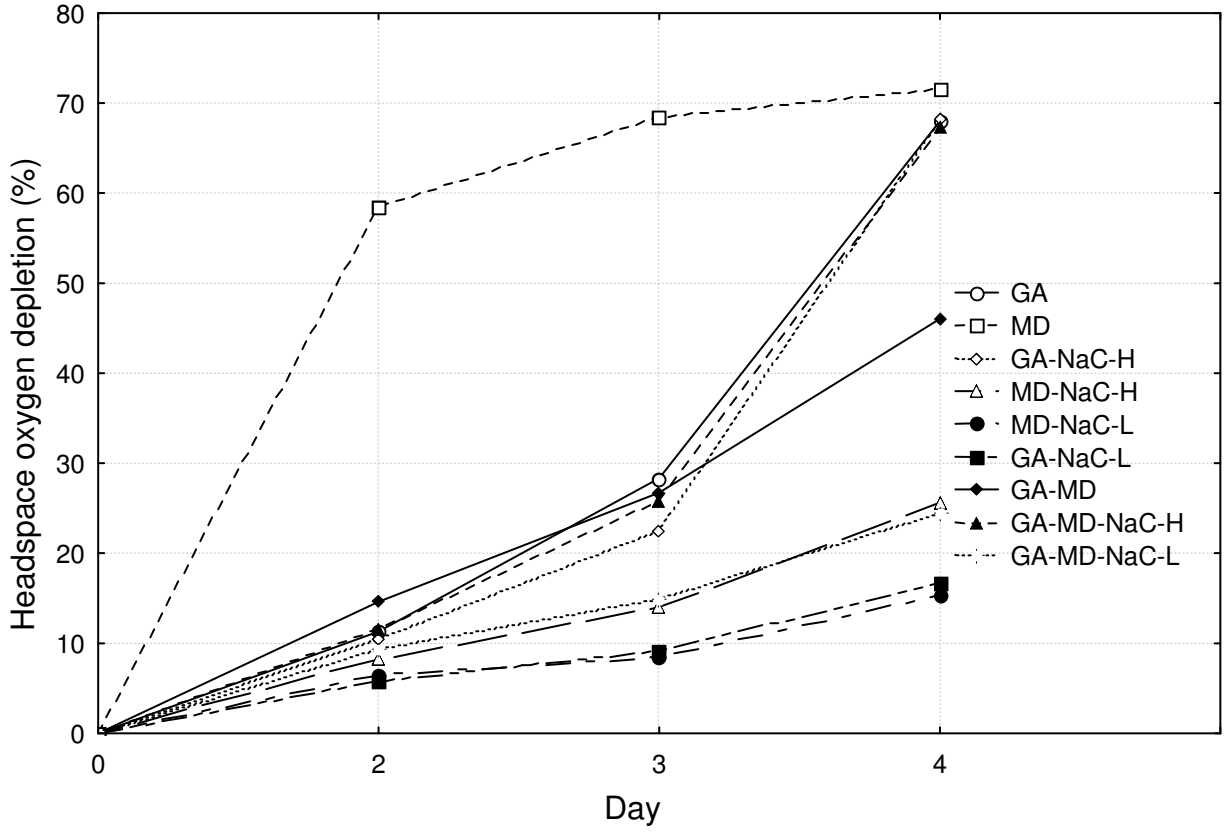

Figure 2. Headspace oxgen deoletion of encapsulated evening primrose oil during storage at 60° C (NaC-H: high NaC, NaC-L: low NaC)

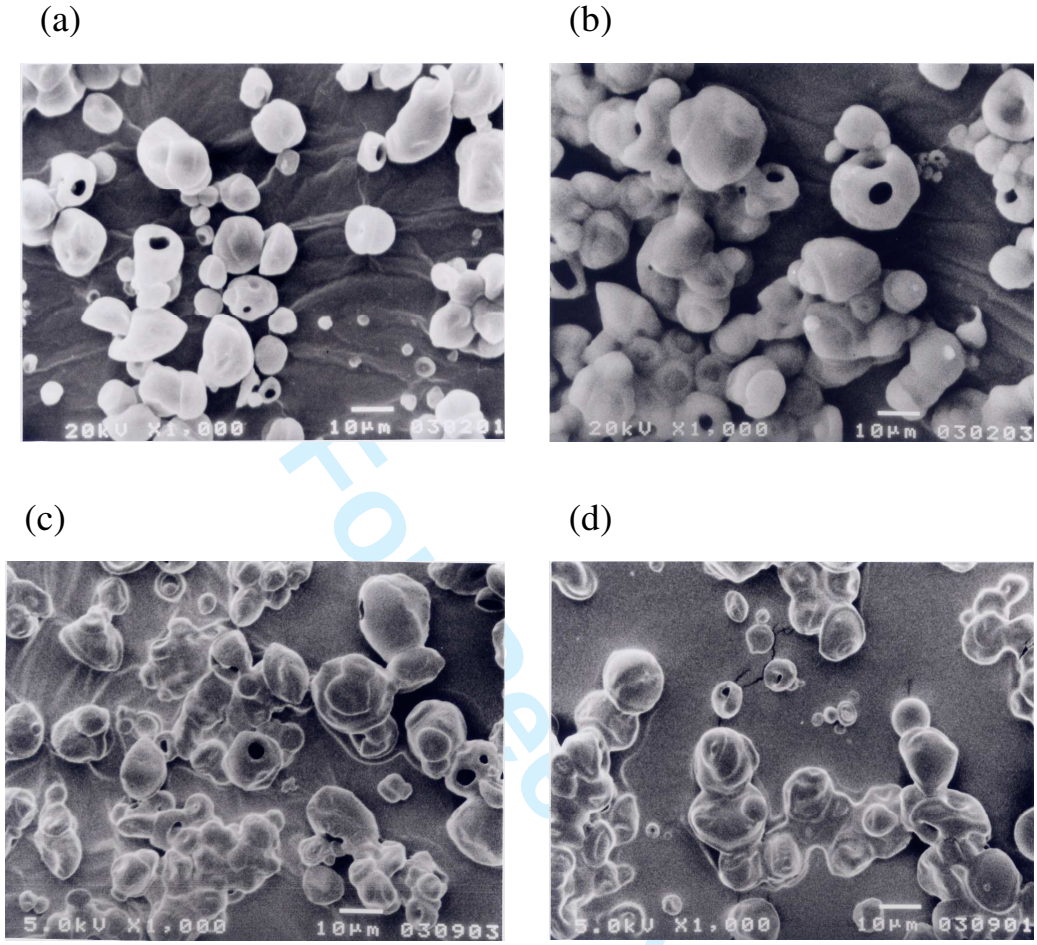

FIGURE 3. OUTER STRUCTURES OF MICROCAPSULES CONSISTING OF (a) GA (b) MD, (c) GA/MD, AND (d) GA/MD/NAC.

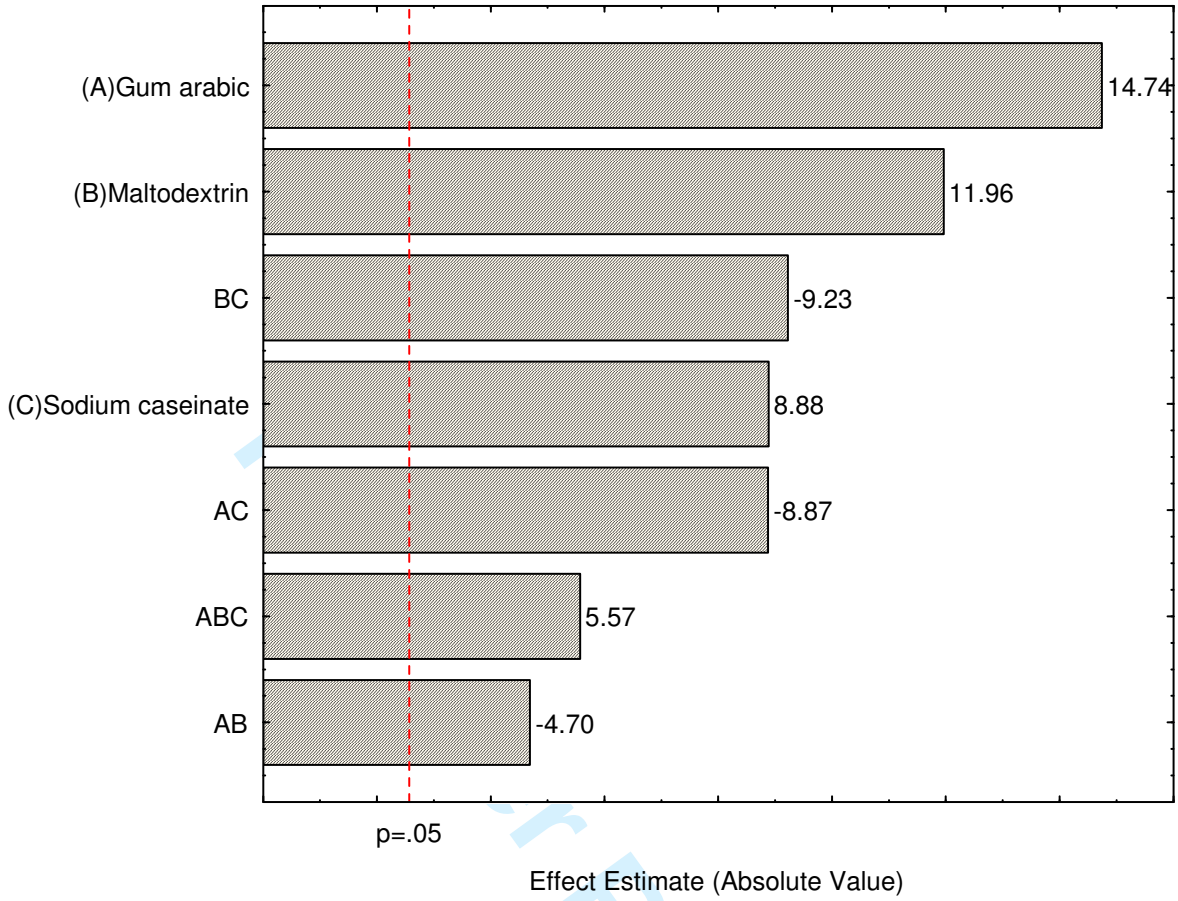

FIGURE 4. PARETO CHART OF THE EFFECTS OF GUM ARABIC, MALTODEXTRIN, AND SODIUM CASEINATE IN THE STANDARDIZED PSEUDO-COMPONENT FORM ON THE HEADSPACE OXYGEN DEPLETION OF THE MICROENCAPSULATED PRIMROSE OIL.