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Effects of Lecithin and Pectin on Riboflavin-Photosensitized Oxidation of Orange Oil in a Multilayered Oil-in-Water Emulsion

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ABSTRACT: The effects of lecithin and pectin on riboflavin-photosensitized oxidation of orange oil in a multilayered oil-in-water emulsion are studied by response surface methodology. Lecithin and pectin contents are two variables studied. Mean oil droplet size, viscosity, and ζ-potential are investigated for evaluation of emulsion stability. Headspace oxygen depletion, increase of conjugated diene value, and released amounts of limonene and carvone are used as responses to evaluate the oxidative stability of orange oil in this emulsion. The results show that both lecithin and pectin contents have significant effects ($p < 0.05$) on the oxidative stability of orange oil in the multilayered emulsion during photosensitized oxidation. No interactive effect ($p < 0.05$) is found between the lecithin and pectin contents. To achieve optimal oxidative stability, the suggested values in ratio for lecithin and pectin contents are 14.1 ± 0.5 and 19 ± 0.7, respectively.

KEYWORDS: Multilayered oil-in-water emulsion, lecithin, pectin, orange oil, response surface methodology, riboflavin, photosensitized oxidation

INTRODUCTION

Citrus flavors are one of the most popular flavors worldwide, of which orange flavor is the favorite of consumers.[1] Orange peel oils are oil soluble; therefore, they must be emulsified in order to be used in aqueous food systems. In addition, flavors formulated as an emulsion have an advantage of rendering food products an appealing opaque appearance to attract consumers, such as that in citrus drinks.[2] Flavor properties in an emulsion are influenced by variables such as emulsion stability, chemical properties of flavor molecules, and interaction of volatile flavor compounds with other molecules in the emulsion system.[3]

In terms of enhancing emulsion stability, using electrostatic repulsion and steric hindrance among dispersed droplets in an emulsion is one of the most effective methods. For example, Katsuda et al.[4] used a double-layered β-lactoglobulin−pectin membrane to enhance oxidative stability of fish oil-in-water (o/w) emulsions. Klinkesorn et al.[5] studied the effect of a double-layered lecithin−chitosan membrane on the stability of tuna oil during encapsulation. Djordjevc et al.[6] also employed a double-layered dodecyl sulfate−chitosan membrane to protect citral and limonene from metal ion induced oxidation. Gu et al.[7] investigated a triple-layered β- lactoglobulin−ι-carrageenan−gelatin membrane to improve the freeze−thaw stability of corn oil o/w emulsions. Ogawa et al.[8] employed a triple (lecithin−chitosan−pectin) membrane to increase the stability of a corn o/w emulsion. Similarly, Akoi et al.[9] reported that a dodecyl sulfate−chitosan−pectin multilayered membrane could stabilize a corn o/w emulsion against environmental stress.

Oxidation is an important factor affecting flavor quality of foods, especially for those containing ingredients susceptible to oxidation. Limonene is a major component in citrus peel oils and sensitive to oxidation.[10] Limonene oxidation would result in the loss of lemon-like odors and the formation of off-flavors described as flowery, piney, and minty.[11] The oxidative stability of multilayered o/w emulsions in the literature was speculated to be determined by interaction of metal ions with the layer materials. The protective effect of the multilayered membranes on lipid oxidation is mainly ascribed to electrostatic complexation or repulsion toward the metal ions thus preventing their access to lipids.[4] However, little information has been available about whether the multilayered membrane has a protective effect on reactive oxygen species such as free radicals or singlet oxygen generated in photosensitized oxidation. The important role of singlet oxygen in the initiation of lipid oxidation has been reported and the reaction rate of singlet oxygen with linoleic acid is about 1450 times greater than that of triplet oxygen.[12] Singlet oxygen can be produced by photosensitizers such as chlorophyll in soybean oil,[13] myoglobin and its derivatives in meat,[14] and riboflavin in milk.[15] Riboflavin is a vitamin already found in green plants and many dairy products such as milk, yoghurt, and cheese,[16] as well as in nutrient-fortified processed foods or beverages.[17]

Orange o/w emulsion is generally prepared by a monolayered membrane emulsion.[2] The information about whether a triple-layered membrane can be used in stabilizing orange oil against photosensitized oxidation has not been available. Moreover, the interactive effects between the materials used in the multilayered membranes have neither been investigated. Response surface methodology (RSM) is a very effective tool when many factors and interactions between factors affect the desirable responses.[18] Therefore, the aims of this study were to investigate the effect of lecithin and pectin on riboflavin photosensitized oxidation of orange oil in the multilayered o/w emulsion and to employ RSM

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to optimize the experimental conditions that can increase the photosensitized oxidative stability of orange oil in the multilayered emulsion.

MATERIALS AND METHODS

Chemicals. Chitosan (85% deacetylated), orange oil, riboflavin, limonene, carvone, and citrus pectin were purchased from Sigma-Aldrich Co. (St. Louis, MO).

Lecithin Preparation. Lecithin was prepared according to the method of Liu et al.[19] Egg was boiled and cooled to room temperature, and then the yolk was separated from the egg white. The yolk was crumbed and freeze-dried. The freeze-dried sample was dissolved in acetone with a ratio of 1/10 (w/v) and stirred for 5 min at 40 °C. This solution was filtered and the acetone insoluble portion was obtained. The acetone insoluble portion was dissolved in ethanol with a ratio of 1/6 (w/v) and stirred for 5 min at 30 °C. The solution was filtered and the ethanol portion was collected. The ethanol was removed by vacuum evaporation and the lecithin was obtained with a purity of about 96%, as analyzed by a thin layer chromatographic method.[20]

Preparation of Emulsion. An acid buffer stock solution was prepared by dissolving 100 mM acetic acid in deionized water and adjusted to pH 3 by 1 M HCl. Chitosan, lecithin, and pectin solutions were prepared separately by adding them into the acid stock solution and sonicating or stirring for complete dissolution. The multilayered emulsion was prepared according to Ogawa et al.[8] with some modifications. A primary emulsion was prepared by homogenizing medium-chain triacylglycerol (containing orange oil) and lecithin solution by a homogenizer (IKA-Werke T20 S1, Staufen, Germany) at 10 000 rpm with cooling to maintain its temperature as that of ambiance for 3 min. The secondary emulsion was prepared by mixing the primary emulsion with the chitosan solution and stock solution. This system was stirred with a magnetic stirrer at ambient temperature for 1 h and then homogenized for 1 min at 24 000 rpm with cooling. The tertiary emulsion was prepared by mixing the secondary emulsion with pectin solution and following the procedures used in the preparation of the secondary emulsion. Because stable emulsions could not be formed in certain experimental designs in our preliminary study by varying the amount of chitosan, the chitosan content was kept constant and the respective ratios of lecithin and pectin to chitosan were used instead. The concentrations of chitosan, medium-chain triacylglycerol, and orange oil were invariably kept at 0.04 wt %, 2 wt %, and 1250 μg/g, respectively, in the tertiary emulsion for each treatment, but the concentrations of lecithin and pectin varied as in Table 1. The values in the table multiplied by the amount of chitosan (0.04 wt %) were the amounts used in the experiments.

Droplet Size Analysis. A gentle rotation of the emulsion container was done before sampling to obtain an even dispersion of the oil droplets. After sampling, the emulsion was added to a sample compartment in the instrument with a sample delivery controller that can automatically adjust the sample concentration and circulate the sample to fully disperse the droplets. When the dispersed sample passes through the detecting cell system in the particle size analyzer (S3000, Microtrac, Montgomeryville, PA), the droplet diameter was determined by a trilaser diffraction method at 780 nm. The droplet diameter and distribution were automatically calculated and reported with the fluid refractive index of 1.33 and particle refractive index of 1.47. Three readings per measurement was recorded and averaged in each analysis. Three rounds of sampling and analyses were performed for each treatment. The droplet diameter was expressed using volume-weighted mode (mv), which can reflect the effect of droplet volume.

ζ-Potential Analysis. Emulsions were diluted with the buffer solution to avoid multiple scattering effects. Diluted emulsions (1: 30) were filled into a polystyrene latex cell and then placed into the Zetamaster instrument (ZN 90, Malvern Instruments, Worcester, U.K.) for analysis. The ζ-potential was determined by measuring the direction and velocity of the droplet movement in a well-defined electric field at 25 °C. Smoluchowski's $f(ka)$ of 1.5 was used for calculation of ζ-potential. The value of ζ- potential was obtained from the average of 10 readings per measurement, and the analysis was performed in triplicate.

Table 1. Central Composite Design of Lecithin and Pectin Contents for the Multilayered Membrane in the O/W Emulsion

	variables[a]	
run	lecithin	pectin
1	8.25	10
2	8.25	20
3	14.25	10
4	14.25	20
5	7.00	15
6	15.49	15
7	11.25	7.92
8	11.25	22.07
9[b]	11.25	15
10	11.25	15
11	11.25	15
12	11.25	15

[a] The values are the ratios of the amount in each variable to that of chitosan (0.04 wt %). [b] Runs 9−12 are repetition for the center point.

Viscosity Analysis. An aliquot of sample (1 g) was taken from the emulsion and the viscosity was determined with a rotational speed of 200 rpm at 25 °C by a cone−plate Brookfield viscometer (RVDV-II +PRO, Middleboro, MA). The analysis was carried out in triplicate.

Photosensitized Oxidation of Emulsion. Emulsions with or without 80 μg/g riboflavin at an amount of 8 g were weighed in a 14-mL glass serum bottle. The bottle was sealed airtight with a septum secured with an aluminum cap. The prepared sample bottles in triplicate were stored with or without light (covered with aluminum foil) for 24 h in a light box as described by Liu and Yang.[21] The temperature in the box was maintained at about 25 °C by an electric fan system venting generated heat and inhaling cool air from an air-conditioned room during illumination. The light intensity of the sample bottles was about 3600 lx on average measured by a digital light meter (model TES-1330A, TES Electrical and Electronic Corp., Taipei, Taiwan).

Headspace Oxygen Analysis. The headspace oxygen was determined by injecting 100 μL (split ratio 20) of headspace air from the sample bottle into a gas chromatograph (GC 7890A, Agilent Technologies, Palo Alto, CA) equipped with a thermal conductivity detector. A molecular sieve 5A PLOT capillary column (30 m × 0.53 mm) (Supelco, Bellefonte, PA) was used. Helium was used as a carrier gas with a column flow rate of 10 mL/min. The temperatures of injector, oven, and detector were 150, 40, and 250 °C, respectively. Computer software (Chemstation, Agilent Technologies, Palo Alto, CA) was used for integration and calculation of the peak areas in the gas chromatograms. Headspace oxygen depletion is expressed as the following: headspace oxygen depletion (%) = [HO_2(before) − HO_2(after)] × 100%/ HO_2(before). Where HO_2(before) is the headspace oxygen content of sample before light treatment and HO_2(after) is the headspace oxygen content of sample after light treatment.

Conjugated Diene Analysis. A modification of the method described by Kiokias et al.[22] was used to determine the amount of conjugated diene hydroperoxides present. The emulsion sample (0.35 g)

Table 2. Effects of Lecithin and Pectin Contents on the Mean Oil Droplet Diameter, ζ-Potential, Heaspace Oxygen Depletion (HS), Increase of Conjugated Diene (CD), and Release of Limonene and Carvone

	effects						
factors[a]	droplet diameter	ζ-potential	viscosity	HS	CD	release of limonene	release of carvone
lecithin (L)	−1.7*[c]	−8.3*	−0.7	7.3*	4.3*	−1 862 781*	−101 121*
lecithin (Q)	1.0*	−0.6	0.1	−2.7*	−2.4*	1 133 151*	53 769*
pectin (L)	−1.0*	−3.5*	11.1*	−4.9*	−4.2*	−2 501 662*	−80 480*
pectin (Q)	1.0*	−0.4	2.5*	−2.3*	−3.4*	1 408 583*	50 683*
lecithin (L) × pectin (L)[b]	−0.3	1.3	−1.1	2.3	1.5	−125 200	−6 362

[a] L, linear term; Q, quadratic term. [b] Interaction. [c] The asterisks denote significance at $p < 0.05$.

was added to a 15 mL centrifugal tube with a mixture of 2 mL of isooctane/2-propanol (2:1 v/v) and 2 mL saturated NaCl solution. This solution was vortexed for 1 min and then centrifuged with a force of 3000*g* at 5 °C for 3 min. The upper layer was used for the measurement. The absorbance was measured at 232 nm using a UV−vis scanning spectrophotometer (Ultrospec 2100 pro, Biochrom Ltd., Cambridge, UK). The increase of conjugated diene value is calculated as the following: increase of conjugated diene value (%) = [CD(after) − CD(before)] × 100%/CD(before), where CD(after) is the conjugated diene value of sample after light treatment and CD(before) is the conjugated diene value of sample before light treatment.

Headspace Volatile Analysis. Emulsions with or without 80 μg/g riboflavin at an amount of 8 g were weighed in a 14-mL glass serum bottle. A SPME fiber (DVB/Carboxen/PDMS, 50/30 μm, Supelco, Bellefonte, PA) was used to extract the headspace volatiles in the sample bottles at 25 °C for 30 min. After sampling the headspace volatiles from the sample bottles, the fiber was inserted into the injection port of a gas chromatograph (GC, model 6890, Agilent Technologies, Palo Alto, CA) equipped with a linear inlet (i.d., 0.75 mm; o.d., 78.5 mm × 6.3 mm, Supelco, Bellefonte, PA). The temperature was set at 250 °C and kept for 3 min at this value for desorption of the volatiles. The GC oven temperature was programmed from 50 to 200 °C (5 min) at 10 °C/min. A capillary column (DB-5, 30 m × 0.25 mm × 0.25 μm, J &W Scientific, Folsom, CA) was used with helium as the carrier gas under a constant flow of 1 mL/min. Flame ionization detector (FID) was used and the temperature was set at 250 °C. The amount of headspace volatiles was quantified by peak areas integrated by a computer system (Chem station, Agilent Technologies, Palo Alto, CA). The instrumental conditions for SPME−GC analysis were monitored using an internal standard compound of pentadecane for comparison and calibration. The volatiles extracted by the fiber DVB/CAR/PDMS were separated and identified by GC−MS using an Agilent 5973 MS system with a quadruple mass analyzer. The column, carrier gas (He) flow rate, and oven temperature conditions used in GC−MS were the same as those used in the GC−FID analysis. The MS operating parameters were the following: electron impact (EI) mode for molecular ionization with a voltage of 70 eV; ion source temperature, 200 °C; total ion scan mode with a scan rate of 4.37 scans/s; and mass scan range of 29−350 m/z The volatiles were identified by comparing their mass spectra with those in the library of MS data system (Wiley 275, G1035A, Agilent Technologies, Palo Alto, CA) and with standard compounds.

Statistical Analysis. A central composite design with two factors and two levels (Table 1) was used to study the effects of lecithin and pectin contents on the mean oil droplet size, ζ-potential, heaspace oxygen depletion, increase of conjugated diene value, and release of limonene and carvone. Regressive analyses were performed to establish appropriate fitting models and equations, and the significant level for comparing different treatments was $p < 0.05$. The statistical designs and analyses were conducted using Statistica for Windows (StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

Effects of Lecithin and Pectin Contents on Physical Stability of O/W Emulsion. Droplet size, electric charge, and viscosity are three important factors in maintaining emulsion stability.[23] The influence of the variables (lecithin and pectin contents) on the droplet size was studied by the analysis of variance (ANOVA) in a second-order regressive model. The degree of the influence exerted by both variables is represented by the statistical term "effect". The effect for the linear effects (marked by the L next to the factor name as in Table 2) can be interpreted as the difference between the average response at the low and high settings for the respective factors. The effect for the quadratic (nonlinear) effect (marked by the Q next to the factor name) can be interpreted as the difference between the average response at the center (medium) settings and the combined high and low settings for the respective factors (Table 2). The higher the value, the more influential the independent variable is. This term is used for data analysis and comparison because it is independent of the units of variables.

In droplet size analysis, the droplet size distribution exhibits a monodispersed pattern with a sharp peak, indicating a narrow range of droplet size distribution (Figure 1). In terms of statistical analysis, the dependent variable is the mean oil droplet diameter and the independent variables are the lecithin and pectin contents. Furthermore, 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 negative value, the smaller the droplet size is in this study. Increasing lecithin content provides additional interfacial areas, thus leading to smaller droplet sizes. However, when the radius of curvature of a droplet reaches a critical value, an increase of lecithin does not seem to energetically favor a further decrease in the droplet size any more. Pectin also has an effect on reducing the droplet size. The reason may be that pectin can provide steric repulsion via a thickening effect and prevents the aggregation or coalescence of the oil drops with unstable secondary layers because of unbalanced chitosan/lecithin ratio in some experimental designs. The aggregation or coalescence of the oil drops would result in an increase of oil droplet size. As with lecithin, increasing the amount of pectin beyond the critical value did not further decrease the droplet size because of the saturation in the interfacial areas. Though both lecithin and pectin have effects on the oil droplet size, no significant interactive effect ($p < 0.05$) is found between the lecithin and pectin contents. The oil droplet size influenced by the amounts of lecithin and pectin is shown in Figure 2 and can be predicted by using the regressive equation in Table 3. The predicted minimal mean oil droplet diameter is

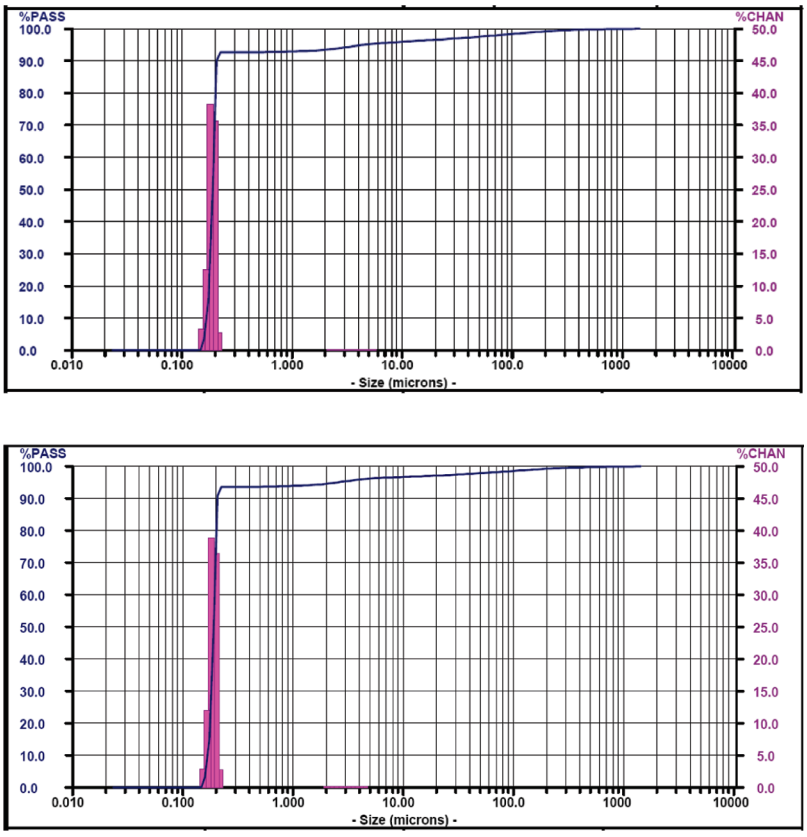

Figure 1. The droplet size distribution at lecithin content of 11.25 and pectin contents of 15 (top) and 22.07 (bottom).

2.86 μm at lecithin and pectin contents of 14.2 and 18.3, respectively. According to the Stokes's law, the creaming rate of an oil droplet is proportional to the reciprocal of the square of the droplet radius. Therefore, the smaller the droplet size, the more stable the emulsion should be.

Since electrostatic repulsion between oil droplets favors emulsion stability, the electric charge state of droplets was acquired by measuring the ζ-potentials of droplets. The ζ-potential for primary emulsion ranges from -27 ± 1 to -40 ± 3 mV, secondary emulsion from $+76 \pm 3$ to $+90 \pm 3$ mv, and tertiary emulsion from -11 ± 2 to -26 ± 3 mV in these designed experiments. This shows a layer-by-layer adsorption of the coating materials on the oil droplets. Because the amount of chitosan, the only positive charge carrier, remains constant, the charge of droplets is mainly determined by lecithin and pectin, the negative charge carriers. Table 2 shows that both lecithin and pectin have significant effects on the ζ-potential, but no interactive effect between them is found at $p < 0.05$. Because only the main effects (linear term) are significant, the regressive model is fitted linearly, and therefore, no convergent point in a response surface is available in terms of the combined effects of lecithin and pectin contents. Namely, ζ-potential of the droplets increases with the increase of amounts in lecithin and pectin. Moreover, lecithin has a greater impact than pectin on the ζ-potential. The reason is likely that lecithin has a greater effect than pectin on reducing the size of oil droplets, which may affect the eletrophoretic mobility of the oil droplets because ζ-potential is proportional to the eletrophoretic mobility of the oil droplets.

Viscosity can enhance emulsion stability by retarding the movement of dispersed droplets and then reducing the frequency of coalescence among the droplets. The viscosity of the emulsions ranges from 3.8 ± 0.2 to 20.5 ± 0.3 cP in these designed experiments. The result in Table 2 shows that pectin has a significant effect ($p < 0.05$) on the viscosity of the emulsion; however, lecithin does not. The increase of viscosity is due to more solutes, i.e., pectin, used in the aqueous solution. Pectin is a high molecular weight polysaccharide with good water-absorbing ability; therefore, it plays a decisive role in determining the viscosity of the emulsion. Although the increase of pectin raises the viscosity, the maximal viscosity of 20.5 cP is about 20 times that of water (about 1 cP at 20 °C) and thus the aqueous phase is not sensibly sticky. Therefore, the increase of viscosity may contribute less than the thickening effect of more pectin molecules adsorbed at the interface of oil droplets.

Photosensitizing Effect on Oxidative Stability of O/W Emulsion. The oxidative stability of the multilayered oil droplets containing orange oil was evaluated by analyses of the depletion of headspace oxygen and the increase of conjugated diene in the oil phase. The depletion of headspace oxygen in the samples with or without riboflavin stored in a dark environment at 25 °C had no change during 24-h storage (data not shown), whereas that of the samples without riboflavin or with riboflavin was 13% and 25%, respectively, an average from all different experimental designs after the same period of storage under illumination. The rapid depletion of headspace oxygen must be due to the photosensitizing effect of riboflavin, which can initialize free radical formation (type I reaction) and the singlet oxygen formation (type II reaction). Triplet oxygen is the atmospheric oxygen and can be converted into singlet oxygen via the type II reaction. Compared with free radical induced oxidation, the reaction rate of singlet

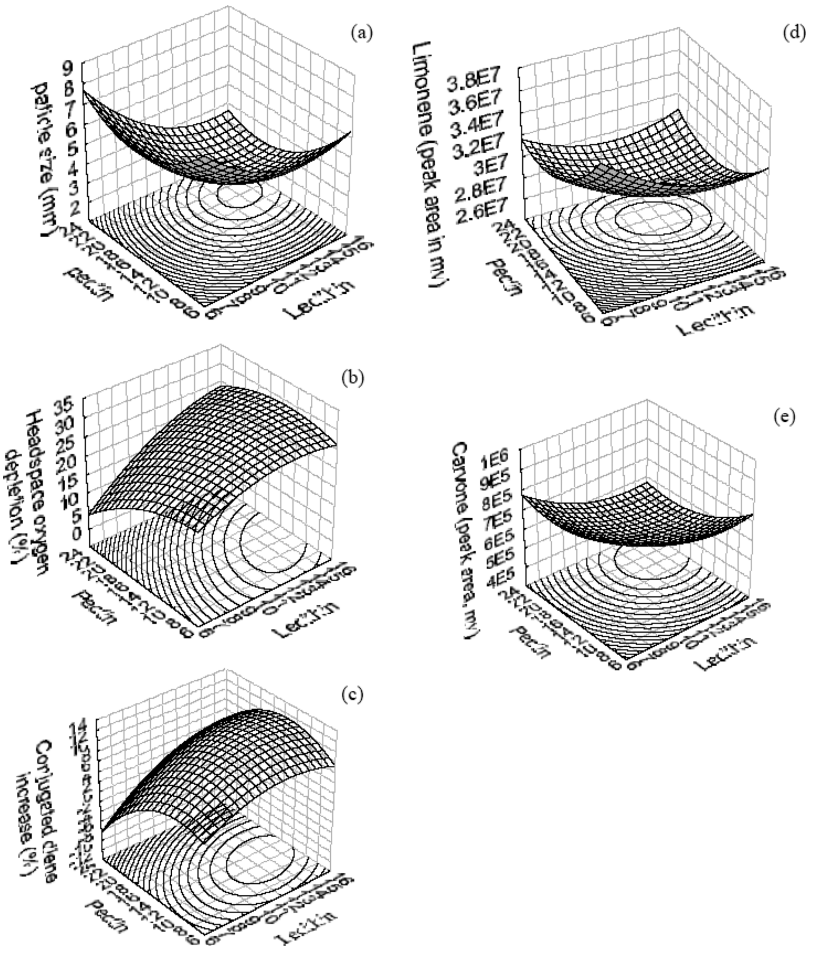

Figure 2. Response surfaces for the mean oil droplet size (a), heaspace oxygen depletion (b), conjugated diene increase (c), release of limonene (d), and carvone (e) with lecithin and pectin contents as variables.

Table 3. Regressive Equations of Lecithin and Pectin Contents and Optimal Responses for the Mean Oil Droplet Size, Heaspace Oxygen Depletion (HS), Increase of Conjugated Diene (CD), and Release of Limonene and Carvone

				responses	
items	regression equations[a]	R^2	critical values (x, y)	predicted	observed
particle size (μm)	$z = 17.5019 - 1.3312x + 0.0543x^2 - 0.5669y + 0.0199y^2 - 0.0114xy$	0.95	14.2, 18.3	2.86[b]	3.03
HS (%)	$z = 4.0219 + 3.3866x - 0.1479x^2 - 0.0138y - 0.0449y^2 + 0.0773xy$	0.91	14.7, 12.5	28.8[c]	29.7
CD (%)	$z = -14.3493 + 2.9096x - 0.1308x^2 + 1.0244y - 0.0668y^2 + 0.05xy$	0.90	13.6, 12.7	11.9[c]	13.1
limonene (peak area, mV)	$z = 49\,408\,836 - 1\,664\,302x + 62\,952x^2 - 1\,048\,366y + 28\,171y^2 - 4\,173xy$	0.93	13.8, 19.6	2 7575 536 [b]	2 8167 007
carvone (peak area, mV)	$z = 1\,463\,312 - 80\,884x + 2\,987x^2 - 36\,072y + 1\,013y^2 - 212xy$	0.92	14.2, 19.2	540 108 [b]	558 175

[a] x, lecithin; y, pectin; z, response. [b] Minimal response value. [c] Maximal response value.

oxygen with compounds that are rich in electrons such as double bonds in unsaturated fatty acids or terpene compounds is so fast that can cause the rapid depletion of headspace oxygen within a short period of time, such as 24 h in this research. Table 2 shows the effects of lecithin and pectin contents on the headspace oxygen depletion. Comparing the main effects (linear term) of lecithin and pectin, we can find that when the amount of lecithin increases, headspace oxygen depletion increases. On the contrary, as the amount of pectin increases, headspace oxygen depletion decreases. Lecithin has a greater effect (7.3) than pectin (−4.9) on the headspace oxygen depletion and there is no interactive effect between the two variables ($p < 0.05$). The maximal headspace oxygen depletion is shown in Figure 2 and can be predicted as 28.8% at 14.7 and 12.5 for lecithin and pectin contents, respectively (Table 3).

Because lecithin contains unsaturated fatty acid moiety such as linoleic acid in its structure,[24] it is likely that the depleted oxygen in the emulsion reacts with lecithin or orange oil in the dispersed oil droplets. Therefore, the conjugated diene (CD) was analyzed for further verification. The effects of lecithin and pectin on CD are shown in Table 2. The main effect (linear term) with a positive value of 4.3 shows that increasing the amount of lecithin

favors the increase of the CD value. Therefore, it can be inferred that part of depleted headspace oxygen reacts with the unsaturated fatty acids in the lecithin. Though the linear term (L) of lecithin gives a positive effect, the quadratic term (Q) of lecithin shows a negative effect with a value of −2.4, which can counteract the unfavorable main effect. This means that when the amount of lecithin increases to a certain level, the CD value does not increase further. This reason is that lecithin may render some antioxidant activity. Phospholipids can be tightly packed at the interface between water and oil in an o/w emulsion and decrease the autoxidation of oil by reducing diffusion of oxygen.[25] Besides, Choe and Lee[26] reported that phosphatidylcholine could quench singlet oxygen by a chemical reaction. Because there are unsaturated fatty acids in the lecithin molecules, the use of more lecithin will simultaneously increase the contents of phosphatidylcholine and unsaturated fatty acids, as well as the packing effect. Phosphatidylcholine and molecular package favor antioxidant effect, whereas, unsaturated fatty acids are unfavorable to the antioxidant effect. Therefore, these factors must be balanced at a certain amount of lecithin used in order to get an optimal antioxidant effect. Pectin also contributes to reduction of the headspace oxygen depletion because an increasing amount of pectin decreases the CD value. The function of pectin may be due to its thickening effect and radical scavenging ability.[27] The maximal increase of CD is shown in Figure 2 and can be predicted as 11.9% at 13.6 and 12.7 for lecithin and pectin contents, respectively (Table 3). These values are very close to those of lecithin and pectin contents in the maximal headspace oxygen depletion. This shows a consistent result in the oxidation process that the more headspace oxygen that is consumed, the more unsaturated fatty acids in the lecithin that are oxidized, leading to the increase of CD.

Photosensitizing Effect on Release of Headspace Volatiles in the O/W Emulsion. The retention of the volatile compounds in the orange oil in the multilayer system was studied by analyzing the released amount of the headspace volatiles. Since limonene is the largest compound in the orange oil, it is used as a representative compound to investigate the release of headspace volatiles. Table 2 shows the effects of lecithin and pectin contents on the release of limonene. Lecithin gives a negative value (−1 862 781) of effect meaning that when the amount of lecithin increases, the released amount of limonene decreases. Similarly, pectin also gives a negative value (−2 501 662) of effect on the release of limonene, showing that its increase can decrease the release of limonene as well. Statistical analysis shows that there is no interactive effect between the lecithin and pectin ($p < 0.05$), although both have effects on the release of limonene. As can been seen from the quadratic terms (Q) of effects in Table 2, the values of 1 133 151 and 1 408 583 are positive for lecithin and pectin, respectively, which will counteract the main effects (linear terms, L) with the negative values, as shown above. This means that increasing the amounts of lecithin and pectin favors the reduction of release of limonene; however, over the optimal values this effect declines. The minimal release of limonene is shown in Figure 2 and the corresponding lecithin and pectin contents are 13.8 and 19.6, respectively (Table 3). Since limonene oxidation can generate oxidative products such as carvone, which is commercially available as a standard compound for identification, carvone is used for evaluating the oxidative stability of orange oil in the multilayered emulsion. As with limonene, both lecithin and pectin have favorable effects with the values of −101 121 and −80 480, respectively, on reducing the release of carvone in terms of increasing the amounts of lecithin and pectin prior to optimal values being reached. The minimal release of carvone is shown in Figure 2 and the corresponding lecithin and pectin contents are 14.2 and 19.2, respectively (Table 3). These values are very close to those of lecithin and pectin contents in the minimal release of limonene. This result supports a reasonable inference that the less the amount of limonene that is released from the multilayered emulsion, the less the chances of exposure to environmental oxidative stress, resulting in lower carvone formation.

When the main effects of lecithin and pectin on the release of limonene and carvone are compared, pectin (−2 501 662) exhibits a greater effect than lecithin (−1 862 781) on limonene release. The reason that may be inferred is that pectin can provide a better thick-layer effect than lecithin. On the contrary, lecithin (−101 121) has a greater effect than pectin (−80 480) on carvone release. It can be suggested that lecithin has antioxidant activity and results in relatively low formation of carvone during photosensitized oxidation. It seems contradictory that a greater amount of lecithin used results in higher CD increase but less limonene oxidation. Our hypothesis is that the depleted oxygen molecules are principally trapped by lecithin and the partially formed hydroperoxides that are relatively polar and oriented toward the o/w interface.[28,29] Therefore, the chance of contact between the polar hydroperoxides and the nonpolar orange oil is reduced. Moreover, the increase of lecithin favors reduction of oil droplet size. It has been reported that oil droplets with a small size have better oxidative stability than those with a large size in terms of a "wedge" effect, which can retard the mobility of oil molecules to access the hydroperoxides at the interface of oil and water.[30]

In conclusion, the photosensitized oxidative stability of the orange oil in the multilayered emulsion mainly depends on the antioxidant activity and the thick-layer effect of lecithin and pectin. Skillfully controlling the amounts of lecithin and pectin can enhance the oxidative stability of orange oil in the multilayered emulsion. The suggested optimal values in ratio for lecithin and pectin contents are 14.1 ± 0.5 and 19 ± 0.7, respectively.

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