Use Optimization of Natural Antioxidants in Refined, Bleached, and Deodorized Palm Olein During Repeated Deep-Fat Frying Using Response Surface Methodology

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ABSTRACT: An optimization study on the use of oleoresin rosemary extract, sage extract, and citric acid added into refined, bleached, and deodorized (RBD) palm olein in deep-fat frying of potato chips was carried out using response surface methodology (RSM). Results showed that oleoresin rosemary extract was the most important factor affecting the sensory acceptability of potato chips. For taste and odor, its effects were highly significant (P < 0.01), while for crispiness and overall acceptability, the effects were significant (P < 0.05). As for sage extract, the level of this antioxidant had a highly significant (P < 0.01) effect on appearance and taste and a significant effect (P < 0.05) on odor and overall acceptability, but had no effect on crispiness. Although there was no significant synergistic correlation between citric acid and oleoresin rosemary extract or sage extract at the first order, its second order was significantly (P < 0.05) related to taste, crispiness, and overall acceptability. An interaction between oleoresin rosemary and sage extracts also significantly (P < 0.05) improved the score of overall acceptability of the potato chips. Contour maps of the sensory scores of potato chips indicated that the optimal points for appearance were achieved using 0.062% oleoresin rosemary extract, 0.066% sage extract, and 0.023% citric acid, while optimal task was achieved with 0.063% oleoresin rosemary extract, 0.075% sage extract, and 0.025% citric acid. With the same sequence of ingredients added into oil, the combinations required to achieve the optimal odor, crispiness, and overall acceptability scores were 0.058-0.046-0.026, 0.060-0.071-0.022, and 0.060-0.064-0.026%, respectively.

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KEY WORDS: Citric acid, deep-fat frying, response surface methodology (RSM), potato chips, RBD palm olein, rosemary, sage, sensory evaluation.

Deep-fat frying is one of the most common methods used world-wide for preparation and production of foods. It is extensively used both at home and on a commercial scale to enhance the organoleptic characteristics of foods. In commercial deep-fat frying operations, a fat is exposed continously to heat, air, and light for hours per day at a temperature of around 180°C, and it may be used to cook a variety of foods (1). Fats or oils undergo a complex series of changes and reactions during deep-fat frying. Many studies have reported

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the physical and chemical changes occurring in oils under frying conditions (2,3). The rate of change occurring during frying is influenced by the frying conditions and the characteristics of the fat (4).

The prime justification for using an antioxidant in oils is to avoid or delay lipid oxidation during frying. The mechanism of lipid oxidation involves free-radical reactions in which attack by molecular oxygen occurs at positions adjacent to the double bonds. This leads to formation of hydroperoxides which decompose further giving rise to a variety of oxidative breakdown products (5). It is believed that antioxidants protect from oxidation during the time oil is exposed to high temperature. Lin et al. (6) stated that antioxidants play an important role in the manufacturing, packaging, and storage of fats and fatty foods, and have been proven to retard oxidation. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are two antioxidants commonly used in oils. Augustin and Berry (2) used BHA and BHT to study effects of antioxidants on palm olein during heating and frying. However, with the increase of consumer interest in "natural" products, the use of natural antioxidants is increasing to replace these synthetic antioxidants, which in some countries are contested or even banned. For frying purposes, a study conducted by Chang et al. (7) showed that BHA and BHT are quite volatile and easily decomposed at high temperature, so they are not satisfactory for such common food products such as French fries and potato chips and not effective in vegetable oils in preventing the development of initial off-flavor.

Among natural antioxidants that have recently been studied intensively are rosemary and sage extracts. They have been reported to have a very good thermal resistance and strong antioxidative characteristics (8–10). Chipault *et al.* (11) reported a study of 32 common spices used as antioxidants in lard and showed that only rosemary and sage are effective. The use of rosemary and sage extracts as antioxidants in foods has been also reported by Chang *et al.* (7), Berner and Jacobson (12), and Nakatani (13). Our recent work found that both rosemary and sage extracts added to palm olein retarded the oil deterioration during 6-d deep-fat frying of potato chips (Che Man, Y.B., and J. Irwandi, unpublished data)). It was also revealed that the two natural antioxidants could improve acceptability of the fried product. Che Man and Tan (14) reported that the use of rosemary and sage extracts at a level of 200 ppm could significantly prolong the storage life of potato chips. Meanwhile, citric acid, together with an antioxidant, could be considered as a synergist for enhancing the antioxidant activity (15).

This study was carried out using response surface methodology (RSM) to investigate the optimal level of oleoresin rosemary extract, sage extract, and citric acid added into refined, bleached, and deodorized (RBD) palm olein for frying of potato chips. RSM is a useful statistical technique that uses quantitative data from appropriate experimental designs to determine and simultaneously solve multivariate equations (16). RSM can be used to study relationships between one or more responses (dependent variables) and a number of factors (independent variables). This technique seems to be a popular method for product optimization within the sensory evaluation field (16,17).

MATERIALS AND METHODS

Materials. RBD palm olein used in this study was purchased from a local refinery. Oleoresin rosemary (Herbalox Brand, Type O) and sage (Herbalox seasoning, Type S-O) extracts, both in liquid form, were donated by Kalsec Inc. (Kalamazoo, MI, through Gulf Chemical Sdn. Bhd., Selangor, Malaysia). Citric acid (food grade) was supplied by Labchem Sdn. Bhd. (Selangor, Malaysia). Fresh potatoes and salt (sodium chloride) were purchased from a local supermarket. All reagents used were of analytical grade and obtained from local suppliers.

Experimental design. RSM was used to investigate the optimal points of combination of oleoresin rosemary extract, sage extract, and citric acid added into RBD palm olein before the frying of potato chips. RSM uses an experimental design such as the central composite design (CCD) to fit a model by the least squares technique. In this study, Echip software was used to provide experimental designs, calculate equations, make statistical evaluations, and print out data (18). The CCD with the quadratic model was used. With ranges of oleoresin rosemary and sage extracts levels from 0 to 0.1% each, and citric acid from 0 to 0.05%, there were 15 combinations of the three antioxidants obtained from the Echips software. All 15 combinations are shown in Table 1.

Frying experiment. Fresh potatoes to be fried were peeled and sliced around 1.5 mm thick manually. The sliced potatoes were then soaked in 2.5% salt (NaCl) solution for 5 min and dried before frying. A combination of rosemary and sage extracts and citric acid, depending on the treatment, were added into RBD palm olein just before frying.

Frying was conducted in batch fryers (Berto's, Model ELT 8B; Hamburg, Germany). Oil (4 kg) was heated to $180 \pm 5^{\circ}$ C in 10 min. Frying was started half an hour after the temperature of the oil reached 180°C. Potato chips (100 g) were fried for 2.5 min. Afterward, the oil temperature was allowed to rise to 180°C again within 30 min. There were 10 fryings conducted every day for five consecutive days. The fryers were left uncovered during the frying operations. For the control

TABLE 1 Combinations of Oleoresin Rosemary Extract, Sage Extract, and Citric

Acid Adde	d into RBD ^a Palm Ole	ein Before Frying	
Trial no.	Rosemary (%)	Sage (%)	Citric acid (%)

	1.	0 、	()
1	0	0.1	0.05
2	0.1	0.05	0
3	0	0.1	0
4	0	0	0.05
5	0.1	0.1	0.05
6	0.05	0.1	0.025
7	0	0.05	0.025
8	0.05	0.05	0.05
9	0	0	0
10	0.1	0	0.025
11	0.1	0.1	0.025
12	0.05	0.1	0
13	0.05	0	0
14	0.1	0.05	0.05
15	0	0.5	0

^aRBD, refined, bleached and deodorized.

sample (combination No. 9), the physicochemical analyses of the oil were conducted. For these analyses, at the end of each day, 200 g oil at 60° C was removed from the fryer, flushed with nitrogen gas, and kept in a cold room at 5°C until the day of analysis. The lid of the fryer was then put on, and the fryer was left overnight for the following day's frying.

After frying, the potato chips were removed from the fryer and toasted. The ninth and tenth batch of potato chips each day was labeled and packed in low-density polyethylene plastic bags for sensory evaluation. The evaluation was carried out on the same day the frying was conducted.

Sensory evaluation. The responses measured in this study were acceptability in appearance, taste, odor, crispiness, and acceptability of the potato chips. All sensory attributes were evaluated using a 7-point hedonic scale (1 = dislike extremely, 4 = moderate, 7 = like extremely) (19) by 20 untrained panelists selected from staff and graduate students of the Faculty of Food Science and Biotechnology, Universiti Putra Malaysia. All panelists are regular participants in sensory evaluation and also regular consumers of potato chips. The procedure of the sensory evaluation was explained to the panelists before testing commenced. The panelists were then asked to read through the instructions and the questions on the sensory form. The meaning of each attribute was explained to the panelists to avoid any misinterpretation.

Physicochemical analysis of oil. This analysis was carried out for the control sample as a reference. Peroxide value (PV), free fatty acid (FFA), iodine value, polymer content, color, and viscosity were determined using AOCS Official Methods (20). The absorbances at 232 and 268 nm and anisidine value were obtained using IUPAC methods (21). The fatty acid composition of the oil was determined by gas chromatography as reported by Berry (22). The oil samples were analyzed on a Hewlett-Packard (Palo Alto, CA) gas chromatograph Type 5890 A using a 15 m × 0.53 mm capillary column. The chromatograph was equipped with a flame-ionization detector. The temperature of the column was 140°C

		After 1 d of frying				After 5 d of frying				
Trial		-			Overall		-			Overall
no.	Appearance	Laste	Odor	Crispiness	acceptability	Appearance	Laste	Odor	Crispiness	acceptability
1	5.2	5.7	4.9	4.3	4.9	5.0	4.4	4.0	4.6	4.6
2	5.0	6.0	5.0	5.0	5.7	5.1	5.2	4.7	5.4	5.4
3	5.1	5.6	4.9	4.6	4.4	5.1	4.3	4.4	4.1	4.1
4	5.2	5.2	4.7	3.5	3.9	4.5	3.7	3.4	3.9	3.9
5	4.3	5.8	4.8	4.9	5.5	5.0	5.1	4.6	5.2	5.2
6	5.0	6.1	4.8	5.3	5.8	5.3	5.4	5.0	5.6	5.6
7	5.4	5.7	5.3	4.8	5.5	5.2	4.8	4.6	5.4	5.4
8	5.0	6.2	5.2	5.4	5.7	5.6	5.5	5.1	5.3	5.3
9	5.0	5.2	4.8	3.8	4.3	4.5	3.8	3.5	3.8	3.8
10	5.0	5.8	5.0	5.6	5.3	5.2	4.9	5.5	4.9	4.9
11	4.4	5.9	4.7	5.8	5.6	5.2	5.4	5.6	5.0	5.0
12	4.9	6.0	5.0	5.0	5.4	5.4	5.2	4.7	5.1	5.1
13	5.2	5.5	5.1	4.6	5.1	4.9	4.9	4.4	4.8	4.8
14	5.0	5.8	4.9	4.4	5.0	5.1	4.6	4.2	4.7	4.7
15	5.5	5.5	4.7	4.5	5.0	5.0	4.7	4.2	4.8	4.8

 TABLE 2

 Effect of Oleoresin Rosemary Extract, Sage Extract, and Citric Acid on Sensory Scores^a of Potato Chips

^aMean of 20 panelists.

and then increased 4°C/min up to 200°C, while the temperature of injector and detector was 250°C. The flow rates were 65, 44, and 440 mL/min for carrier gases nitrogen, hydrogen, and air, respectively.

of more than 0.75 are relatively adequate for prediction purposes.

RESULTS AND DISCUSSION

Sensory scores of potato chips after one-day of frying. Table 2 shows the sensory scores of potato chips fried on day 1, while the regression coefficients for the five sensory attributes evaluated with their coefficients of determinations (R^2) and probability (P) of F values are shown in Table 3. It is clearly shown that, except for crispiness, all sensory responses gave R^2 greater than 0.900. The R^2 values were 0.941, 0.962, 0.922, and 0.948 for appearance, taste, odor, and overall acceptability, while the R^2 for crispiness was 0.848. This meant that the R^2 values were satisfactory and considered accurate enough for prediction purposes. The closer the R^2 value to unity, the better the empirical model fits the actual data. According to Henika (17), R^2 values

From Table 3 it is also seen that for all responses, some models or equations could be developed with confidence; some of them could even be developed with high confidence. For appearance, odor, and crispiness attributes, the *P* of *F* values obtained were less than 0.05, meaning that models or equations could be developed confidently (P < 0.05), while lower *P* of *F* values for taste and overall acceptability (P < 0.01) indicated that models for the two attributes could be developed with high confidence.

From the significance test on the estimates, as also seen in Table 3, oleoresin rosemary extract was the most important factor affecting the sensory acceptability of potato chips. For both taste and odor, its effects were highly significant (P < 0.01), while for crispiness and overall acceptability the effects were significant (P < 0.05). As for sage extract, level of this antioxidant had a highly significant (P < 0.01) effect on appearance and taste and a significant effect (P < 0.05) on odor and overall acceptability but had no effect on crispiness.

TABLE 3

Regression Coefficients, R^2 , and P or Prob. Values^a for Sensory Evaluation of Potato Chips After One Day of Frying

		Sensory attributes						
Coefficients	Appearance	Taste	Odor	Crispiness	Overall acceptability			
β_0 (intercept)	6.154	6.097	5.562	5.634	6.159*			
$\beta_1^{a,b}$	3.130*	5.683**	-4.433**	7.398*	5.410*			
β_2	3.363**	6.097**	-2.646*	4.201	5.712			
β_3	0.864	-4.621	-1.292	-3.681	-3.760			
β ₁₂	-29.599	-1.454	-65.506*	-58.839	0.217*			
β_{13}	-37.938	-77.438	-35.084	8.856	-90.613*			
β_{23}	-5.865	143.757	-11.012	15.656	187.101*			
β_1^2	-108.130*	-237.243**	-21.440	-142.659	-187.612*			
β_2^2	-84.217*	-191.769**	-87.902*	-72.972	-211.529**			
β_3^2	-177.493	-645.721*	-61.202	-1062.450*	-762.964*			
R^2	0.941	0.962	0.922	0.848	0.948			
P or Prob.	0.013	0.0049	0.026	0.113	0.0099			

^aP or Prob., meaning Probability of level of significance.

^b1, Oleoresin rosemary extract; 2, sage extract; 3, citric acid. **Significant at 0.01 level, *Significant at 0.05 level.

On the other hand, although there was no significant correlation between level of citric acid and sensory attributes evaluated at the first order, its second order was significantly (P < 0.05) related to taste, crispiness, and overall acceptability. An interaction between citric acid and oleoresin rosemary extract

or between citric acid and sage extract significantly (P < 0.05) improved the score of overall acceptability of potato chips. It is also shown that the interaction between oleoresin rosemary and sage extracts was significant (P < 0.05) to the odor attribute, but not significant to the rest of attributes.



FIG. 1. Contour maps on the effects of oleoresin rosemary extract, sage extract and citric acid on sensory attributes of deep-fat fried potato chips after one day of frying.

		Frying time (day)								
Characteristic	0	1	2	3	4	5				
Peroxide value (meq oxygen/kg)	0.91 ^d	6.55 ^c	7.80 ^b	8.06 ^b	8.47 ^b	11.70 ^a				
Anisidine value	0.96 ^d	31.53 ^c	36.06 ^{bc}	40.17 ^b	51.41 ^a	54.97 ^a				
lodine value (g l ₂ /100 g oil)	56.07 ^a	52.02 ^b	48.81 ^c	45.25 ^d	43.39 ^e	41.88 ^f				
Free fatty acid (%)	0.05 ^e	0.14 ^d	0.19 ^d	0.27 ^c	0.42 ^b	0.52 ^a				
Polymer content	0.01 ^f	0.71 ^e	1.00 ^d	1.29 ^c	1.55 ^b	1.97 ^a				
Color (red unit)	0.53 ^c	1.10 ^b	1.20 ^{ab}	1.20 ^{ab}	1.30 ^a	1.35 ^a				
Color (yellow unit)	5.93 ^c	13.15 ^b	13.20 ^b	13.75 ^a	13.90 ^a	14.10 ^a				
Viscosity (centipoise)	50.22 ^e	53.17 ^f	54.19 ^d	57.30 ^c	62.11 ^b	66.40 ^a				
E ^{1%} 1cm at 232 nm	1.71 ^d	3.91 ^c	5.06^{b}	8.22 ^a	8.90 ^a	9.01 ^a				
$E^{1\%}_{1 cm}$ at 268 nm	0.41 ^c	1.75 ^b	1.86 ^b	1.96 ^a	1.98 ^a	2.08 ^a				
$C_{18:2}/C_{16:2}$ ratio	0.29 ^a	0.26 ^b	0.26 ^b	0.24 ^c	0.21 ^d	0.20 ^d				

IABLE 4	
Quality Changes	^{<i>x</i>} in Control RBD ^{<i>y</i>} Palm Olein During Deep-fat Frying

^xMean of three replicates.

^yRBD, refined, bleached and deodorized.

^{a-f}Means within a column with different letters are significantly different (P < 0.05).

Contour maps for the sensory scores of potato chips (Fig. 1a-e) indicate that the use of a moderate level of each ingredient was preferred by the panelists. The optimum points for all attributes evaluated were achieved by using oleoresin rosemary extract at range of 0.057-0.062%, sage extract at 0.046-0.075%, and citric acid at 0.022-0.026%. The optimum points for appearance were achieved using 0.062% oleoresin rosemary extract, 0.066% sage extract, and 0.023% citric acid, while optimum taste was achieved with 0.063% oleoresin rosemary extract, 0.075% sage extract, and 0.025% citric acid. The combination required to achieved the optimum odor score was 0.058% oleoresin rosemary extract, 0.046% sage extract and 0.026% citric acid, while 0.060% oleoresin rosemary extract, 0.071% sage extract, and 0.022% citric acid, achieved the optimum crispiness score. For optimum overall acceptability, a combination of 0.060% oleoresin rosemary extract, 0.064% sage extract, and 0.026% citric acid was needed.

Effect of frying time on sensory scores. Table 2 also shows the changes in sensory scores of potato chips after 5 days of frying. The results illustrate that the scores for all attributes evaluated generally decreased. For example, the appearance score of sample No. 9 (control) decreased from 5.0 at day 1 to 4.5 at day 5. Its score for taste dropped from 4.1 to 3.8, while its odor score fell from 5.0 to 4.1. The scores for crispiness and overall acceptability of control sample were also down from 4.8 to 3.5 and 4.3 to 3.8, respectively. This indicated that during five days of frying, the quality changes in the oil used, as shown in Table 4, significantly influenced the acceptability of the product.

From the statistical analyses, including regression coefficients, coefficients of determinations (R^2), and probability (P) of F values for five sensory attributes examined in potato chips at day 5 (Table 5), it is shown that the effect of three antioxidants used—oleoresin rosemary extract, sage extract, and

TABLE 5

Regression coefficients, R , and r of r values for sensory evaluation of rotato emps after rive Days of riv	Regression	Coefficients, R ²	, and P of F Val	ues for Sensory	Evaluation of Potat	o Chips afte	r Five Days of Fry	ing
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-									
	Sensory attributes								
Coefficients	Appearance	Taste	Odor	Crispiness	Overall acceptability				
β_0 (intercept)	5.570	5.709	5.373	5.405	5.912				
β_1^a	1.809	6.088*	7.640*	5.463*	3.937				
β_2	2.728*	5.483*	2.996	2.650	5.360*				
β_3	1.076	-3.446	-2.693	-3.179	-2.091				
$\hat{\beta_{12}}$	-53.092	1.313	-66.974	-40.815	1.583				
β ₁₃	23.056	-53.968	14.126	69.192	-117.841				
β ₂₃	-39.518	82.625	-27.282	-1.630	136.500				
β_1^{-2}	-121.854*	-231.857*	-125.178	-183.568	-193.486*				
β_2^{2}	-92.586*	-156.959*	-51.468	73.363*	-234.634*				
β_3^{-2}	-264.825	-544.361	1132.880*	-790.973*	-750.156				
R ²	0.909	0.926	0.849	0.902	0.893				
P of F	0.036	0.023	0.111	0.044	0.052				

^a1, Oleoresin rosemary extract; 2, sage extract; 3, citric acid.

**Significant at 0.01 level.

*Significant at 0.05 level.

citric acid—on the sensory acceptability also slightly decreased. While oleoresin rosemary extract had significant effects on appearance, crispiness, and overall acceptability, and highly significant effects on taste and odor scores at day 1, this antioxidant only had significant (P < 0.05) correlations to the scores of taste, crispiness, and odor of the product at the end of frying period, and had no effect on appearance and overall acceptability. As for sage extract, its effects were sig-



Citric acid = 0.026%

FIG. 2. Contour maps on the effects of oleoresin rosemary extract, sage extract, and citric acid on sensory attributes of deep-fat fried potato chips after five days of frying.

nificant (P < 0.05) on appearance, taste, and overall acceptability. There were no combination or synergistic effects among the two antioxidants used and citric acid; however, for citric acid alone, its second order was significant (P < 0.05) for crispiness and odor scores of the product. The decrease in the effects of the antioxidants and citric acid on the sensory quality of potato chips in this study showed that although oleoresin rosemary and sage extracts are high-temperature stable antioxidants, their effectiveness was also slightly reduced when applied continuously in deep-fat frying operations.

However, the results from Table 5, also revealed that models or equations that could be developed from the antioxidants and citric acid used to predict the sensory qualities of the product are still statistically considered adequate. R^2 values for the fried product were 0.909, 0.926, 0.849, 0.902, and 0.893 for appearance, taste, odor, crispiness, and overall acceptability, respectively.

Figures 2a–e show the contour maps for sensory responses of the potato chips fried at day 5. In general, for every response examined, the pattern of the maps are quite similar to the optimum maps obtained for samples at day 1 (Figs. 1a-e). However, except for overall acceptability, there was little change in the optimum level of antioxidants and citric acid. For taste and odor attributes, the combinations required to achieved the optimum points were 0.065% oleoresin rosemary extract, 0.064% sage extract, and 0.018% citric acid, and 0.072% oleoresin rosemary extract, 0.068% sage extract and 0.022% citric acid, respectively. For appearance, a combination of 0.062% oleoresin rosemary extract, 0.066% sage extract, and 0.023% citric acid was required. For crispiness scores, the use of 0.063% oleoresin rosemary extract, 0.075% sage extract, and 0.025% citric acid was the optimum combination, while the optimum overall acceptability was achieved by using a combination of 0.060% oleoresin rosemary extract, 0.064% sage extract, and 0.026% citric acid. The optimum combination obtained for overall acceptability at day 5 was exactly the same as the optimum combination at day 1. This meant that although the average scores given by panelists for overall acceptability attribute decreased after 5 days of frying, the decrease was relatively proportional to each sample examined. The constant combination of variables obtained in this case also meant that to achieve the same score of overall acceptability, the level of the two antioxidants and citric acids might have to be increased.

Physico-chemical changes in oil during five days of frying. The quality changes in oil occurring during deep-fat frying obviously have an effect on the fried products. Oil is picked up by the food during frying and forms an integral part of deep-fried products (24). During frying, oil is continously exposed to high temperatures in the presence of air and moisture. These conditions result in rapid deterioration of the frying oil. The reaction involved results in the formation of numerous decomposition products which not only affect the functional and sensory and qualities of the oil (25) but also the fried products (26). Table 4 shows the changes in physicochemical quality of control oil during five days of deep-fat frying of potato chips. This table illustrates that the quality of RBD palm olein used gradually decreased during the five days of frying. The changes in PV and FFA could contribute to the decrease in some sensory parameters, as described before. Although in this study we did not investigate the relationship between sensory scores and physico-chemical analyses, our previous work indicated that the increases in PV and FFA had a good correlation to the decrease in taste and odor (14,26). Idris et al. (27) also revealed that there seemed to be an inverse linear relationship between quality evaluation by sensory panels and FFA content from chemical analysis. It is also reported that flavor intensity scores were related to FFA content of RBD palm olein. On the other hand, slightly better correlations between PV, anisidine value and totox value, and flavor intensity scores were also found, even though at lower coefficient of determinations (R^2).

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