

# Synergistic Effects of Rosemary, Sage, and Citric Acid on Fatty Acid Retention of Palm Olein During Deep-fat Frying

Irwandi Jaswir<sup>a,b</sup>, Yaakob B. Che Man<sup>a,\*</sup>, and David D. Kitts<sup>b</sup>

Departments of <sup>a</sup>Food Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor DE, Malaysia, and

<sup>b</sup>Food Science, University of British Columbia, Vancouver, British Columbia, V6T 1W5 Canada

**ABSTRACT:** A study to optimize the use of oleoresin rosemary extract, sage extract, and citric acid in refined, bleached, and deodorized (RBD) palm olein during deep-fat frying of potato chips was performed using response surface methodology. Results showed that the natural antioxidants used in this study retarded oil deterioration, as evidenced by retention of fatty acid profiles. The linoleic to palmitic (C18:2/C16:0) ratio was chosen as the parameter for optimizing the use of natural antioxidants in RBD palm olein during deep-fat frying. Linoleic ( $R^2 = 0.946$ ) and palmitic ( $R^2 = 0.825$ ) acids were found to be the most important dependent variables, giving highest  $R^2$  values to various antioxidant treatments after 25 h of frying. All three antioxidants had independent significant ( $P < 0.05$ ) effects on the C18:2/C16:0 ratio. In fact, significant effects on the C18:2/C16:0 ratio of RBD palm olein were also given by a second-order form. A combination of 0.076% oleoresin rosemary extract, 0.066% sage extract, and 0.037% citric acid produced the optimal retention of the essential fatty acid C18:2. In addition, a synergistic effect among these antioxidants on the fatty acid ratio of RBD palm olein was found.

Paper no. J9277 in *JAOCS* 77, 527–533 (May 2000).

**KEY WORDS:** Citric acid, optimization, palm olein, response surface methodology, rosemary, sage.

A number of physical and chemical changes involving a complex pattern of thermolytic and oxidative reactions occur in fats and oils during deep-fat frying (1). Under commercial deep-fat frying operations, fat is continuously exposed to air and light for extended periods at temperatures approaching 180°C. The lipid composition of food systems cooked by deep-fat frying will promote chemical changes within the frying oil (2). These reactions result in significant changes in fatty acid composition and cause a rapid deterioration of the frying oil. Ultimately, the functional, sensory, and nutritional quality of the oil can be affected (3).

To avoid or delay lipid oxidation during food processing, antioxidants (AO) are added to protect the oil from oxidation when exposed to high temperature. Considerable interest in the use of natural AO for frying purposes has been expressed recently owing to safety concerns about synthetic AO, e.g.,

butylated hydroxyanisole (4). Extracts of many plants have been reported to have varying degrees of AO activities in fats and oils (5–7). Results of these studies indicated clearly that the majority of the spices tested possessed some AO activity, with rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) being the most potent. Rosemary and sage are two plant sources of AO that have been studied intensively and proven effective for stabilizing frying oils (8). Both AO sources have very good thermal resistance and strong AO characteristics (9,10). Recently, Che Man and Irwandi (11) found that rosemary and sage extracts, when added to palm olein, effectively retarded oil deterioration during a 30-h deep-fat intermittent frying of potato chips. Acceptable sensory characteristics of fried food were observed when rosemary and sage extracts were added to the frying oil (12).

Monitoring of fatty acid changes in oils during deep-fat frying is an effective method to assess thermal oxidative changes in the oils. Thomson and Aust (13) found that after 100 h of intermittent frying, total linoleic and linolenic acids decreased by 50%. Miller and White (14) reported a decrease in linoleic and linolenic acids and a corresponding increase in relative amounts of saturated fatty acids in soybean oils heated for 40 h at 180°C. Augustin *et al.* (15) found that the change in C18:2/C16:0 ratio was an effective parameter for assessing oxidation and quality of frying oil.

The purpose of this study was to use response surface methodology (RSM) (16,17) to determine the synergistic AO effect of oleoresin rosemary (OR), sage (OS), and citric acid (CA) on changes in fatty acid composition in refined, bleached, and deodorized (RBD) palm olein during 25 h of intermittent deep-fat frying.

## MATERIALS AND METHODS

**Materials.** RBD palm olein was obtained from Ngo Chew Hong (M) Sdn. Bhd., Selangor, Malaysia. OR (Herbalox Brand, Type O) OS (Herbalox seasoning, Type S-O) extracts were kindly donated by Kalsec Inc. USA (Gulf Chemical Sdn. Bhd., Selangor, Malaysia), whereas CA was purchased from a local supplier in Selangor, Malaysia. Fresh potatoes and sodium chloride were obtained from a local supermarket. All reagents were of analytical grade.

**Experimental design.** RSM was used to investigate the ef-

\*To whom correspondence should be addressed.

E-mail: yaakub@fsb.upm.edu.my

fect of OR, OS, and CA and their different combinations on changes in fatty acid composition of RBD palm olein and to determine the optimal combinations during 25 h of intermittent deep-fat frying. Echip software (Echip Inc., Hockessin, DE) (18) was used in this study to provide initial experimental designs, calculate multiregression equations, and provide statistical evaluations. RSM basically uses an experimental design such as the central composite design (CCD) to fit a model by least squares analysis (18). Initial concentrations of OR and OS ranged from 0 to 0.1% each, and CA from 0 to 0.05%, according to Jaswir and Che Man (12). A total of 15 different combinations of the three AO (Table 1) established from the Echip software were tested to evaluate effectiveness in preserving the fatty acid composition of the RBD palm olein in potato chip frying experiments. Each experiment was performed in three replications.

**Frying experiment.** All 15 frying experiments conducted in this study were similar to that reported by Jaswir and Che Man (12). In short, RBD palm oil was heated to  $180 \pm 5^\circ\text{C}$  in batch fryers (Berto's, Model ELT 8B, Montegrotto, Padova, Italy). Ten fryings equivalent to a period of 5 h were completed every day for five consecutive days using the following protocol; 100 g of freshly sliced potatoes were fried for 2.5 min, the oil temperature was allowed to return to  $180^\circ\text{C}$  within 30 min, and the frying repeated. The fryers were left uncovered during the frying operations.

At the end of each day, 200 g of oil at a temperature of  $60^\circ\text{C}$  was removed from the fryer, flushed with nitrogen gas, and kept in a cold room at  $4^\circ\text{C}$  until analysis. Analysis of oil was carried out immediately after the frying experiment and completed within 8 d. The fryers were covered with a lid and left overnight for the following day's frying. No fresh oil or AO was added to the frying vessel. At the end of this study, a frying experiment with optimal AO combination was also performed. This sample was used to validate data obtained from the software (predicted data) and to evaluate effects of

AO on other oxidative parameters during frying. For the physico-chemical analyses, the oil sample for 0 h was obtained by adding AO to the oil and heating the oil to  $60^\circ\text{C}$ .

**Gas chromatography analysis for fatty acid composition.** The fatty acid composition of the oil was determined by gas chromatography as reported by Berry (19), and the fatty acid methyl esters (FAME) dissolved in the hexane were dried over  $\text{Na}_2\text{SO}_4/\text{NaHCO}_3$  (4:1, w/w). FAME samples were quantified using a Shimadzu Model GC-17A flame-ionization gas chromatograph (Mandel Scientific Co. Ltd, Guelph, Canada) containing a fused-silica capillary column (Omega wax 320<sup>TM</sup>) of 30 m length, 0.32 mm internal diameter, and 0.25  $\mu\text{m}$  film thickness. The initial column temperature of  $140^\circ\text{C}$  was increased to  $200^\circ\text{C}$  at a rate of  $4^\circ\text{C}/\text{min}$ , and a temperature of  $250^\circ\text{C}$  was used for the injector and detector. The flow rates for carrier gas, hydrogen, and air were 65, 44, and 440 mL/min, respectively. A fatty acid standard containing C12:0, C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, and C20:0 (Sigma-Aldrich Canada Ltd., Oakville, ON) was used.

**Analyses of oil.** Peroxide value (PV), free fatty acid (FFA), and iodine value (IV) were all determined using PORIM test methods (20). The oil color was measured in a 1-in cell in a Lovibond tintometer (Salisbury, United Kingdom) (20). Polymer content was analyzed according to the method of Peled *et al.* (21). The absorbances at 232 and 268 nm and the anisidine value (AnV) were obtained using IUPAC methods (22). Each reported value is the mean of three replications.

**Statistical analyses.** For the optimization purposes, based on fatty acid composition results, mathematical models or equations developed in this study were reported as follows:

$$\text{response} = \beta_0 + \beta_1(\text{OR}) + \beta_2(\text{OS}) + \beta_3(\text{CA}) + \beta_{12}(\text{OR})(\text{OS}) + \beta_{13}(\text{OR})(\text{CA}) + \beta_{23}(\text{OS})(\text{CA}) + \beta_1^2(\text{OR})^2 + \beta_2^2(\text{OS})^2 + \beta_3^2(\text{CA})^2 \quad [1]$$

where response = percentage of each fatty acid,  $\beta_0$  = intercept,  $\beta_{1,2,3}$  = coefficient for each antioxidant at the first-order form,  $\beta_{12,13,23}$  = coefficient for each interaction among AO,  $\beta_1^2, \beta_2^2, \beta_3^2$  = coefficient for each AO at the second-order form, (OR) = concentration of oleoresin rosemary extract in oil, (OS) = concentration of oleoresin sage extract in oil, and (CA) = concentration of citric acid in oil.

Statistical analyses of the effects of each AO and interactions on fatty acid profiles were provided by the Echip software (18). In addition to the Echip software used for optimization purpose, data of physicochemical analyses of oil were also statistically analyzed by one-way analysis of variance procedure using SAS (23). Significant differences ( $P < 0.05$ ) between treatment means were further determined by Duncan's multiple-range test.

## RESULTS AND DISCUSSION

Table 2 shows the effect of addition of OR, OS, and CA on fatty acid composition of RBD palm olein after 5 and 25 h of deep-fat frying of potato chips, compared with the fatty acid profile of fresh RBD palm olein. Oleic (C18:1), palmitic

**TABLE 1**  
Combinations of Oleoresin Rosemary Extract, Sage Extract, and Citric Acid Added into RBD<sup>a</sup> Palm Olein Before Frying

Trial no.	Rosemary (%)	Sage (%)	Citric acid (%)
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	0.05	0

<sup>a</sup>RBD, refined, bleached and deodorized.

**TABLE 2**  
**Fatty Acid Compositions of Fresh RBD Palm Olein and Natural Antioxidant-Treated RBD Palm Olein after 5 and 25 h of Deep-Fat Frying<sup>a</sup>**

	Trial no.	Type of fatty acid (%)								
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	Others
Fresh oil	—	1.18	39.60	0.14	4.09	42.33	10.66	0.15	0.52	1.333
After 5-h frying	1	1.16	41.13	0.16	4.34	44.00	8.53	0.12	0.47	0.09
	2	1.16	41.76	0.15	4.21	43.08	8.98	0.11	0.41	0.14
	3	1.13	41.96	0.18	3.89	43.60	8.69	0.11	0.37	0.07
	4	1.16	41.62	0.15	4.42	43.55	8.38	0.11	0.39	0.22
	5	1.13	40.49	0.17	4.30	44.17	8.77	0.13	0.36	0.48
	6	1.14	40.76	0.16	4.02	44.25	8.77	0.07	0.36	0.47
	7	1.18	42.49	0.18	3.84	43.07	8.78	0.10	0.33	0.03
	8	1.20	41.77	0.18	4.00	43.27	8.94	0.08	0.33	0.23
	10	1.15	41.32	0.19	4.29	43.33	8.51	0.13	0.39	0.69
	11	1.15	40.58	0.18	4.27	44.16	8.57	0.13	0.40	0.56
	12	1.15	41.18	0.19	4.35	43.90	8.60	0.10	0.38	0.15
	13	1.16	41.17	0.18	4.26	43.83	8.62	0.14	0.41	0.23
	14	1.18	40.75	0.17	4.38	44.09	8.64	0.13	0.39	0.27
	15	1.16	41.24	0.16	4.40	43.56	8.53	0.13	0.41	0.41
		9 (control)	1.18	43.27	0.17	4.24	42.37	8.23	0.09	0.41
$R^2$		0.699	0.717	0.479	0.387	0.631	0.729	0.488	0.693	—
After 25-h frying	1	1.15	42.42	0.20	4.52	44.22	7.07	0.05	0.27	0.10
	2	1.16	41.86	0.16	4.63	43.94	7.40	0.08	0.26	0.51
	3	1.13	42.94	0.18	4.40	43.63	7.00	0.08	0.26	0.38
	4	1.16	43.23	0.22	4.57	43.63	6.66	0.05	0.24	0.24
	5	1.13	41.82	0.18	4.52	44.25	7.43	0.09	0.26	0.32
	6	1.13	42.94	0.16	4.07	43.47	7.97	ND	0.21	0.05
	7	1.13	42.94	0.18	4.40	43.13	7.00	0.08	0.25	0.89
	8	1.18	42.76	0.21	4.10	43.70	7.73	ND	0.26	0.06
	10	1.14	42.20	0.19	4.52	43.99	7.31	0.05	0.26	0.34
	11	1.14	42.53	0.20	4.47	43.53	7.45	0.09	0.26	0.33
	12	1.14	41.80	0.19	4.40	44.58	7.27	0.05	0.27	0.30
	13	1.16	42.74	0.21	4.39	43.86	7.08	0.05	0.28	0.23
	14	1.18	41.98	0.18	4.38	44.12	7.64	0.06	0.26	0.20
	15	1.16	42.95	0.16	4.52	43.60	6.86	0.05	0.25	0.45
		9 (control)	1.17	44.25	0.22	4.62	43.42	5.97	0.03	0.27
$R^2$		0.721	0.825	0.735	0.815	0.793	0.946	0.662	0.712	—

<sup>a</sup>Mean of three replications. ND, not detected. For other abbreviation see Table 1.

(C16:0), linoleic (C18:2), and stearic (C18:0) acids are four major fatty acids present in RBD palm olein, with relative proportions of 42.33, 39.57, 10.66, and 4.09%, respectively. Other fatty acids found in this oil were myristic (C14:0, 1.18%), arachidic (C20:0, 0.52%),  $\alpha$ -linolenic (C18:3, 0.15%), and palmitoleic (C16:1, 0.14%).

*Effect of natural AO on fatty acid composition after 5-h of frying.* The relative proportions of C16:0, C16:1, and C18:1 for all 15-trial samples evaluated increased in the oil, while the relative compositions of C14:0, C18:2, C18:3, and C20:0 decreased after 5 h of frying. The proportion of C16:0 in the oil ranged from 40.49 (Trial 5) to 43.27% (Trial 9; control), representing an increase in proportion of 2.27 to 9.27%. This finding is in accordance with the results reported by Augustin and Berry (24), who reported that the marked increase in C16:0 proportion following frying correlated with the breakdown of double bonds of fatty acids with higher carbon numbers. Our data extend this finding by showing that the addition of rosemary, sage, and citric acid to the oil reduced the thermal oxidation of unsaturated fatty acids as early as the first 5 h of the frying, thus minimizing the increase in C16:0.

After 5 h of frying, a marked decrease in C18:2 proportion in the oil was observed. The range of the decrease in C18:2 was 15.74 (Trial 2) to 22.77% (Trial 9).

After 5 h of frying, the correlations (Table 2) between different combinations of AO tested and either C18:2 or C16:0 proportions were markedly better than between AO combinations and other fatty acids (e.g., C14:0, C20:0, and C18:1).  $R^2$  values for responses of both C18:2 and C16:0 were 0.729 and 0.717, respectively.  $R^2$  values for different fatty acids after 5 h of frying, however, were insufficient to adequately ( $R^2 = 0.75$ ) predict the fatty acid composition based on concentration of each AO used (16,17).

*Effect of natural AO on fatty acid composition after 25-h of frying.* The changes in fatty acid composition of RBD palm olein after 25 h of frying had the same pattern of relative change observed after 5 h of frying. C16:0 and C16:1 gradually increased, while other fatty acids, except for C18:0, decreased (Table 2). For some trial samples, C18:3 was not detected following repeated frying. Four fatty acids, namely, C18:2, C18:0, C16:0, and C18:1 were observed to have  $R^2$  values greater than 0.75.

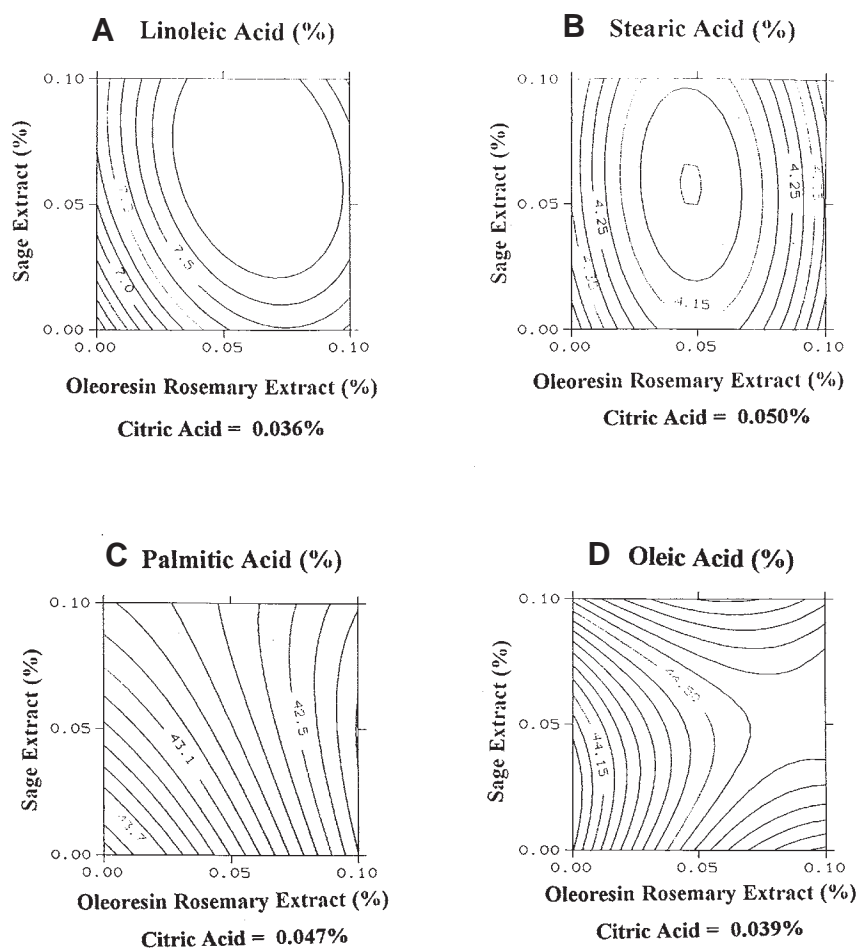


FIG. 1. Contour maps of the effects of oleoresin rosemary extract, sage extract, and citric acid on individual fatty acids after 25 h of frying.

Contour maps used for prediction purposes of these four fatty acids at optimal levels of CA are shown in Figure 1A–1D), and individual regression coefficients are given in Table 3. After 25 h of frying, OR had a significant ( $P < 0.05$ ) effect on C18:2, C16:0, and C18:1. Second-order form also

produced a significant ( $P < 0.05$ ) effect on C18:0. However, OS and CA were only significant ( $P < 0.05$ ) on retaining C18:3, while the second-order form of CA level had a significant ( $P < 0.05$ ) effect for C18:1.

*Optimization of use of natural AO based on C18:2/C16:0*

TABLE 3  
Regression Coefficients for Some Fatty Acids of Natural Antioxidant-Treated RBD Palm Olein After 25 h of Frying

Coefficients <sup>a</sup>	Type of fatty acid			
	C16:0	C18:0	C18:1	C18:2
$\beta_0$ (intercept)	42.814	4.050	44.478	7.8179
$\beta_1$	-11.395*	0.584	4.838*	5.6899**
$\beta_2$	-5.217	-0.517	1.875	3.9508*
$\beta_3$	-1.497	-3.533	2.473	6.4391*
$\beta_{12}$	106.285	13.080	-67.263	58.6182
$\beta_{13}$	138.427	-54.832	-36.597	-7.4236
$\beta_{23}$	75.543	19.214	-13.099	-50.143
$\beta_{12}$	-21.944	131.682**	-63.7844	174.358*
$\beta_{22}$	47.344	35.715	92.108	-95.7583
$\beta_{32}$	-614.420	177.801	749.540*	-284.164

<sup>a</sup>Subscripts: 1, oleoresin rosemary extract; 2, sage extract; 3, citric acid. For abbreviations see Table 1. \*\*, significant at 0.01 level; \*, significant at 0.05 level.

**TABLE 4**  
Regression Coefficients and  $R^2$  for C18:2/C16:0 Ratio of Natural Antioxidant-Treated RBD Palm Olein After 5 and 25 h of Frying<sup>a</sup>

Coefficients	Frying time (h)	
	5	25
$\beta_0$ (intercept)	0.2140	0.1807
$\beta_1$	0.0784*	0.1812***
$\beta_2$	0.0778*	0.1052**
$\beta_3$	0.0399*	0.1508*
$\beta_{12}$	-0.4207	-1.7521*
$\beta_{13}$	-0.8349	-0.7094*
$\beta_{23}$	0.1452	-1.4602*
$\beta_1^2$	-1.6161	-3.4877**
$\beta_2^2$	-1.6962	-2.4329*
$\beta_3^2$	0.5865	-2.1472
$R^2$	0.832	0.872

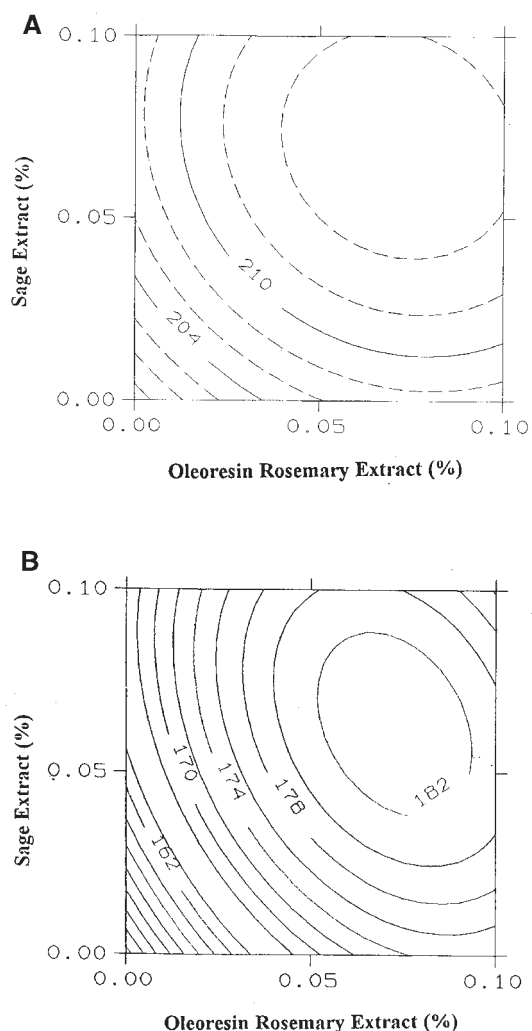
<sup>a</sup>For subscripts see Table 3 and for abbreviations see Table 1.

\*\*\*, significant at 0.001 level; \*\*, significant at 0.01 level; \*, significant at 0.05 level.

**TABLE 5**  
Effect of Oleoresin Rosemary Extract, Sage Extract, and Citric Acid on C18:2/C16:0 Ratio of RBD Palm Olein Samples During Deep-fat Frying<sup>a</sup>

Trial no.	Frying time (h)				
	5	10	15	20	25
1	0.207	0.198	0.185	0.174	0.167
2	0.215	0.199	0.186	0.182	0.177
3	0.207	0.199	0.189	0.173	0.163
4	0.201	0.182	0.175	0.170	0.154
5	0.217	0.205	0.192	0.191	0.178
6	0.215	0.210	0.195	0.185	0.183
7	0.207	0.207	0.196	0.181	0.163
8	0.214	0.205	0.202	0.186	0.181
9	0.190	0.178	0.164	0.153	0.135
10	0.206	0.201	0.200	0.193	0.173
11	0.211	0.200	0.191	0.185	0.175
12	0.209	0.205	0.204	0.183	0.174
13	0.209	0.197	0.191	0.169	0.166
14	0.212	0.205	0.203	0.199	0.182
15	0.207	0.205	0.195	0.179	0.160

<sup>a</sup>Mean of three replications. For abbreviations see Table 1.



**FIG. 2.** Contour maps on the effects of oleoresin rosemary extract, sage extract, and citric acid on C18:2/C16:0 ratio ( $\times 10^{-3}$ ) after (A) 5 and (B) 25 h of frying. In A, citric acid content is 0.038%; in B, 0.037%.

ratio. Based on fatty acid composition obtained after 5 and 25 h of frying, a C18:2/C16:0 ratio was used to predict the optimal amounts of OR, OS, and CA for controlling RBD palm olein during frying. The use of C18:2/16:0 ratio as a quality parameter in fat and oil analysis was first reported by Augustin *et al.* (15) and used by Che Man and Tan (7) to determine the quality changes of RBD palm olein during frying. In this study, the natural AO had higher  $R^2$  values (0.972 and 0.832, respectively) after both 5 and 25 h of frying (Table 4).

The changes in the C18:2/C16:0 ratio of 15-trial samples examined during 25 h of frying are shown in Table 5. After 5 h of frying, the C18:2/C16:0 ratio of all samples ranged from 0.190 (Trial 9; control) to 0.217 (Trial 5). As a comparison, the C18:2/C16:0 ratio for fresh RBD palm olein was 0.269. This result indicated that the AO added to the oil before frying effectively retarded lipid oxidation as early as the first day of frying. Over 25 h of frying, the C18:2/C16:0 ratio gradually decreased, with the greatest decrease (29.02%) exhibited in samples without added AO (Trial 9). Conversely, the least decrease (14.20%) observed in C18:2/C16:0 ratio occurred in samples containing 0.1% rosemary, 0.05% sage, and 0.05% citric acid (Trial 14).

Table 4 shows the regression coefficients and  $R^2$  for C18:2/C16:0 ratio of RBD palm olein heated with natural AO after 5 and 25 h of frying. After 5 h of frying, all three AO produced a significant effect ( $P < 0.05$ ) on the C18:2/C16:0 ratio. No interactive effect among the three AO was found. The model developed from the C18:2/C16:0 ratio for the first day of frying was significant ( $R^2 = 0.832$ ,  $P < 0.05$ ). The contour map for this response (Fig. 2A) showed that a combination of 0.072% OR, 0.078% OS, and 0.038% CA was required to reach the optimum after 5 h of frying.

Also, Table 4 shows the effect of using all three natural AO on the C18:2/C16:0 ratio after 25 h of deep-fat frying. A predictive equation for estimation of C18:2/C16:0 ratio change was developed with high confidence ( $R^2 = 0.972$ ). OR and OS



**TABLE 6**  
Predicted vs. Experimental Fatty Acid Profiles of Optimal Antioxidant Combination Treatment After 25 h of Frying<sup>a</sup>

Fatty acid	Composition (%)	
	Predicted	Experimental
C14:0	1.13	1.10
C16:0	41.62	42.00
C16:1	0.17	0.15
C18:0	4.13	4.21
C18:1	44.71	44.01
C18:2	7.89	7.90
C18:3	0.03	0.03
C20:0	0.25	0.28
Others	0.07	0.32
C18:2/C16:0 ratio	0.189	0.188

<sup>a</sup> $R^2 = 0.9998$ .

produced a significant ( $P < 0.001$ ;  $P < 0.01$ , respectively) effect on the C18:2/C16:0 ratio, while the CA effect on the ratio was significant at a 0.05 confidence level. The results indicated that there were synergistic effects among these AO on the C18:2/C16:0 ratio after repeated oil frying. Interactions between OR and OS, OR and CA, and OS and CA also were significant ( $P < 0.05$ ). For OR and OS, significant effects on the C18:2/C16:0 ratio were found at both first-order and second-order. The second-order form of OR was significant at  $P < 0.01$ , while the OS's second-order had a significant effect at the 0.05 level. From the contour map obtained in this study (Fig. 2B), an optimal point for the retention of C18:2/C16:0 was achieved by combining 0.076% OR with 0.066% OS and 0.037% CA. To validate these optimal data, a frying experiment using the AO combination was performed. Data on fatty acid profiles after 25 h of frying experiment are compared in

Table 6 with predicted data from the Echip software. There is a very high correlation ( $R^2 = 0.9998$ ) between these two data sets, thus the optimization study supports the use of RSM for predicting addition levels of natural AO during deep-fat frying of RBD palm olein.

*Changes of oil during repeated frying.* Table 7 shows the quality changes of control oil (Trial 9) and sample prepared from optimal AO combination (treatment sample) during 25-h deep-fat frying of potato chips. During 25 h of frying, all quality parameters of RBD palm olein decreased. The relative changes in PV, AnV, FFA and polymer content, viscosity, and IV, all provided good indices of lipid deterioration rate of the control sample, as evidenced by the significant ( $P < 0.05$ ) changes in these parameters compared to oil sample containing AO. However, in terms of color, no significant ( $P > 0.05$ ) difference was found between samples with or without AO, although the redness and yellowness indices of both samples significantly ( $P < 0.05$ ) increased during 25-h deep-fat frying.

Similar to the PV, the absorbance at 232 nm measures the degree of primary oxidation. In general, results obtained in this study were closely related to the PV as described above. There was a trend of increased diene content with increased frying days. The absorbance of the sample treated with AO was also significantly ( $P < 0.05$ ) different from the control sample.

There was also a significant ( $P < 0.05$ ) effect of the use of AO and days of frying on the absorbance at 268 nm. The absorbance of the control oil sample was significantly ( $P < 0.05$ ) greater than that of the sample treated with AO. In addition, the longer the frying time, the higher the absorbance readings from these oils. These phenomena agree with the AnV, al-

**TABLE 7**  
Quality Changes in RBD Palm Olein During Deep-fat Frying<sup>a</sup>

Characteristics	Sample	Frying time (h)					
		0	5	10	15	20	25
Peroxide value (meq oxygen/kg)	Control	0.91 <sup>D,a</sup>	6.55 <sup>c,A</sup>	7.80 <sup>b,A</sup>	8.06 <sup>b,A</sup>	8.47 <sup>b,A</sup>	11.70 <sup>a,A</sup>
	Treatment	0.73 <sup>D,a</sup>	1.84 <sup>c,B</sup>	2.20 <sup>c,B</sup>	2.73 <sup>c,B</sup>	4.11 <sup>b,B</sup>	6.14 <sup>a,B</sup>
Anisidine value	Control	0.96 <sup>D,a</sup>	31.53 <sup>c,A</sup>	36.06 <sup>b,c,A</sup>	40.17 <sup>b,A</sup>	51.41 <sup>a,A</sup>	54.97 <sup>a,A</sup>
	Treatment	0.96 <sup>E,a</sup>	26.10 <sup>d,B</sup>	29.11 <sup>d,B</sup>	36.32 <sup>c,B</sup>	41.45 <sup>b,B</sup>	44.88 <sup>a,B</sup>
Iodine value (g I <sub>2</sub> /100 g oil)	Control	56.07 <sup>A,a</sup>	52.02 <sup>b,B</sup>	48.81 <sup>c,A</sup>	45.25 <sup>d,B</sup>	43.39 <sup>e,B</sup>	41.88 <sup>f,B</sup>
	Treatment	55.67 <sup>A,a</sup>	54.35 <sup>a,A</sup>	49.25 <sup>b,A</sup>	46.70 <sup>c,A</sup>	44.04 <sup>d,A</sup>	43.65 <sup>d,B</sup>
Free fatty acid (%)	Control	0.05 <sup>E,a</sup>	0.14 <sup>d,A</sup>	0.19 <sup>d,A</sup>	0.27 <sup>c,A</sup>	0.42 <sup>b,A</sup>	0.52 <sup>a,A</sup>
	Treatment	0.05 <sup>E,a</sup>	0.11 <sup>d,A</sup>	0.15 <sup>c,d,A</sup>	0.19 <sup>c,B</sup>	0.24 <sup>b,B</sup>	0.35 <sup>a,B</sup>
Polymer content (%)	Control	0.01 <sup>F,a</sup>	0.71 <sup>e,A</sup>	1.00 <sup>d,A</sup>	1.29 <sup>c,A</sup>	1.55 <sup>b,A</sup>	1.97 <sup>a,A</sup>
	Treatment	0.01 <sup>F,a</sup>	0.41 <sup>e,B</sup>	0.70 <sup>d,B</sup>	1.11 <sup>c,B</sup>	1.33 <sup>b,B</sup>	1.44 <sup>a,B</sup>
Color (red unit)	Control	0.53 <sup>C,a</sup>	1.10 <sup>b,A</sup>	1.20 <sup>a,b,A</sup>	1.20 <sup>a,b,A</sup>	1.30 <sup>a,A</sup>	1.35 <sup>a,A</sup>
	Treatment	0.57 <sup>C,a</sup>	1.10 <sup>b,A</sup>	1.14 <sup>b,B</sup>	1.16 <sup>b,A</sup>	1.25 <sup>a,B</sup>	1.30 <sup>a,A</sup>
Color (yellow unit)	Control	5.93 <sup>C,a</sup>	13.15 <sup>b,A</sup>	13.20 <sup>b,A</sup>	13.75 <sup>a,A</sup>	13.90 <sup>a,A</sup>	14.10 <sup>a,A</sup>
	Treatment	5.91 <sup>C,a</sup>	12.94 <sup>b,A</sup>	13.01 <sup>b,A</sup>	13.26 <sup>a,b,B</sup>	13.65 <sup>a,A</sup>	14.02 <sup>a,A</sup>
E <sub>1cm</sub> <sup>1%</sup> at 232 nm	Control	1.71 <sup>D,a</sup>	3.91 <sup>c,A</sup>	5.06 <sup>b,A</sup>	8.22 <sup>a,A</sup>	8.90 <sup>a,A</sup>	9.01 <sup>a,A</sup>
	Treatment	1.70 <sup>D,a</sup>	2.40 <sup>c,B</sup>	3.66 <sup>b,B</sup>	4.05 <sup>b,B</sup>	4.57 <sup>a,b,B</sup>	5.12 <sup>a,B</sup>
E <sub>1cm</sub> <sup>1%</sup> at 268 nm	Control	0.41 <sup>C,a</sup>	1.75 <sup>b,A</sup>	1.86 <sup>b,A</sup>	1.96 <sup>a,A</sup>	1.98 <sup>a,A</sup>	2.08 <sup>a,A</sup>
	Treatment	0.40 <sup>D,a</sup>	0.50 <sup>c,B</sup>	0.63 <sup>c,B</sup>	0.88 <sup>b,B</sup>	1.24 <sup>b,B</sup>	1.47 <sup>a,B</sup>

<sup>a</sup>Mean of three replicates. <sup>a-f</sup>Means within a row with lowercase different letters are significantly different ( $P < 0.05$ ). <sup>A-B</sup>Means within a column for each parameter with different uppercase letters are significantly different ( $P < 0.05$ ). For abbreviation see Table 1.

though the basic principle of determination of absorbance at 268 nm and anisidine analyses is not totally similar. Although the two analyses are based on secondary oxidation of oil, the absorbances at 268 nm measure the diethylenic ketones, whereas ketones are not monitored in the anisidine test (24).

C18:2 and C16:0 fatty acids were found to be the most important fatty acids for predicting changes in oil quality after frying.  $R^2$  values for C18:2 and C16:0 after 25 h of frying were 0.946 and 0.825, respectively. However, for optimization purposes, the use of the C18:2/C16:0 ratio best predicted the efficacy of natural AO in preserving RBD palm olein during deep-fat frying. Further analysis showed that after 25 h of frying, all three AO had a significant effect on the C18:2/C16:0 ratio, given by second-order forms. It was also clear that there were synergistic effects among these AO on this fatty acid ratio. Based on these results, a combination of 0.076% OR extract, 0.066% OS extract, and 0.037% CA can be recommended for use in RBD palm olein before deep-fat frying.

## ACKNOWLEDGMENTS

The authors wish to thank Universiti Putra Malaysia for providing research grant IRPA No. 03-02-04-003 and the Natural Sources and Engineering Council of Canada.

## REFERENCES

- White, P.J., Methods for Measuring Changes in Deep-Fat Frying Oils, *Food Technol.* 45 (2):75–80 (1991).
- Gwo, Y.Y., G.J. Flick Jr., and H.P. Dupuy, Effect of Ascorbyl Palmitate on the Quality of Frying Fats for Deep Frying Operations, *J. Am. Oil Chem. Soc.* 62:1666–1671 (1985).
- Chang, S.S., R.J. Peterson and C.T. Ho, Chemical Reactions Involved in the Deep-Fat Frying of Foods, *Ibid.* 55:718–727 (1978).
- Kitts, D.D., Toxicity and Safety of Fats and Oil, in *Bailey's Industrial Oil and Fat Products*, 5th edn., cited by Y.H. Hui, Wiley-Interscience, New York, 1996, Vol. 1. pp. 215–280.
- Zhang, K.Q., Y.D. Bao, P. Wu, R.T. Rosen, and C.T. Ho, Antioxidative Components of Tanshen (*Salvia miltiorrhiza* Bung), *J. Agric. Food Chem.* 38:1194–1197 (1990).
- Kim, S.Y., J.H. Kim, S.K. Kim, M.J. Oh, and M.Y. Jung, Antioxidant Activities of Selected Oriental Herb Extracts, *J. Am. Oil Chem. Soc.* 71:633–640 (1994).
- Che Man, Y.B., and C.P. Tan, Effects of Natural and Synthetic Antioxidants on Changes in Refined, Bleached, and Deodorized Palm Olein During Deep-Fat Frying of Potato Chips, *Ibid.* 76:331–339 (1999).
- Chang, S.S., B. Ostric-Matijasevic, O.A.L. Hsieh, and L.H. Cheng, Natural Antioxidants from Rosemary and Sage, *J. Food Sci.* 42:1102–1106 (1977).
- Saito, Y., Y. Kimura, and T. Sakamoto, Studies on the Antioxidant Properties of Spices, *J. Japan Soc. Food Nutr.* 29:404–409 (1976).
- Houlian, C., and C.T. Ho, Natural Antioxidants, in *Flavor Chemistry of Fats and Oils*, edited by D. Min and T. Smouse, American Oil Chemist's Society, Champaign, 1985, pp. 117–173.
- Che Man, Y.B., and J. Irwandi, Effect of Rosemary and Sage Extracts on Frying Performance of Refined, Bleached and Deodorized (RBD) Palm Olein during Deep-Fat Frying, *Food Chem.* 69:301–307 (2000).
- Jaswir, I., and Y.B. Che Man, Use Optimization of Natural Antioxidants in Refined, Bleached and Deodorized Palm Olein during Repeated Deep-Fat Frying Using Response Surface Methodology, *J. Am. Oil Chem. Soc.* 76:341–348 (1999).
- Thomson, L.U., and R. Aust, Lipid Changes in French Fries and Heated Oils During Commercial Deep-Fat Frying and Their Nutritional and Toxicological Implications, *Can. Inst. Food Sci. Technol. J.* 16:23–28 (1983).
- Miller, L.A., and P.J. White, High Temperature Stability of Low-Linoleic, High Stearate and Common Soybean Oils, *J. Am. Oil Chem. Soc.* 65:1324–1327 (1988).
- Augustin, M.A., T. Asap, and L.K. Heng, Relationships Between Measurements of Fat Deterioration During Heating and Frying in RBD Olein, *J. Am. Oil Chem. Soc.* 64:1670–1675 (1987).
- Giovanny, M., Response Surface Methodology and Product Optimization, *Food Technol.* 37 (11):41–45, 83 (1983).
- Henika, R.G., Use of Response Surface Methodology in Sensory Evaluation, *Food Technol.* 36 (11):96–101 (1982).
- Wheeler, B., *Echp—Version 6.0 Windows. Reference Manual*, Echp Inc., Hockessin, Delaware (1993).
- Berry, S.K., Cyclopropene Fatty Acids in Some Malaysian Edible Seeds and Nuts: I. Durian (*Durio zibethinus*, Murr.), *Lipids* 15:452–455 (1980).
- PORIM, *PORIM Test Methods*, Palm Oil Research Institute of Malaysia, Ministry of Primary Industries, Kuala Lumpur, Malaysia, 1995, pp. 72–101.
- Peled, M., T. Gutfinger, and A. Letan, Effect of Water and BHT on Stability of Cottonseed Oil During Frying, *J. Sci. Food Agric.* 26:1655–1666 (1975).
- IUPAC, *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 6th edn., edited by C. Paquot, International Union Pure and Applied Chemistry, Commission on Oils, Fats and Derivatives, 1979, pp. 138–146.
- SAS, *Statistical Analysis System User's Guide: Statistics*, SAS Institute Inc., Cary, 1989, pp. 125–154.
- Augustin, M.A., and S.K. Berry, Effectiveness of Antioxidants in Palm Olein During Heating and Frying, *J. Am. Oil Chem. Soc.* 60 (1):105–107 (1983).

[Received June 23, 1999; accepted January 25, 2000]