Original Article

Performance of phytochemical antioxidant systems in refined-bleached-deodorized palm olein during frying

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Antioxidants are important inhibitory compounds against the oxidative deterioration of food. This study investigated the effects of various phytochemical antioxidant systems [oleoresin rosemary (OR), oleoresin sage (OS) and citric acid (CA)] on the physico-chemical characteristics of refined, bleached and deodorized (RBD) palm olein during the frying of potato chips. The effects of various mixtures of the antioxidants on the oil was also studied in repeated deep frying. The response surface methodology was used to optimize the composition of mixed antioxidants used. A comparative study was carried out with synthetic antioxidants. Samples of the oil after frying were analyzed for different physical and chemical properties. OR and OS were found to be effective phytochemical antioxidants protecting RBD palm olein against oxidative deterioration during frying.

Key Words: rosemary, sage, phytochemical antioxidant , deep frying, palm oil, oxidation

Introduction

Many lipids are labile when exposed to heat, air and light. In heating or frying, both thermal and oxidative decomposition are accelerated.[1,2] Excessive heating of oils or fats can cause the formation of compounds with anti-nutritional properties, such as enzyme inhibitors[3], and accelerated loss of the antioxidant vitamins, such as vitaminE[4,5], leading to growth and histologic changes in the gastrointestinal tissues.[6,7] Moreover, oxidized lipids enhance peroxidation of the membrane macromolecules[8], contributing to their mutagenicity[9], genotoxicity[10] and angiotoxicity.[11] These cellular aberrations induced by thermally oxidized oils have been linked to growth retardation,[12] colon carcinogenesis[13] and reproductive disorders.[14] Notwithstanding these potential adverse health effects, lipid oxidation also decreases the acceptability of fried products.[15] Thus, to prevent undesirable changes in oil during storage and frying, antioxidants are required.[16]

This study investigated the oxidative behavior of various mixtures of natural antioxidants - rosemary, sage and citric acid - in palm olein systems in order to optimize the use of natural antioxidant mixtures in frying.

Materials

Refined, bleached and deodorized (RBD) palm olein was obtained from a local refinery in Selangor, Malaysia. Oleoresin rosemary (OR; Herbalox Brand, Type O) and oleoresin sage (OS; Herbalox seasoning, Type S-O) extracts were kindly donated by Kalsec Inc. USA, and citric acid (CA) was purchased from a local supplier in Selangor, Malaysia. All the other chemicals used were of analytical grade.

Experimental design

The response surface methodology (RSM) was used to investigate the effectiveness of OR, OS and CA and their different combinations as antioxidants. An RSM-based computer program - Echip software (Echip Inc., Hockessin, Delaware, USA)[17] - was used to provide the initial experimental designs, calculate the multi-regression equations and do the statistical analyses. RSM basically uses an experimental design, such as the central composite design (CCD), to fit a model by the method of least squares. The initial concentrations of OR and OS were 0 to 0.1% each, and CA 0 to 0.05% as per Irwandi and Che Man.[18] A total of 15 different combinations of the three antioxidants (Table 1) established from the Echip software were tested on palm olein. The experiments were performed in triplicate.

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Table 1. Combinations of rosemary, sage and citric acid added into RBD 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

[1]RBD=Refined, bleached and deodorized

For the purpose of optimization using the results from both analyses, the mathematical models, or equations, developed in this study were:

$$\text{Response} = \beta_0 + \beta_1(OR) + \beta_2(OS) + \beta_3(CA) + \beta_{12}(OR)(OC) + \beta_{13}(OR)(CA) + \beta_{23}(OS)(CA) + \beta_1^2(OR)^2 + \beta_2^2(OS)^2 + \beta_3^2(CA)^2$$

Where:

β_0 = intercept

$\beta_{1,2,3}$ = coefficient for each antioxidant at the first order form

$\beta_{12,13,23}$ = coefficient for each interaction among antioxidants

$\beta_{1^2,2^2,3^2}$ = coefficient for each antioxidant at the second order form

(OR) = concentration of OR extract in oil

(OS) = concentration of OS extract in oil

(CA) = concentration of CA in oil

Frying experiment

Fresh potatoes were hand-peeled and sliced to a thickness of 1.5 mm. The slices were soaked in 2.5% NaCl solution for 5 min, allowed to drain dry and then dried with paper napkins. OR, OS and CA were added to the RBD palm olein just before frying. The antioxidants were only added once at the beginning of the study.[18]

The frying was done in two batches (replicates) as per Che Man and Irwandi.[19] in a batch fryer (Berto's, Model ELT 8B, Italy) with 3 kg oil maintained at 60°C. About 10 min before the frying, the oil was heated to 180 ± 5°C with frying started 30 min later. One hundred grams of the sliced potato were then fried for 2.5 min, and the oil allowed 30 min to equilibrate back to 180°C before the next batch. Ten batches were fried daily for 5 consecutive days without any fresh oil added. The fryers were uncovered during the frying. For the physico-chemical analyses, 200g oil at 60°C were sampled from the fryer at the end of each day and stored in a cold room at 5°C. The fryer lid was then closed and the fryer left overnight for continuation of the experiment the next day. The fried chips were drained of excess oil. Daily, the ninth and tenth batches were packed in low-density polyethylene plastic bags for later sensory evaluation the same day.

Analyses of oil

The peroxide value (PV), free fatty acid (FFA) content and iodine value (IV) were all determined using PORIM test methods.[20] The oil color was measured in a one-inch cell in a Lovibond Tintometer (Salibury, United Kingdom),[20] and the viscosity by a Brookfield viscometer (Stoughton, MA).[20] The oil polymer content was analyzed by the method of Peled *et al.*[21] The absorbances at 232 and 268mm and the anisidine value (AnV) were obtained using IUPAC methods.[22] The fatty acid profile of the oil was determined by gas chromatography (Hewlett Packard gas chromatography Type 5890) as per Berry[23] using a 15 m X 0.53 mm capillary column and a flame ionization detector. The temperature of the column was initially 140°C, and programmed to increase at 4°C/min to 200°C. The temperature of both the injector and detector was 250°C. The flow rates for the carrier gasses, namely nitrogen, hydrogen and air were 65 mL/min, 44 mL/min and 440 mL/min, respectively. Each reported value was the mean from three replicates.

Statistical analysis

The data from physico-chemical analyses of the oil and sensory evaluation of the chips were analyzed by one-way analysis of variance using the SAS software to determine the effect of frying time on the quality of oil and fried chips. Significant differences ($P<0.05$) between means were further analysed by Duncan's multiple-range test. In addition, the SAS program was also used to derive linear regressions between the oil quality and sensory responses to the fried chips.

Results and Discussion

The physico-chemical characteristics of the fresh RBD palm olein are given in Table 2. The oil was of good initial quality, as evidenced by its low PV of 0.91 meq/kg and FFA content of 0.05%. Together with its IV of 56.07g I_2/100 g oil, it was well within the standards for Malaysian palm olein.[24] Table 3 shows the effects of adding OR, OS and CA on the fatty acid composition (FAC) of the RBD palm olein after one and five days of deep frying. Oleic (C18:1), palmitic (C16:0), linoleic (C18:2) and stearic (C18:0) acids are the four major fatty acids in palm olein at 42.33, 39.57, 10.66 and 4.09%, respectively. Other fatty acids in lesser amounts are myristic (C14:0, 1.18%), arachidic (C20:0, 0.52%), α-linolenic (C18:3, 0.15%) and palmitoleic (C16:1, 0.14%).

Effects of the natural antioxidants on the fatty acid composition (FAC) of palm olein after one day's frying

C16:0, C16:1, C18:1 in all the 15 samples increased while C14:0, C18:2, C18:3 and C20:0 decreased. C16:0 ranged from 40.49% (Trial 5) to 43.27% (Trial 9, control), representing increases of 2.27 to 9.27%. This is in accordance with the results of Augustin and Berry[25] who found a marked increase in C16:0 after frying, consistent with the breaking of double bonds in the unsaturated fatty

Table 2. Physico-chemical characteristics[1] of fresh RBD palm olein

Parameter	Value
Peroxide value (meq/kg oil)	0.91 ± 0.02
Iodine value (g I2/100g oil)	56.07 ± 0.15
Anisidine value	0.96 ± 0.03
FFA content (%)	0.05 ± 0.01
Polymer content (%)	0.01 ± 0.00
$E^{1\%}_{1cm}$ at 232 nm	1.71 ± 0.05
$E^{1\%}_{1cm}$ at 268 nm	0.41 ± 0.04
Red Colour (Lovibond unit)	0.53 ± 0.01
Yellow colour (Lovibond unit)	5.93 ± 0.26
Viscosity (centipoise)	50.22 ± 0.13
C18:2/C16:0 ratio	0.29 ± 0.01

[1] Means of three determinations

acids and their carbon chain lengths. The addition of rosemary, sage and citric acid reduced the oxidation of unsaturated fatty acids, minimizing the increase in C16:0. Conversely, there was a marked decrease in C18:2, ranging from 15.74 (Trial 2) to 22.77% (Trial 9).

Table 3 also shows the correlations obtained between the concentrations of antioxidants used (all the 15 combinations) with the compositions of the individual fatty acids in the RBD palm olein after frying. The correlations with C18:2 and C16:0 were markedly better than those with the other fatty acids (e.g. C14:0, C20:0 and C18:1). Their R^2-values were 0.729 and 0.717, respectively. The correlations with the other fatty acids were insufficiently strong ($R^2 \leq 0.75$) to be able to predict their proportions from the concentration of anti-oxidants used.[26,27]

Table 3. Fatty acid compositions of fresh RBD[1] palm olein and natural antioxidant-treated RBD palm olein after 1 and 5 days of frying[2]

	Trial	Fatty Acid (%)								
	No.	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	1.33
After 1-d 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	0.04
R^2		0.699	0.717	0.479	0.387	0.631	0.729	0.488	0.693	-
After 5-d 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[3]	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	0.05
R^2		0.721	0.825	0.735	0.815	0.793	0.946	0.662	0.712	-

Effects of natural antioxidants on fatty acid composition (FAC) after five days' frying

The changes in FAC of RBD palm olein after five days' frying were largely a continuation of the changes after one day's frying. C16:0 and C16:1 continued to increase, while the other fatty acids, except C18:0, decreased (Table 3). In some of the samples, C18:3 was not even detectable. Four fatty acids - C18:2, C18:0, C16:0 and C18:1 – had R^2 values > 0.75.

Table 4. Regression coefficients on some fatty acids from natural antioxidant-treated RBD[1] palm olein after 5 days' frying

Coefficient	Fatty Acid			
	C16:0	C18:0	C18:1	C18:2
β_0 (intercept)	42.814	4.050	44.478	7.8179
β_1	-11.395*	0.584	4.838*	5.6899**
β_2	-5.217	-0.517	1.875	3.9508*
β_3	-1.497	-3.533	2.473	6.4391*
β_{12}	106.285	13.080	-67.263	58.6182
β_{13}	138.427	-54.832	-36.597	-7.4236
β_{23}	75.543	19.214	-13.099	-50.143
β_1^2	-21.944	131.682**	-63.7844	174.358*
β_2^2	47.344	35.715	92..108	-95.7583
β_3^2	-614.420	177.801	749.540*	-284.164

1RBD = Refined, bleached and deodourized;, Subscript: 1 = OR extract; 2 = OS extract; 3 = CA; ** = significant at 0.01 level; * = significant at 0.05 level.

Contour maps for prediction of these four fatty acids at optimum levels of CA are shown in Figure 1 (A-D) with the individual regression coefficients given in Table 4. OR had a significant (P<0.05) effect on C18:2, C16:0 and C18:1. The 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 C18:3, while the second order form of the CA level had a significant (P<0.05) effect on C18:1.

Optimizing the use of natural antioxidants based on the C18:2 n-6/C16:0 ratio

Based on the FAC results after one and five days' frying, the C18:2/C16:0 ratio was used to predict the optimal amounts of OR, OS and CA for RBD palm olein during frying. The ratio, as a quality parameter in fat and oil analysis, was first suggested by Augustin *et al.*,[28] and has been used by Che Man and Tan[29] to determine the quality changes of RBD palm olein during frying. In this study, the natural antioxidants yielded high R^2 values (0.972 and 0.832, respectively) after one and five days' frying.

The changes in the C18:2/C16:0 ratio of all the oil samples after five days' frying are shown in Table 5. After one day, it was down to 0.190 (Trial 9, control) to 0.217 (Trial 5) from the fresh RBD palm olein value of 0.269. Thus, the antioxidants had retarded lipid oxidation even on the first day of frying. After five days, the ratio had further decreased, the most (29.02%) in the sample with-out added antioxidant (Trial 9). Conversely, the lowest decrease (14.20%) occurred with 0.1% OR, 0.05% OS and 0.05% CA (Trial 14).

Table 6 shows the regression coefficients and R^2 for the C18:2 n-6/C16:0 ratio of RBD palm olein heated with natural antioxidants after 1 and 5 d frying. After 1d, all the three antioxidants had a significant effect (P<0.05) on the ratio. No interactive effect between the three anti-oxidants was found. The model developed from the C18:2/C16:0 ratio for the first day of frying was signi-ficant (R^2=0.832, P<0.05). The contour map for this response (Fig. 2A) showed that the combination of 0.072% OR, 0.078% OS and 0.038% CA reached the optimum point after 1 d frying.

Table 6 also shows the effects of using all three natural antioxidants on the C18:2/C16:0 ratio after 5 d frying. An equation for estimating the ratio was developed with high confidence (R^2=0.972). OR produced a highly significant (P<0.001) effect on the ratio. OS also had a significant (P<0.01) effect, while the CA effect was only significant

Table 5. Effect of OR extract, OS extract and CA on the C18:2/C16:0 ratio of RBD[1] palm olein after deep-fat frying of potato chips[2]

Trial	Frying Time (h)				
	5	10	15	20	25
1	0.207±0.006	0.198±0.005	0.185±0.005	0.174±0.006	0.167±0.005
2	0.215±0.005	0.199±0.004	0.186±0.006	0.182±0.005	0.177±0.006
3	0.207±0.006	0.199±0.004	0.189±0.006	0.173±0.006	0.163±0.005
4	0.201±0.008	0.182±0.004	0.175±0.007	0.170±0.004	0.154±0.004
5	0.217±0.002	0.205±0.005	0.192±0.007	0.191±0.004	0.178±0.004
6	0.215±0.003	0.210±0.006	0.195±0.004	0.185±0.003	0.183±0.007
7	0.207±0.005	0.207±0.010	0.196±0.002	0.181±0.004	0.163±0.007
8	0.214±0.006	0.205±0.006	0.202±0.002	0.186±0.004	0.181±0.009
9	0.190±0.006	0.178±0.007	0.164±0.002	0.153±0.002	0.135±0.007
10	0.206±0.003	0.201±0.001	0.200±0.005	0.193±0.004	0.173±0.007
11	0.211±0.004	0.200±0.005	0.191±0.004	0.185±0.004	0.175±0.006
12	0.209±0.009	0.205±0.004	0.204±0.005	0.183±0.001	0.174±0.005
13	0.209±0.005	0.197±0.004	0.191±0.006	0.169±0.007	0.166±0.005
14	0.212±0.006	0.205±0.009	0.203±0.006	0.199±0.004	0.182±0.005
15	0.207±0.003	0.205±0.004	0.195±0.006	0.179±0.005	0.160±0.005

[1] RBD = Refined, bleached and deodorized

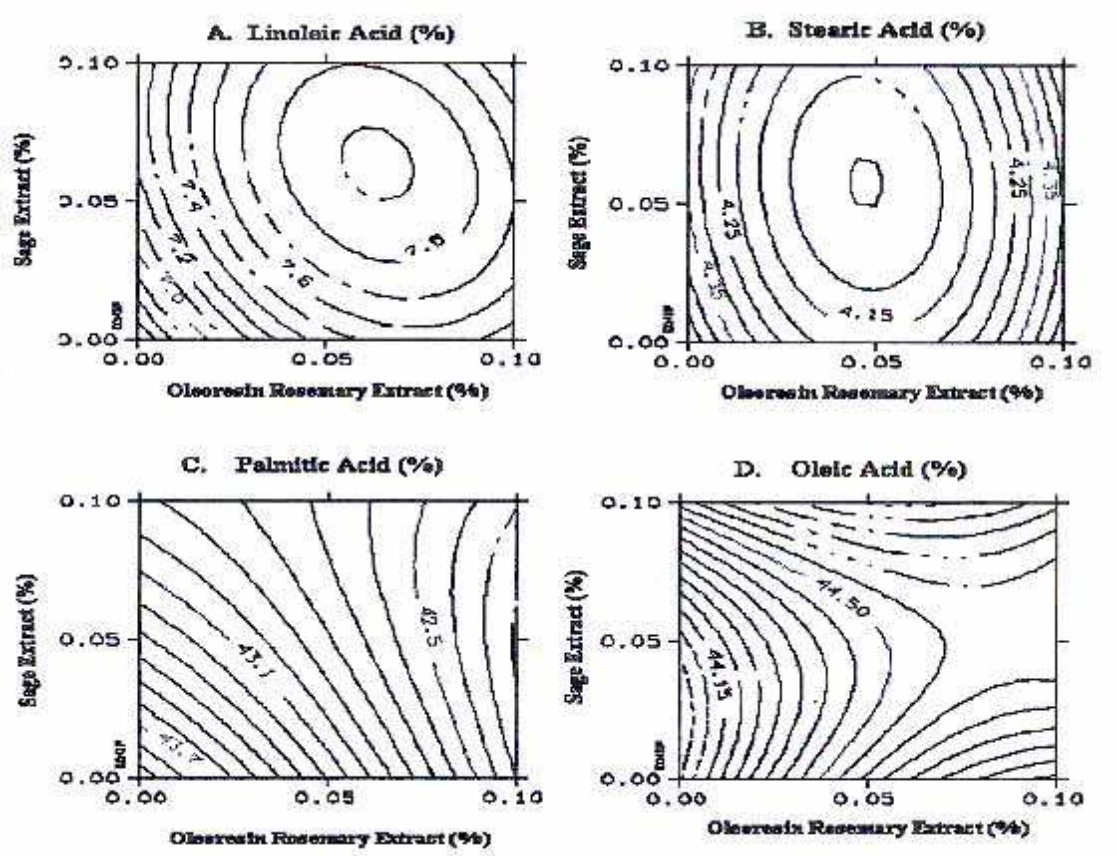

Figure 1. Contour maps of the effects of OR and OS at optimum level of CA on individual fatty acids after 5 days of frying (Levels of CA for A=0.036%, B=0.050%, C=0.047% and D=0.039%).

at the 0.05 confidence level. The results also showed synergism between the antioxidants on the ratio after repeated frying. The interactions between OR and OS, OR and CA, and OS and CA were also significant (P<0.05). For OR and OS, the significant effects were at both the first and second orders. The second order form of OR was significant at P<0.01, while the OS second order was only significant at the 0.05 level. From the contour map obtained (Fig. 2), the optimum point for retention of C18:2/C16:0 was 0.076% OR, 0.066% OS and 0.037% CA. To verify this finding, a frying experiment using the antioxidant combination was performed. The fatty acid profiles after 5 d frying and predicted by the Echip software are compared in Table 7. A very high correlation (R^2=0.9998) was obtained, indicating that

Table 6. Regression coefficients and R^2 for C18:2/ C16:0 ratio of natural antioxidant-treated RBD[1] palm olein after 1 and 5 d of frying

Coefficient	Frying Time (d)	
	1	5
β_0 (intercept)	0.2140	0.1807
β_1	0.0784*	0.1812***
β_2	0.0778*	0.1052**
β_3	0.0399*	0.1508*
β_{12}	-0.4207	-1.7521*
β_{13}	-0.8349	-0.7094*
β_{23}	0.1452	-1.4602*
β_1^2	-1.6161	-3.4877**
β_2^2	-1.6962	-2.4329*
β_3^2	0.5865	-2.1472
R^2	0.832	0.972

[1]RBD = Refined, bleached and deodourized; Subscripts: 1 = OR extract; 2 = OS; 3 = citric acid; *** = significant at 0.001 level; ** = significant at 0.01 level; * = significant at 0.05 level

Table 7. Predicted vs. experimental fatty acid profiles of the optimum antioxidant combination treatment after 5 d of frying

Fatty Acid	Composition (%)	
	Predicted	Experimental
C 14:0	1.13	1.10
C 16:0	41.62	42.00
C 16:1	0.17	0.15
C 18:0	4.13	4.21
C 18:1	44.71	44.01
C 18:2	7.89	7.90
C 18:3	0.03	0.03
C 20:0	0.25	0.28
Others	0.07	0.32
C18:2/C 16:0 ratio	0.189	0.188

R^2 = 0.9998

optimizing the natural antioxidants to be added to RBD palm olein for frying by the RSM was feasible.

Changes in the oil quality during frying

Table 8 shows the changes in the oil over 25 h frying of potato chips. All the antioxidant treatments significantly (P<0.05) reduced the oxidation rate of the oil from the common indicators used. AnV, PV, FFA, polymer content, colour units, viscosity, and adsorbances at 232 and 268 nm gradually increased, while IV and the C18:2/C16:0

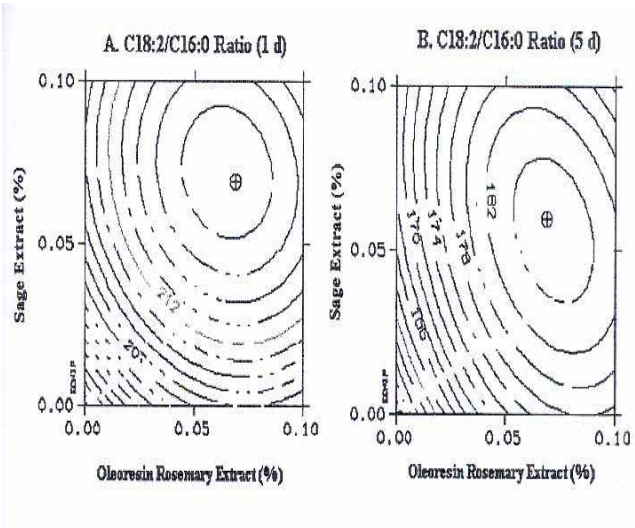

Figure 2. Contour maps of the effects of OR and OS at optimum level of CA on c18:2/C 16:0 (x 10^{-3}) ratio after 5 days of frying (Levels of CA for A=0.038%, B=0.037%).

ratio decreased. For almost all the quality parameters, adding OR, OS, CA or their combinations retarded the quality deterioration of RBD palm olein during frying. PV is a measure of the amount of peroxide formed in fats and oils through oxidation. Indirectly, it indicates the initial oxidation of the oil/fat. On day 1, the control (Trial 9) PV was 6.55 meq/kg, while the treatment PVs were 4.23 to 6.04 meq/kg. On Day 5, the control value had risen to 11.70 meq/kg while the treatment values were 6.10 to 10.89 meq/kg. For all the treatments, only one sample (Trial 4) had PV >10 meq/kg, while the rest were <7.11 meq/kg. The results in Table 8 also indicate that for all the samples, PV increased gradually until Day 5. Augustin and Berry[25] reported that hydroperoxides, the product of primary oxidation, degrade to secondary products, of which the aldehydic components are measured by the anisidine test. This test has an enhanced sensitivity for unsaturated aldehydes, especially 2,4-dienals, but does not measure the ketonic secondary products of oxidation.[25] In this study, there was a marked increase in AnV on the first day to 31.53 for the control, while the antioxidant-treated samples had values of 27.10 to 30.10. Also, in all the treatments the time of frying significantly increased AnV. On Day 5, the control reached 54.97 and the treatment samples 42.23 to 52.22.

IV is a measure of the number of double bonds in the oil. The decrease in IV of an oil after frying indicates its more oxidized sate. A big decrease in IV occurs with excessive deterioration of the oil.[25] The antioxidants used in this study significantly (P<0.05) decreased the fall in IV which corresponded to both PV and AnV During frying, the IV of all the treatments decreased significantly (P<0.05), from 54.10 - 55.70 on Day 1 to 41.92 - 47.21 g I_2/100 g on Day 5. The control IV decreased from 54.02 to 41.88 g I_2/100 g.

The changes in the FFA content of the oil during frying are shown in Table 8. The FFA contents all increased gradually with frying time. The increase could have been due to hydrolysis of the triacylglyceols by water introduced into the frying system from the potato chips. The natural antioxidants significantly (P<0.05) reduced the FFA contents of the oils during frying compared with the control.

The polymer content increased with frying time, with the increase mitigated by the natural antioxidants (Table 8). On Day 1, all the treatment samples had 0.41 to 0.61% polymer and the control 0.71%. On the final day, the control content was 1.97% and the treatment contents 1.39 to 1.89%. The increase was due to decomposition polymerizing[30], eg. the free radicals from the hydrolysis of hydroperoxides reacting to form polymers and other complex products.[31]

The colour changes of the thermally processed oils are also shown in Table 8. On Day 0, the treatment samples were darker than the control due to the colour of the added antioxidants. However, during frying the control became significantly (P<0.05) darker than most of the treatment samples. Lovibond units of 1.10 red and 13.15 yellow were recorded for the control on Day 1. The increases in red and yellow colour, due mostly to polymerization,[28] were similar for all the treatments. However, not all the darkening was due to oxidative deterioration of the oil, but also from colour absorbed from the fried food.

The changes in viscosity of the oils during frying are presented in Table 8. The control viscosity ranged from 53.17 cp on Day 1 to 66.40 cp on Day 5. The oil treated with OR and OS had narrower ranges. On Day 0, the treatment viscosities were 50.06 to 52.17 cp and increased to 58.50 to 65.95 cp at the end of frying. The addition of antioxidants generally significantly (P<0.05) reduced the viscosity although the effect was not significant in Sample 4 (CA added alone). With frying time, the viscosity increased. Berger[32] reported that the rate of oxidation of unsaturated fatty acids was directly related to the increase in oil viscosity. In addition to this explanation, it should be noted that polymeric materials are mainly responsible for the increase in oil viscosity.

The absorbance at 323 nm is similar to PV since it also measures the degree of primary oxidation. It was therefore not surprising that the absorbance results (Table 8) were closely related to those for PV. There was an increasing diene content with duration of frying. The absorances of the treatment samples were significantly (P<0.05) different from that of the control. The absorbance at 268 nm, which indicates formation of conjugated trienes increased gradually with frying time. There was a significant (P<0.05) effect of the antioxidants and frying time on absorbance of the oil (Table 8). The absorbance of the control oil was significantly (P<0.05) higher than those of the samples containing rosemary or sage extracts. The trend in absorbance was similar to that for AnV, which also measures the secondary oxidative products in

Table 8. Physico-chemical changes of RBD palm olein during deep-fat frying

Parameter	Frying Days	Trial Number														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Anisidine	1	28.52	28.20	29.05	30.10	28.05	27.10	29.15	28.06	31.53	28.65	28.10	27.99	28.05	28.00	29.10
Value	5	47.58	45.22	48.00	52.22	44.08	42.23	48.10	44.10	54.97	47.71	45.77	46.10	47.85	43.88	48.25
Peroxide Value	1	5.05	4.44	5.21	6.04	4.50	4.32	4.82	4.70	6.55	4.81	4.55	4.39	4.55	4.23	5.00
(meq/kg)	5	6.52	6.24	6.91	10.89	6.24	6.14	6.83	6.20	11.70	6.41	6.29	6.35	6.60	6.10	7.11
Iodine Value	1	54.15	54.95	54.65	54.10	55.10	54.80	55.05	55.70	54.02	55.01	54.99	55.20	55.13	54.65	54.85
(g I_2/100 g oil)	5	45.22	46.10	44.20	41.92	46.22	47.21	44.22	46.11	41.88	45.10	45.57	45.44	45.00	47.10	43.78
Free Fatty	1	0.12	0.12	0.13	0.14	0.12	0.12	0.13	0.11	0.14	0.13	0.12	0.12	0.12	0.11	0.13
Acid (%)	5	0.41	0.36	0.43	0.49	0.34	0.33	0.42	0.33	0.52	0.40	0.36	0.36	0.42	0.34	0.43
Polymer	1	0.49	0.44	0.56	0.61	0.42	0.40	0.53	0.41	0.71	0.45	0.46	0.49	0.52	0.40	0.55
Content (%)	5	1.53	1.44	1.57	1.89	1.42	1.39	1.54	1.40	1.97	1.49	1.47	1.47	1.54	1.40	1.58
Red Color	1	1.10	1.07	1.10	1.10	1.08	1.10	1.10	1.05	1.10	1.10	1.10	1.08	1.10	1.05	1.10
	5	1.31	1.30	1.33	1.35	1.27	1.28	1.32	1.25	1.35	1.32	1.30	1.30	1.34	1.25	1.35
Yellow	1	12.90	12.75	12.95	13.08	13.05	12.80	13.10	13.15	13.15	12.95	12.95	12.90	12.85	12.95	12.90
Color	5	14.05	13.90	14.05	14.10	13.90	14.00	14.10	14.00	14.10	14.00	13.95	14.00	14.10	14.00	14.10
Viscosity	1	52.84	52.40	52.61	53.01	51.89	52.17	52.81	52.26	53.17	52.50	52.44	52.66	52.62	52.62	52.71
(centipoise)	5	61.02	59.22	61.10	65.95	58.21	58.44	61.10	59.04	66.40	60.70	59.87	60.10	60.90	58.50	62.00
$E^{1\%}_{1cm}$	1	2.65	2.46	2.80	3.82	2.44	2.50	2.72	2.48	3.91	2.62	2.62	2.67	2.71	2.45	2.93
at 232 nm	5	5.40	4.95	6.10	9.00	4.80	4.84	6.10	4.85	9.01	5.25	5.00	5.10	5.80	4.80	6.25
$E^{1\%}_{1cm}$	1	0.72	0.65	0.91	1.53	0.63	0.62	0.90	0.63	1.75	0.71	0.71	0.70	0.73	0.63	0.75
at 268 nm	5	1.56	1.41	1.70	2.05	1.38	1.37	1.65	1.38	2.08	1.50	1.49	1.50	1.62	1.37	1.70
C18:2/C16:0	1	0.207	0.215	0.207	0.201	0.217	0.215	0.207	0.214	0.190	0.206	0.211	0.209	0.209	0.212	0.207
Ratio	5	0.167	0.177	0.163	0.154	0.178	0.183	0.163	0.181	0.135	0.173	0.175	0.174	0.166	0.182	0.160

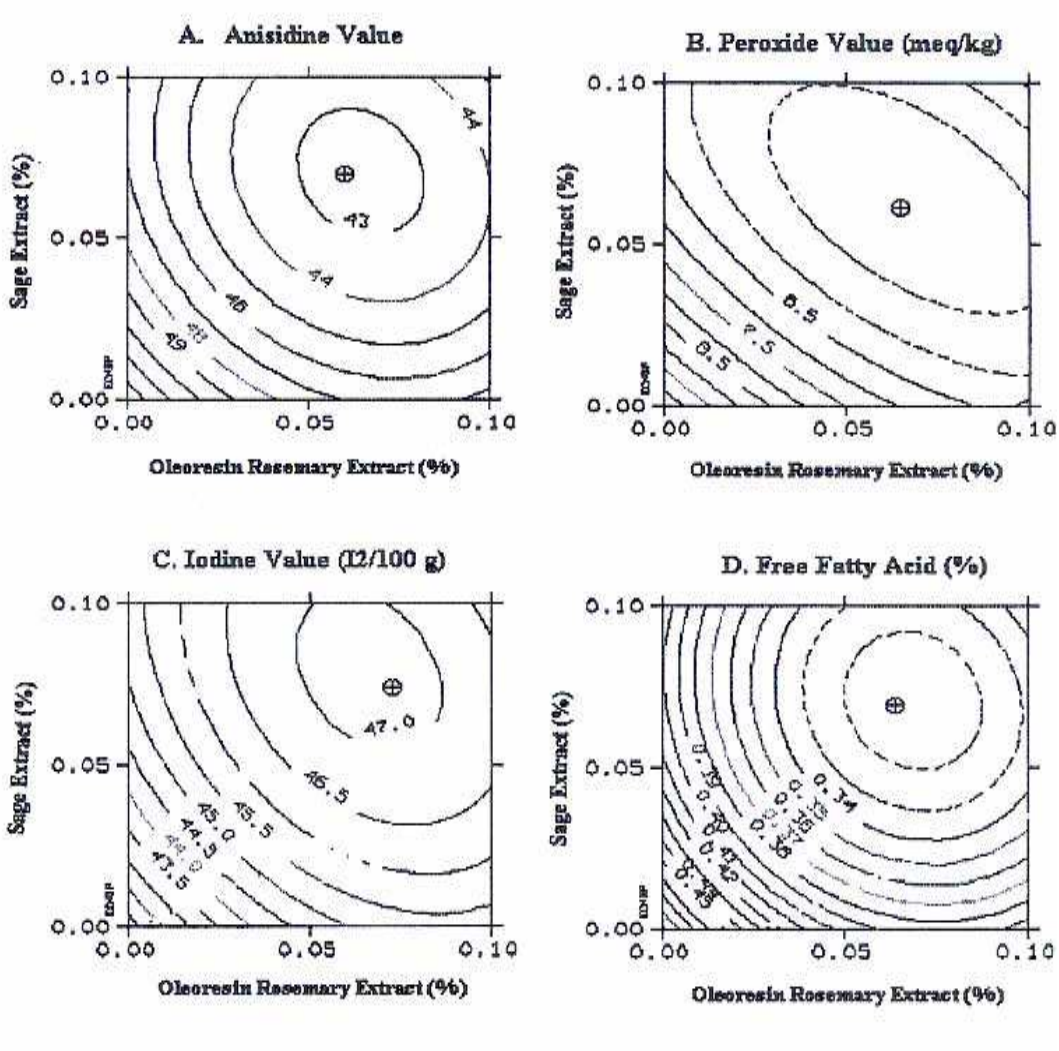

Figure 3. Contour maps of the effects of OR and OS at optimum level of CA on AnV, PV, IV and FFA after 5 days of frying (Levels of CA for A=0.025%, B=0.026%, C=0.049% and D=0.043%).

oil. The only difference is that absorbance at 268nm also measures ketones, particularly diethylenic ketones, which are not monitored by AnV.[25]

Optimizing the physico-chemical properties of theRBD palm olein after day 5 of frying

The regression coefficients for all the physico-chemical parameters of the oil for five days' frying of potato chips are summarized in Tables 9 and 10. The high coefficients of determination (R^2) indicated that the regressions fitted the data well and could, therefore, be used for prediction and optimization of the antioxidant mixtures. All the parameters had R^2 >0.90, with FFA content having the highest value (0.994), followed by $E^{1\%}_{1cm}$ at 268 nm (0.990), $E^{1\%}_{1cm}$ at 232 nm (0.975) and the C18:2/C16:0 ratio (0.972). The R^2 values for AnV, PV, IV, polymer content and viscosity were 0.945, 0.904, 0.961, 0.960 and 0.961, respectively, while red and yellow colours had R^2 of 0.932 and 0.903, respectively. Any R^2 >0.75 is considered sufficiently accurate for predicting the changes in oil quality.[26, 27]

From the tests of significance, OR and OS were once again found to be the most important factors influencing all the physico-chemical characteristics (Tables 9 and 10). OR had highly significant (P<0.001) effects on FFA, absorbances at 232 and 268 nm and the C18:2/C16:0 ratio. OR was highly significant (P<0.01) on AnV, IV, polymer content, yellow colour and viscosity, and significant (P<0.05) on PV and red colour. OR, in the second order term, in fact, produced highly significant (P<0.001) effects on the FFA content, absorbance at 268 nm and the C18:2/C16:0 ratio (P<0.01). Similarly, the level of OS had highly significant (P<0.001) effects on FFA content and absorbance at 268 nm. The second order terms of OS also produced a highly significant effect (P<0.001) on FFA content and absorbance at 268 nm (P<0.01). Significant (P<0.05) effects of OS were seen on absorbance at 232 nm, viscosity, C18:2/C16:0 ratio, AV, IV and polymer content.

CA alone only had a significant effect on FFA (P<0.01) and absorbance at 268 nm (P<0.05). No effect of the second order term for CA was found. However, the interaction between CA and OS gave a significant (P<0.05) effect on absorbance at 268 nm and the C18:2/C16:0 ratio. Tables 9 and 10 also show that, except for AV and yellow colour, the interaction between OR and OS was significant (P<0.01) for all the parameters examined. Figure 3 shows the response contours for three the parameters examined - AnV, PV, IV and Free fatty

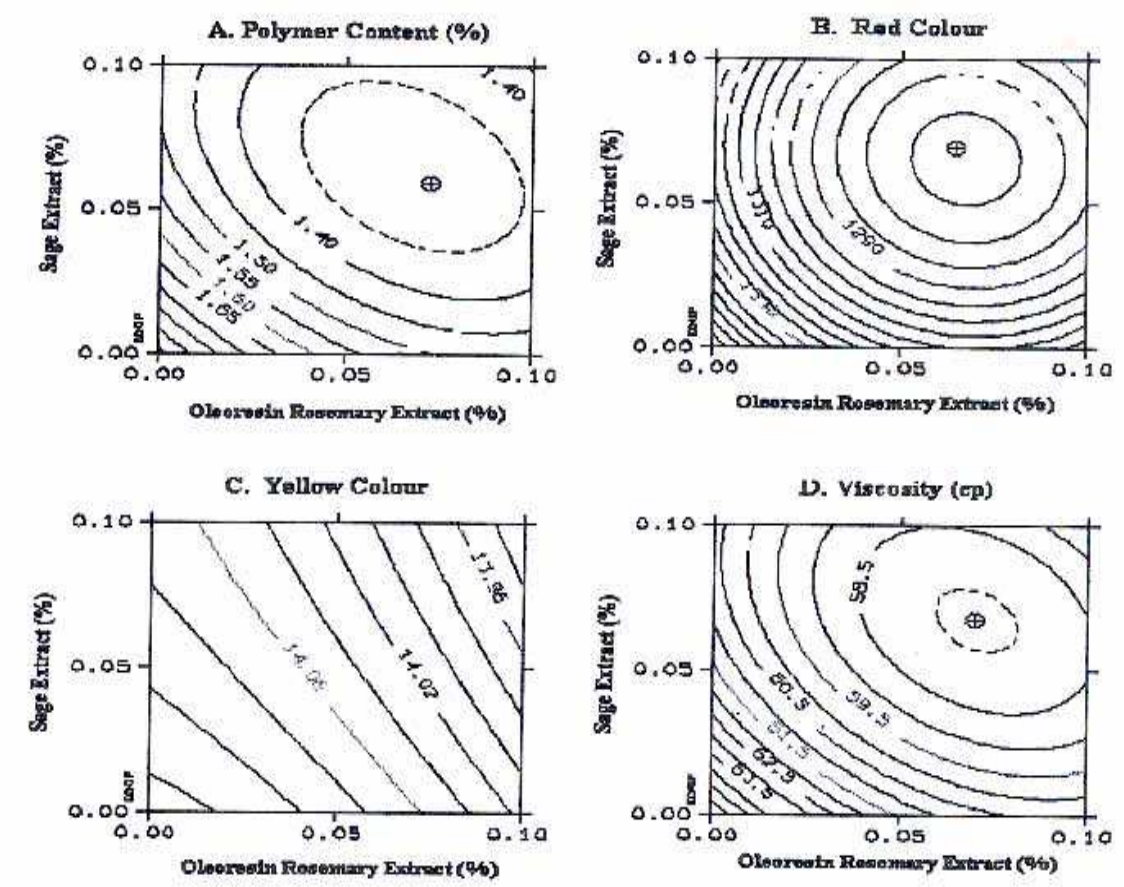

Figure 4. Contour maps of the effects of OR and OS at optimum level of CA on polymer content, colors and viscosity after 5 days of frying (Levels of CA for A=0.034%, B=0.025%, C=0.025% and D=0.025%).

acids (FFA). Figure 4 gives the contours for polymer content, colours and viscosity, while the contours for absorbances at 232 and 268 nm are shown in Figure 5. Except for yellow colour, the contours clearly indicate the optimum combinations for all the parameters - all within the ranges of the antioxidants used. For many of the responses, using only a moderate level of antioxidant sufficed to produce the optimum points. For AnV and PV, the optimum antioxidant combinations were 0.061% OR, 0.070% OS and 0.025% CA, and 0.067% OR, 0.062% OS and 0.026% CA, respectively.

Table 9. Regression coefficients, R^2 and P of F values for AnV, PV and IV, polymer content and colours of RBD palm olein after 5 days of frying

	FFA	$E^{1\%}_{1cm}$ at 232 nm	$E^{1\%}_{1cm}$ at 268 nm	Viscosity
β_0 intercept	0.33	4.57	1.36	58.50
β_1	-0.77***	-18.11***	-3.17***	-31.54**
β_2	-0.66***	-16.24**	-2.36***	-28.95**
β_3	-0.49**	-2.17	-1.29*	-11.15
β_{12}	3.80*	295.00**	38.82**	386.25*
β_{13}	-1.07	-7.57	-0.61	-195.99
β_{23}	-0.41	-243.33	-35.69*	-324.83
β_1^2	18.84***	307.77*	58.34**	611.50*
β_2^2	14.37***	305.57*	56.14**	604.00*
β_3^2	11.00	398.95	56.82	586.88
R^2	0.994	0.975	0.990	0.961
P of F	0.0	0.002	0.0	0.005

Subscripts: 1 = OR extract; 2 = OS extract; 3 = CA; ** = significant at 0.01 level; *= significant at 0.05 level

For IV and FFA, the CA levels for the optimum combinations were slightly higher - 0.073% OR, 0.073% OS and 0.049% CA and 0.065% OR, 0.0714% OS and 0.043% CA, respectively. For polymer content, 0.073% OR, 0.064% OS and 0.034% Ca were required (Figure 4). From Figure 4, the optimum combinations for red colour and viscosity were 0.069% OR, 0.071% OS and 0.025% CA, and 0.070% OR, 0.065% OS and 0.025% CA, respectively, and for absorbances at 232 and 268 nm, 0.069% OR, 0.076% OS and 0.037% CA, and 0.069% OR, 0.066% OS and 0.042% CA, respectively.

As mentioned earlier, FFA was the most important dependent variable, giving the highest R^2-value to natural antioxidants after 5 days' frying. To verify the results, a frying experiment was run using the optimum combination for FFA (0.065% OR, 0.071% OS and 0.043% CA). The physico-chemical changes of RBD palm olein after 5 days' frying were compared with predicted data from the Echip software (Table 11). There was a very high correlation (R^2 = 0.999) between the two data sets, and thus the optimization study once again supports the use of RSM for predicting the levels of natural antioxidants to be added to RBD palm olein for frying.

Conclusion

The addition of OR, OS and CA effectively retarded the deterioration of RBD palm olein over five days of frying potato chips. C18:2 and C16:0 were the most important fatty acids contributing the changes in the oil quality after

Table 10. Regression coefficients, R^2 and P of F-values for FFA, adsorbances at 232 and 268 nm, viscosity and C18:2/C 16:0 ratio

	AnV	PV	IV	Polymer Content	Red Colour	Yellow Colour
β_0 (intercept)	43.35	5.44	46.57	1.35	1.28	14.05
β_1	-37.32**	-18.63*	21.64**	-2.06**	-0.37*	-1.31**
β_2	-39.26**	-19.47*	16.18**	-1.87**	-0.29*	-0.76*
β_3	-28.65	0.04	11.28	-0.73	-0.76	0.15
β_{12}	338.57	440.51*	-241.36*	33.88*	1.12*	-3.24
β_{13}	-136.22	-0.67	42.00	-5.28	5.68	13.00
β_{23}	-68.19	-234.99	275.96	16.23	-0.42	0.18
β_1^2	1056.05*	352.29	480.45*	45.05*	10.15*	-6.93
β_2^2	842.64*	424.87	-328.67*	46.80*	8.54	1.99
β_3^2	964.49	710.04	-308.91	67.70	-6.07	-33.93
R^2	0.945	0.904	0.961	0.960	0.932	0.903
P of F	0.011	0.041	0.005	0.005	0.019	0.043

Table 11. Predicted vs. experimental physico-chemical characteristics[a] of RBD palm olein with optimal combination treatment after 5 days of frying

Characteristic	Predicted	Experimental
Peroxide value (meq/kg)	5.29	5.22
Anisidine value	42.42	42.96
Iodine value (g I_2/100g oil)	47.13	47.29
Free fatty acid (%)	0.31	0.31
Polymer content (%)	1.32	1.39
Colour (red unit)	1.25	1.19
Colour (yellow unit)	14.00	13.90
Viscosity	57.75	58.20
$E^{1\%}_{1cm}$ at 232 nm	4.26	4.30
$E^{1\%}_{1cm}$ at 268 nm	1.29	1.36
C 18:2/C 16:0 ratio	0.189	0.183

R^2 = 0.999, [a] Mean of three replication

frying. R^2 values for C18:2 n-6 and C16:0 after 5 d of frying were 0.946 and 0.825, respectively. For optimizing the amounts of antioxidants to be added, the C18:2/C16:0 ratio was the best predictor as after five days' frying, all the three antioxidants had a significant effect on the ratio as given by the second order forms. There were also clear synergistic effects between the anti-oxidants on this fatty acid ratio. Based on the results obtained, a combination of 0.076% OR, 0.066% OS and 0.037% CA is recommended for adding to RBD palm olein for protection during frying.

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Performance of phytochemical antioxidant systems in refined-bleached-deodorized palm olein during frying

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抗氧化剂是对食物氧化损伤有重要抑制作用的一类化合物。本次研究将探讨多种植物化学抗氧化体系[迷迭香油树脂（OR）、香紫苏油树脂(OS)和柠檬酸(CA)]对薯片油炸过程中精制的漂白-除臭(RBD)棕榈油酸甘油酯的物理化学特性的影响，同时也研究了反复油炸过程中各种复合抗氧化剂对油的影响。效应面优化法用于优选所使用的抗氧化剂复合物成分, 同时用合成抗氧化剂作为对照研究,取煎炸后的油进行各种物理化学特性分析。研究结果表明，OR 和 OS 是有效的植物化学抗氧化剂，它可保护 RBD 棕榈油酸甘油酯在油炸过程中免受氧化破坏。

关键词：迷迭香油、香紫苏油、植物化学抗氧化剂、深度油炸、棕榈油