Optimization of Physicochemical Changes of Palm Olein with Phytochemical Antioxidants During Deep-Fat Frying

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ABSTRACT: Response surface methodology (RSM) was used to optimize the amounts of rosemary and sage extracts together with citric acid as synergist antioxidants in stabilizing refined, bleached, and deodorized palm olein during repeated deep-fat frying of potato chips. For all physicochemical properties studied, these phytochemical antioxidant treatments significantly ($P < 0.05$) reduced the oxidation rate of the oil. During 5 d of frying, anisidine value, peroxide value, free fatty acid, polymer content, color units, viscosity, and absorbances at 232 and 268 nm gradually increased, whereas iodine value and ratio of 18:2/16:0 decreased. Further statistical analyses, including coefficient of determination (R^2) and probability of F values, indicated that mathematical models for each physicochemical parameter could be developed confidently in this study, with R^2 for all parameters greater than 0.90. These results suggested that an optimal mixture of phytochemical antioxidants derived from rosemary and sage together with citric acid could be produced using RSM for stabilizing thermally processed oil. For many physicochemical parameters examined, the use of moderate levels of antioxidants could result in optimal responses.

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KEY WORDS: Antioxidants, optimization, potato chips, response surface methodology, rosemary, sage.

Lipid oxidation is one of the major deteriorative reactions in frying oils and fried foods, and often results in a significant loss of quality. It is well established that lipid oxidation leads to changes in functional, sensory, and nutritive values as well as in the safety of fried foods (1). For these reasons, antioxidants are added to fats, oils, and foods containing fats. With awareness concerning the use of commercial synthetic antioxidants in the food system, many food manufacturers have shown considerable interest in the use of natural sources of antioxidant during the last few years (2).

Rosemary and sage are two plant sources of antioxidants that have been reported among herbs and spices for use as antioxidants in fat and oil systems (3). These plant-derived antioxidants have been studied extensively and proven to be effective for stabilizing frying oils, while having very good thermal resistance (4). When used in palm olein for frying potato chips, Irwandi and Che Man (5) found that both of these antioxidants retarded oil deterioration during frying and increased the acceptability of fried product.

Response surface methodology (RSM) is a useful statistical technique that uses quantitative data from appropriate experimental designs to determine and simultaneously solve multivariate equations. Haryati *et al.* (6) reported optimization of chemical transesterification of palm oil using RSM. Shieh *et al.* (7) used RSM for optimizing enzymatic transesterification of triolein with capric acid, whereas Huang and Akoh (8) used the program for the optimization of enzymatic transesterification of vegetable oils with ethyl caprylate. Application of this method also was reported by Cho *et al.* (9) for the formulation of partially interesterified canola/palm blends. Thus, RSM has been used successfully for fat and oil research.

The present study was performed to optimize the use of rosemary and sage extracts together with citric acid (CA) as a synergist antioxidant in stabilizing refined, bleached, and deodorized (RBD) palm olein during repeated deep-fat frying of potato chips using RSM.

MATERIALS AND METHODS

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

Experimental design. RSM was used to investigate the effect of OR, OS, and CA and different combinations on changes in the physicochemical properties of RBD palm olein and to determine the optimal combinations during 5 d of repeated deep-fat frying. An RSM-based computer program, namely, the Echip software (10), was used in this study to provide initial experimental designs, calculate multiple regression equations, and provide statistical evaluations. RSM basi-

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01TABLE 1
Physicochemical[a] Changes of RBD Palm Olein During Deep-Fat Frying

		Trial number														
		9 (Control)	1	2	3	4	5	6	7	8	10	11	12	13	14	15
Parameter		Percentage of additives[b] added														
	OR	0	0	0.1	0	0	0.1	0.05	0	0.05	0.1	0.1	0.05	0.05	0.1	0
	OS	0	0.1	0.05	0.1	0	0.1	0.1	0.05	0.05	0	0.1	0.1	0	0.05	0.05
	CA	0	0.05	0	0	0.05	0.05	0.025	0.025	0.05	0.025	0.025	0	0	0.05	0
	Frying days															
Anisidine value	Fresh	1.0														
	0	1.0a	1.0a	1.0a	0.9a	1.0a	1.0a	0.9a	1.0a	0.9a	0.9a	0.9a	1.0a	1.0a	1.0a	1.0a
	1	31.5a	28.5b	28.2b,c	29.1b	30.1b	28.1b,c	27.1c	29.2b	28.1b,c	28.7b	28.1b,c	28.0b,c	28.1b,c	28.0b,c	29.1b
	2	36.1a	35.3a,b	36.5a	36.9a	36.5a	35.2a,b	34.7b	36.1a	35.3a,b	35.5a,b	34.8b	33.7c	34.2b	35.1b	36.7a
	3	40.2a,b	40.1a,b	40.4a,b	41.1a	41.2a	39.9a,b	38.8b	42.1a	40.1a,b	41.9a	39.5a,b	39.9a,b	38.6a,b	38.8b	39.5a,b
	4	51.4a	44.6b,c	42.6c	44.2b,c	46.9b	42.5c	40.3c	45.8b	42.7c	44.5b,c	43.2b,c	44.2b,c	45.2b,c	41.4c	42.0c
	5	55.0a	47.6b	45.2c	48.0b	52.2a,b	44.1c,d	42.2d	48.1b	44.1c,d	47.7b	45.8c	46.1c	47.9c	43.9c,d	48.3b
Peroxide value (meq/kg)	Fresh	0.91														
	0	0.9a	0.9a	0.9a	0.9a	0.9a	0.9a	0.9a	0.9a	0.9a	0.9a	0.9a	0.9a	0.9a	0.9a	0.9a
	1	6.6a	5.1b	4.4c	5.2b	6.0a	4.5b,c	4.3c	4.8b,c	4.7b,c	4.8b,c	4.6b,c	4.4c	4.6b,c	4.2c	5.0b
	2	7.8a	5.2c	4.6d	5.4c	7.1b	5.3c	5.1c,d	5.2c	5.2c	5.5c	5.00d	4.7d	4.9d	4.9d	5.4c
	3	8.1a	5.6b	5.3b	5.7b	7.9a	5.8b	5.7b	5.8b	5.6b	5.9b	5.3b	5.3b	5.5b	5.7b	5.9b
	4	8.5a	6.2b	5.8c	6.3b	8.4a	6.00b,c	6.0b,c	6.1b,c	5.9b,c	6.3b	5.8c	5.9c	6.2b	5.9b,c	6.4b
	5	11.7a	6.5b	6.2b	6.9b	10.9a	6.2b	6.1b	6.8b	6.2b	6.4b	6.3b	6.4b	6.6b	6.1c	7.1b
Iodine value (g I_2/100 g oil)	Fresh	56.07														
	0	56.0b	56.1a,b	56.1a,b	56.1b	56.1b	56.1a,b	56.0a	56.1a,b	56.1a,b	56.2a	56.2a	56.2a	56.1b	56.1a,b	56.1b
	1	54.0c	54.3c	55.0a,b	54.7b	54.1c	55.1a	54.8b	55.1a,b	55.7a	55.01a,b	55.0a,b	55.2a	55.1a	54.7b	54.9b
	2	48.8c	52.1b	53.1a	52.7a,b	51.8b,c	52.3b	52.4b	53.2a	53.3a	53.7a	52.4b	53.2a	52.6a,b	52.5b	52.0b,c
	3	45.3e	50.2a	50.2a	49.5b	47.3d	49.9a,b	50.1a,b	49.1b	51.0a	51.3a	49.7b	48.8c	49.9a,b	49.5b	48.8c
	4	43.4e	48.1a	48.2a	47.1b	44.2d	47.1b	48.2a	46.5c	48.7a	47.9a	47.8a,b	46.2c	47.5b	48.2a	46.6c
	5	41.9d	45.2b	46.1a,b	44.2c	41.9d	46.2a,b	47.2a	44.2c	46.1a,b	45.1b	45.6b	45.4b	45.0b,c	47.1a	43.8c
Free fatty acid (%)	Fresh	0.1														
	0	0.1a	0.1a	0.1a	0.1a	0.1a	0.1a	0.1a	0.1a	0.1a	0.1a	0.1a	0.1a	0.1a	0.1a	0.1a
	1	0.1a	0.1a,b	0.1a,b	0.1a	0.1a	0.1a,b	0.1a,b	0.1a	0.1b	0.1a	0.1a,b	0.1a,b	0.1a,b	0.1b	0.1a
	2	0.1a	0.2b	0.6b	0.2a	0.8a	0.6b	0.2b	0.2a,b	0.6b	0.9a,b	0.8a	0.9a	0.8a	0.2b	0.2a
	3	0.3a	0.2b,c	0.2b,c	0.2b,c	0.2b	0.8c	0.2c	0.2b	0.7c	0.2b	0.2b,c	0.2b,c	0.2b,c	0.2b,c	0.2b
	4	0.4a	0.3b,c	0.2d	0.3b,c	0.3b	0.2d	0.2e	0.3b,c	0.2d	0.3b,c	0.3b,c	0.2d	0.3d	0.2e	0.3c
	5	0.5a	0.4c	0.4d	0.4c	0.5b	0.3e	0.3e	0.4c	0.3e	0.4c	0.4d	0.4d	0.4d	0.3e	0.4c
Polymer content (%)	Fresh	0.01														
	0	0.01b	0.01a	0.01a	0.02a	0.03b	0.02a,b	0.01b	0.01b	0.02a,b	0.01b	0.01b	0.01b	0.02a	0.02a	0.02a,b
	1	0.7a	0.5d	0.4d,e	0.6c	0.6b	0.4e	0.4e	0.5c	0.4e	0.5d,e	0.5d,e	0.5d	0.5c	0.4e	0.6c
	2	1.0a	0.7b,c	0.6c	0.7b,c	0.8b	0.5c,d	0.5d	0.7b,c	0.5c,d	0.60c	0.6b,c	0.6b,c	0.6b,c	0.6c,d	0.7b,c
	3	1.3a	0.9c	0.9c,d	1.0b,c	1.1b	0.8d	0.7e	0.8d	0.8e	0.8d,e	0.9c,d	0.8d	0.9c	0.7f	0.9c
	4	1.6a	1.3c	1.3c,d	1.3c	1.5b	1.2d	1.0f	1.3c	1.0e	1.2d	1.2d	1.3c,d	1.3c,d	1.1e	1.3c
	5	2.0a	1.5b	1.4c	1.6b	1.9a	1.4d	1.4d	1.5b	1.4c,d	1.5c	1.5c	1.5c	1.5b	1.4c,d	1.6b

(*continued*)

TABLE 1 (Continued)

Parameter		Trial number														
		9 (Control)	1	2	3	4	5	6	7	8	10	11	12	13	14	15
		Percentage of additives[b] added														
	OR	0	0	0.1	0	0	0.1	0.05	0	0.05	0.1	0.1	0.05	0.05	0.1	0
	OS	0	0.1	0.05	0.1	0	0.1	0.1	0.05	0.05	0	0.1	0.1	0	0.05	0.05
	CA	0	0.05	0	0	0.05	0.05	0.025	0.025	0.05	0.025	0.025	0	0	0.05	0
	Frying days															
Red color	Fresh	0.5														
	0	0.6c	0.7b	0.7b,c	0.7b	0.6c	0.7a	0.7a	0.7b	0.7b	0.7b,c	0.7a	0.7a	0.6b	0.7a	0.6b
	1	1.1a	1.1a	1.1b	1.1a	1.1a	1.1b	1.1a	1.1a	1.1c	1.1a	1.1a	1.1b	1.1a	1.1c	1.1a
	2	1.2a	1.1c	1.1c	1.1b,c	1.1c	1.1c	1.1b	1.1c	1.1d	1.1c	1.1c	1.1b,c	1.1c	1.1c	1.2a
	3	1.2b	1.2b,c	1.2c	1.2b	1.2c	1.2c	1.2b,c	1.2c	1.1d	1.2b	1.2b	1.2c	1.2b	1.2c	1.3a
	4	1.3a	1.2c	1.2c	1.3b	1.2b,c	1.2c	1.2c	1.3b	1.2c	1.3b	1.3b	1.2b,c	1.3b	1.2c	1.3a
	5	1.4a	1.3b	1.3b	1.3a,b	1.4a	1.2b,c	1.3b,c	1.3a,b	1.3c	1.3a,b	1.3b	1.3b	1.3a	1.3c	1.4a
Yellow color	Fresh	5.9														
	0	6.1d	7.1a,b	7.1b	7.0b	6.1d	7.25a	7.15a	6.9c	7.0b	7.0c	7.2a	7.1b	6.7c	7.1b	6.9c
	1	13.2a	12.9b,c	12.78c	13.05b	13.1a	13.1a,b	12.8c	13.1s	13.1a,b	13.0b	13.0b	12.9b,c	12.9b,c	13.0b	12.9b,c
	2	13.2a,b	13.0d	13.0d	13.0d	13.2a,b	13.2a,b	13.0d	13.3s	13.2b	13.1c	13.1c	13.1c	13.0d	13.1c,d	13.1c
	3	13.8a	13.6b,c	13.4d	13.5c	13.6b,c	13.5c	13.4d	13.7b	13.6b	13.5c	13.4d	13.4d	13.3e	13.4d	13.4d
	4	13.9a	13.8a,b	13.8b	13.8b	13.9a	13.8b	13.7b,c	13.9a	13.8a,b	13.9a	13.7b,c	13.7c	13.7b,c	13.7b,c	13.8a,b
	5	14.1a	14.1a	13.9b	14.1a	14.1a	13.9b	14.0a,b	14.1a	14.0a,b	14.0a,b	14.0b	14.0a,b	14.1a	14.0a,b	14.1a
Viscosity (cp)	Fresh	50.2														
	0	50.2a	50.2a	50.3a	50.2a	50.2a	50.2a	50.2a	50.2a	50.2a	50.2a	50.2a	50.2a	50.1a	50.1a	50.2a
	1	53.2a	52.8b	52.4c	52.6b	53.0a	51.9d	52.2c	52.8b	52.3c	52.5b,c	52.4c	52.7b	52.6b	52.6b	52.7b
2	54.2a	53.3c	53.0c	53.0c	54.1a	52.8d	53.0c	53.4c	53.5b,c	54.0a	53.7b,c	53.0c	53.1c	53.0c	53.6b,c	53.6b,c
	3	57.3a	55.7b	54.6c	55.2b	56.5a	53.9c	54.0c	55.1b	54.5b,c	56.9a	55.8b	55.0b,c	54.8b,c	54.0c	56.8a
	4	62.1a	59.6b	56.3c,d	58.2b,c	60.2b	55.2d	56.1c,d	57.2c	56.5c,d	57.3c	57.1c	57.7c	57.8c	56.4c,d	58.6b,c
	5	66.4a	61.0b	59.2c	61.1b	66.0a	58.2c	58.4c	61.1b	59.0c	60.7b	59.9b,c	60.1b	60.9b	58.5c	62.0b
$E^{1\%}_{1cm}$ at 232 nm	Fresh	1.7														
	0	1.7a	1.8a	1.8a	1.7a	1.7a	1.7a	1.7a	1.7a	1.7a	1.8a	1.7a	1.8a	1.7a	1.7a	1.7a
	1	3.9a	2.7b,c	2.5d	2.8b	3.8a	2.4d	2.5d	2.7b	2.5d	2.6c	2.6c	2.7b,c	2.7b	2.5d	2.9b
	2	5.1a	3.0d	2.9d	3.0d	4.2b	2.7e	2.8d,e	3.6c	3.0d	2.8d	2.8d,e	2.8d	3.1c	2.7e	3.5c
	3	8.2a	3.1d,e	3.1e	3.4c,d	5.9b	3.5c,d	3.2d	4.6c	3.4d	3.4d	3.2d,e	3.2d	3.9c	3.3d	4.1c
	4	8.9a	4.2d,e	4.2d,e	4.2d,e	7.1b	4.3d	4.6d	5.1c	4.0e	4.6c,d	4.3d	4.3d,e	4.7c	4.2d,e	5.0c
	5	9.0a	5.4b,c	5.0c	6.1b	9.0a	4.8c	4.8c	6.1b	4.9c	5.3b,c	5.0c	5.1c	5.8b	4.8c	6.3b
$E^{1\%}_{1cm}$ at 268 nm	Fresh	0.4														
	0	0.4a	0.4a	0.4a	0.4a	0.4a	0.4a	0.4a	0.4a	0.4a	0.4a	0.4a	0.4a	0.4a	0.4a	0.4a
	1	1.8a	0.7d	0.7e	0.9c	1.5b	0.6e	0.6e	0.9c	0.6e	0.7d	0.7d	0.7d	0.7d	0.6e	0.8d
	2	1.9a	0.9d	0.8e	1.0d	1.7b	0.7e	0.8e	1.1c	0.8e	0.8e	0.9d	0.9d,e	0.9d	0.8e	1.0d
	3	2.0a	1.1d	1.1e	1.2c	1.8b	1.1d	1.1d	1.3c	1.0e	0.9e	1.2c	1.2c	1.1d	0.8e	1.2c
	4	2.0a	1.3c	1.2c	1.4b	1.9a	1.2c	1.2c	1.4b,c	1.2d	1.3c	1.4b,c	1.4b,c	1.4b,c	1.2c	1.5b
	5	2.1a	1.6c	1.4d	1.7b	2.1a	1.4d	1.4d	1.7b	1.4d	1.5c	1.5c	1.5c	1.6b	1.4d	1.7b
18:2/16:0 ratio	Fresh	0.29														
	0	0.25b	0.3a	0.26a	0.26a	0.25b	0.26a	0.26a	0.26a	0.26a	0.26a	0.27a	0.26a	0.26a	0.26a	0.26a
	1	0.19c	0.21a,b	0.22a	0.21a,b	0.20b	0.22a	0.22a	0.21a,b	0.21a	0.21a,b	0.21a	0.21a	0.21a	0.21a	0.21a,b
	2	0.18c	0.20b	0.20b	0.20b	0.18c	0.21a	0.21a	0.21a	0.21a	0.20b	0.20b	0.21a	0.20b	0.21a	0.21a
	3	0.16d	0.19b,c	0.19b,c	0.19b	0.18c	0.19b	0.20b	0.20b	0.20a	0.20a	0.19b	0.20a	0.19b	0.20a	0.20b
	4	0.15e	0.17d	0.18b	0.17d	0.17d	0.19a,b	0.19b	0.18b,c	0.19b	0.19a	0.19b	0.18b	0.17d	0.20a	0.18c
	5	0.14d	0.17b	0.18a	0.16b	0.15c	0.18a	0.18a	0.16b	0.18a	0.17b	0.18a,b	0.17a,b	0.17b	0.18a	0.16b

[a]Each value in the table represents the mean of three replications. Means within each row with different letters are significantly ($P < 0.05$) different.
[b]Additives: OR = oleoresin rosemary extract, OS = sage extract, CA = citric acid.

cally uses an experimental design such as the central composite design (CCD) to fit a model by least squares analysis (10). Initial concentrations of OR and OS ranged from 0 to 0.1% each, and CA from 0 to 0.05%, according to Irwandi and Che Man (5). A total of 15 different combinations of the three additives (Table 1), established from the Echip software, including a control that contained no added antioxidants or CA, was tested to evaluate effectiveness in preserving the fatty acid composition of the RBD palm olein in frying experiments with potato chips. The experiment was performed in three replications.

Frying experiment. Fresh potatoes for frying were manually peeled and sliced to 1.5-mm thickness. The sliced potatoes were then soaked in 2.5% sodium chloride solution for 5 min, filtered, and surface-dried using paper napkins before frying. OR, OS, and CA were added into RBD palm olein immediately prior to frying.

All 15 frying experiments conducted in this study were similar to that reported by Che Man and Irwandi (11). Fryings were conducted in batch fryers (Model ELT 8B; Berto's, Padova, Italy). Oil (4 kg) was heated to 60°C, and 200 g was removed from the fryer to represent a day 0 sample. Heating was continued to a temperature of 180 ± 5°C in 10 min, and frying was started 30 min after the temperature of the oil reached 180°C. Fresh sliced potatoes (100 g) were fried for 2.5 min. Following this process, the oil temperature was allowed to return to 180°C within 30 min. Ten fryings with a total period of 5 h were completed every day for five consecutive days. The fryers were left uncovered during the frying operations. Fryings were conducted in three replications, with all replications for each frying experiment carried out at the same time. At the end of each day, 200 g oil at a temperature of 60°C was removed from the fryer, flushed with nitrogen gas, and kept in a cold room at 4°C until analysis. Analysis of oil was made 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. Fresh oil and additives were not added to the frying vessel. At the end of this study, a frying experiment with an optimal combination of OR, OS, and CA, obtained from the physicochemical characteristics with the highest R^2, was also performed. This sample was used to validate data obtained from the software (predicted data) and to evaluate effects of these additives on other oxidative parameters during frying.

Analyses of oil. Peroxide value (PV), anisidine value (AV), free fatty acid (FFA), and iodine value (IV) were determined using PORIM test methods (12), numbered p2.3, p2.4, p2.5, and p3.2, respectively. The AV was determined by measuring absorbances at 350 nm. The oil color was measured in one-inch cells in a Lovibond tintometer (Salisbury, United Kingdom) using PORIM test method No. p4.1 (12). Polymer content was analyzed according to the method of Peled *et al.* (13). The absorbances at 232 and 268 nm were obtained using IUPAC's No. 2.505 method (14). Each reported value is the mean of three replications. The ratio of 18:2/16:0 was obtained from analysis of fatty acid composition. The fatty acid profile of the oil was determined by gas chromatography (Hewlett-Packard gas chromatography Model 5890; Palo Alto, CA) as reported by Berry (15) using a 15 m × 0.53 mm capillary column and a flame-ionization detector. The temperature of the column was 140°C, set to increase at 4°C/min to 200°C. The temperature of the injector and detector was 250°C. Flow rates for carrier gas nitrogen, hydrogen, and air were 65, 44, and 440 mL/min, respectively. 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 are as follows (10):

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

where response = concentration of each parameter examined; 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 oleoresin rosemary extract in oil; (OS) = concentration of oleoresin sage extract in oil; (CA) = concentration of citric acid in oil.

Statistical analyses of the effects of each antioxidant and CA and their interactions on the physicochemical properties were provided by the Echip software (10). 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 (16). Significant differences between treatment combinations were further determined by Duncan's multiple-range test.

RESULTS AND DISCUSSION

The initial physicochemical characteristics of fresh RBD palm olein used in this study are given in Table 1. The fresh RBD palm olein was of good quality, as evidenced by its initial low PV of 0.9 meq/kg and an FFA content of 0.1%. With the IV of 56.1 g I_2/100 g oil, the fresh oil employed in this study was in accordance with the Malaysian palm olein standard (17).

Changes in oil quality during deep-fat frying. Table 1 also shows the changes in the oil characteristics for 15 treatment combinations during 5 d of potato chip frying. Results showed that for all physicochemical properties, the addition of antioxidants and CA significantly ($P < 0.05$) reduced the oxidation rate of the oil. During 5 d of frying, parameters AV, PV, FFA, polymer content, color units, viscosity, and absorbances at 232 and 268 nm all gradually increased, whereas IV and the ratio of 18:2/16:0 decreased. Results showed that for almost all quality parameters examined, the addition of rosemary, sage, CA, or their combinations into RBD palm olein during frying retarded the deterioration of the oil.

PV is a measure of the amount of peroxides formed in fats and oils through autoxidation and oxidation processes. Indi-

rectly, this measure indicates the degree of initial oxidation of fats and oils. At day 1, the PV for the control (Trial No. 9) sample was 6.6 meq/kg, whereas treatment samples ranged from 4.2 to 6.0 meq/kg. At day 5, PV for the control was 11.7 meq/kg, whereas treatment samples ranged from 6.1 to 10.9 meq/kq. It should be noted that for the treatment samples, only one sample (Trial No. 4) had a PV greater than 10 meq/kg, whereas PV for the remainder of samples were lower than 7.1 meq/kg at day 5.

Results from Table 1 also indicate that for all samples, PV increased gradually until day 5 of frying. Augustin and Berry (18) reported that hydroperoxides, the product of primary oxidation, react to form secondary products of which 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 (18). In this study, there was a marked increase in AV on the first day. After 1 d of frying, the AV for control was 31.5, whereas for additive-treated samples, AV varied from 27.1 to 30.1. For all treatments, the length of frying significantly influenced the AV. At day 5, the AV of the control reached 55.0, whereas for treatment samples, values ranged from 42.2 to 52.2.

IV is a measure of the total number of unsaturated double bonds present in the oil. The differences in IV of the oil during frying also are indicative of the increased rate of oxidation during frying. A significant ($P < 0.05$) change in IV can be observed when excessive deterioration of the oil occurs (18). Both antioxidants, as well as CA used in this study, significantly ($P < 0.05$) affected IV, which corresponded to both PV and AV (Table 2). During frying, the IV of all treatments increased significantly ($P < 0.05$), from 54.1 to 55.7 at day 1 and from 41.9 to 47.2 g I_2/100 g at day 5. Corresponding, control samples had a decrease in the IV from 54.0 to 41.9 g I_2/100 g.

The changes in percentage of FFA of the oil during frying are shown in Table 1. The FFA content for all treatments increased gradually from day 1 to day 5 of frying. The increase in the FFA content could be caused by the increase in rate of triacylglycerol hydrolysis when water was introduced into the frying system from the potato chips. Results also showed that the natural antioxidants significantly ($P < 0.05$) reduced the FFA contents of the oils during frying when compared with the control.

Results of polymer content analysis of the oils during frying revealed that both the duration of frying and the use of the natural antioxidants in this study were significant ($P < 0.05$) variables on the oil degradation products (Table 1). The polymer content of all treatment samples at day 1 ranged from 0.4 to 0.6%, compared to a control sample value of 0.7%. After the final day of frying, the polymer content of the control reached 2.0%, whereas samples with antioxidants and/or CA treatments ranged from 1.4 to 1.9%. The increase in polymer content during frying occurred because the longer the frying time, the greater the amount of decomposition products which would lead to polymer formation (19). Free radicals from hydrolysis of hydroperoxides, for example, can react to form polymers and other complex products (20).

The changes in red and yellow color units of thermally processed oils are also shown in Table 1. At day 0, samples treated with antioxidants or CA were significantly ($P < 0.05$) darker than the control, caused by the color of the added antioxidants. During the frying period, the color of oils treated with OR, OS, and CA were comparable to that of the control. However, after 5 d of frying, the control sample was significantly ($P < 0.05$) darker than most of the treated samples. The Lovibond color units of 1.1 red and 13.2 yellow were recorded for the control during the frying period at day 1. The increase in red and yellow color units during frying up to day 5 were relatively similar for all treatments, where the color of all samples gradually darkened. This observation can be explained by the formation of polymers which promote the darkening of oils (21).

The viscosity values of oils during frying are presented in Table 2. For the control, oil viscosity ranged from 50.1 cp at

TABLE 2
Regression Coefficients, R^2, and Probability (P) of F Values for Physicochemical Parameters (after 5 d of frying)

	Parameter										
Coefficient[a]	Anisidine value	Peroxide value	Iodine value	Polymer content	Red color	Yellow color	Free fatty acid	$E^{1\%}_{1cm}$ at 232 nm	$E^{1\%}_{1cm}$ at 268 nm	Viscosity	18:2/16:0 ratio
β_0 (intercept)	43.4	5.4	46.6	1.4	1.3	14.1	0.3	4.6	1.4	58.5	0.2
β_1	–37.3**[b]	–18.6*	21.6**	–2.1**	–0.4*	–1.3**	–0.8***	–18.1***	–3.2***	–31.5**	0.2***
β_2	-39.3**	–19.5*	16.2**	–1.9**	–0.3*	–0.8*	–0.7***	–16.2**	–2.4***	–29.0**	0.1**
β_3	–28.7	0.04	11.3	–0.7	–0.7	0.2	–0.5**	–2.2	–1.3*	–11.2	0.6*
β_{12}	338.6	440.5*	–241.4*	33.9*	1.1*	–3.2	3.8*	295.0**	38.8**	386.3*	–1.8*
β_{13}	–136.2	–0.7	42.0	–5.3	5.7	13.0	–1.1	–7.6	–0.6	–196.0	–0.7*
β_{23}	–68.2	–235.0	276.0	16.2	–0.4	0.2	–0.4	–243.3	–35.7*	–324.8	–1.5*
β_1^2	1056.1*	352.3	–480.5*	45.1*	10.2*	–6.9	18.8***	307.78*	58.3**	611.5*	–3.5**
β_2^2	842.6*	424.9	–328.7*	46.8*	8.5	2.0	14.4***	305.6*	56.1**	604.0*	–2.4*
β_3^2	964.5	710.0	–308.9	67.7	–6.1	–33.9	11.0	399.0	56.8	586.9	–2.2
R^2	0.945	0.904	0.961	0.960	0.932	0.903	0.994	0.975	0.990	0.961	0.972
P of F values	0.011	0.041	0.005	0.005	0.019	0.043	0.0	0.002	0.0	0.005	0.002

[a]Subscripts: 1 = OR; 2 = OS; 3 = CA. See Table 1 for abbreviations.
[b]*** = Significant at $P \leq 0.001$ level; ** significant at $P \leq 0.01$ level; * = significant at $P \leq 0.05$ level.

day 0 to 66.4 cp at day 5. For oil samples treated with rosemary and sage, the viscosity ranges were slightly narrower. At day 0, the viscosity of treatment samples ranged from 50.1 to 50.3 cp and reached as high as 58.4 to 66.0 cp at the end of the frying. The statistical analysis indicated that the addition of the antioxidants or CA into oils significantly ($P < 0.05$) reduced the viscosity; however, there was no significant difference between the treatment sample No. 4 (sample with presence of CA alone) and the control. There was a gradual increase in the viscosity of the samples with increased frying days. Similarly, Berger (22) reported that the rate of oxidation of unsaturated fatty acids in palm olein was directly related to the increase in oil viscosity.

The absorbance at 232 nm measures the degree of primary oxidation. In general, the results obtained in this study (Table 1) were closely related to the PV. There was a trend of increasing diene content with progress in frying times. The absorbance of samples treated with antioxidants and/or CA was also significantly ($P < 0.05$) different from the control oil sample without added antioxidants.

There was a significant ($P < 0.05$) effect of the use of OR, OS, or CA on the absorbance of cooked oil at 268 nm, an indicator of the formation of conjugated triene, during 5 d of frying (Table 1). The absorbance of the control oil sample was significantly ($P < 0.05$) greater than samples containing rosemary or sage extracts. Meanwhile, the longer the frying time, the higher the absorbances at 268 nm of oils. The trend in 268 nm values of different oils was similar to that seen with AV, which also measures secondary oxidative products in oil. Although the absorbance at 268 nm measures particularly the diethylenic ketones, ketones are not monitored in the AV test (18).

There was a significant ($P < 0.05$) difference between oils with added antioxidants or CA and control in the 18:2/16:0 ratio (Table 1). This result was not surprising because the changes in 18:2/16:0 ratio occur as a result of double-bond oxidation (23). A marked decrease in 18:2/16:0 ratio was found during the 5-d frying, which was consistent with the increase in the deterioration in oil quality under these conditions.

Optimization of natural antioxidants after day 5 of frying. The regression coefficients required to build a mathematical model or equation as formulated in the "statistical analysis," for all parameters evaluated after 5-d frying of potato chips, are summarized in Table 2. The mathematical models can be used to predict physicochemical properties of RBD palm olein during frying with OR, OS, or CA as dependent variables. Statistical analyses, including coefficients of determination (R^2) and probability (P) of F values, indicated that mathematical models for each dependent variable could be developed confidently in this study (Table 3). Thus, models or equations developed could be used for prediction and optimization of mixtures of the antioxidants and CA. All parameters examined had R^2 values >0.90, with the FFA content having the highest value (0.994). Giovanny (24) reported that $R^2 > 0.75$ are considered statistically accurate for predicting changes in oil quality.

From the significance tests in estimates given in Table 2, OR and OS were once again found to be the most important factors influencing all physicochemical characteristics evaluated. OR had highly significant ($P < 0.001$) effects on FFA, absorbances at 232 and 268 nm, and 18:2/16:0 ratio. The effect of OR was also highly significant ($P < 0.01$) on AV, IV, polymer content, yellow color, and viscosity and produced significant ($P < 0.05$) effects on PV and red color. OR, at the second-order term, in fact, produced highly significant ($P < 0.001$) effects on the FFA content, as well as on absorbance at 268 nm and 18:2/16:0 ratio ($P < 0.01$). Similar to OR, the level of OS used also produced highly significant ($P < 0.001$) effects on FFA content and absorbance values taken at 268 nm. The second-order terms of sage 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 the presence of sage were seen with such parameters as absorbance at 232 nm, viscosity, 18:2/16:0 ratio, AV, IV, and the polymer content.

CA, meanwhile, when present alone, had a significant effect only on FFA ($P < 0.01$) and absorbance at 268 nm ($P < 0.05$). No effect of the second-order term of CA on each parameter measured was found. However, the interaction between CA and OS gave a significant ($P < 0.05$) effect on absorbance at 268 nm and 18:2/16:0 ratio. Table 2 also shows that, except for AV and yellow color, the interaction between OR and OS was significant ($P < 0.01$) for all parameters examined.

Figure 1 exhibits response contours for the FFA parameter that gave the highest R^2 value. Further analysis showed that to reach the optimal FFA, a combination of 0.065% OR, 0.071% OS, and 0.043% CA was required. The Echips' results for contours of other parameters (contours not shown) revealed that, except for yellow color response, optimal combinations for all parameters could be clearly determined. These results indicated that the optimal points were within the levels of ranges of each antioxidant or CA used. For many responses, the results revealed that the use of moderate levels of the three additives could produce optimal points. In the case of yellow color, the optimal point was out of the ranges of antioxidant or CA levels. However, the model developed for this response (Table 3) was still able to be used for prediction purposes or to evaluate trends of the response (24).

TABLE 3
Predicted vs. Experimental Physicochemical Characteristics[a] of Refined, Bleached, Deodorized Palm Olein with Optimal Combination Treatment (after 5 d of frying)

Characteristic	Predicted	Experimental
Peroxide value (meq/kg)	5.3	5.2
Anisidine value	42.4	43.0
Iodine value (g I_2/100 g oil)	47.1	47.3
Free fatty acid (%)	0.3	0.3
Polymer content (%)	1.3	1.4
Color (red unit)	1.3	1.2
Color (yellow unit)	14.0	13.9
Viscosity	57.8	58.2
$E^{1\%}_{1cm}$ at 232 nm	4.3	4.3
$E^{1\%}_{1cm}$ at 268 nm	1.3	1.4
18:2/16:0 ratio	0.19	0.18

[a]Mean of three replications. $R^2 = 0.999$.

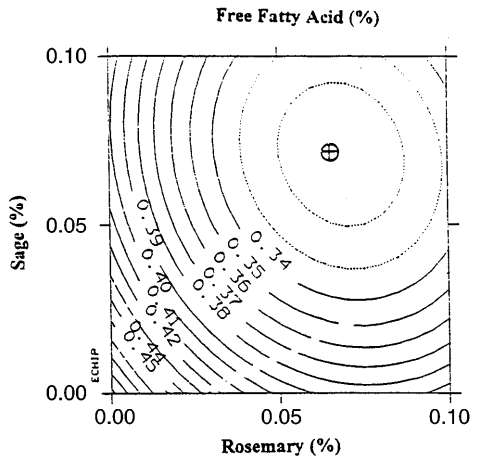

FIG. 1. Contour map illustrating the effect of oleoresin rosemary extract, sage extract, and citric acid (0.043%) on free fatty acid content.

For AV and PV, the combinations required to achieve the optimal conditions for use were 0.061% OR, 0.070% OS, and 0.025% CA; and 0.067% OR, 0.062% OS, and 0.026% CA, respectively. For IV and absorbance at 268 nm, the amount of CA required to achieve the optimal combinations was slightly greater. The optimal combinations for IV and the absorbance were 0.073% OR, 0.073% OS, and 0.049% CA; and 0.069% OR, 0.066% OS, and 0.042% CA, respectively. The optimal combination obtained for polymer content was 0.073% OR, 0.064% OS, and 0.034% CA acid; for the red color was 0.069% OR, 0.071% OS, and 0.025% CA; for viscosity was 0.070% OR, 0.065% OS, and 0.025% CA; for absorbance at 232 nm was 0.069% OR, 0.076% OS, and 0.037% CA; and for the retention of 18:2/16:0 ratio was 0.076% OR, 0.066% OS, and 0.037% CA.

As mentioned earlier, FFA was the most important dependent variable, giving the greatest R^2 value for natural antioxidants and CA treatments after 5 d of frying. To validate the optimal data, a frying experiment was performed using the optimal combination for FFA, 0.065% OR, 0.071% OS, and 0.043% CA. Data on physicochemical changes of RBD palm olein after 5 d of frying are compared in Table 3 with values predicted from the Echip software. There was a very high correlation ($R^2 = 0.999$) between these two data sets; thus the optimization study supports the use of RSM for predicting additional levels of natural antioxidants as well as CA during deep-fat frying of RBD palm olein. The results of this study further show that an optimal mixture of phytochemical antioxidants derived from rosemary, sage, and citric acid could be produced for stabilizing thermally processed oil using RSM.

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