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Effect of Addition of Antioxidants on the Oxidative Stability of Refined Bleached and Deodorized Palm Olein

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ABSTRACT

The addition of 50, 100, 150 and 200 ppm of butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), propyl gallate (PG) and α-tocopherol to refined, bleached and deodorized palm olein resulted in the retardation of the oxidation of the oil when stored at 65°C for 13 days. The extent of oxidation was shown by determination of weight gain, peroxide value and thiobarbituric acid value (TBAR). TBHQ was found to be the most effective antioxidant and α-tocopherol was the least effective antioxidant.

Key words: tert-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, peroxide value, thiobarbituric acid

INTRODUCTION

Fats, oils and lipid-based foods deteriorate through several degradation reactions both on heating and on long-term storage. The main deterioration processes are oxidation reactions and the decomposition of oxidation products, which result in decreased nutritional value and sensory quality. To prevent oxidation of fats and oils, antioxidants are widely used in foods and cosmetics (Cuvelier *et al*., 1996). Palm oil is derived from the mesocarp of the palm fruit (*Eliaeis guineesis*). It is extracted in the oil mill and then fractionated, bleached and deodorized in the refinery. Crude palm oil is one of the major sources of vitamin E and contains high quantities of tocopherols and tocotrienols in the range of 600-1000 ppm (Goh *et al*., 1985). Vitamin E is a natural antioxidant. It is the most efficient antioxidant for breaking free radical chain reactions and provides some natural oxidative protection to the oil (Ruperez *et al*., 2001). Palm olein is the liquid fraction obtained by fractionation of palm oil after crystallization at control temperature. Palm olein is not only odourless and more stable to oxidation but also being of vegetable origin is preferable from many points of view. Inclusion of a permitted antioxidant (BHA, BHT and TBHQ) is highly recommended.

Optimum concentrations and combinations of antioxidants may be used to improve the oxidative stability of edible oil. Several chemicals and sensory techniques are commonly used to monitor the oxidation of foods and to predict their shelf life stability. These techniques can be used to evaluate the effectiveness of antioxidants in different lipid systems (King *et al*., 1995). A number of accelerated oxidation tests have been used to examine the oxidative stability of edible oils and

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thus predict their shelf life. The Schaal oven test, used in this study, involves placing the samples in a forced-air oven, while the temperature is maintained between 60 and 70°C (Frankel *et al.*, 1997; Malcomson *et al.*, 1994). It has been observed that one day of storage, under Schaal oven conditions at 65°C, is equivalent to one month's storage at room temperature (Abou-Gharbia *et al.*, 1996). Furthermore, flavor scores of edible oils stored at 60°C for four days corresponded with those kept at ambient temperatures for four months (Warner *et al.*, 1989). Therefore, the objectives of this study were to determine the effects of BHA, BHT, TBHQ, PG and α-tocopherol on the oxidative stability of refined, bleached and deodorized palm olein (RBDPO).

MATERIALS AND METHODS

Material

Refined, bleached and deodorized palm olein (RPDPO) was provided by the Chumphon Palm Oil Industry Company (Ltd). BHA, BHT, TBHQ, PG and α-tocopherol were purchased from Fluka. Chemicals used were analytical grade.

Method

Preparation of samples

A range of concentrations (0, 50, 100, 150, 200 ppm) of BHA, BHT, TBHQ, PG and α-tocopherol were dissolved in a minimum amount of absolute ethanol, subsequently added to the oil (200 g) and mixed for 10 minutes. The control sample contained only the same amount of absolute ethanol that was used to dissolve antioxidants.

Oxidative stability test

The oxidative stability of each sample was evaluated by determining the weight gain, PV and TBAR. The samples were kept at 65°C in an oven (Memert model 7000) and were removed from the oven after 0, 2, 5, 9 and 13 days for oxidative stability determination (Khan and Shahidi, 2001). A 25 ml of each sample was stored separately under the same conditions in a small, open glass container for performing PV and TBAR determination. A sample of each treatment was removed after 0, 2, 5, 9 and 13 days, flushed with nitrogen, covered with aluminum foil and stored at -20°C until further analysis was carried out.

Weight gain determination (Evan *et al.*, 1973)

To monitor the weight gain during oxidation, 2 g of each sample (in triplicate) was prepared as mentioned above and placed in a glass petri dish, which was kept in a vacuum oven overnight at 35°C to remove any traces of moisture. The sample was reweighed and stored in a forced-air oven at 65°C. The rate of oxidation in terms of weight increase was recorded on day 0, 2, 5, 9 and 13.

Peroxide value determination (AOCS, 1994)

A 0.1 g sample was weighed in a 100 ml Erlenmeyer flask. A solution of 5 ml of 3:2 acetic acid:chloroform and saturated KI solution were added and left for 1 min. Then 5 ml of distilled water was added before titration with 0.001 N $Na_2S_2O_3$. A few drops of starch solution were added. The end point occurred when the blue color disappeared.

Thiobabituric acid substances determination (AOCS, 1994)

A 50-200 mg sample was accurately weighed into a 25 ml volumetric flask and dissolved in a small volume of 1-butanol and made up to volume with 1-butanol. Then 0.5 ml of the sample solution was transferred to a dry test tube and 5 ml of reagent solution was added. The test tube was closed with a ground-glass stopper, mixed thoroughly and placed in a thermostated bath at 95°C. After 120 min, the test tube was removed from the thermostated bath and cooled under

running tap water for about 10 min until it reached room temperature. The absorbance of the reaction solution was measured in a 10 mm cuvette at 530 nm using distilled water in the reference cuvette. A reagent blank was prepared at the same time as the sample. The reading of the blank determination should not exceed 0.1 in a 10-mm cuvette. The results were calculated using Equation 1:

$$\text{TBAR value} = [50 \times (A-B)]/m \quad (1)$$

where:

A = absorbance of the test solution,
B = absorbance of the reagent blank,
m = the weight (g) of the test portion.

Statistical analysis

The data obtained from the study were analyzed using analysis of variance (ANOVA) and the means were separated by Duncan's new multiple range test (Steel and Torie, 1980). The statistical analysis was computed by SAS (1998).

RESULTS AND DISCUSSION

The results and discussion are divided into three sections. The first section discusses the effect of the addition of antioxidants on weight gain of RBDPO. The second section discusses the effect of the addition of antioxidants on the peroxide value. The last section describes the effect of the addition of antioxidants on thiobarbituric acid.

Effect of addition of antioxidants on weight gain of RBDPO

The effect of the addition of antioxidants on weight gain of RBDPO is shown in Figures 1 to 4. The initial percent weight gain in all samples was zero. The weight of RBDPO without the addition of antioxidant was gradually increased with storage time and it was found to be higher than that of RBDPO containing antioxidants. The increasing trend was expected because the longer the storage time, the greater the amount of decomposition products which would lead to the polymer formation. The maximum weight gain in RBDPO containing antioxidant was obtained after storage for 13 days. The polymer content was reduced in the presence of antioxidant (Che Man and Jailong, 1999), as the formation of polymers was believed to require the presence of conjugated dienes. The conjugated dienes were the primary oxidation products of the unsaturated fatty acids. From Figure 1, it can be seen that α-tocopherol was less effective than BHA, BHT and TBHQ. However, the weight gains in RBDPO containing

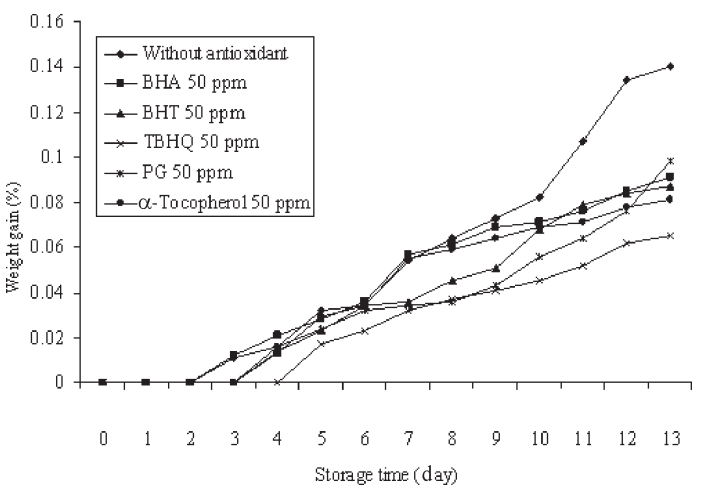

Figure 1 Effect of addition of antioxidants (50 ppm) on weight gain of RBDPO.

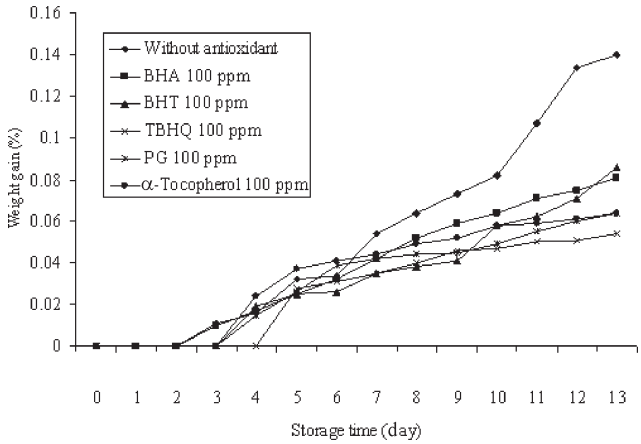

Figure 2 Effect of addition of antioxidants (100 ppm) on weight gain of RBDPO**.**

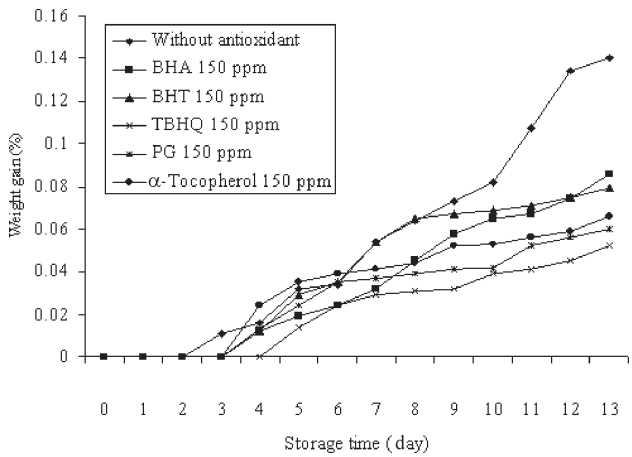

Figure 3 Effect of addition of antioxidants (150 ppm) on weight gain of RBDPO.

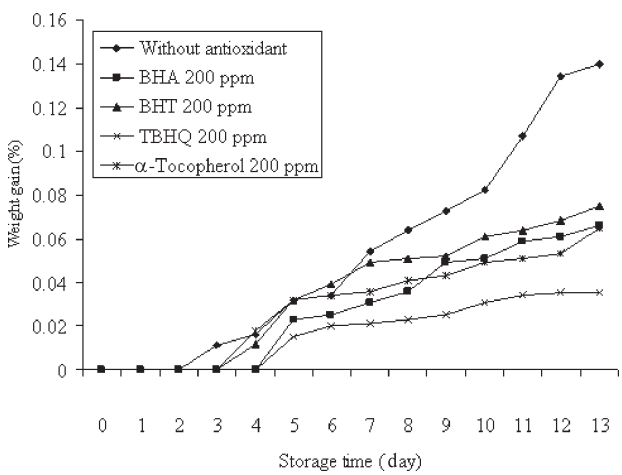

Figure 4 Effect of addition of antioxidants (200 ppm) on weight gain of RBDPO.

BHA, BHT and TBHQ, were not significantly different. Edible oil increased in weight during the early stages of lipid oxidation as fatty acids combined with oxygen during the formation of hydroperoxides. However, decomposition of the hydroperoxides led to a weight reduction, with the fat being severely oxidized at the end of the induction time (Gordon, 2001). Bera *et al*. (2006) studied natural antioxidants in the stabilization of flexseed oil using a comparison with synthetic antioxidants. It was found that TBHQ showed higher thermal stability than that of BHT. In this study, a change of color was observed, with a darkening of all samples throughout the study. This could have been due to the oxidation of phenolic antioxidants while heating. Augustin *et al*. (1989) reported that this darkening was not a good indicator of the deterioration of the palm olein. It would lead to the lower acceptability of the oils.

Effect of addition of antioxidants on peroxide value of RBDPO

The peroxide value was an indicator of the state of primary oxidation (Augustin and Berry, 1983). Table 1 shows the effect of the addition of antioxidants on the peroxide value. As an overall observation, the addition of antioxidants decreased the peroxide value of RBDPO. The peroxide value of fresh RBDPO was 0.86 meq/kg oil. From the results obtained, the peroxide value increased with

Table 1 Effect of antioxidants on peroxide value of RBDPO.

Storage at 65°C (Days)	Antioxidant concentrations (ppm)	With out antioxidant	BHA	BHT	TBHQ	PG	α-Tocopherol
0		0.86±0.04e	-	-	-	-	-
2	0	2.51±0.47d	-	-	-	-	-
	50		1.56±0.75cd	1.38±0.74c	1.17±0.28b	2.27±0.89dc	1.86±0.48dc
	100		1.21±1.41cd	1.84±0.32bc	1.21±0.45b	2.14±0.46dc	1.87±0.17dc
	150		1.91±1.09cd	1.24±0.87c	1.14±0.21b	1.90±0.61d	1.33±0.80dc
	200		1.85±1.30cd	1.16±0.26c	1.07±0.37b	1.67±0.29d	1.21±1.74e
5	0	4.74±1.17c					
	50		2.21±1.27cb	2.23±0.85bc	2.36±0.52ba	3.81±1.28bac	3.92±1.34de
	100		2.51±1.2cb	1.95±0.45bc	2.21±0.47ba	2.84±0.54bac	3.85±1.62de
	150		2.85±0.94d	1.82±0.67c	2.13±0.83ba	2.59±0.53bac	3.75±1.78de
	200		2.24±0.29d	1.76±0.29bc	1.84±0.85ba	2.31±0.92ac	3.87±1.95de
9	0	6.97±1.84b					
	50		4.46±2.72a	4.98±1.56a	2.78±1.03a	5.92±1.28ac	4.34±1.34bc
	100		4.45±1.24a	3.94±0.84a	2.78±0.74a	4.13±0.76bac	4.51±1.54bc
	150		4.86±0.87a	3.23±1.45a	2.14±0.58a	3.98±1.15ac	4.24±2.17bc
	200		4.24±1.85a	2.84±.0.96ba	1.87±0.85ba	3.45±1.59bac	4.84±1.57dc
13	0	8.56±1.51a					
	50		4.09±2.18a	4.45±1.18a	3.89±1.27a	6.89±1.65a	4.97±1.90a
	100		4.54±1.76a	3.84±1.05a	2.84±0.65a	5.67±1.59a	4.48±2.39ba
	150		4.74±2.83a	2.47±1.14a	2.35±1.28a	5.13±2.87a	4.16±1.46ba
	200		4.84±2.84a	2.75±1.42a	1.85±0.79ba	4.98±1.37ba	4.74±2.27a

Data are means of three determinations.

Mean values followed by different letters within the same column are significantly different ($P<0.05$).

storage time. For RBDPO, without the addition of antioxidant, the peroxide value significantly increased from 0.86 to 8.56. The peroxide value of RBDPO containing antioxidants was lower than that of RBDPO without antioxidants. TBHQ was found to be the most effective antioxidant, since there was a slight increment in the peroxide value, while BHT, BHA, PG and α-tocopherol had a lower retardation effect. This experiment indicated that TBHQ was the most effective and α-tocopherol was the least effective antioxidant, which were similar results to those reported by Augustin and Berry (1983). Che Man and Jailong (1999) studied the effect of TBHQ and α-tocopherol on the quality characteristics of RBDPO during deep-fat frying. It was reported that both TBHQ and α-tocopherol significantly improved the oxidative stability of RBDPO during frying.

Effect of antioxidant on thiobarbituric acid

The TBAR of RBDPO containing antioxidants is shown in Table 2. The initial value of RBDPO was 7.95 mgMAD/kg. The value was significantly increased for RBDPO without antioxidant, while for RBDPO containing BHA, BHT and PG, the TBAR was not significantly different but it increased. Hydroperoxides are considered to be the most important initial reaction products obtained from lipid oxidation. They are

Table 2 TBAR of RBDPO containing antioxidants.

Storage at 65°C (Days)	Antioxidant concentrations (ppm)	With out antioxidant	BHA	BHT	TBHQ	PG	α-Tocopherol
0	0	7.95±1.57c					
2	0	9.58±2.65d					
	50		9.58±2.78a	10.13±2.14a	8.34±1.66b	10.15±1.32a	10.54±1.98ba
	100		9.17±1.38a	9.24±1.28a	7.24±2.86b	9.87±2.11a	9.95±1.10b
	150		9.57±1.54a	9.21±2.98a	7.53±1.34b	9.30±1.52a	9.44±1.64b
	200		9.06±2.95a	9.63±1.64a	7.21±2.05b	9.12±1.59a	9.84±1.45b
5	0	11.67±2.14c					
	50		10.32±1.55a	10.11±1.89a	8.79±1.15a	11.97±2.53a	11.36±1.43ba
	100		9.78±2.05a	9.79±2.96a	8.58±2.10a	10.92±1.24a	10.98±0.58ba
	150		9.63±1.90a	9.53±1.47a	8.12±1.13a	10.52±2.17a	11.87±2.14ba
	200		9.47±1.59a	9.31±1.26a	8.41±1.54a	10.58±1.39a	10.87±1.12ba
9	0	18.89±1.49b					
	50		11.54±1.87a	11.32±1.68a	8.92±1.23a	12.47±1.87a	13.27±1.32ba
	100		11.29±1.52a	10.68±1.88a	8.75±2.34a	12.24±1.44a	12.82±1.35ba
	150		11.09±2.54a	10.56±2.16a	9.63±1.13a	11.24±1.32a	12.12±1.45ba
	200		10.95±0.84a	10.07±2.75a	9.58±1.86a	11.05±1.76a	12.56±1.02ba
13	0	22.25±2.84a					
	50		12.22±2.45a	11.64±2.21a	10.46±2.14a	12.76±2.11a	15.24±2.53a
	100		12.58±2.90a	11.27±2.62a	9.85±2.33a	12.57±1.95a	14.78±1.84a
	150		12.45±2.17a	10.37±2.16a	9.83±2.11a	11.43±2.18a	14.26±2.47a
	200		11.84±2.39a	10.89±2.57a	9.72±2.14a	11.23±2.29a	14.95±2.18a

Data are means of three determinations.

Mean values followed by different letters within the same column are significantly different ($P<0.05$).

a labile species, which undergoes changes in deterioration with the radicals. Their breakage causes secondary products such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (MDA) (Juana *et al*., 1997). The TBAR of RBDPO containing TBHQ was increased when the storage time was increased. However, it was lower than that of RBDPO containing BHA, BHT, TBHQ, PG and α-tocopherol. An increment in the TBAR value was due to malonaldehyde that may be formed from polyunsaturated fatty acids with at least three double bonds. The concentration of this product may be assessed by reaction with thiobarbituric acid, which reacts with malonaldehyde to form a red condensate (Gordon, 2001). Farkas *et al*. (1997) studied the oxidation kinetics of Menhaden oil with TBHQ. It was found that TBAR was increased when the storage time was increased. The effectiveness of TBHQ in retarding oxidation was evident, as induction periods increased with increases in the TBHQ concentration.

CONCLUSIONS

The addition of the antioxidants BHA, BHT, TBHQ, PG and α-tocopherol to PBDPO at the level of 200 ppm resulted in the retardation of oxidative deterioration. TBHQ was found to be the most effective and α-tocopherol was the least effective antioxidant.

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