Effectiveness of Antioxidants in Refined, Bleached and Deodorized Palm Olein

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ABSTRACT

The addition of antioxidants butylated hydroxytoluene (BHT), propyl gallate (PG), tertiary butylhydroquinone (TBHQ), dilaurylthiodipropionate (DLTDP), and trihydroxybutyrophenone (THBP) at a level of 200 ppm to refined, bleached and deodorized (RBD) palm olein resulted in the retardation of the oxidative deterioration of the oil when stored at 60 C for a period of 10 weeks. The extent of oxidative deterioration was determined by measuring the peroxide and anisidine values and $E^{1\%}_{1cm}$ at 232 nm and 268 nm of the oil. Butylated hydroxyanisole (BHA) proved to be a relatively ineffective antioxidant, whereas TBHQ afforded the most protection for the RBD olein.

INTRODUCTION

Palm oil is derived from the mesocarp of the palm fruit (*Elaeis guineensis*). It is extracted and purified in the oil mill, fractionated, refined, bleached and deodorized in the refinery. It is usually fractionated into a solid phase, stearin, and a liquid phase, olein. The process of fractionation extends and diversifies the product range of the oil. Palm oil, palm olein and palm stearin are extensively used in the food industry (1). However, like other unsaturated oils, palm oil and its fractions, olein in particular, are subject to oxidative reactions which lead to rancidity and the accompanying decreased organoleptic quality of the oil during storage (2).

The oxidative state of an oil or fat is usually ascertained by measuring the peroxides and anisidine reactive compounds. The experiments are more often conducted under accelerated conditions (temperature 60 C or above), as a study of the oxidative stability of oils at room temperature would require an unreasonable length of time (3,4). However, the peroxide values do not represent the absolute state of oxidation of an oil because of the transitory nature of the peroxides (5). The AOCS iodometric method for peroxide value has inherent errors, for the absorption of iodine at the unsaturation bonds of the oil and the release of iodine from potassium iodide by oxygen present in the solution affect the results. This method also fails to measure low peroxide values adequately, due to difficulty in determining the endpoint.

Various antioxidants have been added to unsaturated vegetable oils in an attempt to delay the onset of rancidity. The effectiveness of antioxidants in stabilizing sunflower, safflower, soybean, cottonseed and palm oils has been studied (3-4, 6-9). These studies generally show that the common antioxidants BHA, BHT, PG and TBHQ improve the stability of the oils. However, no correlation between the relative effectiveness of the antioxidants in different vegetable oils was observed (4). This could have been attributed to the differing balance between prooxidant and antioxidant factors inherent in different oils, which gives each oil its own characteristic stability towards oxidative degradation. There is, therefore, a need to evaluate the ability of an antioxidant to retard oxidative deterioration in each type of oil to ascertain the effectiveness of the antioxidant. In view of the lack of such data for palm olein systems, the present study was set up to evaluate the effectiveness of a range of antioxidants in stabilizing RBD olein. In addition to the common antioxidants BHA, BHT, PG and TBHQ, the effectiveness of two other antioxidants, DLTDP and THBP, was also examined.

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EXPERIMENTAL PROCEDURES

Preparation of Samples

RBD palm olein was obtained from two local refineries (refineries 1 and 2). In these two refineries, palm olein was obtained from the detergent process of fractionation. However, refineries 1 and 2 carried out the fractionation on refined palm oil and crude palm oil, respectively. The characteristics of the RBD olein from each refinery are given in Table I. These oils were used for experimentation within a week of being fractionated.

The antioxidants BHA, BHT, PG, TBHQ, DLTDP and THBP were of analytical grade obtained through Supelco Inc., Bellefonte, PA. Each antioxidant, at a level of 200 ppm, was added directly to the oil which was stirred at ca. 70 C for one hr to ensure complete dissolution of the antioxidant in the oil. The oil was then transferred into brown glass bottles. Each bottle was filled with 95 ± 5 g of the oil so that the initial head space in each bottle was ca. 45 cm^3. The bottles were stoppered with brown glass stoppers and kept in a convection oven at a constant temperature of 60 C throughout the storage period. They were only removed from the oven and unstoppered at the time of sampling, when a constant volume (10 mL) of the oil was pipetted out from each bottle.

TABLE I

Characteristics of RBD Olein from Local Refineries

Characteristics	Refinery 1	Refinery 2
Peroxide value, meq/kg	0.6	0.4
p-Anisidine value	4.7	2.1
$E^{1\%}_{1\ cm}$ at 232 nm	2.3	1.9
$E^{1\%}_{1\ cm}$ at 268 nm	0.9	0.8
Iodine value	58	57
Acid value	0.1	0.1
Cu, ppm[a]	<0.05	<0.05
Fe, ppm	0.08	0.08
Tocopherol, ppm[b]	207	214
Fatty acid composition, area %		
12:0	1.4	0.2
14:0	1.4	1.0
16:0	37.8	39.5
16:1	0.4	0.3
18:0	4.3	4.3
18:1	43.1	43.4
18:2	10.4	11.1
18:3	0.1	0.2
20:0	0.4	0.4

[a]Values given by refineries.

[b]Determined by high pressure liquid chromatography.

Analysis of the Oils

Peroxide and iodine values were determined in accordance with the AOCS Official Methods (10). Anisidine and acid values and $E^{1\%}_{1cm}$ at 232 and 268 nm were obtained using the IUPAC methods (11). The ultraviolet absorbances of the oil were measured on a Pye Unicam SP 8-150 spectrophotometer using ca. 0.2% solution of the oil in spectroscopy grade iso-octane (Merck, Darmstadt, West Germany).

The fatty acid composition of the oil was determined on a Pye Unicam Series 204 gas chromatograph fitted with flame ionization detectors. Details of this operation are given elsewhere (12).

The iron and tocopherol contents of the fresh RBD olein samples were determined by the Palm Oil Research Institute of Malaysia.

RESULTS AND DISCUSSION

The oxidative stability data for RBD olein from refinery 1 in the presence and absence of antioxidants are given in Tables II and III. The values presented in these tables are the average of three determinations. The possibility of interference by the antioxidants in the test methods used was checked to avoid the presentation of spurious results. The absence of discernible changes in the peroxide and anisidine values, and absorbances at 232 nm and 268 nm in fresh RBD olein before and after the addition of antioxidants confirmed that each of the antioxidants had no significant effect on the values obtained. The peroxide value and $E^{1\%}_{1cm}$ at 232 nm are indicators of the state of primary oxidation in the oil, while the anisidine value and $E^{1\%}_{1cm}$ at 268 nm reflect the degree of secondary oxidation (5,13).

TABLE II

Peroxide and Anisidine Values of RBD Olein Containing Antioxidants

Storage at 60 C (days)	Peroxide[a] (PV) and anisidine[b] (AV) values													
	Without antioxidants		BHA		PG		TBHQ		BHT		THBP		DLTDP	
	PV	AV	PV	AV	PV	AV	PV	AV	PV	AV	PV	AV	PV	AV
0	0.6	4.7	0.6	4.7	0.6	4.7	0.6	4.7	0.6	4.7	0.6	4.7	0.6	4.7
7	5.6	5.1	5.8	5.1	2.2	4.5	1.1	4.2	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	3.9	5.2	2.7	5.1	2.4	4.7
14	19.7	5.3	20.4	5.1	5.4	4.5	1.9	4.4	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	8.1	5.1	5.4	4.8	5.6	5.0
21	32.7	6.5	30.9	6.2	6.4	5.2	2.4	4.9	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—	10.9	5.4	7.0	5.5	7.2	5.1
28	42.3	6.2	45.8	6.5	8.0	5.1	2.8	4.6	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—	13.0	5.1	8.1	4.4	8.7	5.3
35	47.7	7.6	51.9	7.7	10.6	5.4	3.1	4.7	19.3	5.4	11.0	5.1	11.7	5.8
42	70.1	8.8	64.2	9.1	13.7	5.5	3.7	4.8	24.6	5.9	13.0	5.1	15.6	6.1
50	83.1	11.8	59.8	10.4	16.8	5.8	4.7	4.7	31.4	6.3	16.1	6.0	23.1	7.4
57	94.3	26.4	80.7	14.4	19.4	6.2	4.6	4.9	37.8	7.4	19.0	6.6	34.6	9.6
68	127.3	45.0	129.7	29.5	19.7	6.6	4.6	4.9	46.0	8.2	22.1	6.5	47.5	12.3

[a]meq/kg.

[b]Anisidine value is 100 times the optical density measured in a 1-cm cell of a solution containing 1 g of the oil in 100 mL mixture of solvent and reagent (11).

TABLE III

$E^{1\%}_{1\ cm}$ at 232 and 268 nm of RBD Olein Containing Antioxidants

Storage at 60 C (days)	$E^{1\%}_{1\ cm}$ at 232 and 268 nm[a]													
	Without antioxidants		BHA		PG		TBHQ		BHT		THBP		DLTDP	
	232	268	232	268	232	268	232	268	232	268	232	268	232	268
0	2.33	0.9	2.33	0.9	2.33	0.9	2.33	0.9	2.33	0.9	2.33	0.9	2.33	0.9
7	2.84	0.9	2.93	0.9	2.53	0.9	2.30	0.9	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	2.59	0.8	2.58	0.9	2.55	0.9
14	4.16	1.0	4.14	0.8	2.62	0.9	2.36	0.9	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	2.97	0.8	2.56	0.8	2.64	0.8
21	6.09	1.1	6.27	1.1	3.00	1.0	2.47	0.9	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—	3.51	0.8	2.97	0.8	3.05	0.8
28	7.05	1.0	7.07	1.0	2.96	0.9	2.39	0.8	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—	3.85	1.0	3.12	1.0	3.32	1.0
35	8.67	1.0	9.23	1.2	3.37	1.0	2.53	1.0	4.25	1.0	3.40	1.0	3.33	0.9
42	9.77	0.9	10.32	0.9	3.55	0.9	2.47	0.8	4.78	0.8	3.58	0.8	3.95	0.9
50	11.80	1.0	11.56	1.0	3.86	0.8	2.57	0.8	5.51	0.9	3.85	0.9	4.78	1.0
57	17.99	1.3	13.80	1.6	4.77	1.2	3.34	1.0	7.56	1.2	5.10	1.5	6.82	1.3
68	23.6	2.8	17.4	2.1	4.75	1.0	3.32	1.1	8.83	1.3	5.28	1.2	9.50	1.5

[a]$E^{1\%}_{1\ cm}$ at 232 and 268 nm = A/(c × d), where A is the absorbance of the solution at the specified wavelength, c is the concentration of the solution in g/100 mL of the solution, and d is the length of the cell in cm (11).

Primary Oxidation

The results on the development of peroxide values in the oil (Table II) clearly showed that the antioxidants improved the oxidative stability of RBD olein. TBHQ was found to be, by far, the most effective antioxidant while BHA showed little retardatory effect on oil oxidation. The order of effectiveness of the antioxidants evident from present data was as follows: TBHQ > PG ~ THBP > DLTDP > BHT > BHA. A rather similar trend in the order of effectiveness of the antioxidants was observed from the increase in absorbances at 232 nm (Table III). A good correlation between peroxide values and $E^{1\%}_{1cm}$ at 232 nm was expected since both these parameters measure the prevalence of primary oxidation in the oxidized oils. However, minor differences were observed between these two parameters. In the case of oil treated with BHA, a smooth increase in absorbance at 232 nm was observed over the storage period of 10 weeks, while there was a sudden decrease in the peroxide value for the same oil on the 50th day of storage, followed by a steady increase thereon until the end of the storage period. A similar anomalous behavior of the peroxide development was also observed in studies done on refined palm oil with added BHA (3). The authors there suggested the possibility of BHA destroying the peroxides rather than retarding the oxidation of the oil (3). The present findings corroborate this suggestion. Furthermore, it is known that conjugated dienes, other than conjugated diene peroxides, e.g., conjugated hydroxydienes, also possess similar extinction coefficients at similar wavelengths of maximum absorbance (13). The possibility of this type of reduction of conjugated diene peroxides to other conjugated diene species must not be disregarded in this context. Therefore, the apparent discrepancy between peroxide values and absorbances at 232 nm may in part be understood based on the fact that peroxide value measures the amount of peroxides present while the absorbance at 232 nm measures the increase in conjugated dienes in the oil. The observed differences in the order of effectiveness of BHA and DLTDP as judged by the peroxide value development and increasing absorbance at 232 nm may also be attributed to the factors discussed above.

Secondary Oxidation

Changes in the anisidine values and absorbances at 268 nm are much smaller than those observed for primary oxidation under these experimental conditions. Hence the changes in the totox value, the overall oxidation value (totox value = 2 peroxide value + anisidine value) are essentially due to the increase of peroxide value during storage.

Comparison of Oxidative Stability of RBD Olein from Two Refineries

Studies on the order of effectiveness of antioxidants in RBD olein from refinery 2 confirmed the order observed for RBD olein from refinery 1. The trends of the increase in peroxide and anisidine values, and absorbances at 232 nm and 268 nm were similar for RBD oleins from both refineries but the absolute experimental values of the measured parameters differed. The apparent anomalous behavior of peroxide development was also observed in the case of RBD olein from refinery 2 with added BHA. However, in contrast to the results obtained for oil from refinery 1, the sudden decrease in peroxide value occurred on the 28th day of storage when a lower peroxide value of 38 meq/kg was attained.

The differences in the absolute values may be due to differences in the initial oxidation state of the oils. Oil from refinery 2 had a higher initial stability as evidenced from the lower initial peroxide and anisidine values and $E^{1\%}_{1cm}$ at 232 and 268 nm (Table I). Over the 10-week storage period, oil obtained from refinery 2 attained a lower degree of oxidation than the oil from refinery 1. This observation is in agreement with the general opinion that the oxidative stability of an oil is dependent to a large extent on its initial oxidation state (6).

Room Temperature Storage of the Oils

Peroxide values of samples kept at room temperature for 10 weeks showed that all the antioxidants used, with the exception of BHA, were effective in retarding the oxidation of RBD olein. The observation that BHA was the least effective antioxidant while TBHQ was the most effective in retarding the oxidative degradation of RBD olein at room temperature was similar to the results obtained at the elevated storage temperature of 60 C. The untreated RBD olein from refinery 1 had a peroxide value of 10 meq/kg at the end of 10 weeks storage at room temperature. This value was similar to that obtained for the oil treated with BHA confirming that, even at room temperature, BHA had hardly any retardatory effect on oil oxidation. The peroxide value of RBD olein with added TBHQ only attained a peroxide value of 1.6 meq/kg over the same storage period. These results from the room temperature storage experiment did not allow us to establish with certainty the order of effectiveness of all the antioxidants, since only a single measurement of peroxide value was obtained at the end of 10 weeks. Moreover, the peroxide values obtained for PG, TBHQ, BHT, DLTDP and THBP were within the narrow range of 1.6-3.6 meq/kg. At these low peroxide values, errors inherent in the test method may be adequate to account for the observed differences. However, at least, this test did confirm that apart from BHA, the antioxidants used were effective both at room temperature and 60 C. It is maintained that the test conditions at 60 C are at least more akin to the normal storage conditions of the oil than AOM stability tests (10) which are carried out at 98 C. This single test at room temperature was carried out as it was borne in mind that certain antioxidants behave differently under different conditions of storage (14,15).

The observation from this study that TBHQ is, by far, the most potent antioxidant for RBD olein is not surprising in view of the reputed effectiveness of this antioxidant in most other vegetable oils and oil- and fat-containing foods (3,4,7,16,17). The effectiveness of TBHQ in RBD olein is especially encouraging since it is known that RBD oils are generally more prone to oxidative degradation (2). TBHQ, therefore, provides a means of effectively extending the shelf-life of this commodity.

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REFERENCES

1. Berger, K.G., in Food Uses of Palm Oil, Palm Oil Research Institute of Malaysia, Occasional Paper No. 2, 1981.
2. Swoboda, P.A.T., in Palm Oil Research Institute of Malaysia Bull. No. 1, 1980.
3. Fritsch, C.W., V.E. Weiss and R.H. Anderson, JAOCS 52:517 (1975).
4. Sherwin, E.R. and B.M. Luckadoo, Ibid. 47:19 (1970).
5. Gray, J.I., Ibid. 55:539 (1978).
6. Sherwin, E.R., Ibid. 55:809 (1978).
7. Luckadoo, B.M. and E.R. Sherwin, Ibid. 49:95 (1972).
8. Mounts, T.L., K.A. Warner, G.R. List, J.P. Fredrich and S. Koritala, Ibid. 45:345 (1978).
9. List, G.R., C.D. Evans and H.A. Moser, Ibid. 49:287 (1972).
10. Official and Tentative Methods of the American Oil Chemists' Society, 3rd edn., AOCS, Champaign, IL, 1974, Methods Cd 8-53, Cd 1-25 and Cd 12-57.
11. Parquot, C., Standard Methods for the Analysis of Oils, Fats

and Derivatives, 6th edn., IUPAC, Appl. Chem. Div., Commission on Oils, Fats and Derivatives, 1979.
12. Berry, S.K., Lipids 15:452 (1980).
13. Swoboda, P.A.T., UV-VIS Spectrophotometric Assays for Palm Oil Quality, Paper presented at Palm Oil Product Technology in the Eighties, Kuala Lumpur, Malaysia, 1981.
14. Swern, D., Bailey's Industrial Oil and Fat Products, Vol. 1, 4th edn., John Wiley & Sons, New York, NY, 1979.
15. Nagy, J.J., F.C. Vibrans and H.R. Kraybill, Oil Soap 21:349 (1944).
16. Carter, D., and M. Pike, Oil Palm News 19:1 (1975).
17. Sherwin, E.R., and J.W. Thompson, Food Technol. 21:912 (1967).

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ERRATA

In the article "Accumulation of Lipids, Proteins, Alkaloids and Anthocyanins during Embryo Development in vivo of *Theobroma cacao* L." appearing in the November issue of *JAOCS* (Wright, Park, Leopold, Hasegawa and Janick 59:475 [1982]), the lower half of Figure 9 should have been aligned directly below the upper half.

In the article "Synergism in Binary Mixtures of Surfactants: II. Some Experimental Data" appearing in the December issue of *JAOCS* (Rosen and Hua 59:582 [1982]), five lines were misplaced. The last five lines on page 583 ("systems showing synergism . . . i.e., the cmc, area") should be omitted, and inserted at the bottom of page 584. The last paragraph on page 584, continuing on page 585, should then read "Currently, there are almost no data in the literature from which calculations of β, β^M, X_c, and X^M can be made on systems showing synergism in this respect. Table III lists some data for the system: $C_{12}H_{25}SO_3K/C_{12}H_{25}N(CH_3)_2O$ (6) in which this type of synergism is present. It also includes data for some hypothetical systems in which the values of C_1^M, C_2^M, A_1°, A_2°, γ_1^M, and γ_2^M (i.e., the cmc, area per molecule and surface tension at the cmc for the individual surfactants) and the value of β^M are identical with those in the real system, while the value of β is changed."