Technical

Stability of Tapioca Chips Fried in RBD Palm Olein Treated with Antioxidants

M.A. AUGUSTIN and **S.K. BERRY**, Department of Food Science, Faculty of Food Science and Technology, Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia

ABSTRACT

The effectiveness of tertiarybutylhydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in stabilizing tapioca chips was assessed by determining the peroxide and *p*-anisidine values, absorbances at 232 nm and 268 nm and the 18:2/16:0 ratios of oil extracted from tapioca chips. The order of effectiveness of the antioxidants in stabilizing the chips was found to be TBHQ > BHT > BHA. The order of effectiveness of antioxidants, TBHQ > BHT > BHA, was maintained for chips from corresponding 1st, 4th and 7th fryings. The loss of antioxidants during storage could not be directly related to oxidation parameters.

INTRODUCTION

Refined, bleached and deodorized (RBD) palm olein is extensively used as cooking and frying oil, either alone or as a blend with other oils. However, its stability toward autoxidation during storage and frying is of concern to both the manufacturer and user. Antioxidants have commonly been used to retard the autoxidative deterioration of oils (1,2). In addition, their incorporation into oils used for deep-fat frying extends to their potential action in stabilizing the fried-food product (3,4).

This study involves an assessment of the relative stabilities of tapioca chips fried in palm olein containing butylated hydroxyanisole (BHA), butylated hydroxytolune (BHT) and tertiarybutylhydroquinone (TBHQ). It also discusses the influence of consecutive fryings on the storage stability of the fried product.

MATERIALS AND METHODS

RBD palm olein was a gift from Lever Bros. (Malaysia) Sdn. Bhd. BHA and BHT used for the preparation of standards for gas chromatographic (GC) analysis were of analytical grade (Supelco Inc., Bellfonte, PA). BHA and BHT added to frying oils were from Sigma Chemical, St. Louis, MO. TBHQ used in frying experiments was obtained from Aldrich Chemical Co. Inc., Milwaukee, WI.

Tapioca tubers *(Manihot utilissima)* were obtained from the farm of Universiti Pertanian Malaysia. They were stored in a cold room (4 C) until the day of use.

Preparation of Tapioca Chips

Tapioca tubers were deskinned and rounds of 2 mm thickness were cut with a mechanical slicer.

In the frying experiment, 2.5 kg RBD olein was put into a Hamilton delux deep-fat fryer. The oil was heated to 70 C and antioxidant was added. The oil was stirred at this temperature for 10 min to dissolve and disperse the antioxidant. In the control experiment, where no antioxidant was added, the oil was also maintained at 70 C, while stirring, for 10 min. The temperature of the oil was then raised to 200 C in 20 min. A batch of 400 g tapioca rounds were fried for 7 min.

The temperature of the oil dropped to 125 C during the 1st frying. At the end of the frying, the excess oil from the chips was drained and the chips were allowed to cool on absorbent tissue. The temperature of the oil was then raised to 200 C in 9-10 min. Second and consecutive fryings were carried out in the same manner. From each batch of 400 g tapioca rounds, we obtained 200 g tapioca chips. A total of 7 fryings were done. The frying temperature decreased slightly with each subsequent frying with the frying temperature being 115 C during the 7th frying. This lower frying temperature may account for the slightly lighter color of the chips obtained from later fryings.

During the course of the fryings, oil samples were taken for analysis of antioxidant concentration in the oil.

Storage Studies

Chips from the 1st, 4th and 7th fryings were kept for storage studies. For each batch, 40 g samples of tapioca chips were loosely packed into clear-glass bottles with screw caps, and these were placed in an oven at 60 C throughout the storage period. A sample from each batch was also kept in a bottle wrapped with Al-foil in a dark cupboard at room temperature (27 C). Bottles were removed from the oven at 2-week intervals to analyze the quality characteristics of the chips and determine antioxidant levels in the chips.

Extraction and Determination of Antioxidants

Five-to-seven g tapioca chips were ground in a grinder for 3 min. The ground chips were extracted 3 times with ethyl acetate (10 mL, 5 mL × 2). The combined extracts were made up to volume in a 25 mL volumetric flask to which 2 mL of internal standard (methyl undecanoate, 144 ppm) had been added. The concentrations of BHA and BHT in the extract were determined using the method of Hartman and Rose (5). The operating conditions for the gas liquid chromatograph (GLC) were as reported previously (6). Recovery studies carried out showed that the percentage of recoveries for BHA and BHT from tapioca chips were 70% and 80%.

An accurate estimation of TBHQ concentration in extracts (5-20 ppm) was not possible as the peak areas for TBHQ were not directly proportional to the concentration of TBHQ in this concentration range.

The BHA and BHT concentration in RBD olein during frying were determined using ca. 10% oil solution in ethyl acetate (6).

Extraction of Oil

Chips (30-40 g) were ground and extracted 3 times with 40 mL volumes of petroleum ether (b.p. 40-60 C). Petroleum ether was removed by flash evaporation at 40 C. The oil was then used to determine quality characteristics. A check on the extraction procedure showed that the peroxide value, *p*-anisidine value and absorbances at 232 nm and 268 nm did not change appreciably during the extraction process.

Analysis of Quality Characteristics

Peroxide, iodine and acid values were determined according to AOCS Official Methods (7). Ultraviolet (UV) absorbances were obtained using ca. 0.2% oil solution in spectroscopy grade isooctane. *p*-Anisidine values were obtained using the IUPAC method (8). The fatty acid composition of

the oil was determined by GC using operating conditions reported previously (9). Fatty acid methyl esters were prepared as described by Timms (10).

The results reported for the quality characteristics are the average of duplicate determinations. The mean errors in the values for the quality characteristics are less than 10%. The absorbances at 232 nm and 268 nm given in the paper are not corrected for triglyceride absorption.

RESULTS AND DISCUSSION

Antioxidants in Frying Systems

The quality characteristics of RBD olein used for frying 7, 400 g batches of tapioca rounds are given in Table I. The changes in the peroxide value and $E^{1\%}_{1cm}$ at 232 nm (indicators of primary oxidation) and *p*-anisidine value and $E^{1\%}_{1cm}$ at 268 nm (indicators of secondary oxidation) during frying were similar for RBD olein without antioxidants, BHA-treated oil and BHT-treated oil. The changes in these indicators of oxidation were least for RBD olein with added TBHQ. The similarity in the magnitude of changes in quality characteristics for RBD olein without antioxidants, BHA-treated oil and BHT-treated oil showed that BHA and BHT were relatively ineffective in retarding the oxidative deterioration of RBD olein during frying. In contrast, TBHQ did appear to afford some protection to oils during frying, as evidenced by the lower values of the oxidation parameters in TBHQ-treated oil compared with RBD olein without antioxidants.

The superiority of TBHQ as a antioxidant at high temperatures has also been observed in AOM stability tests on antioxidant-treated oils (11). These AOM stability tests on soy oil indicated that the order of effectiveness of the antioxidants was TBHQ > BHT > BHA both for soy oil subject to heat alone and to the combined effects of heat and steam. The relatively better antioxidant potency of BHT over BHA exhibited in soy oil was not evident in the RBD olein system. Both antioxidants appeared to be equally ineffective under the conditions used in the frying experiments. The relative ineffectiveness of BHA and BHT as antioxidants for RBD olein at high temperatures was also observed during the frying of potato chips (6).

Loss of antioxidants during frying has often been regarded as a factor contributing to the loss of antioxidant potency, but that the antioxidant remaining in the frying medium (Table II) does not show any protective effect on the rate of oxidation of the oil is surprising. Furthermore, TBHQ is also lost during frying (11) but appears to retard the oxidative deterioration reactions in the oil. Other factors, e.g., change in reaction pathways and interactions of food components may play a major role in influencing antioxidant potency under frying conditions (12).

The limited number of fryings may not have been sufficient to differentiate the effects of BHA and BHT. Whether or not the antioxidant potency of these antioxidants would be displayed when the oil is subjected to harsher conditions and a larger number of repeated fryings cannot be predicted with certainty from these experiments.

Only a limited degree of oxidation occurred during these frying experiments. The iodine value of the oils changed by only 1 unit during frying and insignificant changes occurred in the 18:2/16:0 ratio (Table I). Significant changes in both these parameters arise only when an oil has been grossly abused (13).

The changes in acid value during frying (Table I) are mainly caused by hydrolysis of triglycerides by water supplied primarily from the food product (14).

TABLE II

Antioxidant Concentrations in RBD Olein and Tapioca Chips

		BHA (ppm)		BHT (ppm)	
		RBD olein	Chips	RBD olein	Chips
Initial antioxidant[a]		215.8		245.0	
1st frying	(before)	199.8		242.7	
	(after)	196.4	38.3	232.9	30.5
2nd frying	(before)	191.2		221.5	
	(after)	184.2		216.1	
3rd frying	(before)	177.5		203.4	
	(after)	168.6		194.3	
4th frying	(before)	161.2		187.4	
	(after)	155.9	28.5	179.3	25.2
5th frying	(before)	151.2		171.3	
	(after)	143.0		164.5	
6th frying	(before)	139.5		154.5	
	(after)	134.6		139.8	
7th frying	(before)	127.2		136.8	
	(after)	122.6	18.4	130.0	19.1

[a]Antioxidant concentration when added to oil at 60 C.

TABLE I

Analytical Data on RBD Olein[a] Used for the Frying of Tapioca Chips

Quality characteristics	No antioxidant		BHA[b]		BHT[c]		TBHQ[d]	
	Before frying	After[e] frying	Before frying	After[e] frying	Before frying	After[e] frying	Before frying	After[e] frying
Peroxide value (meq/kg)	2.5	4.4	3.2	4.0	3.3	4.1	5.3	5.0
p-Anisidine value	1.4	21.7	1.3	22.2	1.5	22.7	2.0	18.5
$E^{1\%}_{1cm}$ at 232 nm	2.27	3.10	2.24	3.07	2.36	3.12	2.30	2.73
$E^{1\%}_{1cm}$ at 268 nm	0.62	1.24	0.62	1.30	0.62	1.26	0.58	1.03
Acid value (mg KOH/g)	0.42	0.53	0.42	0.53	0.42	0.54	0.47	0.53
Iodine value	61.2	60.3	61.4	60.3	63.6	63.3	62.8	62.5
18:2 (%)	11.41	11.20	11.16	10.95	11.30	11.00	11.40	11.28
18:2/16:0	0.297	0.293	0.289	0.281	0.296	0.284	0.298	0.295

[a]RBD olein used for frying was obtained from the same can. Frying was carried out over a period of 3 weeks.
[b]Initial [BHA] = 215.8 ppm.
[c]Initial [BHT] = 245.0 ppm.
[d]Initial [TBHQ] = 201.5 ppm.
[e]A total of 7 fryings were done.

CHARACTERISTICS OF CHIPS

The characteristics of chips were related to the extent of oxidation in oil extracted from tapioca chips. The oil content of the chips averaged 18%.

Effect of Fry Number on Characteristics of Chips on Day 0

A definite relationship appears to exist between the fry number and the characteristics of tapioca chips fried in oil with the same antioxidant treatment. In all cases, the expected general trend of increasing oxidation values with subsequent fryings occurs (Table III). Obvious increases were found in *p*-anisidine value and $E^{1\%}_{1cm}$ at 232 nm and 268 nm with subsequent fryings for chips with the same antioxidant treatment. A tendency for peroxides to increase with increase in fry number for chips fried in RBD olein without antioxidants, with BHT and with TBHQ was found, but a trend is not apparent for chips fried in BHA-treated oils. Why no trend in peroxide values was obtained for chips fried in BHA-treated RBD olein is not entirely clear. An explanation based on the unstable nature of peroxides and their intermediary status in oil oxidation is not completely satisfactory in view of the trends observed in other cases. A possible contributing factor for this apparent discrepancy is the suggested role of BHA in decomposing peroxides (15).

Effect of Antioxidants on Characteristics of Chips on Day 0

The extent of oxidation in chips fried in RBD olein with TBHQ was generally lower than those fried in RBD olein without antioxidants, BHA-treated oil and BHT-treated oil for chips with corresponding fry numbers (Table III). This is evidenced by the lower peroxide and *p*-anisidine values and $E^{1\%}_{1cm}$ at 232 nm and 268 nm for chips with corresponding fry numbers. One exception is the comparable peroxide value of chips from the 7th frying in RBD olein containing TBHQ. The chips were, however, indistinguish-

TABLE III

Characteristics of Chips During Storage at 60 C

Treatment	Days of storage	Fry number	Antioxidant (ppm)	Peroxide value (meq/kg)	*p*-Anisidine value	$E^{1\%}_{1cm}$ at 232 nm	$E^{1\%}_{1cm}$ at 268 nm	Ratio 18:2/16:0
No antioxidant	0	1		4.6	7.2	2.5	0.8	0.30
		4		5.5	13.8	2.7	1.0	0.29
		7		5.8	18.9	3.1	1.2	0.29
	14	1		20.5	6.9	4.0	0.8	0.30
		4		20.3	13.3	4.0	1.0	0.29
		7		21.4	17.0	4.5	1.2	0.29
	28	1		56.2	7.4	7.7	0.9	0.28
		4		49.5	12.7	6.8	1.0	0.28
		7		50.6	18.4	7.6	1.2	0.28
	43	1		79.4	7.6	11.4	1.0	0.28
		4		79.0	12.9	12.9	1.2	0.27
		7		72.5	17.0	10.7	1.3	0.27
BHA[a]	0	1	38.3	5.6	8.6	2.8	0.9	0.30
		4	28.5	5.9	14.8	3.0	1.1	0.29
		7	18.4	5.5	18.9	3.3	1.3	0.29
	17	1	37.8	21.8	5.6	4.2	0.9	0.29
		4	27.5	22.5	12.3	4.3	1.0	0.28
		7	18.4	21.0	17.0	4.7	1.2	0.28
	29	1	36.0	48.6	7.0	6.9	0.9	0.29
		4	25.0	31.7	12.2	5.6	1.1	0.29
		7	16.5	46.4	17.8	6.6	1.2	0.29
	43	1	25.5	87.9	7.6	10.7	1.1	0.27
		4	21.7	93.7	12.9	9.9	1.2	0.27
		7	13.4	80.1	17.3	9.6	1.3	0.27
BHT[a]	0	1	30.5	5.5	9.2	2.6	0.9	0.30
		4	25.2	5.9	16.4	3.0	1.1	0.30
		7	19.1	6.2	21.2	3.2	1.2	0.29
	15	1	29.0	11.5	7.2	3.1	0.8	0.29
		4	23.6	8.0	13.5	3.2	1.0	0.28
		7	18.5	12.6	17.5	3.7	1.2	0.28
	29	1	28.8	20.8	6.9	4.2	0.9	0.29
		4	21.8	22.2	12.6	4.8	1.1	0.28
		7	17.7	20.2	17.1	4.7	1.2	0.29
	43	1	18.0	42.0	6.1	6.1	0.9	0.29
		4	13.5	36.1	11.5	6.0	1.1	0.29
		7	9.2	39.8	15.7	6.5	1.3	0.29
TBHQ[a]	0	1		3.5	6.4	2.2	0.7	0.29
		4		5.1	11.9	2.5	0.9	0.29
		7		5.7	16.4	2.9	1.1	0.29
	14	1		5.9	5.9	2.4	0.7	0.30
		4		6.1	11.3	2.6	0.9	0.30
		7		7.9	15.3	3.0	1.1	0.30
	29	1		7.4	5.0	2.5	0.7	0.30
		4		8.7	9.3	2.8	0.9	0.30
		7		9.0	13.5	3.2	1.0	0.30
	43	1		6.6	4.7	2.8	0.8	0.30
		4		7.8	9.1	3.1	1.0	0.30
		7		10.8	13.4	3.8	1.1	0.29

[a]Initial concentrations of antioxidants in oil are 215.8 ppm for BHA, 245.0 ppm for BHT and 201.5 ppm for TBHQ.

able in terms of their organoleptic quality at this stage.

The amount of antioxidant taken up by the chips depended on the type of antioxidant and the level of antioxidant in the frying medium at the time of frying. The concentration of antixoidant in the chips decreased in chips obtained from later fryings (Table III). A higher percentage of BHA compared with BHT was absorbed by the chips. The relatively better absorption property of BHA is amplified when recoveries of antioxidants from tapioca chips is considered. Recoveries from tapioca chips were 70% and 80% for BHA and BHT. The percentage of recoveries of antioxidants from oil were 94% for BHA and 96% for BHT. This superior adsorption property of BHA compared with BHT has been observed previously (16).

STORAGE OF TAPIOCA CHIPS

Effect of Antioxidants on Stability of Chips

The stability of tapioca chips fried in RBD olein with different antioxidant treatments were compared for chips stored over a period of 6 weeks at 60 C. A comparison of the oxidation characteristics of the chips with the same fry number showed that peroxide and *p*-anisidine values and $E^{1\%}_{1cm}$ at 232 nm and 268 nm were consistently lower for chips fried in RBD olein containing TBHQ than those fried in oil with other antioxidant treatments (Table III). Further, the lower oxidation values for chips fried in RBD olein containing BHT compared with those fried in oil with BHA indicates the better protection afforded the chips by BHT. The same order of effectiveness, TBHQ > BHT > BHA, was observed for chips obtained from the 1st, 4th and 7th fryings. In addition, the similarity in the magnitude of the changes in oxidation parameters during storage for chips from 1st, 4th and 7th fryings suggests that the effectiveness of these antioxidants were not directly proportional to antioxidant concentration at the levels incorporated in the chips.

The analysis of a variety of oxidation characteristics of the chips also allowed the inference that primary oxidation predominated over secondary oxidation during the storage of chips. This is evidenced by the more pronounced changes in peroxide value and $E^{1\%}_{1cm}$ at 232 nm than those in *p*-anisidine value and $E^{1\%}_{1cm}$ at 268 nm. Furthermore, the similarities in the trends of peroxide value and $E^{1\%}_{1cm}$ at 232 nm allude to the correlation between these 2 indicators of primary oxidation in food systems. The existence of a relationship between peroxide value and $E^{1\%}_{1cm}$ at 232 nm has been observed for food systems before (17). However, the $E^{1\%}_{1cm}$ at 232 nm has not been as widely used as the peroxide value for monitoring lipid oxidation in food systems.

The *p*-anisidine value, which is indicative primarily of the presence of α,β unsaturated aldehydes, shows a tendency to decrease during storage. This may be attributed to further oxidation of the aldehydes to yield other oxidation products. The possibility also remains of involvement of the aldehydes in nonenzymic browning reactions. Slight browning of the tapioca chips was observed during storage. In view of this latter reaction pathway for aldehydes, the *p*-anisidine value may not necessarily reflect the degree of secondary oxidation, especially in food systems where nonenzymic browning takes place. As such, the usefulness of the *p*-anisidine value as an indicator of the degree of oxidation in some food systems is questionable.

The analysis of oxidation parameters also incorporated an estimation of the 18:2/16:0 ratio. This ratio did not change appreciably during storage. The values of the 18:2/16:0 ratio varied between 0.30 and 0.27 during the period of storage for chips fried in RBD olein without antioxidants and BHA-treated oil. The corresponding values for chips fried in RBD olein with TBHQ or BHT varied between 0.30 and 0.28. The insignificant differences in the changes of 18:2/16:0 did not allow a discrimination of the relative effectiveness of the antioxidants. It did indicate that gross deterioration of lipids in tapioca chips did not take place during the period of storage.

Comparison of Antioxidant Effects in Other Systems

The order of effectiveness for tapioca chips fried in RBD olein with different antioxidant treatments, TBHQ > BHT > BHA, has been found in a number of other deep-fried products. The same order of effectiveness was observed for potato chips fried in hydrogenated cottonseed oil and refined palm oil (15). Schaal oven tests of potato chips fried in soy oil also showed the same trend of antioxidant effectiveness (11). These findings support the superior efficacy of TBHQ as an antioxidant for deep-fried snacks and suggest that TBHQ has good stability characteristics. Stability, in this context, is taken to mean the stability imparted to the fried products.

Although BHA has been found to be an effective antioxidant for baked and fried foods prepared with animal fats, its potency does not seem to extend to foods prepared with vegetable oils. In addition, food components themselves and products formed by the interaction of food components with antioxidants or other constituents of the food may have a role in influencing the antioxidant effectiveness in a particular system.

In food systems where nonenzymic browning occurs, the possibility also exists of these components taking part in antioxidation pathways (18) and the added effect of differing processing steps and storage conditions for different food products. The complexity of lipid oxidation, in conjunction with the role of food components in influencing the mechanism of antioxidation, make predicting antioxidant effectiveness in food systems extremely difficult.

Antioxidant Loss in Chips During Storage

The change in antioxidant levels in chips during storage are given in Table III. The decreasing levels of BHA and BHT during storage may be attributed to the reactions of antioxidant with reactive species in the food or the oil. Although the accepted mechanism of action of phenolic antioxidant in vegetable oils involves the interaction of the phenolic antioxidant with a fatty radical (2), that this reaction accounted for the major part of the loss of BHA is unlikely in view of its relative ineffectiveness as an antioxidant in this system.

Other workers found that increases in oxidation product levels in potato flakes were inversely related to antioxidant concentration (19). This relationship was not evident in tapioca chips.

Storage at Room Temperature

In an attempt to relate the results of stability tests at 60 C to normal conditions of storage, chips were kept at room temperature for a period of 8 wk. However, insignificant differences were found in peroxide, *p*-anisidine, and 18:2/16:0 values and absorbances at 232 nm and 268 nm for chips with differing antioxidant treatments in 8 wk. This test, therefore, did not allow discrimination in the relative order of antioxidant effectiveness at room temperature. The loss of antioxidants in chips during storage at room temperature for 8 wk averaged 4-5 ppm for BHA and BHT.

ACKNOWLEDGMENTS

P.A.T. Swoboda provided helpful comments, Chan Tin Wan assisted

with GLC analyses and Universiti Pertanian Malaysia supported the research.

REFERENCES

1. Sherwin, E.R., JAOCS 49:468 (1972).
2. Sherwin, E.R., Ibid. 55:809 (1978).
3. Chipault, J.R., in Autoxidation and Antioxidants, Vol 2, edited by W.O. Lundberg, Interscience Pub., NY, 1962.
4. Furia, T.E., Handbook of Food Additives, The Chemical Rubber Co., Cleveland, Ohio, 1968, Chap. 5.
5. Hartman, K.T. and L.C. Rose, JAOCS 47:7 (1970).
6. Augustin, M.A. and S.K. Berry, Ibid. 60:1520 (1983).
7. Official and Tentative Methods of the American Oil Chemists' Society, 3rd edn., Champaign, IL, 1973.
8. Standard Methods for the Analysis of Oils, Fats and Derivatives, IUPAC, App. Chem. Div., Commission on Oils, Fats and Derivatives, 6th edn., edited by C. Paquot.
9. Berry, S.K., Lipids 15:452 (1980).
10. Timms, R.E., Aust. J. Dairy Tech. 33(1):4 (1978).
11. Buck, D.F., JAOCS, 58:275 (1981).
12. Labuza, T.P., CRC Reviews in Food Tech. 355 (1971).
13. Peers, K.E. and P.A.T. Swoboda, J. Sci. Food. Agric. 33:389 (1982).
14. Roth, H. and S.P. Rock, Bakers Digest 46:38 (1972).
15. Fritsch, C.W., V.E. Weiss and R.H. Anderson, JAOCS 52:517 (1975).
16. Dugan, J.R., Jr., L. Marx, C.E. Weir and H.R. Kraybill, Amer. Meat Instit. Foundation Bull. No. 18 (1954).
17. St. Angelo, A.J., R.L. Ory and L.E. Brown, JAOCS 52:34 (1975).
18. Vandewalle, L. and A. Huyghebaert, FSTA 15(2):2A 132 (1983).
19. Sapers, G.M., O. Panasiuk, F.B. Talley and R.L. Shaw, J. Food Sci. 40(4):797 (1975).

[Received August 15, 1983]

Identification and Quantification of Cholesterol Oxides in Grated Cheese and Bleached Butteroil

E. TERRY FINOCCHIARO, K. LEE and **T. RICHARDSON,** Department of Food Science, University of Wisconsin-Madison, Madison, WI 53706

ABSTRACT

Butteroil samples bleached with benzoyl peroxide (BP) and 17 commercial cheeses were screened for oxidized sterols by thin layer chromatography (TLC). Ungrated cheeses made from bleached milk and freshly bleached butteroil contained no detectable oxidized sterols. Oxidized sterols were detected in stored, bleached butteroils and in grated cheeses. Four major oxidation products were the isomeric 5,6-epoxycholesterols and the epimeric 7-hydroxycholesterols identified by TLC, high performance liquid chromatography (HPLC) and mass spectrometry (MS). Additional sterol oxides (tentatively identified and not quantified) present in these samples included low levels of 7-ketocholesterol and cholesta-3,5-dien-7-one. The epimeric 7-hydroxycholesterols were detected in bleached butteroils stored in air (BP-A) and nitrogen (BP-N) for 22 days at 15 C. Butteroil, after 90 days of storage at 15 C, had 30 (BP-N) and 60 (BP-A) μg total oxides/g of bleached oil and, after 1-year at −20 C, had 70 (BP-N) and 180 (BP-A) μg/g butteroil. A grated, unbleached cheese packaged in clear glass contained the most oxidized sterols (44 μg/g). Sterol oxides were not detected in bleached cream using a simulated industrial process.

INTRODUCTION

The toxicity of dietary oxidized cholesterol has been studied using both in vivo (1,2) and in vitro (3-6) systems. The 25-hydroxy cholesterol and the isomeric 5,6-epoxides are common autoxidation products of cholesterol that have received considerable attention in recent years (7). The epoxides as well as other cholesterol oxides are formed by attack of several oxygen species (7), some of which occur in food systems (8). Although little is known about the biological effects of the β-epoxide (1,9,7,10), many studies have linked the α-epoxide to the development of atherosclerosis and cancer (1-4,7,11,12).

Smith's comprehensive review (7) on the autoxidation of cholesterol and biological effects of cholesterol oxides illustrates the need to characterize and quantify sterol oxides in processed foods. Recent reviews (7,13) have documented the probable occurrence of common sterol autoxidation products in food. Two studies that have clearly identified cholesterol epoxide(s) (14,15) involved dried egg products. Some studies have used cholesterol-rich foods subjected to high oxidative stress, which would rarely occur in foods. For example, Chicoye et al. (14) identified the β-epoxide and other sterol oxides in spray-dried egg yolks exposed to direct sunlight for 5 hours. Tsai et al. (15) found 1-33 μg/g α-epoxide in commercial dried-egg products (7).

Although autoxidation of sterols in most foods will occur in time, little is known of the rate of oxidation, the quantities and distribution of the oxidation products and the factors that accelerate sterol oxidation in foods. Attention should be given to foods rich in cholesterol that have been processed with prooxidants, which could generate sterol oxides. Benzoyl peroxide (BP), a widely used free-radical generating agent (16), is allowed in the US to bleach flour and in milk (at 20 μg/g) to make Blue, Swiss and Italian cheeses (17). Although BP itself or flour treated with it, and bread baked from BP-treated flour, carry no significant carcinogenic hazard (18), recent studies using mice have shown BP to be a promoter of skin tumors, possibly from the generation of free radicals (16). Thus, residual BP in foods should be of concern to the food industry. This research detected, and quantified, oxidized sterols in butteroil and in cream treated with BP and in commercial cheese samples purchased from local sources.

MATERIALS AND METHODS

Bleaching of Butteroil and Cream

Anhydrous butteroil was prepared from fresh, unsalted butter (University of Wisconsin Dairy Laboratory) by melting it at 60 C, separating it by centrifugation, washing it once with distilled water and drying it over anhydrous sodium sulfate. Butteroil samples were bleached (in duplicate) with BP (Aldrich Chemical Co., Milwaukee, WI, 99.2% peroxide by analysis (19)). Sufficient BP dissolved in 50.0 mL ethyl acetate was added to 1,440 g of oil to give a final concentration of 500 μg/g. After being stirred (in darkness) for 2 hr at 60 C, 40 mL portions of bleached oil were stored (in darkness) in test tubes sealed with Teflon-lined screw caps (Scientific Products, Inc., McGraw Park, IL). Samples were stored either under N_2 (BP-N) or air (BP-A) at 15 C for 90 days, then an additional yr at −20 C. Controls were treated only with solvent and stored as