Tocopherol and Fluorescence Levels in Deep-Frying Oil and Their Measurement for Oil Assessment

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This study examined how tocopherol retention is affected by the presence or absence of food coatings, and also tested the measurement of fluorescent substance levels in cooking oil to evaluate oil deterioration. Potato slices were tempura-fried (with a coating) or french-fried (without a coating). The three tocopherol isomers decreased with heating time, and better retention was found in the tempura process. The decomposition rates of tocopherol were in the order $\gamma > \delta \geqq \alpha$ for the three isomers for both processes over repeated fryings. The fluorescence of frying oil increased 15- and 17-fold after tempura- and french-frying, respectively, for 32 consecutive times. Changes in the amounts of tocopherol and the fluorescence correlated well with the changes found by the chemiluminescent intensity and five conventional methods of oil quality measurement. These results indicated that tocopherol retention is affected by the food coating, and that measurements of vitamin E loss and fluorescence increase in oil should be useful for assessing the progressive deterioration of frying oil with its repeated usage.

KEY WORDS: Assessment of frying oil, fluorescence level, tocopherol.

Vitamin E (tocopherol) which is labile to oxygen, heat and light is a natural antioxidant for preventing oxidation of unsaturated fatty acid (1,2). The major source of human tocopherol intake is oils and fats with the highest amounts being found in vegetable oils (3), although the amounts vary considerably (4). Vegetable oils also contain large amounts of unsaturated fatty acids such as linoleic acid (5). When such oil is used for frying, the vitamin E is lost with oxidation of unsaturated fatty acids during heating (6,7). This frying oil is absorbed by the food during cooking (8). Thus, the quality of the cooking oil affects the net intake of tocopherol. Polyunsaturated fatty acids are readily oxidized (9,10) and the conjugated dienes which form show increased absorption at 233 nm (11,12). The degradation products of lipids give off a fluorescence (13,14), and interestingly, the fluorescent components of the human tissues are known to increase with aging (15). However, the changes in fluorescence of frying oil during heating have not been studied well. In considering the effects of oxidized lipids on vitamin E nutrition and toxicity to animals (16,17), it is important to study the loss of vitamin E from cooking oil and its relationship to oil deterioration during the frying process.

The present study was undertaken to determine whether or not having a coating can affect the loss of tocopherol in fried food during prolonged heating. Two deep-frying processes, tempura- and french-frying, were examined, the former used a coating on the food and the latter did not. The tocopherol losses were studied in relation to the oxidation occurring in the frying oil: the changes in fluorescence, chemiluminescence, absorption at 233 nm and values from other common oil assessment methods (the color, refractive index, acid value and iodine value).

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EXPERIMENTAL PROCEDURES

Preparation of frying oil. Peeled potatoes, sliced 0.5 cm thick and 5 cm in diameter, were deep-fried by two frying processes often used in Japanese household kitchens: tempura-frying with a coating of wheat flour, egg and water (88, 44 and 106 g per 400 g potatoes, respectively), and french-frying without a coating. Potatoes of the same variety were purchased from a local store, and 400 g each was deep-fried at 180 ± 10°C over a total of 17 min in every frying test. After cooling, the frying process was repeated 32 times using new potatoes in the same oil (total time of heating: 17 min × 32 times = 9 hr 4 min). The deep fryer was filled with 1.7 kg of commercial oil composed of a mixture of soybean and rapeseed oils, and the oil level was adjusted after each frying to compensate for the loss due to absorption by the potatoes. The oil was sampled before and during heating at every fourth frying and stored in sealed bottles at 6°C until analysis.

Determination of tocopherol amounts and fluorescence of the frying oil. Tocopherol isomers (α, γ and δ) and fluorescent substance (FS) were separated by high-performance liquid chromatography (HPLC) and were measured fluoremetrically using a Hitachi FL203 fluorescence detector with a xenon discharge lamp (Ex 298 nm, Em 325 nm) and a Hitachi 234 pump (18). A LiChrosorb SI60 (5 μm) column (4 mm i.d. × 250 mm) was used with isopropanol in n-hexane (0.2/100, v/v) as the mobile phase moving at the rate of 1.8 mL/min. An adequate volume of oil sample dissolved in n-hexane was injected with an internal standard of 2,2,5,7,8-pentamethyl 6-hydroxy chroman (19). The HPLC of the fresh oil and deep-fried oil are shown in Figure 1 with FS appearing as the first peak. The amount of FS was measured in relation to the value for fresh oil.

Determination of chemiluminescence of frying oil. The chemiluminescence (CL) was measured by the method of Kaneda *et al.* (20) using Chemiluminescence Analyzer QX 7C with a synchronous single photon counting apparatus (Tohoku Electronic Industries Co., Ltd., Sendai, Japan). An oil sample was placed on a stainless-steel plate and analyzed at 37°C for 10 min. The chemiluminescent intensity was expressed in terms of average counts for 10 sec of the 10-min measurement and corrected for background current.

Chemical and physical analysis of frying oil. The color of oil (L, La and Lb) was measured using a Model 101D Digital Color and Color Difference Meter (Nippon Denshoku Co. Ltd., Osaka, Japan). ΔE can be obtained from the following formula: $\Delta E = \sqrt{(\Delta L)^2 + (\Delta La)^2 + (\Delta Lb)^2}$. The refractive index (RI) of each oil was determined with

an Abbe refractometer at 20°C. The conjugated dienes to assess deterioration of frying oil (11,12) were separated by silica gel thin-layer chromatography with hexane-diethyl ether (93:7) and determined at 233 nm using a Shimazu dual wavelength thin-layer chromatography scanner CS900 (21). The acid value (AV) and iodine value (IV) were estimated according to the Japan Oil Chemists' Society's Standard Method for Analysis of Fats and Oils (22).

RESULTS AND DISCUSSION

Tocopherol retention in frying oil. Deep-frying reduced the tocopherol content in the frying oil with heating time and the three tocopherol isomers decreased at different rates with oxidation in the two cooking methods studied (Table 1). The initial contents of α-, γ- and δ-tocopherol in the oil were 10.7, 70.7 and 13.6 mg per 100 g oil, respectively. After the 32nd frying, 16.8% α-tocopherol had been lost from tempura oil but 52.5% from french-frying oil. The difference seemed to be due to the use of the coating material in the tempura process. The retention level also differed among the isomers in tempura oil with the highest retention recorded for α-tocopherol. The levels at the 32nd frying were 83.2%, 49.7% and 66.3%, respectively, for α-, γ- and δ-tocopherol. In french-frying oil, the retention levels for these isomers were 47.5%, 24.9% and 50.3%, respectively, at the 32nd frying.

The decomposition rates of tocopherols were $\gamma > \delta \geqq \alpha$ for the three isomers for both processes over repeated fryings in our present study. It has been reported in the literatures that the decomposition of tocopherols is due to oxidation and that the order of antioxidant activity is $\alpha > \gamma > \delta$ tocopherol. On the other hand, Lea (23) showed that the order of antioxidant activity changed with the oil for the experiment and Parkhurst *et al.* (24) reported the order of $\gamma > \delta > \alpha$ for lard. The order of the decomposition rates of tocopherols differs with the heating time and oil used($\gamma > \alpha$ with 26-hr heating and $\alpha > \gamma$ with 103-hr heating in peanut oil and $\gamma > \alpha$ with 26-hr and 103-hr heating in cottonseed oil (6), and $\alpha > \gamma$ with 2-hr heating and $\gamma > \alpha$ with 23-hr heating in a partially hydrogenated soybean oil (7)).

Carlson and Tabacch (7) suggested that the practice of adding frying oil over the frying period can reduce the vitamin E loss of the oil in actual food service operations. We also found that when additional oil was not used to compensate for the loss due to absorption by food being fried, the decomposition rates of α-tocopherol increased in the order of $\gamma = \alpha > \delta$ instead of the $\gamma > \alpha = \delta$ found with 3-hr heating (25). The order of the antioxidant effect for peroxide values (POV) in lard depends on the concentration of tocopherol ($\gamma > \alpha > \delta$ below 0.03% of tocopherol, $\delta > \gamma > \alpha$ above 0.045%) but the decomposition rates were $\alpha > \gamma > \delta$ for both concentrations of tocopherol (26). The changes in the decomposition rates of tocopherol in different oils may be due to different levels of the tocopherol isomers in each oil ($\gamma > \delta > \alpha$ in soybean oil (7) $\alpha = \gamma$ in cottonseed oil and $\alpha > \gamma$ in peanut oil (6)).

Complex reactions can occur such as oxidation, polymerization, and hydrolysis between oil and the food during cooking (27). Thus, the decomposition rate of tocopherol is not the same as the antioxidant activity in every case. The differences in the amount of vitamin E loss from experiment to experiment were probably due to differing experimental conditions such as oil and food used for frying, heating conditions and so on. As vegetable oils significantly contribute to the daily intake of tocopherol, this variation in tocopherol content not only with the original content of the oil but also with the cooking process should be noted.

Fluorescence of frying oil. The fluorescence level of the oil was measured for comparison with the tocopherol content and the results of other common methods of determining oil deterioration. The fluorescence increased with frying time in both tempura- and french-frying

TABLE 1

Retention Level of Tocopherol and the Increase of Fluorescent Intensity of Tempura- and French-Frying Oils over a Period of 32 Successive Frying

Frying method	Number of fryings	Tocopherol retention (%)			Fluorescent intensity (- fold)
		α-toc	γ-toc	δ-toc	
Tempura-frying	0	100.0	100.0	100.0	1.0
	4	90.1	80.7	87.4	3.1
	8	89.6	70.6	83.5	7.7
	12	93.8	61.2	76.4	11.1
	16	92.0	55.7	75.8	14.8
	20	87.3	53.7	70.7	12.2
	24	85.5	50.8	73.1	14.2
	28	87.8	50.4	69.0	13.2
	32	83.2	49.7	66.3	14.7
French-frying	0	100.0	100.0	100.0	1.0
	4	86.2	82.1	91.0	2.4
	8	85.9	74.0	87.1	4.1
	12	83.1	67.6	80.3	4.9
	16	75.9	59.3	74.3	7.0
	20	71.9	49.6	67.8	9.0
	24	64.5	41.6	63.7	11.9
	28	60.9	33.5	51.5	13.6
	32	47.5	24.9	50.3	16.7

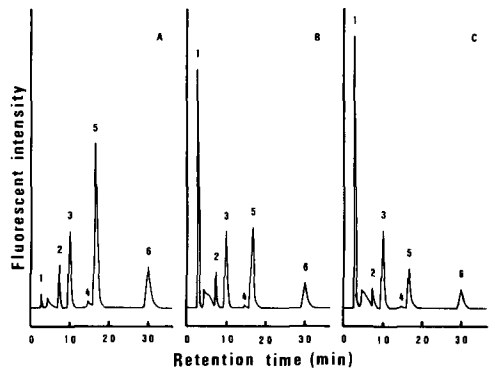

FIG. 1. High-performance liquid chromatogram of fresh oil (A) and deep-fried oil; tempura-frying oil (B) and french-frying oil (C), with internal standard. 1: fluorescent substance, 2: α-tocopherol, 3: 2,2,5,7,8-pentamethyl 6-hydroxychroman (internal standard), 4: β-tocopherol, 5: γ-tocopherol, 6: δ-tocopherol.

processes as shown in Table 1 and Figure 1. This suggested that the fluorescence represents the degradation products of unsaturated fatty acid formed by the reaction to molecular oxygen during lipid oxidation. The fluorescence increased 15- to more than 17-fold after 32 fryings for tempura- and french-frying, showing that it can be a sensitive indicator of frying oil.

Chemiluminescence in frying oil. As shown in Table 2, the level of CL in oil increased with frying time during both tempura- and french-frying processes. The CL level increased 15- and 6-fold after the oil had been used 32 times, respectively. These results confirmed that CL represents the oxidized products formed during frying and that it can be employed to assess the quality of used frying oil (20), and showed that it can be a sensitive indicator of oil deterioration.

Physical and chemical evaluation of frying oil. Five conventional physical and chemical analysis methods used to assess the quality of frying oil were compared. As can be seen from Table 2, all methods correctly ranked the oils according to the length of frying time. The values from ΔE, RI, A233 and AV increased and L and IV decreased with frying time. During heating, darkening of the oil from light yellow to dark brown (ΔE value), an increase in AV, and a decrease in IV agreed with the results found for rice bran oil and palm olein in experiments conducted without using foodstuff (28). The relationships between oil heating and measurements, ultraviolet (UV) absorbance at 233 nm, AV and IV, agree with the reports of other workers (6,7,12,28,29). Comparing the effects of the frying processes on the changes in the specific constituents measured by each method showed three patterns of changes. First, the changes in RI and A233 in tempura-frying were lower than those in french-frying, which agreed with the changes found with the three tocopherol isomers shown in Table 1. On the other hand, the rates of change in ΔE, L and AV showed different trends, and the values for tempura-frying were higher than those for french-frying, which agreed with the changes found with the level of CL. Both frying processes gave almost the same IV values. These results supported the suggestion that no single oil assessment could give an accurate evaluation of oil deterioration (30). Because of the complex reactions between the oil and the food during heating (27), the usefulness of each assessment method is limited to the monitoring of a simple operation, such as where frying time is the only variable. Accurate assessment of used frying oil would require the development of a suitable correlation formula.

Evaluation of tocopherol loss and fluorescent substance level for assessing oil deterioration with repeated heat treatments. Retention of tocopherol and the increase of FS in oil used for deep-frying were compared with other common analytical values for assessing the quality of frying oil. Table 3 summarizes the correlation coefficients (r) obtained from the values shown in Tables 1 and 2. Good correlation among the methods was found among the values for each frying process. As the changes in tocopherol and FS correlated well with the results of

TABLE 2

Changes in Physical and Chemical Values of Tempura- and French-Frying Oils over a Period of 32 Successive Fryings

Frying method	Number of fryings	CL	Color		RI	A233	AV	IV
			ΔE	L				
Tempura-frying	0	2700	0.0	103.0	1.4721	28.8	0.10	123.2
	4	8900	14.7	97.6	1.4722	65.1	0.18	122.1
	8	13530	25.5	89.7	1.4724	88.4	0.38	121.8
	12	22670	36.4	79.8	1.4725	102.8	0.62	120.0
	16	29910	40.7	78.2	1.4725	121.3	0.78	118.0
	20	37630	44.1	73.9	1.4726	116.7	0.93	117.0
	24	34118	44.7	73.4	1.4727	122.5	1.06	117.0
	28	33020	48.2	68.5	1.4727	122.1	1.22	110.0
	32	40580	50.8	64.8	1.4728	98.0	1.33	102.3
French-frying	0	2700	0.0	103.0	1.4721	28.8	0.10	123.2
	4	2870	5.1	102.6	1.4723	70.3	0.08	121.2
	8	3580	7.4	102.0	1.4726	112.4	0.14	119.0
	12	4280	10.6	101.0	1.4728	138.2	0.16	117.3
	16	6220	13.7	101.1	1.4728	167.6	0.21	115.5
	20	9550	17.1	100.1	1.4730	200.0	0.20	115.4
	24	12800	20.3	99.5	1.4732	194.2	0.27	108.5
	28	15280	23.5	99.0	1.4733	212.2	0.42	107.3
	32	16420	26.2	98.3	1.4734	242.2	0.58	105.5

TABLE 3

Correlation Coefficients (r values) Between Tocopherol Level and the Results of Other Methods of Analyzing Frying Oil Deterioration in Tempura- and French-Frying

		Tocopherol retention (%)					Color					
Frying method	Analysis	α-toc	γ-toc	δ-toc	FS	CL	ΔE	L	RI	A233	AV	IV
Tempura-frying	α-toc	1.000	0.800	0.815	-0.682	-0.777	-0.789	0.767	-0.820	-0.669	-0.776	0.708
	γ-toc	0.800	1.000	0.973	-0.967	-0.948	-0.994	0.960	-0.954	-0.955	-0.914	0.679
	δ-toc	0.815	0.973	1.000	-0.933	-0.919	-0.979	0.968	-0.962	-0.878	-0.932	0.793
	FS	-0.682	-0.967	-0.933	1.000	0.940	0.970	-0.947	0.933	0.929	0.905	-0.667
	CL	-0.777	-0.948	-0.919	0.940	1.000	0.966	-0.973	0.955	0.855	0.959	-0.772
	ΔE	-0.789	-0.994	-0.979	0.970	0.966	1.000	-0.984	0.972	0.927	0.945	-0.740
	L	0.767	0.960	0.968	-0.947	-0.973	-0.984	1.000	-0.987	-0.857	-0.983	0.826
	RI	-0.820	-0.954	-0.962	0.933	0.955	0.972	-0.987	1.000	0.843	0.977	-0.815
	A233	-0.669	-0.955	-0.878	0.929	0.855	0.927	-0.857	0.843	1.000	0.792	-0.455
	AV	-0.776	-0.914	-0.932	0.905	0.959	0.945	-0.983	0.977	0.792	1.000	-0.873
	IV	0.708	0.679	0.793	-0.667	-0.772	-0.740	0.826	-0.815	-0.455	-0.873	1.000
French-frying	α-toc	1.000	0.983	0.972	-0.985	-0.949	-0.982	0.968	-0.952	-0.945	-0.925	0.967
	γ-toc	0.983	1.000	0.992	-0.979	-0.945	-0.998	0.985	-0.989	-0.982	-0.882	0.970
	δ-toc	0.972	0.992	1.000	-0.982	-0.963	-0.996	0.986	-0.979	-0.967	-0.902	0.972
	FS	-0.985	-0.979	-0.982	1.000	0.984	0.986	-0.985	0.962	0.940	0.943	-0.986
	CL	-0.949	-0.945	-0.963	0.984	1.000	0.959	-0.963	0.926	0.890	0.925	-0.971
	ΔE	-0.982	-0.998	-0.996	0.986	0.959	1.000	-0.990	0.987	0.977	0.893	-0.977
	L	0.968	0.985	0.986	-0.985	-0.963	-0.990	1.000	-0.986	-0.964	-0.905	0.975
	RI	-0.952	-0.989	-0.979	0.962	0.926	0.987	-0.986	1.000	0.981	0.855	-0.964
	A233	-0.945	-0.982	-0.967	0.940	0.890	0.977	-0.964	0.981	1.000	0.824	-0.921
	AV	-0.925	-0.882	-0.902	0.943	0.925	0.893	-0.905	0.855	0.824	1.000	-0.918
	IV	0.967	0.970	0.972	-0.986	-0.971	-0.977	0.975	-0.964	-0.921	-0.918	1.000

common methods of judging oil quality, these measurements can also be considered to be indicators of the degree of deterioration of frying oil.

If the nutritional quality of an oil is to be measured together with its deterioration level, measuring the amount of tocopherol can be useful. As shown in Figure 1, the oxidized compounds have short retention time in comparision with tocopherol isomers. During deep-fat frying, the first peak (FS) increased markedly and was followed by many other peaks that increased with heating time. These oxidized compounds may affect quantitation of the α-tocopherol in more highly oxidized oils. Of the three tocopherol isomers, γ-tocopherol is the most abundant and therefore the easiest to measure. Also, as shown in Table 1, the changes in γ-tocopherol during the heating were the greatest. Therefore, for assessing used frying oil, γ-tocopherol should be better than α- and δ-tocopherol. Of course, this method is limited to the monitoring of oils which contain tocopherol. These results show that measurements of tocopherol and FS levels offer a combination of good sensitivity, simplicity and rapidity and thus should be useful for assessing used frying oil.

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