Factors Affecting the Content of Tocopherol in Soybean Oil

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The state of soybeans prior to extraction affected the tocopherol content of crude soybean oils. Soybean flakes with a thickness of 0.16–0.33 mm had higher extracted oil yield but a slightly lower tocopherol content of the oils than did cracked beans and thicker bean flakes. High-moisture content and long storage of soybeans resulted in lower tocopherol content in the crude oils, with moisture content being more important than storage time at decreasing the tocopherol content of oils. Soybean oil from stored beans with 15 ± 1% moisture content led to a more significant decrease in the tocopherol content than did oil from stored beans with low (12%) or high (18%) moisture contents. Soybean flakes contaminated with oxidized oil had a significant effect on the decrease of the tocopherol content in crude oils. The high amount of phospholipids in crude soybean oil might result in a smaller decrease in the tocopherol content of oil during heating.

KEY WORDS: Moisture, phospholipids, soybean oils, tocopherol.

The most important natural antioxidants in fats and oils are tocopherols, which consist mainly of α, β, γ and δ forms (1,2). Among these tocopherols, γ- and δ-tocopherols, shown by some researchers to have better antioxidant activities than the others (3), are the major forms in soybean oil (4,5). The autoxidation of unsaturated oils is a free-radical chain process (6,7). Tocopherols are easily oxidized; in particular, γ-tocopherol is readily oxidized to chroman-5,6-quinone, which has poor antioxidant characteristics (8). Oil processing, especially deodorization, is mainly responsible for a decreased tocopherol content in oil products (4), which leads to reduced oil stability (9). Other factors affecting the tocopherol content of soybean oils include soybean quality, duration of storage, content of damaged beans and storage conditions of refined, bleached and deodorized (RBD) oils. Thus, the purpose of this study was to evaluate factors affecting the tocopherol content of soybean oils, and to determine ways to improve the quality of RBD soybean oil.

MATERIALS AND METHODS

Materials. Raw soybeans (FGIS Grade No. 2; FWU SOW Grain Products Co., Ltd., Taichung, Taiwan) were imported from the United States and had a moisture content of *ca.* 12%. Whole intact soybeans were selected from these beans. Tocopherol isomers (α, β, γ and δ) were purchased from E. Merck (Darmstadt, Germany). Solvents used in the analyses by high-performance liquid chromatography (HPLC) were HPLC-grade and included *n*-hexane, isopropanol and alcohol. The *n*-hexane used for extraction of soybean oil was of commercial grade.

Soybean preparation. The soybeans used in these experiments were prepared as follows: (i) Soybeans were prepared in several forms, including soybean flour (40 mesh), cracked beans and three different thicknesses of flakes (0.16–0.33, 0.67–0.83 and 1.30–1.90 mm) and (ii) cracked and intact soybeans were prepared to specific moisture contents in the range of 12–18% by adding the required amount of water and then equilibrating for one week and storing for another one to four weeks before extraction.

Soybean oil processing. Soybean oil processing, including extracting, refining, bleaching and deodorizing, was based on the procedure described by Chu and Lin (10). Soybean preparation and processing were conducted in triplicate for each test. To measure the effects of the presence of oxidized oil from a contaminated extractor on the tocopherol contents of soybean oils during extraction, crude oils were extracted from 80 g of soybean flakes in the extractor, where different amounts (0–2.5 mL) of oxidized oils with 15% total polar compounds had been added *in* advance. The oxidized oils were prepared by flushing RBD soybean oil with compressed air at 60 L/h during heating at 120°C for 6 h. The total polar compounds and peroxide values of these oxidized oils were 15% and 74.5 meq/kg, respectively.

Heating tests of crude and RBD soybean oil. Three replicates (500 mL each) of crude and RBD soybean oil, processed from intact soybeans, were subjected to continuous heating at 60°C in a forced draft oven for at least 600 h. Oil (10 mL) was removed every day for further analyses, including tocopherol content and phospholipid composition.

Analysis. The tocopherol contents in crude and in RBD soybean oils were determined by HPLC (ABI Analytical Kratos Division, Ramsey, NJ) according to the procedure described by Lai *et al.* (11). The phospholipid composition was determined by thin-layer chromatography according to the American Oil Chemists' Society Method Ja 7-86 (12). The induction period (IP) of the oils was determined by Rancimat (Metrohm Herisau, Schweiz, Suisse, Switzerland), an apparatus for determining the oxidation stability (proneness to rancidity) of products containing oils and fats, and followed the procedure described by Zurcher and Hadorn (13). The total polar compounds were determined by liquid chromatography methods according to the procedure described by Paquot and Hautfenne (14). The peroxide value of the oil was measured by AOCS Method Cd 8-53 (12). All test results are the average of duplicate analyses of each replicate.

Statistical analysis. Paired Student's *t*-test was used for statistical comparison, and the calculation was done with the Statistical Analysis System software package (15).

RESULTS AND DISCUSSION

State of soybeans prior to oil extraction. The effects of soybean flour, cracked beans, different thicknesses of bean flakes and extraction time on the total oil yield are shown in Table 1. Higher amounts of oil were extracted from flour and from thin flakes (0.16–0.33 mm) than from cracked beans or medium (0.67–0.83 mm) and thick (1.30–1.90 mm) flakes. The oil extraction from either bean flour or thin flakes was almost complete within 1 h. As the extraction time was extended to 2–3 h, the respective oil yields from bean flour, medium and thin flakes were nearly all in the range of 19–20%. Cracked beans and thick flakes had

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TABLE 1

Time of Extraction of Soybean Flour[a], Cracked Beans[a] and Different Thicknesses of Bean Flakes[a] in Relation to the Total Oil Yield[b] and the Tocopherol Content[b] of the Oils

Time of extraction (h)	Flour[c]	Cracked beans	Flakes[d] Thick	Flakes[d] Medium	Flakes[d] Thin
Oil yield (% w/w)					
1	20.6 ± 0.4^{e}	10.5 ± 0.3^{h}	12.3 ± 0.3^{g}	18.2 ± 0.1^{f}	20.1 ± 0.5^{e}
2	20.8 ± 0.2^{e}	11.6 ± 0.5^{h}	13.9 ± 0.1^{g}	19.1 ± 0.2^{f}	20.1 ± 0.1^{e}
3	20.8 ± 0.5^{e}	14.1 ± 0.1^{h}	15.2 ± 0.1^{g}	19.0 ± 0.3^{f}	20.2 ± 0.1^{e}
Tocopherol content (ppm)					
1	1450 ± 80^{e}	1419 ± 14^{e}	1388 ± 22^{e}	1351 ± 15^{e}	1284 ± 33^{f}
2	1507 ± 47^{e}	1458 ± 10^{e}	$1410 \pm 28^{e,f}$	$1415 \pm 25^{e,f}$	1326 ± 21^{g}
3	1531 ± 36^{e}	1483 ± 19^{e}	$1456 \pm 33^{e,f}$	1408 ± 16^{f}	1334 ± 27^{g}

[a]Soybeans at 12% moisture content; cracked beans were obtained from cracking rolls; flakes were obtained from cracking and flaking rolls.
[b]Values represent the average of duplicate analyses of three replicates, mean ± SD.
[c]Particle size of soybean flour was smaller than 40 mesh.
[d]Thickness of flakes: thin, 0.16–0.33 mm; medium, 0.67–0.83 mm; thick, 1.30–1.90 mm.
Means in the same horizontal row bearing different superscripts (e–h) differ significantly at a level of $P < 0.05$.

lower oil yields (14–15%), even after 3-h extraction. The high extraction rate of thin flakes (0.16–0.33 mm) is generally why soybeans are flaked to 0.254–0.305 mm in a conventional solvent extractor (16). Much thinner flakes (<0.200 mm) have been reported to reduce the bed percolation rate and to limit the ability of the solvent to get through the flake bed (17), which is undesirable in a commercial process. Although bean flour had greater oil extraction yields in our studies and superior qualities as proposed by Nieh and Snyder (18), there is still no commercial extraction process designed for bean flour.

Aside from oil yields, the effect of bean state and extraction time on the tocopherol content of the oils is also shown in Table 1. In the first and second hour of extraction, there were no significant ($P > 0.05$) differences in the tocopherol content of the oils from bean flours, cracked beans and all types of flakes, except for the thin ones. After 3-h extraction, the oils from flour, cracked beans and thick flakes had significantly ($P < 0.05$) higher amounts of tocopherol than did those from thin ones. However, among the bean flour, cracked beans and thick flakes, the tocopherol contents of the extracted oils were not significantly ($P > 0.05$) different from each other. The high amounts of tocopherol in the oils from bean flour most likely resulted from the immediate subjection of the flour to oil extraction without too much exposure to air. Although cracked beans had a high amount of tocopherol, they had the lowest extraction rate among all the bean types, indicating that they were not suitable for oil extraction.

Concerning different thicknesses of bean flakes, thick and medium ones had no significant difference ($P > 0.05$) in the tocopherol content of the oils. However, thin flakes had a significantly lower tocopherol content ($P < 0.05$) in the oil than did the thick and medium ones. The loss of tocopherol in the oils from thin flakes might result from the oxidation of tocopherol through enzymatic reactions. Several papers (10,11) have reported that enzyme action is responsible for the reduction of tocopherol content in soybean oil from beans of poor quality, such as broken beans after long storage, and from bean flakes with high moisture contents (10,11). Recently, the relationship between enzyme activity and flake thickness has been reported by List *et al.* (19), who studied the effect of flaking soybeans on phospholipase D activity. They found that the enzyme activity increased as flake thickness decreased. These previous studies implied that the lower tocopherol content in the oils from thin flakes might also result from the higher enzyme activity, as well as from more increased surface area for tocopherol oxidation in these flakes than available in thick ones. The tocopherol content of oils increased as bean flake thickness increased, while the extracted oil yield decreased as bean flake thickness increased. To obtain a high oil yield and high levels of tocopherol in the extracted oil, bean flakes with optimum thickness were required for oil extraction.

Effects of moisture content of soybeans and bean breakage on the tocopherol content of oils. Intact soybeans at different moisture contents, prepared as just described, were stored for three weeks. Figure 1 shows that

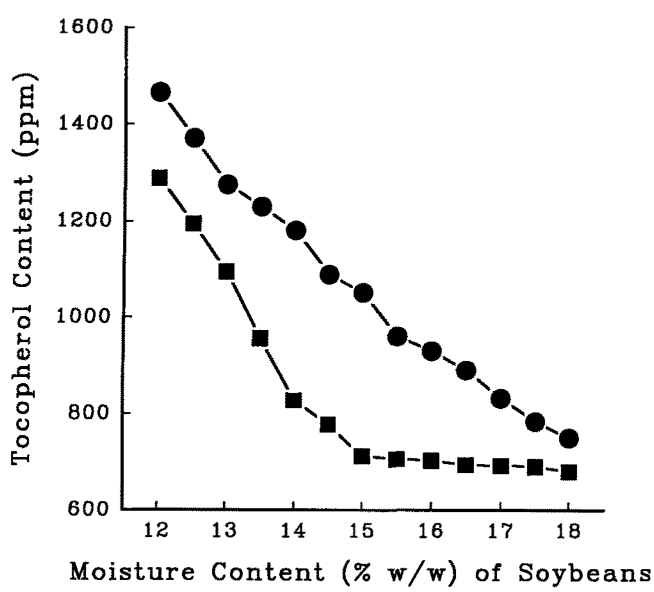

FIG. 1. Tocopherol content of crude soybean oil extracted from intact soybeans held at different moisture contents. (●) One-week equilibration; (■) one-week equilibration plus three-week storage. Values are the average of duplicate analyses of two replicates.

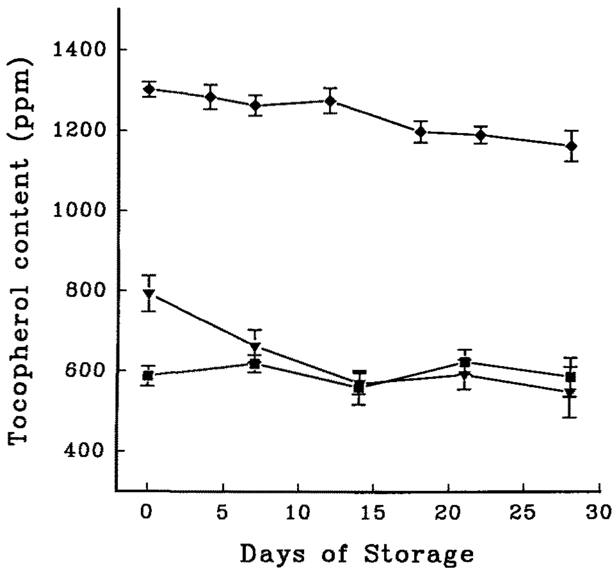

FIG. 2. Effects of moisture content and storage time of cracked soybeans on the tocopherol content of crude soybean oils. Cracked soybeans at different moisture contents; (◆, 12%; ▼, 15%; ■, 18%) were equilibrated for one week before storage. Each data point is the average of three samples, with the standard deviation shown by the error bars.

the total tocopherol content of crude soybean oil decreased as the moisture content of beans increased. A decrease of 25% or more in tocopherol content was found in the oil from 3-wk stored beans with 14–16% moisture content as compared to oils from beans equilibrated but not stored. However, when the moisture content of soybeans exceeded 17%, the difference in the tocopherol content between oils from equilibrated and stored beans became smaller because of a great loss of tocopherol from these beans during equilibration.

The oils from cracked beans with 15 and 18% moisture content had lower tocopherol contents than did those from beans with 12% moisture (Fig. 2). A 10% decrease in the tocopherol content was found in the oil from cracked beans with 12% moisture between day zero and 4 wk of storage. These results indicate that oils with a high amount of tocopherol can be obtained if the bean moisture was maintained at 12% when the beans were broken. For cracked beans with 18% moisture, the mean value and standard deviation of the tocopherol content in the oils from all beans stored for different times were 595 ± 36 ppm, which fluctuated within a 6% range (Fig. 2). A great loss of tocopherol during equilibration resulted in the low tocopherol content (587 ± 25 ppm) of oils from beans at day zero of storage, the value of which is close to the mean value (595 ppm) of all stored beans. These results indicate that, as the bean moisture content increased to 18%, the length of bean storage time had no effect on the reduction of tocopherol content of the extracted oil. For oils from beans with 15% moisture content, however, a 16% decrease in the tocopherol content was found between day zero and 1-wk storage. As the length of storage for beans with 15% moisture content increased to four weeks, the decrease in the tocopherol content reached 31%, which was greater than for beans with 12 and 18% moisture contents. The results were similar to those shown in Figure 1, in which a greater (32%) decrease occurred in the tocopherol content of the oils from intact beans with 15% moisture content than from beans with other moisture contents when comparing a 1-wk equilibration with no storage to a 1-wk equilibration plus a 3-wk storage.

Table 2 shows that the tocopherol content of RBD soybean oil was affected by the moisture content of soybeans regardless of whether the beans were intact or cracked. Oils from cracked beans contained significantly ($P < 0.05$) less tocopherol than did those from intact beans with the same moisture content. RBD soybean oil from intact and cracked beans with 12% moisture content had a longer IP than did oils from beans with 15 or 18% moisture contents, as measured by the Rancimat (Table 2). There was no significant difference ($P > 0.05$) in the IP between the oils from cracked and intact beans with either 12 or 18% moisture contents, but a significant difference ($P < 0.05$) occurred in the IP between the oils from cracked and intact beans with 15% moisture contents. Beans with different moisture contents caused a significant difference in the IP ($P < 0.05$) of the oils. The lower the moisture content of the soybeans, the longer the IP of soybean oil and the higher the tocopherol content. To maintain good oil stability and to retain the most tocopherol in soybean oils, it is important to have a low moisture content in the beans prior to oil extraction.

The effects on tocopherol content of oils from intact beans with 12, 15 and 18% moisture content containing 0, 10, 15 and 20% cracked beans, which are depicted as 12-0, 12-10, 12-15, etc., are shown in Table 3. The bean moisture content had a great impact on the tocopherol content of the oils. For beans at the initial storage period and after 1-wk storage, the tocopherol content of the extracted oils significantly decreased as bean moisture

TABLE 2

Tocopherol Content[a] (Toc) and Induction Periods[a] (IP) of Refined, Bleached and Deodorized Soybean Oil at Different Moisture Contents

	Moisture content (% w/w)					
	12%		15%		18%	
	A[b]	B[b]	A	B	A	B
IP (h)	3.22 ± .07[c]	3.20 ± .03[c]	2.61 ± .05[d]	2.38 ± .08[e]	2.19 ± .02[f]	2.13 ± .06[f]
Toc (ppm)	1348 ± 65[c]	1113 ± 45[d]	748 ± 33[e]	533 ± 20[f]	475 ± 22[g]	398 ± 35[h]

[a]Values represent the average of duplicate analyses of three replicates, mean ± SD.
[b]A, intact soybeans; B, cracked soybeans.
Means in the same horizontal row bearing different superscripts (c–h) differ significantly at a level of $P < 0.05$.

TABLE 3

Effects of Bean Moisture Content and Levels of Cracked Beans on the Tocopherol Content (ppm)[a] of Crude Soybean Oils

Group[b]		Initial period	1-wk Storage	2-wk Storage
12%	0%	1476 ± 30^{c,h}	1387 ± 35^{c,h}	1322 ± 57^{c,h}
12%	10%	1444 ± 67^{c,h}	1362 ± 26^{c,h}	1302 ± 70^{c,h}
12%	15%	1421 ± 35^{c,h}	1352 ± 30^{c,h,i}	1284 ± 56^{c,i}
12%	20%	1400 ± 62^{c,h}	1305 ± 60^{c,h,i}	1255 ± 61^{c,i}
15%	0%	991 ± 44^{d,h}	841 ± 35^{d,i}	786 ± 32^{d,i}
15%	10%	984 ± 37^{d,h}	835 ± 42^{d,i}	776 ± 22^{d,i}
15%	15%	951 ± 27^{d,h}	797 ± 36^{d,e,i}	717 ± 31^{d,e,j}
15%	20%	937 ± 23^{d,h}	749 ± 21^{e,i}	694 ± 28^{e,f,j}
18%	0%	706 ± 35^{e,h}	699 ± 23^{f,h}	675 ± 27^{e,f,h}
18%	10%	687 ± 47^{e,f,h}	673 ± 36^{f,g,h}	641 ± 25^{f,g,h}
18%	15%	668 ± 33^{e,f,h}	657 ± 33^{f,g,h}	616 ± 21^{g,h}
18%	20%	633 ± 25^{f,h}	626 ± 33^{g,h}	601 ± 22^{g,h}

[a]Values represent the average of duplicate analyses of three replicates, mean ± SD.
[b]Group: The first column represents the moisture content (% w/w) of soybeans, and the second column represents the level of cracked beans (% w/w).

Means in the same vertical column bearing different superscripts (c–g) differ significantly at a level of $P < 0.05$. Means in the same horizontal row bearing different superscripts (h–j) differ significantly at a level of $P < 0.05$.

increased. When the beans were stored for two weeks, the oils from treatments 15-15, 15-20 and 18-0 were not significantly different ($P > 0.05$) in their tocopherol contents. Similar results were obtained for treatments 15-20, 18-0 and 18-10, which indicates that the tocopherol content of the oil from beans containing a high level of cracked beans at medium to high moisture content (*e.g.*, 15-20) was similar to that of oil from beans with high moisture content containing no cracked beans after long storage (*e.g.*, 2 wks).

In addition to bean moisture, the effects are illustrated for different levels of cracked beans among the intact beans at each moisture content during storage on the tocopherol content of the extracted oil (Table 3). For soybeans with 12% moisture content at day 0 (initial period), 1- and 2-wk storage, the tocopherol content of the oils from all levels of cracked beans (12-0 to 12-20) was not significantly different ($P > 0.05$). When a high level of cracked beans was included (*e.g.*, 12-15, 12-20), however, a significantly lower ($P < 0.05$) tocopherol content of the oils was found in the beans stored for two weeks than in those stored for one week. These results indicate that a high level (≥15%) of cracked beans in the intact beans after long storage (>1 wk) still affected the tocopherol content of the oils, even when the bean moisture was low (*e.g.*, 12%).

For soybeans with 15% moisture content, the levels of cracked beans among the intact beans at the initial period had no significant effect on the reduction of tocopherol content in the extracted oils. But when the beans were stored for one and two weeks, respectively, the oils from beans with 20% cracked beans (15–20) had significantly lower ($P < 0.05$) tocopherol content than did those from beans with 0–10% cracked beans. However, no significant difference ($P > 0.05$) was found in the tocopherol content of the oils from treatments 15-15 and 15-20. Within a storage period, insignificant differences also were observed among oils from treatments 15-0, 15-10 and 15-15 ($P > 0.05$) (stored for 1 or 2 wk, respectively). By comparing the storage effect of beans at each level of cracked beans, it was seen that 1-wk of storage caused a significant reduction in tocopherol content. For beans with a high level of cracked beans (15-15, 15-20), the tocopherol content of the oil from beans after 1-wk storage was significantly higher ($P < 0.05$) than that from oil after 2-wk storage.

As the bean moisture content increased to 18%, the oil from beans containing 20% cracked beans (18-20) had a significantly lower ($P < 0.05$) tocopherol content than did oil from intact beans (18-0). There was no significant difference ($P > 0.05$) between the tocopherol content of treatments 18-15 and 18-20 for each bean storage period. The length of storage also made no significant difference ($P > 0.05$) in the tocopherol content of the oils from the beans with the same levels of cracked beans. These results indicate that a high level of cracked beans (≥20%) among the beans with high moisture content (18%) was more important to tocopherol content than was the length of bean storage.

Effect of oxidized soybean oil contamination on the tocopherol content of crude soybean oils. The effect of oxidized soybean oil present in the extractor on the tocopherol content of total extracted oil during oil extraction of soybean flakes is shown in Figure 3. The tocopherol content in the extracted oils decreased as the amount of oxidized soybean oil (15% total polar compounds) present in the extractor increased. The autoxidation of unsaturated lipids is a free-radical chain process (6). Thus, the presence of oxidized oil in the extractor during extraction of soybean flakes might promote the propagation of free radicals initiating from unsaturated lipids. The behavior of tocopherols in the production of free radicals from a chain-propagating reaction is to suppress and terminate the production of lipid-peroxyl radicals (20,21). This might explain the decrease in the tocopherol content of total extracted oil when the amounts of oxidized soybean oil present in the extractor increased. A reduction of 25% tocopherol content occurred in crude soybean oil extracted from soybean flakes in the extractor when it contained 1.5 mL oxidized soybean oil. To retain a maximum amount of tocopherol in soybean oil and, thus, a high-quality RBD

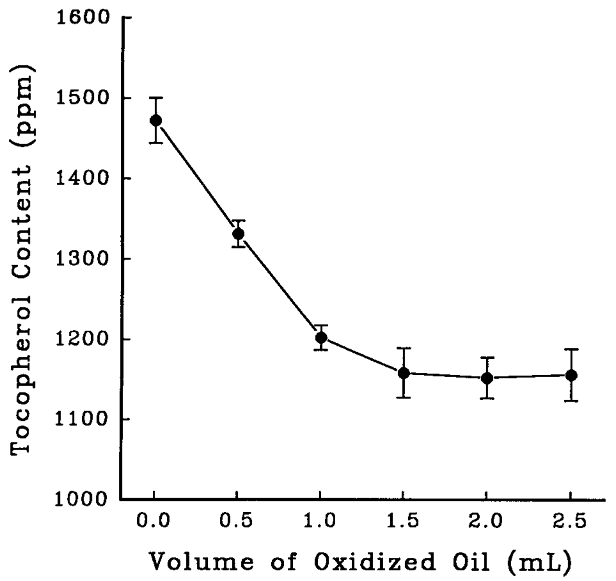

FIG. 3. Effect of the presence of oxidized oil (total polar compounds, 15%) in the extraction on the tocopherol content (±SD) of crude soybean oil. Each extractor data point is the average of three samples, with the standard deviation (SD) shown by the error bars.

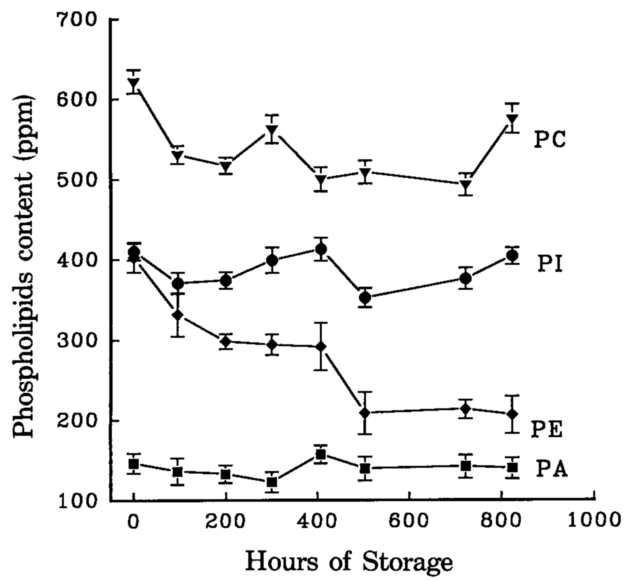

FIG. 5. Change in phospholipid composition of crude soybean oil during storage at 60°C. PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PA, phosphatidic acid. Each data point is the average of three samples, with the standard deviation shown by the error bars.

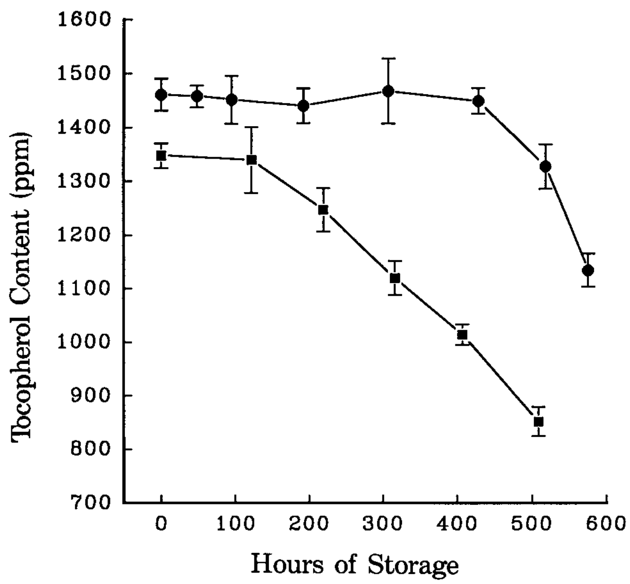

FIG. 4. Change in tocopherol content of crude (●) and refined, bleached and deodorized (■) soybean oils during storage at 60°C. Each data point is the average of three samples, with the standard deviation shown by the error bars.

soybean oil, the removal of oxidized oils or impurities in the extractor is important.

Change in tocopherol content of crude and RBD soybean oils during hot storage. Both crude and RBD soybean oils were stored at 60°C for more than 600 h. The tocopherol content of RBD soybean oil was significantly lower ($P < 0.05$) than that of crude soybean oil before and during storage (Fig. 4). The analysis of phospholipids in the crude oil (Fig. 5) showed that only the content of phosphatidylethanolamine (PE) decreased gradually during storage, whereas the contents of phosphatidylcholine, phosphatidylinositol and phosphatidic acid fluctuated during storage. PE clearly has been shown to act synergistically in cooperation with tocopherols and some of their derivatives as antioxidants (22), likely as a result of the strong proton-donating ability of PE as compared to the other phospholipids (22). Phosphoric acid has been reported to regenerate tocopherol by providing protons (23). Thus, a greater maintenance of tocopherol content of crude soybean oil as compared to RBD soybean oil during storage might result from PE or its decomposition products, which could donate a proton and react with radicals when the formation of tocopherol radicals or lipid-peroxyl radicals occurred. The lesser amount of phospholipid in RBD soybean oil (10 ppm), than that in crude oil, might allow a greater decrease in the tocopherol content of RBD soybean oil. In this study, the tocopherol content in the crude soybean oil decreased only slightly during 400 h of storage.

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