SHORT COMMUNICATION

Hydroperoxide Formation in Soybean Seeds During Storage[1]

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'Forrest' soybeans were stored for two years, and the extracted lipids were assayed for hydroperoxide content. The crude lipid was separated by high-performance liquid chromatography (HPLC), and three hydroperoxide peaks plus the triglyceride peak were measured every two months. There was a lag in hydroperoxide production for the first nine months followed by a steady increase for the remaining twenty-two months. This method of measuring changes in lipid oxidation should be useful for monitoring seed changes in germination and vigor during storage.

KEY WORDS: HPLC, hydroperoxide, lipids, oxidation, peroxidation, soybeans.

Hydroperoxides are products of lipid oxidation and can break down to form secondary oxidation products. Secondary volatile products have off-flavors and are undesirable in vegetable oil used for food. Although hydroperoxides and their secondary products can be removed during refining, the refined oil coming from oxidized crude oil is of low quality (1). Thus, it would be advantageous to use crude oils that are low in hydroperoxides.

Lipid peroxidation is thought to occur in seeds and to be responsible for seed deterioration during storage, causing reductions in vigor or germination (2-4). Secondary volatile products of oxidation have been shown to inhibit the germination of soybean seeds (3). Most studies on seed deterioration measure lipid decrease rather than hydroperoxides, and the seeds have had accelerated aging rather than natural aging, which is not the same (4). Wilson and McDonald's (4) lipid peroxidation model of seed deterioration indicates that seed damage occurs after imbibition during germination due to secondary products from hydroperoxides. Though membrane lipids are considered a sensitive and damaging site of lipid peroxidation, storage lipids in soybeans might be more important to seed germination and vigor because of their large quantities (4).

In this investigation, relative quantities of hydroperoxides were measured in crude soybean oil extracted from soybeans over 31 mon storage.

EXPERIMENTAL PROCEDURES

Arkansas-certified 'Forrest' soybeans grown in 1987 were obtained and stored at room temperature (23-26°C) in plastic bags for two years. 'Forrest' soybeans harvested in 1990 were also obtained and stored in the same manner for 3 mon. The storage bags held 1 kg bean samples, and bean moistures were below 10%. A UDY Cyclone Sample Mill (UDY Corp., Fort Collins, CO) was used to grind 60-g portions for oil extraction. Oil was extracted from 1-g flour samples by means of a five-minute equilibrium method (5) with three replications at each sampling time.

Hydroperoxide detection. Components in crude soybean oil were separated by high-performance liquid chromatography (HPLC). A 50-μL sample loop was used to inject approximately 400 μg of crude soybean oil onto a Beckman Model 322 liquid chromatograph (Beckman Instruments, Fullerton, CA). A 15 × 0.46 cm normal phase Beckman column packed with 5-μm Ultrasphere silica particles was used. The solvent system was 0.75% isopropyl alcohol in hexane, and the solvent flow was 2 mL/min. Detection was by a Hitachi 155-00 spectrophotometer (Hitachi Co., Tokyo, Japan) at 233 nm. Areas of the three largest hydroperoxide peaks (compounds with maximum absorbance at 233 nm) eluting between 3 and 5 min and the triglyceride peak were measured (6).

RESULTS AND DISCUSSION

During the HPLC separation of freshly extracted lipids from various lots of soybeans, we noticed large differences in the amounts of hydroperoxides in the oil. Factors investigated that may have been responsible for the variability included flour moisture, preheat treatment of the beans, presence of hulls, extraction temperature, grinding temperature, cultivars, method of extraction, and flakes vs flour. None of these factors could explain the large differences in hydroperoxides.

When new soybeans were purchased for laboratory analyses, we saw a large decrease in hydroperoxides compared to older soybeans. The soybeans were nine months

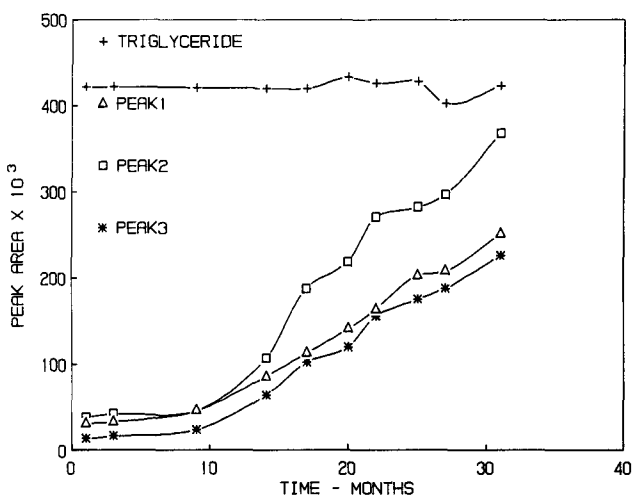

FIG. 1. Increases in area of three hydroperoxide peaks in freshly extracted crude soybean oil over a period of 31 mon of seed storage.

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old when obtained, but relatively low hydroperoxide peaks were found. Analyzing freshly extracted oil every other month indicated that there was an increase in the amount of hydroperoxides extracted as the beans aged compared to the triglyceride peak (Fig. 1). When fresh 1990 beans were analyzed 1 and 3 mon after harvest, low hydroperoxide peaks were found, indicating that they were present at bean maturity and formation was slow. The rapid equilibrium extraction procedure extracts only stored lipids rather than membrane lipids as indicated by the very low phospholipid content (5). The hydroperoxides, therefore, were coming only from the stored lipids.

The several compounds absorbing at 233 nm (peaks 1, 2 and 3) may be hydroperoxides that differ in *cis-,trans* and positional isomerization. Neff *et al.* (7) separated monohydroperoxide isomers of trilinoleoylglycerol with an HPLC system similar to ours and obtained similar chromatographs. We speculate that the first hydroperoxide peak represents the *cis,trans*-13-hydroperoxides located in the 2-position on trilinoleoylglycerol and the second peak the *cis,trans*-13-hydroperoxides in the 1(3)-position. The third peak shows probably *trans,trans* hydroperoxides and the fourth peak, which was not monitored, the *cis,trans*-9-hydroperoxides.

The results of this investigation indicate a slow accumulation of hydroperoxides over time in soybean seeds. The highest quality crude oil and hence the highest quality refined oil would come from soybeans that have been stored for the least time.

The direct measure of hydroperoxides by HPLC in oil extracted from soybean seeds should be a useful technique in studying seed deterioration during storage.

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