Effects of Lipid Peroxidation During Storage on Raw and Roasted Peanut Proteins[1]

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ABSTRACT

The development of peroxidation in several varieties of peanuts stored for 12 months at 4°C was measured spectrophotometrically. Samples of raw and roasted peanuts were extracted with two solvent systems: hexane and chloroform/methanol. Proteins were extracted from the meals with buffered sodium chloride; protein recoveries, solubilities, and electrophoretic mobilities were compared. Intact raw peanuts were stored for at least 5 months at 4°C without appreciable peroxidation, but thereafter, peroxidation proceeds slower than that of roasted peanuts.

Storage conditions for quality retention in raw peanuts have been investigated for many years (Young and Holley, 1965; Holley and Hammons, 1968; Woodroof, 1973). According to Woodroof (1973), to maintain high quality during storage, the temperature and relative humidity should be low, the atmosphere should be free of odors, and air should be circulated through the stored peanuts. Temperatures of 32-40°F are recommended for storage of shelled peanuts, but information relating temperature to storage life (i.e., how long peanuts can be stored without loss of edible quality) is still sought by manufacturers of peanut products who must store peanuts for long periods.

While Woodroof's data on storage of raw peanuts might be interpolated for certain uses, they lack information on the development of peroxidation in raw (or roasted) peanuts caused by enzymic (or nonenzymic) catalysts. The purpose of this research was to examine the effects of 12-months cold storage on peroxidation of lipids in peanuts, the effects of polar and nonpolar solvents on extraction of lipids, on solubility and electrophoretic mobility of proteins, and finally, the effects of enzymic and nonenzymic catalyzed peroxidation on properties of the peanut proteins.

Materials and Methods

Runner, Spanish, and Virginia (1972 crop) peanuts were obtained from commercial suppliers. The raw peanuts, plus corresponding samples roasted for 25-30 minutes at 150° to 175° in a forced draft oven, were stored in closed glass jars (one large jar per sample) at 4°C for a year. Duplicate samples for analyses were removed every four months. Raw and roasted peanuts were defatted by extraction with spectral grade hexane or chloroform/methanol (3/1) with a mortar and pestle. The deoiled meal was further extracted with pH 7 buffered 10% sodium chloride (NaCl) for 1 hour at room temperature (24°C), and the mixture centrifuged at 12,000 x g for 15 minutes. The salt-insoluble fraction was resuspended in deionized water, then both the salt-soluble and insoluble fractions were dialyzed against deionized water, and lyophilized. All fractions, i.e., meal, salt-soluble and insoluble fractions were analyzed for nitrogen by the macro-Kjeldahl method. (Protein is reported as N X 5.46.) The salt-soluble fraction was dissolved in 0.1 M Tris buffer, pH 7.2. The salt-insoluble fraction was suspended in 1% sodium dodecyl sulfate (SDS) buffered with 0.1 M sodium phosphate, pH 7.2, and incubated overnight at 37°C. The suspension was then centrifuged and the clear supernatant was saved for further analyses. Salt-soluble and insoluble fractions were analyzed for their protein and lipid banding patterns by polyacrylamide gel electrophoresis (St. Angelo and Ory, 1975). The degree of lipid peroxidation was determined by measuring the increase in conjugated diene hydroperoxide (CDHP) according to the method of St. Angelo, et al. (1972), except that larger samples (1.5 - 2.5g) of whole peanuts were analyzed.

Results and Discussion

A preliminary study of the effects of different organic solvents on proteins extracted from peanuts showed that certain proteins become more insoluble, partially dissociated, and have different elution patterns after DEAE-cellulose chromatography (Neucere and Ory, 1968). To investigate lipid-protein interaction and the effects of polar and nonpolar solvents on properties of specific proteins, peanuts were extracted with a nonpolar solvent, hexane, and a polar solvent system, chloroform/methanol, that is generally used for extraction of lipids from animal tissues. Results are compared in Table 1. The polar solvent extracted 51% lipids while hexane extracted 49%. Since hexane extracts primarily neutral glycerides, the difference may be due to the extraction of phospholipids, glycolipids, and other complex lipids by the polar solvent. The recovered meals differed by 2% but the percent protein in both meals was the same, 44%. However, the meal prepared by hexane extraction was a fine, white flour, while that from the polar solvent system was clumpy,

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Table 1. Effect of Organic Solvents on Recoveries of Lipid and Protein Content of Raw Peanuts.

	$CHCl_3$MeOH	Hexane
Oil Extracted	51.1 (%)	49.2 (%)
Meal Recovered	48.9	50.8
Protein Content of Meal*	44.8	44.4
NaCl Soluble Extract**	36.2	41.3
NaCl Sol. Protein	86.3	84.7
NaCl Insol. Extract**	40.7	36.0
NaCl Insol. Protein	28.9	39.3
Total Recovery***	76.9	77.2

* N X 5.46

** After Dialysis and Lyophilization

*** Total of Soluble plus Insoluble Extracts

protein) were obtained by extracting with hexane.

Polyacrylamide gel electrophoresis of the salt-soluble and salt-insoluble fractions from both hexane and chloroform/methanol deoiled meals showed identical protein bands and lipid-protein patterns (Figure 1). The salt-soluble fraction had four major proteins and several minor constituents (Figure 1-I, A and B). Lipids appear to be associated with only 3 of these proteins (Figure 1-I, C and D). The association of lipid with only 3 of these proteins confirmed earlier results that lipids are associated only with the arachin fraction and not with the conarachin fraction of peanuts (St. Angelo and Ory, 1975).

Electrophoresis of salt-insoluble proteins of the two deoiled meals on 0.1% SDS gels showed several major protein bands, each associated with a small amount of lipid. Again the banding patterns were identical, suggesting that the electrophoretic mobilities of neither fraction were affected by the two different solvents. Since the protein yield from hexane extraction was greater and the meal

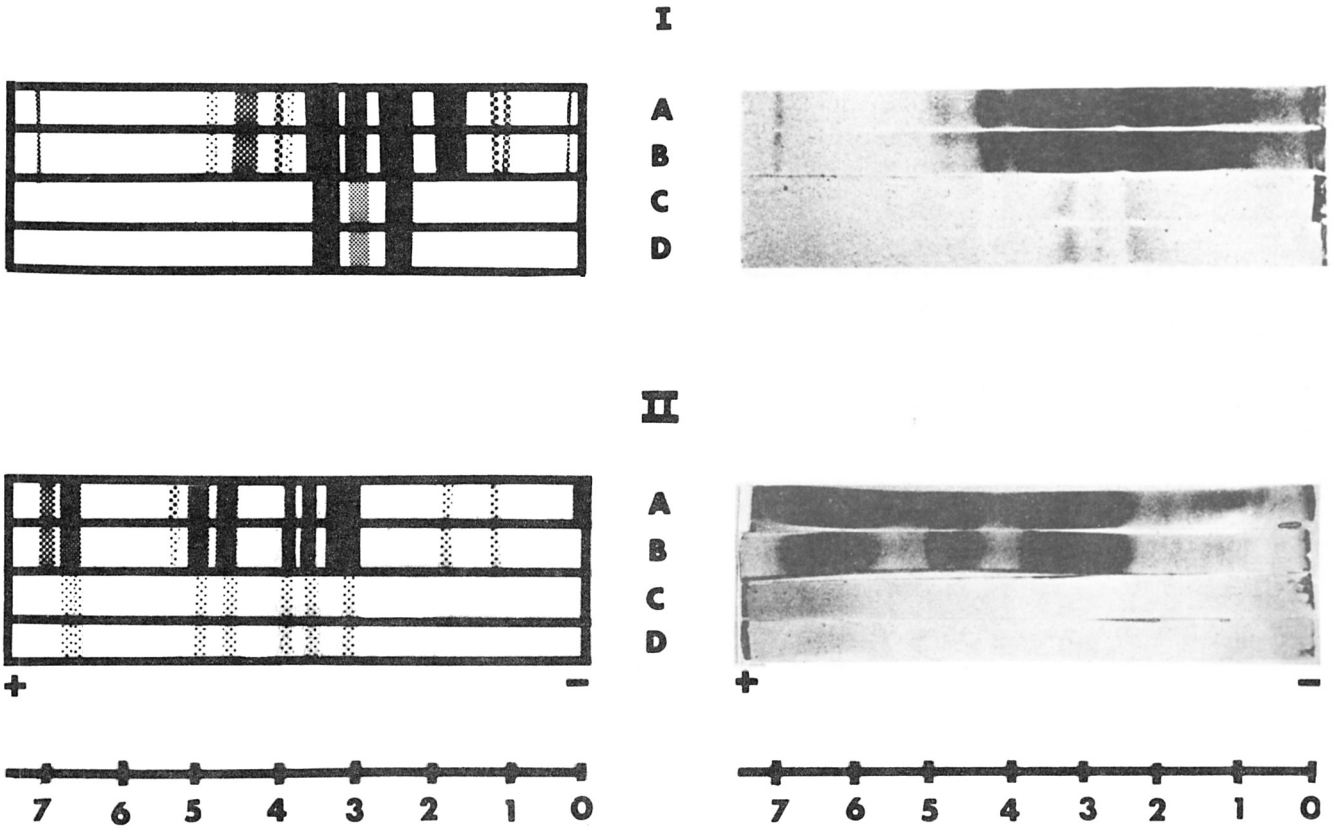

Fig. 1 Photographs and sketches of polyacrylamide gel electrophoretic patterns of salt-soluble (I) and salt-insoluble (II) proteins from raw peanuts. Peanuts were deoiled with chloroform/methanol (A and C) or hexane (B and D). Samples A and B were stained for protein with Amido Black; samples C and D were stained for lipid with Oil Red O. Protein concentartion applied to each gel was approximately 0.4 mg. Migration was towards the anode.

hard, and very difficult to pulverize, and would be less desirable for commercial use.

The total yields of salt-soluble plus insoluble extracts from both meals were the same (77%) but 4% more soluble proteins (41.3% yield containing 84.7% protein *vs.* 36.2% yield containing 86.3% protein) and 2.4% more insoluble material (36% yield, 39.3% protein *vs.* 40.7% yield, 28.9% had better physical characteristics than proteins after extraction with chloroform/methanol, hexane was used for defatting peanuts thereafter.

The effect of cold-storage on raw peanuts is shown in Figure 2. The results from 12 different samples from 3 cultivars combined, fit into 3 general curves: (A) those that showed no sign of peroxidation over the first 28 weeks and whose

CDHP values remained about 3μMoles/g of peanuts during refrigeration; (B) those that showed peroxidation in progress, but whose initial CDHP values were also about 3; and (C) those that showed peroxidation already taking place, with slightly higher initial CDHP values, averaging 8.5. These data suggest that the peanuts obtained from commercial suppliers (Curves B and C) were probably old and had already undergone some peroxidation prior to initiation of these experiments. Unfortunately, the exact age and history of these peanuts could not be obtained. By coincidence, if the 3 curves were placed end to end, disregarding the "absolute" time in storage, the initial value for Curve B fit Curve A at about the same value obtained after 20-weeks storage. By placing Curve C at the end of Curve B in similar fashion, it appeared that Curve C could be a continuation of the process illustrated in Curve B. The composite results of these three curves suggest that raw shelled peanuts may be safely stored at 4°C for at least 5-6 months before physiological changes occur in the seeds and slow but constant oxidation of the oil begins. Apparently, once this oxidative mechanism was activated, simply storing raw peanuts at 4°C did not inhibit or retard its progress.

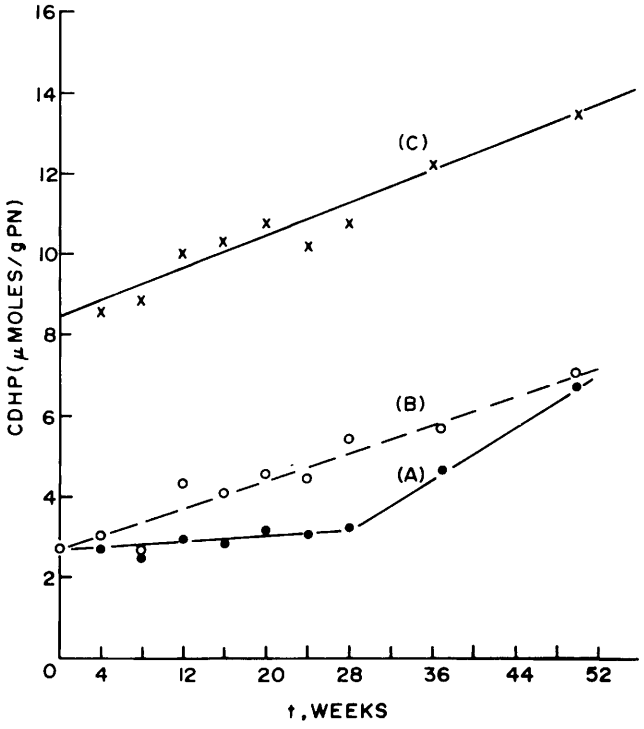

Fig. 2. Effect of cold-storage on peroxidation of fatty acids in raw peanuts. Peroxidation was measured by observing the change in conjugated diene hydroperoxide content for 12 different lots of peanuts separated into 3 groups: A, B, and C.

The most likely catalyst for lipid oxidation in raw peanuts is the enzyme, lipoxygenase. Apparently the enzyme is sufficiently active at 4°C to promote oxidation after a lag period of several months. Ericksson (1974) reported that this enzyme was still active in green peas stored for several months at -20°C.

Roasted peanuts stored in sealed glass jars at 4°C, as expected, showed a rate of oxidation that increased greatly during 12 months, from an initial low of 8 CDHP units to 40 in 50 weeks; after 2 years, this value increased to 61 CDHP units. Figure 3, Curve A, illustrates the results from averages of 12 samples stored 50 weeks at 4°C. Since peanut lipoxygenase is destroyed by heating at 40°C (St. Angelo and Ory, 1972), this peroxidation apparently was not catalyzed by an active lipoxygenase, but possibly by metals or metalloproteins (e.g., peroxidase, tyrosinase) as reported on stored peanut butters (St. Angelo *et al.*, 1972; St. Angelo and Ory, 1973). Soybean lipoxygenase was reported to contain 1 mole of iron per 100,000 molecular weight (Chan, 1972, 1973; Roza and Francke, 1973). If the peanut enzyme also contains iron, it might cause peroxidation of the unsaturated fatty acids in roasted peanuts, but through a different mechanism (nonenzymic metal catalysis rather than by an active lipoxygenase). Nevertheless, storage of roasted peanuts at 4°C in jars containing air apparently would not retard peroxidation. Sealing under nitrogen or vacuum, as is done with commercially packed roasted nuts, probably would be a more efficient method for preventing peroxidation.

Curve B of Figure 3 is a composite plot of all CDHP values from the same samples of raw peanuts but, in order to compare the greater rate of peroxidation for roasted peanuts, the scale for CDHP values was reduced to the same order of magnitude as that for roasted peanuts. During the first 24 weeks, there was no significant peroxidation in raw peanuts. When oxidation did begin, the rate was still very much slower (1/8) than that for roasted peanuts.

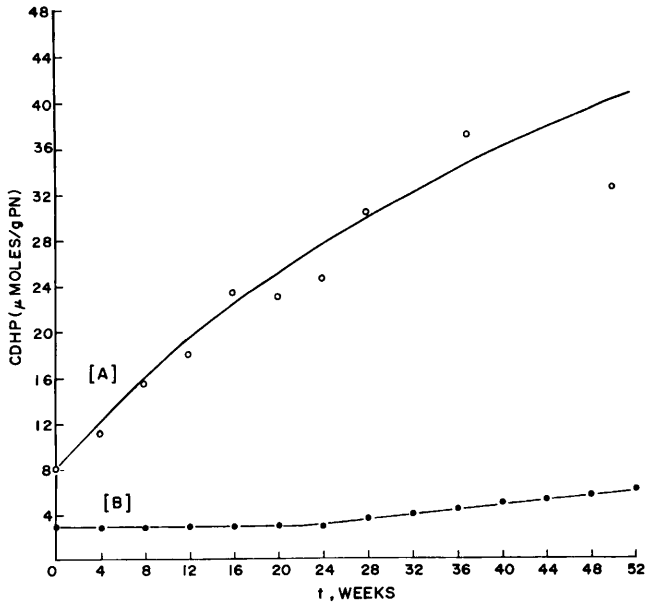

Fig. 3. Comparison of rates of peroxidation of fatty acids in roasted (A) and raw (B) peanuts during cold storage. Each value represents the average for the 12 lots of peanuts.

As previously reported with increasing peroxidation, recovery of salt-soluble fractions increased and salt-insoluble residues decreased correspondingly (St. Angelo and Ory, 1974). This phenomenon was further investigated with intentionally damaged peanuts that were ground in a food blender and stored three different ways: (1) sealed jar, at 4°C: (2) jar covered with cheesecloth, at 30°C; (3) ground peanuts blended with 10% rancid vegetable oil to enhance lipid-protein interaction, placed in a jar, covered with cheesecloth, and stored at 30°C. After 4½ months, the hexane-defatted meals prepared from these samples were extracted with buffered sodium chloride, the extracted oil was assayed for CDHP absorption at 234 nm, and the yields of soluble and insoluble fractions determined. The results showed that sample 1, stored at 4°C (Figure 4, Curve A) had an absorbance of 0.55 units; 480 mg of salt-soluble material was recovered from 1.5 g. For samples 2 and 3, peroxidation and salt-soluble material increased; absorbance readings were 1.23 and 1.90 and weights of soluble material were 550 and 88 mg, respectively. Conversely, in Curve B, as peroxidation increased, the salt-insoluble residues recovered from the 3 corresponding samples were 562, 537, and 515 mg, respectively.

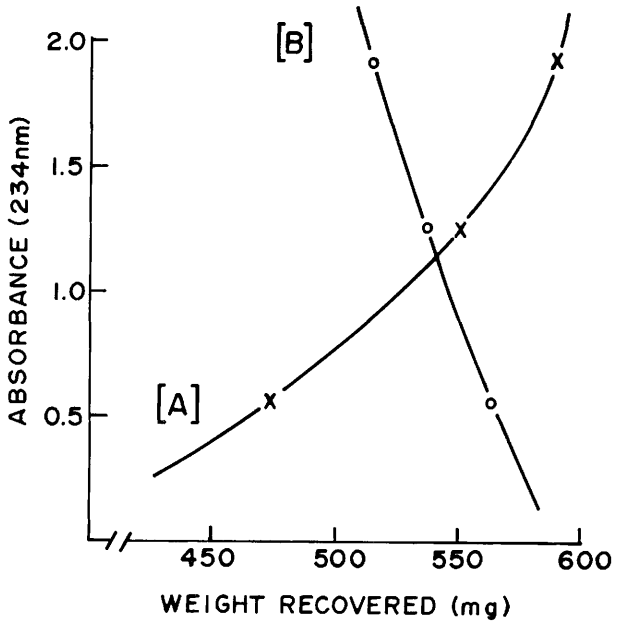

Fig. 4. Effects of peroxidation on the total recoveries of residues from salt-soluble (A) and salt-insoluble (B) extracts from 1.5 g. deoiled peanut meals. Peanut samples and storage conditions are described in the text.

In mammalian tissues, peroxidized or autoxidized lipids were reported to form insoluble complexes with proteins (Tappel, 1965; Roubal and Tappel, 1966). The apparent increase in salt-solubility of peanut proteins after interaction with peroxidized lipids is still under investigation.

In conclusion, the results of this 12-month study suggest that raw undamaged shelled peanuts can be safely stored at 4°C (40°F) for at least 5-6 months before enzymatically catalyzed peroxidation of the polyunsaturated fatty acids begins to occur slowly. For roasted peanuts, even refrigeration will not retard peroxidation in the presence of air. Packing under nitrogen or vacuum as above commercially should be the best means of retarding peroxidation.

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