# Lipoxidase Deactivation to Improve Stability, Odor and Flavor of Full-Fat Soy Flours[1]

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## Abstract

Oxidation of soybean lipids catalyzed by lipoxidase was prevented by heat treatment of soybean meats, which were then ground to give a full-fat soy flour free of rancid odor and flavors. Our previous studies showed that lipids in cracked, dehulled, soybeans rapidly oxidized after the lipoxidase system was activated by increasing moisture content to 20%. A series of experiments are reported in which various heat treatments were evaluated for effectiveness of lipoxidase deactivation. Dry heat to 212 F, steaming, or both, deactivated lipoxidase to give flours that had low values of peroxide, conjugated diene and free fatty acid and had good flavors after 2 years' storage. Wet heat alone was also an effective treatment, whereas wet heat preceded by dry heat at 180 F gave poor flavor stability after 2 years. Gas liquid chromatography studies gave evidence that the rapid formation of volatiles in full-fat soy flours was catalyzed by an enzyme system. A 10 member taste panel was able to detect significant flavor and odor differences between oxidized and nonoxidized samples.

## Introduction

During the past 3 years, the Agency for International Development, U.S. Department of State, supported in part a research program at the Northern Laboratory on converting soybeans to high-protein foods with emphasis on full-fat soy flour. The basic problems of upgrading the soybean to human food can be listed as follows: (a) inactivating growth inhibitors, (b) producing a bland and palatable product and (c) obtaining good storage stability. Storage stability of full-fat soy flour is related to its highly unsaturated fat content (11) and represents a problem that has been a deterrent to its greater use in foods.

Several workers have proposed mechanisms to explain lipoxidase-catalyzed oxidation of soybean lipids (3–10,14,17,18). The enzyme catalyzes the oxidation of unsaturated fatty acids by molecular oxygen and reportedly is specific for fatty acids possessing methylene group-interrupted *cis* double bonds (17), such as linoleic acid. The development of conjugated dienes during the oxidation of linoleic acid and related substances has been demonstrated (4,6,9,10). Dolev et al. (5), at this Laboratory, found that crystalline lipoxidase (Nutritional Biochemical Corporation) exerts high specificity, with the exclusive formation of $C_{13}$ hydroperoxide of linoleic acid, and that the oxygen molecule in lipoxidase oxidation originates with ambient air.

Since lipoxidase was suspected as a key factor in full-fat soy flour stability, experiments were carried out to study the lipoxidase deactivation of cracked soybeans by various heat treatments and to study the effectiveness of these procedures for controlling or improving shelf life of the flour. Information developed in this report should be useful for control of quality during the production of full-fat soybean products.

## Experimental Procedures

### Materials

Hawkeye soybeans, 1966 crop, 6% moisture, were used in all the experiments. Soybeans were prepared by cracking through 6 in. corrugated rolls and then dehulled by passing over a shaking screen with use of aspiration.

### Method and Procedure

Peroxide values were determined on the extracted oil from the full-fat flour by the standard AOCS procedure (2); conjugated diene on the extracted oil by measuring ultraviolet absorption at 233 mμ in isooctane according to AOCS methods (2); urease activity on the defatted meals by the official AACC method (1); nitrogen solubility index (NSI) on the defatted meals for the measurement of water-soluble protein by a modified procedure (pH 7.2) of Smith and Circle (15) and lipoxidase deactivation of the full-fat flours by a modification of the technique of Smith (16).

The GLC procedure as reported by Mattick et al. (12) and Wilkins et al. (19) was followed to correlate the development of volatiles with heat treatment. In their study, Mattick and Wilkins suggested that lipoxidase could account for off-flavor development in soy milk. Samples of soy flour were suspended in water and the resulting mixture was distilled under vacuum at 80–90 C in a rotary flask evaporator. The volatiles were condensed in an ice bath and the distillate was extracted with carbon disulfide. The $CS_2$ extract was concentrated and then chroma-

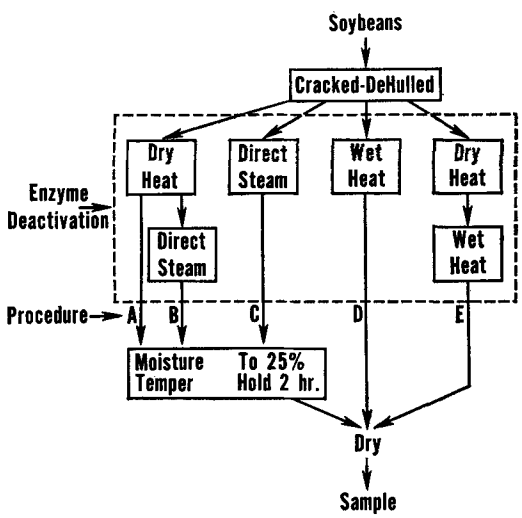


FIG. 1. Experimental procedures for lipoxidase deactivation of soybeans.

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TABLE I

Effect of Heat Treatments on Enzyme Activity, Oxidation Indices, NSI and Flavor Scores of FF Soyflours

| Sample | Procedure | Heat treatment | Lipoxidase deactivation[a] | Peroxide value meq/kg oil[a] | Diene conjugation,[a] % | FFA,[a] % | Urease activity pH change[a] | Nitrogen solubility index (water),[a] % | Flavor rancidity score (5),[b,c] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | A | Dry heat to 180 F | 52.1 | 17.0 | 0.3 | 0.16 | 2.0 | 77 | 2.2[d] |
| 2 | A | Dry heat to 212 F | 84.1 | 1.7 | 0.2 | 0.12 | 1.6 | 63 | 8.0 |
| 3 | B | Dry heat to 150 F + 5 min steaming[e] | 93.3 | 0.5 | 0.15 | 0.14 | 1.2 | 52 | 7.0 |
| 4 | B | Dry heat to 180 F + 5 min steaming[e] | 98.4 | 0.8 | 0.13 | 0.08 | 1.1 | 49 | 8.0 |
| 5 | C | Direct steaming, high rate[f] | 98.4 | 1.1 | 0.09 | 0.29 | 0.6 | 39 | 7.7 |
| 6 | C | Direct steaming, low rate[g] | 99.2 | 1.0 | 0.16 | 0.23 | 0.0 | 28 | .... |
| 7 | D | Wet heat | 96 | 3.9 | 0.22 | 0.33 | 0.2 | 31 | 7.3 |
| 8 | E | Dry heat + wet heat initial dry heat level = 180 F | 97[h] | 11.4 | 0.38 | 0.36 | 0.0 | 23 | 3.7[d] |
| 9 | E | Dry heat + wet heat initial dry heat level = 212 F | 96 | 0.6 | 0.14 | 0.32 | 0.0 | 14 | 8.0 |
| 10 | | Pilot plant continuous dry-heat treatment in paddle conveyor | 98.4 | 0.7 | 0.13 | 0.21 | 1.1 | 76.5 | .... |
| 11 | | Raw wet control (no enzyme deactivation) | ..... | 19.9 | 0.36 | 0.40 | 2.0 | 79.2 | 1.0[d] |

[a] Zero storage.
[b] Rancidity score by trained panel of six tasters; samples stored 2 years in glass bottles at room temperature (77 F ± 8 F).
[c] Rancid, 0; nonrancid, 10.
[d] Significantly lower scores (95% level).
[e] Temperature reached 212 F after 5 min steaming; moisture before steaming, 7.5%; after steaming, 9.1%.
[f] Temperature reached 212 F after 2 min steaming; moisture before steaming, 6.0%; after steaming, 23.0%.
[g] Temperature reached only 184 F in 25 min steaming; moisture before steaming, 6.0%; after steaming, 28.7%.
[h] Wet heat increased deactivation from 52% (occurring from original dry-heat treatment) to 97%.

tographed. For a blank control the soy flour was omitted.

Other tests reported here, unless otherwise designated, were conducted by standard AOCS procedures (2).

To test the development of rancidity in enzyme active (unheated) soybeans or cracked soy meats, the samples were either moistened or water-soaked and held for various times. An unmoistened control was also run. Treated samples were dried at room temperature for 16 hr in a forced-air drier. All samples were then analyzed for peroxide, FFA values and checked for rancid odors.

Five deactivation procedures, shown in Figure 1, were investigated.

*A. Dry Heat Studies.* Dehulled soybeans were heated by indirect steam in a ribbon-blender cooker to a predetermined meal temperature and immediately discharged and air-cooled. Temperatures evaluated were 180, 190, 200 and 212 F. Heat-up times for these temperatures varied from 28 min (180 F) to 62 min (212 F). The samples were then moistened to 25% moisture and tempered for 2 hr (to initiate oxidation by any residual lipoxidase not destroyed by heat). The samples were finally air-dried at room temperature for 16 hr and analyzed. This moistening and drying procedure was also used after test Procedures B and C (Fig. 1), but only air drying was used after test Procedures D and E since moisture

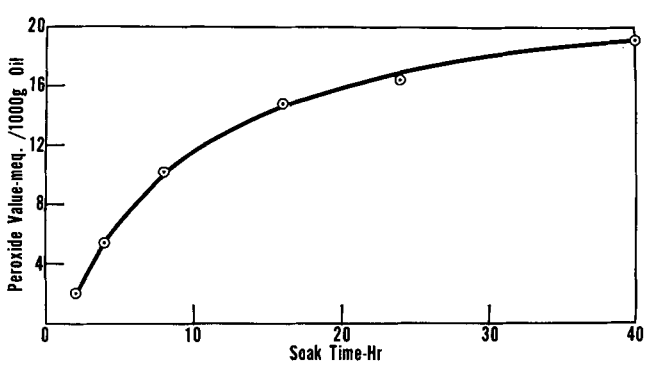


FIG. 2. Increase in peroxide value of whole soybeans with water-soak time.

incorporated in the treatment would have already activated the residual enzymes.

*B. Dry Heating Followed by Steaming.* Same as A except that the dry heat step at either 150 F or 180 F was followed by direct steaming for 5 min.

*C. Direct Open Steaming.* Steam was introduced at a high rate to reach a temperature of 212 F in 5 min (Sample 5, Table I) or at a lower rate so that meal temperature reached only 184 F in a total steaming period of 25 min (Sample 6, Table I).

*D. Wet-Heat Treatment.* Meal was first tempered to 25% moisture, then heated to 200 F, held 20 min, cooled, held 2 hr and finally dried in a forced-air drier at room temperature.

*E. Dry Heat.* Procedure A was followed by the wet-heat treatment (Procedure D). For Sample 8 (Table I) the dry-heat temperature reached was 180 F and for Sample 9 (Table I) the dry-heat temperature was 212 F.

*F. Continuous Dry Heat.* Soy meats were passed through a two-stage jacketed paddle conveyor (heated indirectly by 40 psig steam) at such a rate that they left the unit at 218–220 F. This procedure was generally comparable to continuous operation of Procedure A. Lipoxidase was deactivated in 6–8 min.

After drying at room temperature, samples were finely ground in a pin mill and evaluated for lipoxidase deactivation, oxidative stability and flavor. Extracted oils from the samples were analyzed for peroxide value, conjugated diene and FFA. Urease activity and NSI were run on the defatted flours. Organoleptic evaluations were made on the flour after 2 years' storage in glass bottles at room temperature (77 F ± 8 F). The flours were diluted with 8 parts of water and tasted as beverages. Scoring was based on a 10 point scale with 10 indicating a nonrancid sample and 0 a highly rancid sample. Volatile off-flavors were measured both organoleptically and by GLC.

## Results and Discussion

Peroxide values of whole beans increased with soaking time in water as shown in Figure 2. Also in a separate experiment peroxide values of moistened

soybean meats (25% $H_2O$) increased markedly after 2 hr storage at 75 F, going from 0.6 to 19.6 meq/kg oil. Rancid odors developed immediately upon wetting samples. A small increase 0.30% to 0.36% in FFA content shows that lipase action was slight, whereas the increase in peroxide value suggests that lipoxidase action was considerably greater.

Peroxide formation in lipoxidase-active soybeans was also reported by Hand (13) who measured a rapid increase during the early stages of wet grinding raw soybeans.

#### Dry Heat

Dry-heat treatment was effective in deactivating lipoxidase and improving oxidative stability as demonstrated by peroxide values, diene conjugation and organoleptic data of treated samples (Fig. 3, Table I).

Dry heat at 180 F or below gave less than 50% deactivation of the enzyme and resulted in high peroxide and diene values. However, as the dry-heat temperature and time was increased, enzyme deactivation was more nearly complete. Nearly complete deactivation (98.4%) was obtained by dry heating to 218 F in the continuous pilot-plant jacketed conveyor.

In organoleptic evaluations conducted after 2 years' storage, the 180 F dry-heated sample with high peroxide value (17.0) was significantly rancid, whereas the 212 F dry-heat sample, which had more complete enzyme deactivation, contained little to no rancidity.

The dry-heat samples retained a high degree of protein dispersibility in water (Samples 1 and 2, Table I) after enzyme deactivation. This property should have a distinct advantage in certain soybean processes or uses.

#### Low Dry-Heat Levels Followed by Steaming

Dry heat to levels of 150 F and 180 F followed by 5 min steaming was also effective for lipoxidase deactivation, although the same dry-heat temperature levels without steaming were inadequate to give good stability. Data in Table I for Samples 3 and 4 show high lipoxidase deactivation and good stability as evaluated by peroxide value, diene conjugation and FFA. Neither Sample 3 nor 4 was rancid by flavor score after 2 years' storage. Condensation of steam in the heat treatment added only about 1% moisture to the meals during treatment.

#### Direct Steaming

Steaming raw soybean meats directly (Samples 5 and 6, Table I) resulted in good enzyme deactivation, although considerable protein denaturation occurred and some moisture condensed on the product. Urease activity was destroyed more completely than in the dry-heat procedures. Organoleptic scores on flour after 2 years' storage indicated that steamed Sample 5 (Table I) was not rancid, thus correlating with its low values of peroxide and FFA.

#### Wet Heat

The wet-heat test (Sample 7) gave effective enzyme deactivation. Although this treatment was somewhat less effective than some of the others, no significant degree of rancidity was found by the taste panel in the stored sample (Table I). Wet heat accelerated the denaturation of protein as shown by the low NSI value as compared with the NSI value of flours subjected only to dry heat.

#### Dry Heat Followed by Wet Heat

Stability data on samples subjected to dry-heat treatment followed by wet-heat treatment are given in Figure 4 and Samples 8 and 9, Table I. Peroxide and diene values correlated with the maximum dry-heat temperature used. FFA values and protein denaturation were higher than with dry heat alone. Enzyme deactivation of the sample subjected to dry heat at 180 F was nearly complete (97%) since the subsequent action of wet heat was in addition to that of the dry-heat effects. However, in this sample enzymatic oxidation occurred during the early stages of wet heat as indicated by the high peroxide value of 11.4. This factor resulted in a significantly low flavor score of 3.7 for the 2 year stored sample. By contrast the sample subjected to dry heat at 212 F showed low peroxide formation and excellent flavor stability after the same storage.

#### Volatile Off-Flavors as Indicated by GLC

GLC was used to measure the formation of volatiles catalyzed by the enzyme systems residual after the various processes. In this measurement, we used the gas chromatogram as a profile without any attempt to identify the compounds, just as Mattick et al. (12) did on soy milk. They suggested that the volatile off-flavors of soybean milk arise predominantly

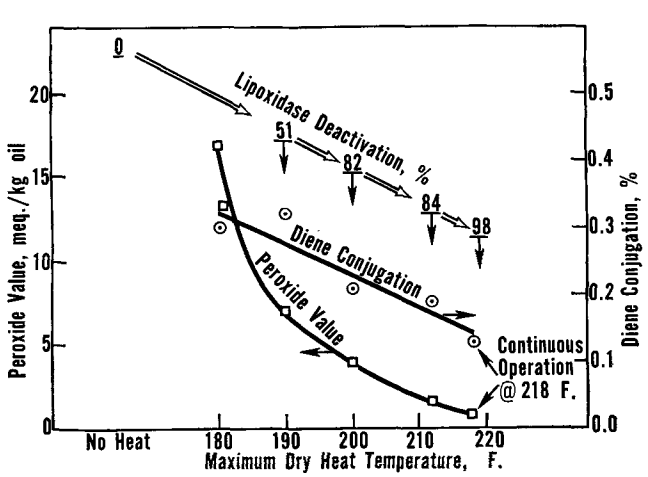


FIG. 3. Effect of dry-heat treatment on peroxide and diene values. All samples rewetted to 25%, moistened for 2 hr at room temperature and dried.

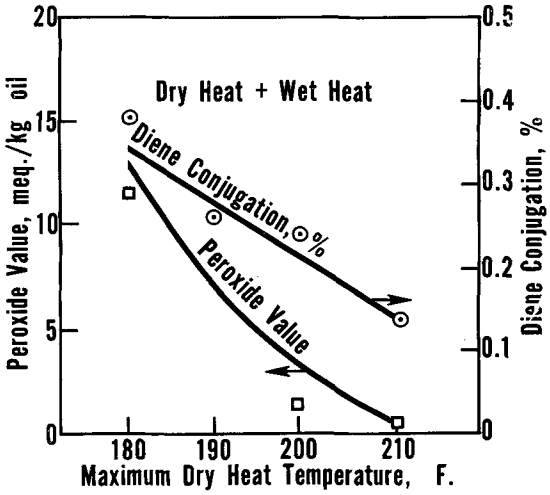


FIG. 4. Influence of combined dry-wet-heat treatments on peroxide and diene values.

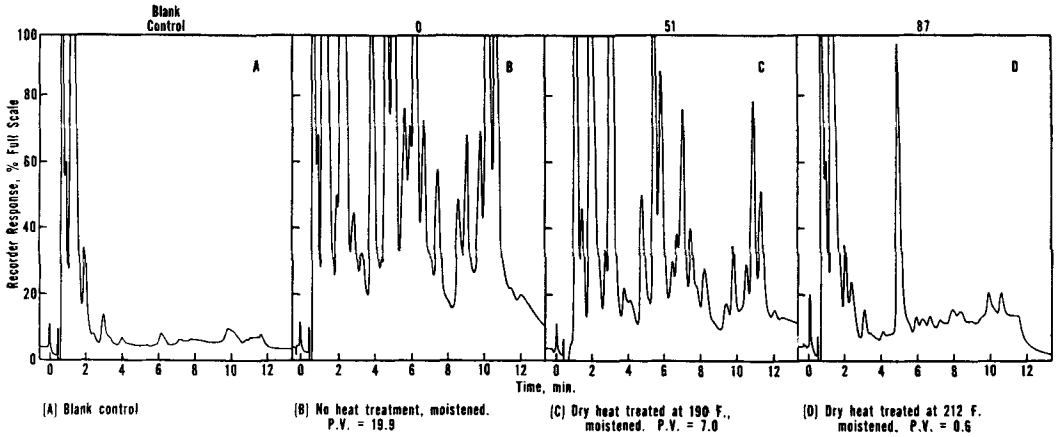


FIG. 5. Chromatogram showing effects of enzyme deactivation.

from the lipid fraction through an oxidative mechanism which is catalyzed by a lipoxidase system.

Samples with and without heat treatment were evaluated. As discussed previously, all samples were subsequently tempered to 25% moisture for 2 hr and air-dried to activate any residual lipoxidase. After the samples were dispersed in water and vacuum distilled, the concentrated $CS_2$ extracts of the condensed volatiles were chromatographed (Fig. 5). For Samples B and C the odor of rancid fat could be detected immediately in the distillates before $CS_2$ extraction.

Mattick reported that high profiles were associated with off-flavor development but that low profiles resulted when no off-flavors developed. Our study corroborated his finding. The chromatograms indicate a high concentration of volatiles for flour samples with no heat (B) or dry heat at 190 F (C). A blank control (A) was used as a reference. The samples with high profiles (B,C) also had strong rancid odors and high peroxide values.

The enzyme-deactivated sample (D, Fig. 5) showed a very low profile in comparison with the nondeactivated samples. Similar low profiles were obtained for direct steam deactivation and continuous dry-heat treatment to 218 F.

By sensory evaluation these samples were free of rancid odor and taste, and they also had low peroxide values. Distillate samples introduced to the GLC instrument, where no enzyme deactivation was carried out, had rancid off-odors. The GLC data, therefore, corroborate other data in this report that show lipoxidase-active soy flours were associated with volatile rancid odors after processing, whereas flours adequately deactivated by proper heat treatment had none.

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REFERENCES

1. American Association of Cereal Chemists, "Cereal Laboratory Methods," 7th Edition, The Association, St. Paul, Minn., 1962.
2. AOCS, "Official and Tentative Methods," Edited by V. C. Mehlenbacher, T. H. Hopper and E. M. Sallee, 3rd Edition, rev. to 1966, Chicago, 1945–1966.
3. Balls, A. K., B. Axelrod and M. W. Kies, J. Biol. Chem. *149*, 491 (1943).
4. Bolland, J. L., and H. P. Koch, J. Chem. Soc. *112*, 445 (1945).
5. Dolev, A., T. L. Mounts, W. K. Rohwedder and H. J. Dutton, Lipids *1*, 293 (1966).
6. Farmer, E. H., H. P. Koch and D. A. Sutton, J. Chem. Soc. *144*, 541 (1943).
7. Hamberg, M., and B. Samuelsson, Biochem. Biophys. Res. Commun. *21*, 531 (1965).
8. Holman, R. T., Arch. Biochem. Biophys. *15*, 403 (1947).
9. Holman, R. T., and S. Bergstrom, in "The Enzymes," Vol. 2, Edited by J. R. Summer and K. Myrback, Academic Press, New York, 1951, p. 559–580.
10. Holman, R. T., W. O. Lundberg and G. O. Burr, J. Am. Chem. Soc. *67*, 1386 (1945).
11. Markley, K. S., "Soybeans and Soybean Products," Vol. 2, Interscience Publishers, Inc., New York, 1951, p. 955.
12. Mattick, L. R., W. F. Wilkins and D. B. Hand, Journal Paper No. 1510, New York State Agricultural Experiment Station, Geneva, N.Y., 1966.
13. Hand, D. B., "Proc. Int. Conf. Soybean Protein Foods," Peoria, Ill., October 1966; USDA Bull. ARS-71-35, May 1967, p. 70.
14. Siddiqui, A. M., and A. L. Tappel, JAOCS *34*, 529 (1957).
15. Smith, A. K., and S. J. Circle, Ind. Eng. Chem. *30*, 1414 (1938).
16. Smith, G. N., Arch. Biochem. *19*, 133 (1948).
17. Tappel, A. L., P. D. Boyer and W. O. Lundberg, J. Biol. Chem. *199*, 267 (1952).
18. Tookey, H. L., R. G. Wilson, R. L. Lohmar and H. J. Dutton, Ibid. *230*, 65 (1958).
19. Wilkins, W. F., L. R. Mattick and D. B. Hand, Food Technol. *21*, 1630 (1967).

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