and in cottonseed-oil chips only a slight decrease in 27 weeks was noticed (Table III).

The fatty acid patterns of cottonseed frying oil sampled at 2, 50 and 98 hr indicate very little change in composition. Chips were stored for 7, 10 and 13 weeks after which the oil was extracted and analyzed. With the exception of 20:1 (icocenoic acid) no change was observed. The patterns of peanut frying oils, sampled at 2, 50 and 98 hr were slightly changed in that the 16:0 (palmitic) and 18:2 (linoleic) acid percentages showed a small downward trend. Peanut-oil chips, stored for 6, 9 and 12 weeks showed no change in composition.

The organoleptic evaluation of the peanut- and cottonseed-oil chips indicated that both products were considered acceptable. It is important to note, however, that the mean panel scores for peanut-oil chips were significantly higher (at 5% level) than those for cottonseed-oil chips throughout the storage period. It must be remembered, however, that in practice, the majority of the chips is flavored. It is therefore doubtful whether differences would be observed between flavored chips.

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Effect of Enzyme Inactivation on the Extracted Soybean Meal and Oil

R.D. RICE, Marfleet Refining Co., Ltd., Hull, England HU9 5NJ, and
L.S. WEI, M.P. STEINBERG and **A.I. NELSON**, Dept. of Food Science, University of Illinois, Urbana, IL 61801

ABSTRACT

The objective was prevention of lipoxygenase activity prior to oil extraction in order to obtain a meal of superior flavor quality and a crude oil of superior oxidative stability. Accordingly, experiments were performed in which soybeans were heated at various moisture contents and times to inactivate the enzyme system. Once the optimal conditions were determined, heat treated and raw beans were extracted in a laboratory system designed to simulate conditions in commercial solvent extraction and the component oil and meal were evaluated. Oxidative stability of the oil from heat-treated beans was increased as determined by the Swift stability test and an organoleptic evaluation. Similarly, organoleptic blandness ratings of the heat-treated meal were also superior to the meal produced from raw beans. It was concluded that steam heat treatment of soybeans prior to extraction was beneficial to quality of both oil and flake.

INTRODUCTION

The soybean has been recognized for many years as a rich source of the enzyme lipoxygenase. The enzyme itself has been recognized since the late 1920s. An extensive review of lipoxygenase in relation to food quality was published by Eskin (1). It has a high turnover number, 2×10^4 mol/mol enzyme/min (2). It is also well known for the variety and intensity of undesirable flavor it can produce from its normal substrate, linoleic acid (3,4). Investigators have identified a range of oxidation products of linoleic acid generated by lipoxygenase action (3,5-7). Examples are ethyl vinyl ketone, propionaldehyde and pentenal. In addition, many other volatile oxidation products have been identified from autoxidized linoleate, and at least in theory, these same compounds can be generated by lipoxygenase action. 3 *cis*-Hexenal, e.g., was identified as an oxidation product of linoleic acid by Hoffman (8) who concluded that its organoleptic threshold was less than 0.1 ppm. This compound has a "green bean" odor, and is one of the initial manifestations of reversion flavor in soy oil.

The specific effects of lipoxygenase activity on the flavor of aqueous suspensions of ground soybeans were investigated by Wilkens et al. (9). They found that the characteristic beany or painty flavor of such preparations was due to enzyme activity, and that the flavors could be improved by using hot water extraction. Mustakas et al. (10) similarly found that the heat treatment of whole beans prior to grinding produced full-fat soy flours which were much blander than flour made from raw soybeans. Nelson et al. (11,12) demonstrated that the off-flavors commonly associated with soybean products occur whenever damaged tissue is exposed to moisture. List et al. (13) noted that quality of both crude and hydrogenated oils was adversely affected by field and storage damage of the soybeans. Such damage clearly triggers enzyme activity. Gardner (14) also noted the importance of tissue rupture. Tissue rupture occurs during preparation of the soybeans for oil extraction. Specifically, the meats are "conditioned" for flaking by heating to 60-65 C with steam so that the moisture content is increased to 10.5-11.0%. It should be noted that this is an average moisture content after equilibration for 20-30 min, so the moisture content on the particle surface, where ruptured tissue is present, must be considerably greater. The meats show an equilibrium relative humidity of 60-70%, a moisture level suitable for enzyme activity (15). The next step, flaking, greatly exacerbates the problem by extensive rupture of the cells. Thus, the moisture content, temperature and time are suitable for at least some degree of enzyme activity in the flakes prior to extraction.

This enzyme activity could be detrimental to both oil and meal. Therefore, the objective of this work was to study the effect of moist heat enzyme inactivation prior to

cracking on stability of the crude oil and flavor acceptance of the meal obtained after solvent extraction.

MATERIALS AND METHODS

Oil Extraction

Several varieties of soybean seed were used in this study. These were dried, cleaned and held in a 1 C storage until used the same year.

Conventional oil extraction was simulated in the laboratory in triplicate from flakes prepared from both enzyme active and inactive soybeans. The dry soybeans were cracked with a fluted roll into 6-8 pieces. The meats were aspirated to remove the hulls, conditioned in a steam chest at 60-65 C for 30 min at 90-95% relative humidity, and finally rolled between preheated smooth rolls to flakes 0.010-0.012 in. thick. The flakes were held for 15 min in an insulated container to simulate the delays between flaking and extraction. One kg flakes was immersed in 5 ℓ hexane and maintained at 55 C for 15 min. The flakes were then drained of miscella and two additional extractions of the same flakes were done in the same manner.

Oil Processing and Evaluation

The combined miscellas were evaporated to a volume of 600 ml prior to vacuum filtration to remove fine particles of soy solids. Final removal of solvent was done on a rotary vacuum evaporator.

The oil was examined for oxidative stability by the Swift stability test (16). Oil was heated in a boiling water bath with air at constant flow rate bubbling through the oil. The exhaust air from the tubes was ducted to "sniffing ports" for organoleptic assessment by a trained panel. Periodic panel evaluation sessions were held at intervals throughout the time that the oils were heated. In addition, samples of oil were withdrawn at intervals for determination of peroxide value using the AOCS method (16).

Meal Processing and Evaluation

The extracted flake was desolventized by evaporation at ambient temperature (20 C), with removal of final solvent traces done by air flushing of thin layers of flake.

Part of the extracted flake was closed in a can and heat-treated to inactivate trypsin inhibitor. For flakes from raw soybeans a process of 120 C for 30 min was found to be adequate as judged by gelatin hydrolysis, a modification of the Kunitz procedure reported by Kwok (17). For the flakes from heat-treated beans, a certain amount of heat inactivation had already occurred, such that a further 10 min at 100 C was all that was needed to completely inactivate the residual trypsin inhibitor.

The extracted flake was evaluated by a trained 15-member organoleptic panel in two ways. The first method involved presentation of a 1:2::ground flake:water paste, colored to mask visual variations. A triangular difference test was used to determine whether panelists could consistently differentiate the 2 flake samples. Preference was recorded of those panelists correctly identifying the odd sample in the triangle test. The second technique was to "dilute" the ground flakes to various degrees with precooked, drum-dried rice. Panelists were again asked to identify the odd sample in triangle comparison tests. Other conditions and analysis of results were as given by Larmond (18).

Enzyme Analyses

Residual lipoxygenase level in the heated soybeans was determined by a technique described by Surrey (19) as modified by Lao (20). The sample being assayed was added to linoleate in buffer and the increase in conjugated dienes absorbed at 234 nm was measured. From this, a rate of increase in dienes was calculated and related to the amount of dry soy solids present/ml of reaction mixture.

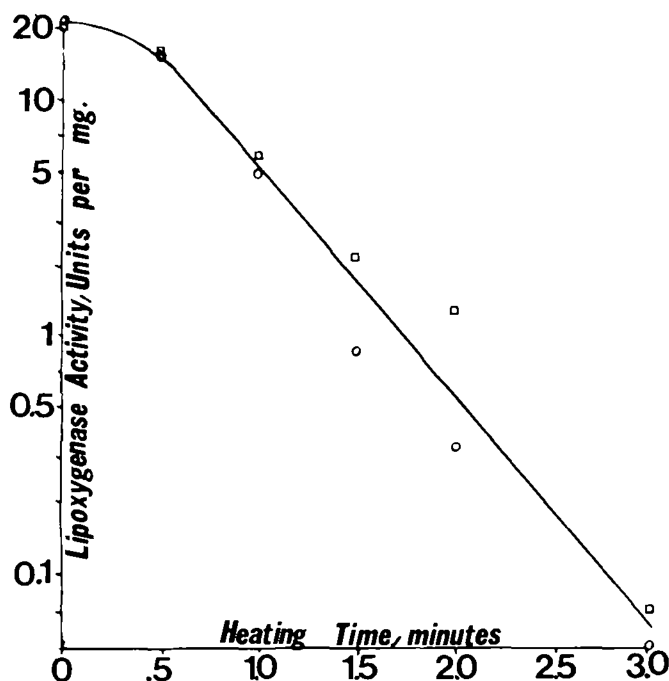

FIG. 1. Effect of time of exposure to steam at 100 C on levels of residual lipoxygenase activity in whole hark soybeans at 8.0% moisture; two replications.

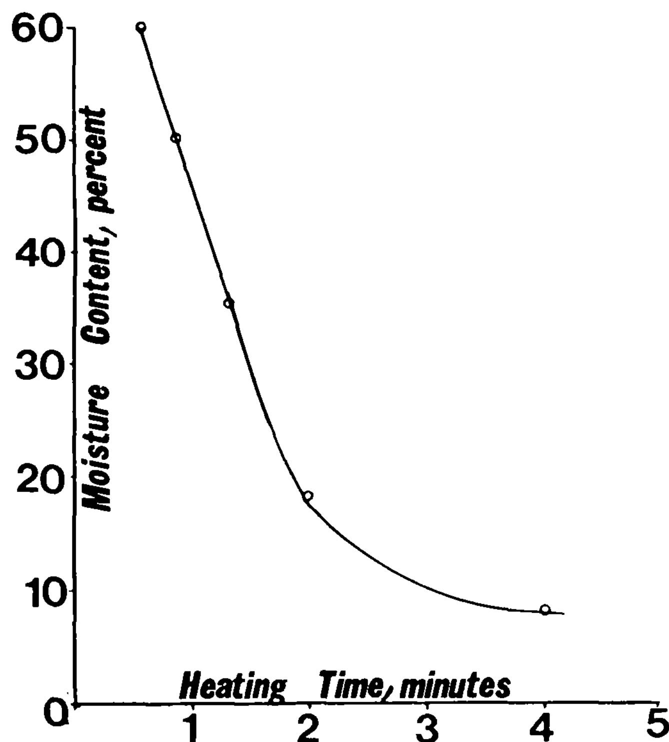

FIG. 2. Effect of moisture content on the time required for complete inactivation of lipoxygenase in whole hark soybeans during steaming at 100 C.

RESULTS AND DISCUSSION

Enzyme Inactivation Studies

Using live steam at atmospheric pressure as the heating medium, an investigation was undertaken to determine the optimal conditions for lipoxygenase inactivation in the whole beans. Three factors were examined: bean variety, bean moisture content and heating time.

Heating Time

Hark variety whole soybeans at 8.0% moisture content were heated for various times using two replications, and residual enzyme activity was determined. The data in Figure 1 are plotted as log activity vs time. This shows a linear decrease in residual activity as time of exposure is increased. This indicates a first order reaction rate, a common feature of the thermal inactivation of biological systems. Similar results were obtained by Farkas and Goldblith (21) and by Budnitskaya et al. (22).

Moisture Content

Hark variety soybeans were rehydrated to various moisture levels, heated for various lengths of time and assayed for lipoxygenase. A linear relationship was obtained between log of residual activity and heating time. Therefore, these data were extrapolated to determine time for complete inactivation at each moisture content. Since the lowest level of lipoxygenase activity that could be measured by this method was 0.01 units/mg soy solids, this level was arbitrarily selected as the "end-point." Based on 20 units originally present, this represents 99.95% inactivation. The resulting times are shown plotted against moisture content in Figure 2. This shows that inactivation time decreases slowly until moisture is increased to above 18% and then decreases rapidly as moisture is further increased.

Variety

Six varieties were obtained from storage at moisture contents ranging from 5.5 to 8.5%. The data in Figure 3 indicate that Clark 63, Amsoy and Bragg contain a more heat-resistant lipoxygenase than the other varieties. However, care must be taken in interpreting these data because the beans differed in moisture content, as shown in the figure legend. If an error was made in the moisture determination for Wayne and the value was actually somewhat higher, inactivation lines would group according to moisture content rather than variety, with high moisture requiring less inactivation time.

Since the results just described showed a powerful effect of moisture content, the experiment was repeated after rehydrating these varieties to 14-20% moisture. The results in Figure 4 show that at these moisture levels varietal differences become insignificant and time of complete lipoxygenase inactivation was drastically reduced to 1.5 min or less.

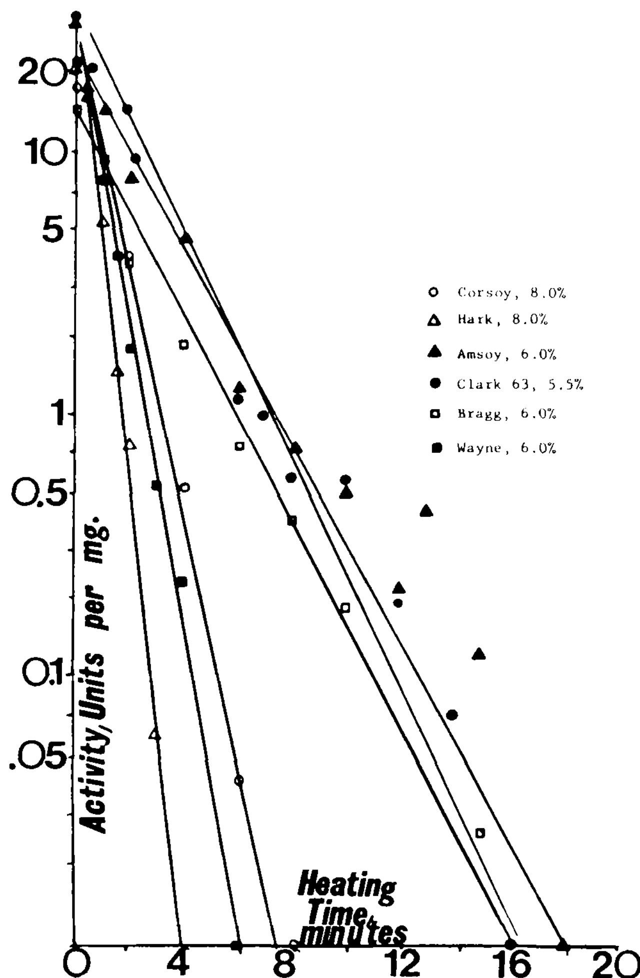

FIG. 3. Inactivation of lipoxygenase in several soybean varieties at low moisture content (indicated after variety name) by heating the whole bean in steam at 100 C.

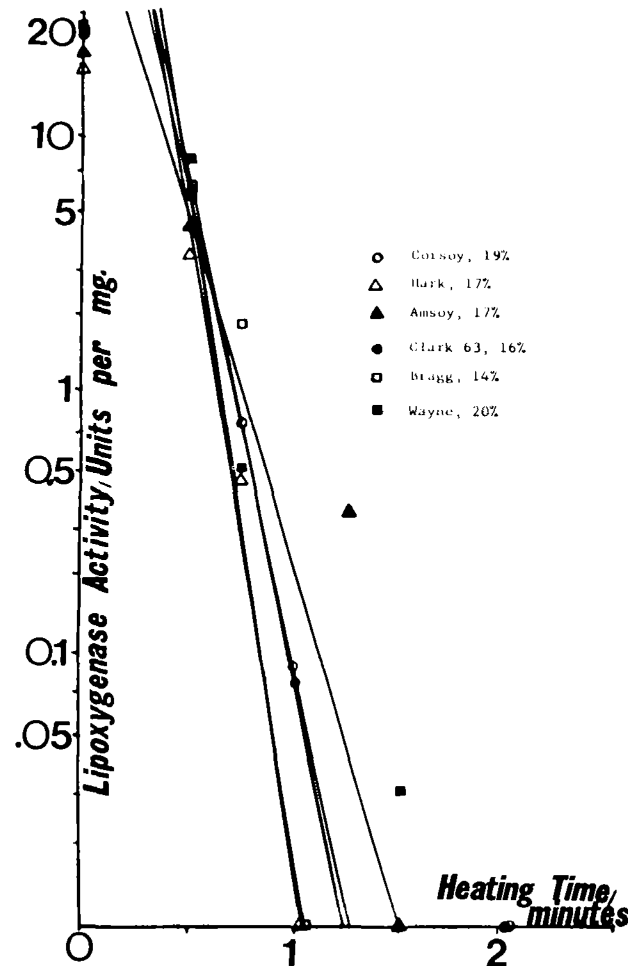

FIG. 4. Inactivation of lipoxygenase in several soybean varieties at increased moisture content by heating the whole bean in steam at 100 C.

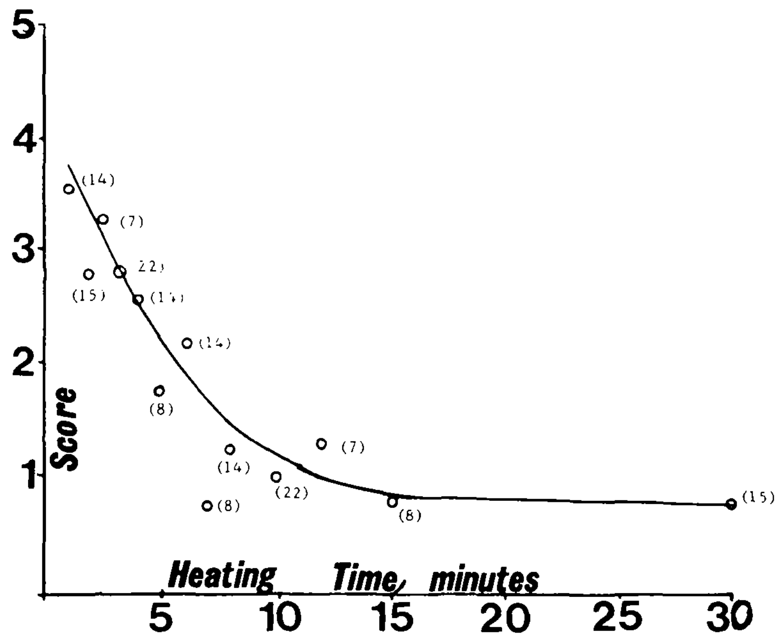

FIG. 5. Mean organoleptic score at 10% solids slurries prepared from hark soybeans at 18% moisture heated in steam at 100 C for various times. Number of judgments in parentheses. A score of 0 indicates no off-flavors and 5 indicates raw off-flavors.

Organoleptic Evaluations

The work described was based on chemical analysis for residual lipoxygenase activity. It was considered prudent to apply organoleptic evaluation as well. A trained panel was presented with samples prepared by heating 18% moisture content Hark variety soybeans for various times and grinding to a 1:10 slurry with water. The panel was instructed to score 5 for the off-flavor in raw beans and 0 for no detectable off-flavor (i.e., as in beans boiled for 30 min). The data are in Figure 5. After 1 min of heating, the Hark soybeans at 18% moisture should have shown complete inactivation according to Figure 4, but the organoleptic data here show that the sample at 1 min heating still contained off-flavor. The off-flavor continued to decrease with heating time and did not reach a minimal value until after 10 min. These results indicate that enzyme activity is still detectable at far longer heating times by taste test than by chemical analysis. Based on these results, the enzyme inactivation procedure used for the oil extraction studies was to hydrate the soybeans to 20% moisture and steam for 10 min at atmospheric pressure.

Oil Evaluation

The extraction technique used was reasonably effective, as judged by the residual oil contents, which averaged 1.4 and 2.0% for the flake from raw beans and heat-treated beans, respectively. Industrially extracted toasted soy meal oil contents are about 1.0 to 1.5% w/w.

The peroxide value data from the Swift stability test on the oils are presented in triplicate in Figure 6. At all times

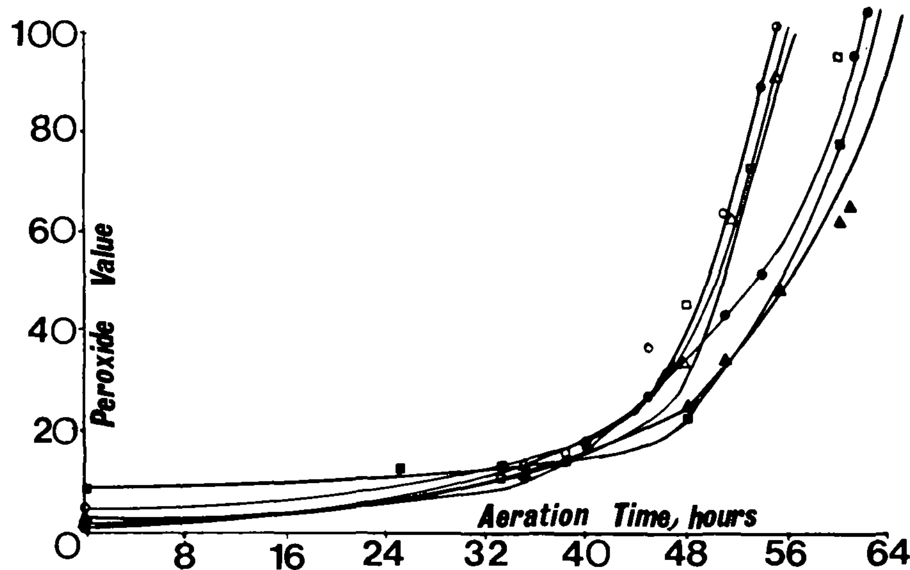

FIG. 6. Peroxide values of soybean oils extracted from raw and blanched beans and aerated at 100 C for various times. Each of the three symbols is a different replication. Open symbols = raw and closed symbols = heated.

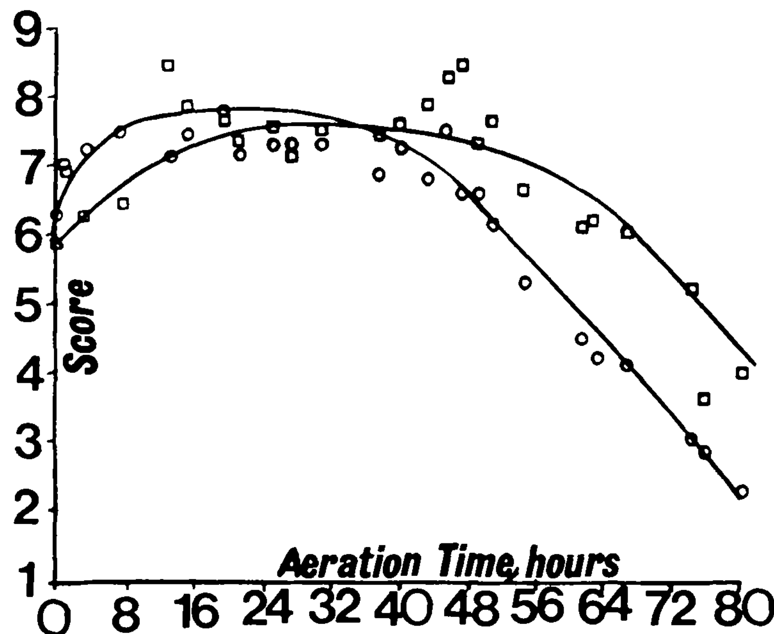

FIG. 7. Mean organoleptic rancidity score (3 replicates) of soybean oils obtained from raw (circles) and heated (squares) soybeans and aerated at 100 C for various times. A score of 9 indicated no rancidity and 1 = very rancid.

after 40 hr, the blanched bean oils show lower peroxide values than the raw bean oils. A peroxide value of 100 is normally considered indicative of a rancid oil (16) and by extrapolating to this level for both lots of oils, values of 56 and 62 hr for raw bean oil and heated bean oil, respectively, are obtained.

The organoleptic assessment of rancidity in the oils by the Swift test is in Figure 7 and shows considerable variability. Perhaps this is due to the inherent variability in subjective analyses. However, when the data are pooled (over 1,200 individual judgments) it is readily possible to discern differences in the two groups of oils. After the initial rise in organoleptic score, both oils reached a plateau during hours 16-40; the blanched bean oils had slightly higher ratings than the raw bean oils. After 40-43 hr heating, the raw bean oils began to drop in score, and thereafter dropped steadily as heating continued, reaching a value of 2.4 after 80 hr heating. The blanched bean oils began a decrease in score after 51-55 hr heating, over 8 hr longer than the raw bean oils. The blanched bean oils fell in score at much the same rate as the raw bean oils, and reached a value of 4.0 when the heating was terminated after 80 hr. It would appear that the blanched bean oils resisted oxidation for 8 or more hr longer than the raw bean oils (51 hr against 43 hr). These results are in agreement with peroxide value data in Figure 6.

In summary, these data indicate a quality advantage for oil from enzyme inactive soybeans. Work with oils from damaged soybeans by List et al. (13) supports this conclusion because damage is known to trigger enzyme-catalyzed oxidation of lipids. In their work, even after refining, the keeping properties of oil from damaged soybeans was poor.

Meal Evaluation

The extracted flake from both raw and blanched beans was examined in a triangle difference test to establish whether a detectable difference in taste existed between them. The ground flake used for this evaluation was prepared from flakes that had been heated (after completion of the extraction process) for 2 min at 100 C, to complete solvent removal. This treatment also inactivated the residual lipoxygenase in the raw bean flakes. Consequently, any flavor differences must have been due to flavors formed prior to oil extraction. Out of a total of 24 judgements, 21 correctly identified the odd sample, demonstrating a significant ($p = 0.001$) difference in flavor. Of the tasters correctly identifying the odd sample, a majority preferred the flake from blanched beans.

The extracted flakes were further evaluated after additional heating required to inactivate residual trypsin inhib-

TABLE I

Triangle Test of Comparison of Blandness of Fat-Free Soy Flour Produced from Raw and Blanched Soybeans: Soy Flour and Rice Mixtures Compared with Rice Alone

Sample	Number of judgments made (total)	Number of correct judgments	Number of correct judgments needed for significance at the 5% level
3% Raw flour	6	1	5
5% Raw flour	19	11	11
6% Raw flour	13	8	8
12% Blanched flour	13	6	8
15% Blanched flour	19	9	11
20% Blanched flour	6	5	5

itor. This was achieved by heating the blanched extracted flakes for 10 min at 100 C. The raw bean flakes required a process of 30 min at 120 C, similar to the conditions used industrially.

The flakes thus processed were evaluated by a taste panel. This involved admixing the flours in varying proportions with precooked, drum-dried rice. Triangle difference tests were used to determine the highest level of incorporation at which the mixtures could not be distinguished from the rice alone. The data in Table I show that 5% raw, defatted soybean flour in the rice adversely affected the taste but as much as 15% of the blanched defatted soybean flour could be incoporated without affecting flavor of the rice ($p = 0.05$).

Economics

There are two cost considerations. One is that the soybeans must be hydrated to about 18% for heat treatment. Since this moisture is too high for oil extraction, the soybeans must be dried to about 12%. The other is that the yield of crude oil from enzyme inactive soybeans was reduced by 0.5 out of about 20%.

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Two Soybean Genotypes Lacking Lipoxygenase-1

D.F. HILDEBRAND and **T. HYMOWITZ**, Department of Agronomy, University of Illinois at Urbana-Champaign, Urbana, IL 61801

ABSTRACT

The U.S. Department of Agriculture soybean germplasm collection (6,499 accessions) was screened for genotypes with greatly reduced or missing lipoxygenase-1 (L-1) [linoleate: O_2 oxidoreductase, EC 1.13.11.12] and lipoxygenase-2 and L-3 (L-2 and L-3) activity. The L-1 assay used linoleic acid dispersed in Tween-20 at pH 9.0 as the substrate (acid assay) and the L-2 and L-3 assay used linoleic acid methyl ester dispersed in ethanol at pH 7.0 as the substrate (ester assay). The spectrophotometric assay based on conjugated diene formation at 234 mm was used in the qualitative screening procedure. Two plant introductions (PI), 133226 from Indonesia and PI 408251 from Korea lacked L-1 activity. Oxygen uptake, electrophoresis and isoelectric focusing confirm the lack of detectable L-1 activity in the seed of these two genotypes. Radial diffusion against soybean seed lipoxygenase antiserum showed that the two genotypes are missing a precipitin band that normal soybean genotypes and purified lipoxygenase from soybean seed exhibit. Neither the L-1 variants nor any other accessions tested had greatly reduced activity with the ester assay.

INTRODUCTION

Lipoxygenase [linoleate: O_2 oxidoreductase, EC 1.13.-11.12] has been implicated as the principal cause of the undesirable flavors of soybean products (1,2) especially soymilk (3). Additionally, the lipid hydroperoxides resulting from lipoxygenase action can lead to loss of nutritive value by the destruction of certain vitamins and protein (4). Also, lipid hydroperoxides and their breakdown products may have toxic effects (4).

In commercial soybean crushing operations, there is usually from 15 to 20 min between flaking of the seeds and extraction of the oil. Thus lipoxygenase has ample time to initiate the oxidation of the linoleic and linolenic acids and their esters in the oil (5). Heat treatment of soybeans prior to oil extraction increases the stability of the oil, presumably due to the inactivation of lipid oxidizing enzymes such as lipoxygenase, but has the undesirable consequence of reducing the solubility of the protein (5-7).

Soybean seeds contain at least three lipoxygenase isozymes, all having molecular weights (MW) of about 100,000. With linoleic acid as the substrate, lipoxygenase-1 (L-1) has a pH optimum at 9.5, lipoxygenase-2 (L-2) has a pH optimum at 6.5 and lipoxygenase-3 (L-3) has a broad pH optimum from 4.5 to 9.0. The isoelectric points of the three isoenzymes also are different; L-1 is the most acidic (8,9). L-1 is the most reactive with free linoleic acid, whereas L-2 and L-3 are most reactive with methyl linoleate or trilinolein (10). On an equal protein basis, L-1 is 2.5 times as active as L-2 at its pH optimum, and L-2 is 2.5 times as active as L-3 or L-3b (11). L-1 is at least 36 times more stable than L-2 at 69 C (12).

Hammond et al. (13) and Chapman et al. (14) investigated the genetic and environmental influences on a number of chemical components of soybean seed that