Effect of Different Extrusion Temperatures and Moisture Content on Lipoxygenase Inactivation and Protein Solubility in Soybeans

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Inactivation of lipoxygenase-1, -2, and -3 and changes in protein dispersibility index (PDI) during extrusion of soybeans at different temperatures and moisture levels were studied. Soybeans were extruded at six different temperatures, ranging from 77 to 121 °C, with or without holding for 30 min immediately after extrusion. Inactivation by extrusion was in the order of lipoxygenase-2 > -1 > -3. Holding for 30 min immediately after extrusion had a significant ($p \leq 0.05$) effect on inactivation of lipoxygenase-3. Essentially 100% inactivation of all three lipoxygenase enzymes in extruded soybeans resulted with a PDI of about 22. Extrusion of soybeans with different moisture contents showed a rapid decrease in PDI from 68.4 to 24.2 as soybean moisture content increased from 9.2% to 16.3%.

INTRODUCTION

Soybeans have been used as a food source in the Orient for centuries and are well-recognized for their good nutritional value. However, frequent development of off-flavors, described as beany and astringent, has long been an obstacle for their increased use in Western countries, except for extracted and purified forms. Beany flavors commonly can occur if raw, ground, wet soybeans are held for prolonged periods without heat processing (Aguilera and Lusas, 1981). By using extrusion cooking, soybeans can be converted into high-quality food ingredients. The short holding time and high temperature in an extruder reduce the damage to nutritional properties but still adequately destroy the enzymes responsible for off-flavor.

Plant lipoxygenases are distributed primarily in legumes, such as soybeans, peas, peanuts, mung beans, and navy beans, in cereal, such as wheat, oat barley, rye, and corn, and in fruits, such as apple, pear, strawberry, and tomato (Gardener, 1980). Lipoxygenases are of practical importance to food scientists for several reasons. These enzymes can have an effect on the color, flavor, texture, and other nutritive properties of foods (Schwimmer, 1981). Off-flavor development in soybeans and soybean products is highly dependent on the action of the various endogenous lipoxygenases, since subsequent decomposition of the resulting hydroperoxides yields rancid flavor (Schwimmer, 1981). Failure to inhibit lipoxygenases leads to flavor deterioration and contributes to the undesirable flavors in soybean (Axelrod, 1974). Initial hydroperoxidation of unsaturated fatty acids, which is catalyzed by lipoxygenase, may lead to formation of short-carbon-chain acids, ketones, and aldehydes (Leoni et al., 1985) that are most likely responsible for the beany flavor of soybean products (Rackis et al., 1979). Three lipoxygenase isozymes have been isolated, lipoxygenase-1, -2, and -3 (Axelrod et al.,

1981). These three isozymes have different effects on the generation of compounds in soybeans with undesirable flavors and aromas (Addo et al., 1993; Hildebrand et al., 1990).

Since the development of off-flavors in soybeans is largely of enzymatic origin, inactivation of the enzymes by a common process like heat might provide an effective means for control. Although heat treatment has effectively inactivated lipoxygenase, it also has denatured and insolubilized the protein (Wolf and Cowan, 1975). Several workers have investigated relationships between soybean protein solubility and lipoxygenase inactivation by various treatments including dry and wet heat (Mustakes et al., 1969), steam heat (Brown et al., 1982), heat with pH adjustment, heat with shearing action by homogenization (Ediriweera et al., 1987), and microwave heat (Wang and Toledo, 1987). All these researchers, except Ediriweera et al. (1987), monitored only lipoxygenase-1 among the three lipoxygenase isozymes. Guzman et al. (1989) studied the properties of soybean–corn mixture processes by low-cost extrusion. Their results showed that extrusion at 127–160 °C completely destroyed the activities of lipoxygenase-1 and -2 in soybeans and different blends of soybean and corn, but they did not study the effect of extrusion temperature on the protein dispersibility indes (PDI) with relation to lipoxygenase-1, -2, and -3.

The present study was undertaken to investigate the effect of extrusion on lipoxygenase enzymes and protein solubility in soybeans. This study was divided into two different phases. In phase 1, the effect of extrusion on protein solubility and deactivation of lipoxygenase isozymes (1, 2, and 3) at different extrusion temperatures was investigated. Whereas in phase 2, the effect of extrusion was observed on protein solubility and inactivation of lipoxygenase enzymes in soybeans of different moisture levels.

MATERIALS AND METHODS

Materials. Food grade soybeans were obtained from Jacob Hartz Seed Co., Inc., Stuttgart, AR. General analysis of the sample showed 31.7% protein, 21.9% lipids, 5.0% ash, and 10.6% moisture (AOCS, 1993).

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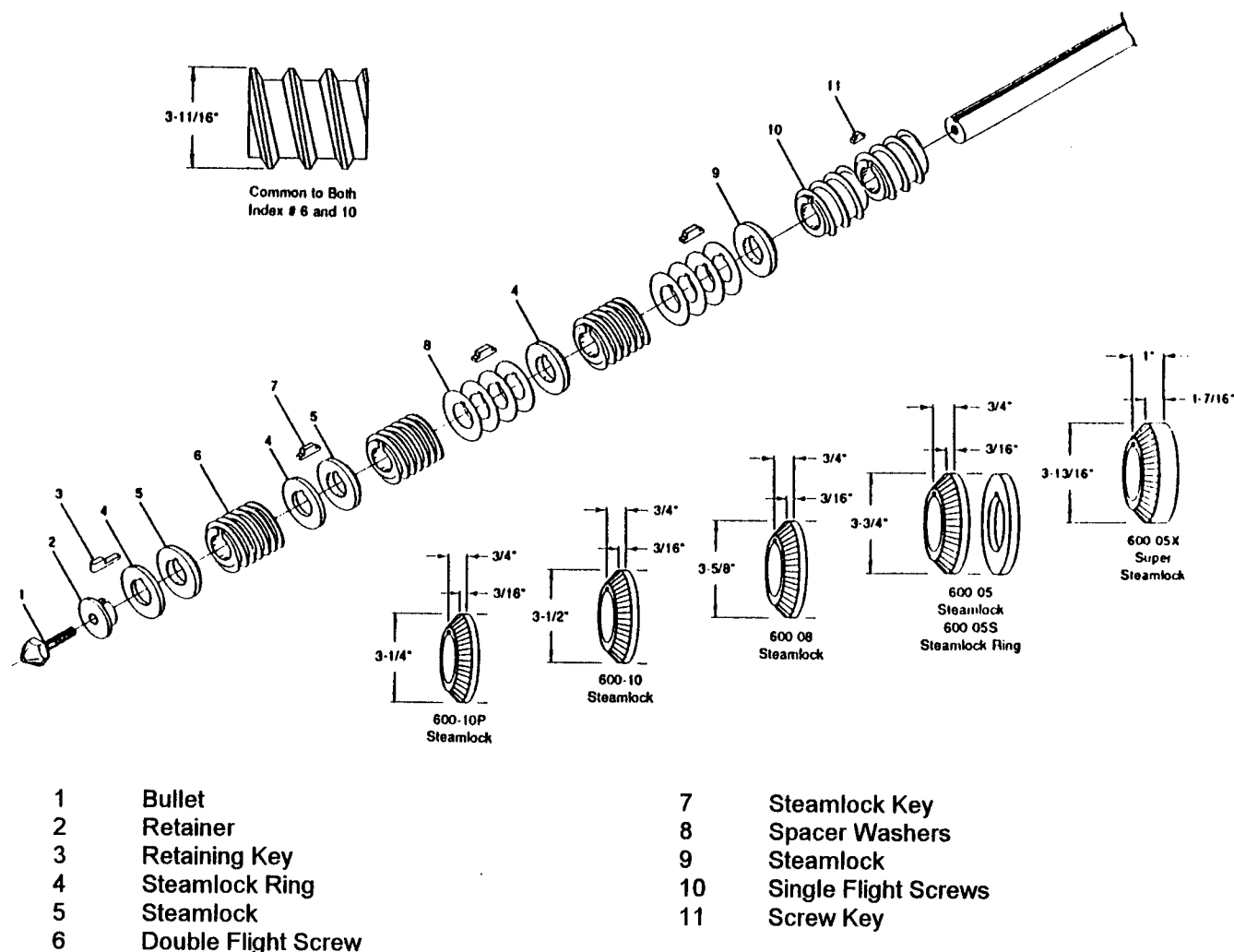[†] Hangzhou Institute of Commerce.
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1	Bullet	7	Steamlock Key
2	Retainer	8	Spacer Washers
3	Retaining Key	9	Steamlock
4	Steamlock Ring	10	Single Flight Screws
5	Steamlock	11	Screw Key
6	Double Flight Screw		

Figure 1. Model 600 JR barrel assembly internal parts (courtesy of Insta-Pro International, Des Moines, IA).

Extrusion at Different Temperatures. In the first phase of this study, whole soybeans (23 kg/batch) were extruded at six different extrusion temperatures (77, 96, 99, 107, 116, and 121 °C). Extrusion was carried out in an Insta-Pro dry extruder (Model 600 Jr. Triple F, Insta-Pro International, Des Moines, IA), with a screw diameter of 9.36 cm and powered by a 50-hp motor. This extruder, with a motor rpm of 1800 and screw rpm of 550, has a rated capacity of approximately 340 kg/h. The estimated residence time within the barrel was stated as 20−25 s when using soybeans. The barrel and screw are made up of segments. The screw segments slip over a central, keyed shaft with steam locks (ringlike restrictions) placed between them. Primary operation adjustments of the Insta-Pro extruder involve the use of steam locks with different diameters, a choice between single-flight and double-flight screws, and the use of different die areas. These restrictions control the temperature profile along the barrel. The different extrusion temperatures in this experiment were achieved by using a combination of single- or double-flighted screws with different steam locks and back pressure on the die by rotating the nose cone located at the center of the die plate and changing the feeding rate (Figure 1). The diameter of the die was 7.94 mm. Feed rate varied from 176 to 310 kg/h depending upon the desired temperature of extrusion. The last thermometer (before the die) was recorded as the extrusion temperature. In each run, the extruder was started with nonexperimental soybeans. Once the extruder temperature was raised to near operation temperature, the experimental soybeans were fed in the extruder. The extrudate samples were divided into two subsamples. The first sample was immediately spread on trays that were kept on dry ice in a cold room to arrest the effect of heating. The second sample was collected in a covered preheated container in order to keep it hot for an extra 30 min in the same room. After 30 min this sample was spread on trays, which were precooled on dry ice in the cold room to stop heating effects immediately.

Extrusion at Different Moisture Contents. In the second phase of this study, soybeans were first dried at 43 °C through a continuous dryer (Wenger Manufacturing Co., Sabetha, KS) to 9.2% moisture. Different amounts of water were added in each 23-kg batch of soybeans to obtain four different moisture levels. Soybeans were kept in a cold room (4.4 °C) for 1 week to get the homogeneous moisture level in all soybeans.

Whole soybeans with different moisture contents (9.2%, 12.2%, 13.6%, and 16.3% on dry weight basis) were extruded at a temperature of 99 °C by an Insta-Pro dry extruder. In each run, the extruder was started with the same pattern as in the first phase. Samples of extrudates were collected after the desired temperature had stabilized for at least 2−3 min. Extrudates were cooled immediately by using trays on dry ice in the cold room for analysis.

Determination of Protein Dispersibility Index (PDI). Protein dispersibility index was measured by the "fast stir" AOCS (1993) method. NSI was calculated using the following formula (Central Soya Company, 1988):

$$PDI = 1.07(NSI) + 1$$

Determination of Lipoxygenase Activity. The spectrophotometric procedures of Axelrod et al. (1981) and Engeseth et al. (1987) were used with minor modifications as described.

Preparation of Sample. Raw soybeans were ground as finely as possible without heating, preferably 100 mesh or smaller by using Miracle Mill (Model MC17B, Yugoslavia). Extra caution was taken during grinding so that the sample would
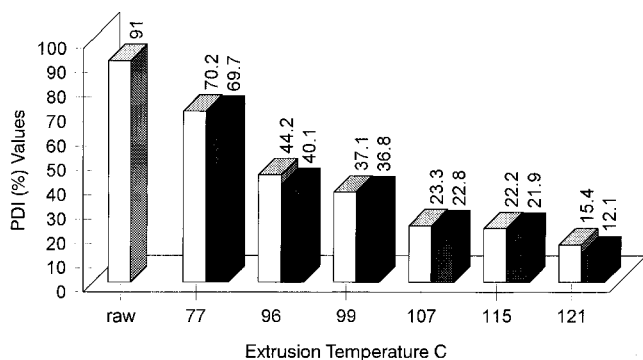
Temperature and Moisture Effects on PDI and Lipoxygenase

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Figure 2. Effect of extrusion temperature and 30-min extra hold time on PDI values of extruded soybeans: open bars, hold time 0 min; solid bars, hold time 30 min.

not exceed 37−40 °C at any time. A small quantity of dry ice (solid CO_2) was added during grinding to keep the temperature low. Extruded samples were used as such.

Preparation of Extracts. Sodium phosphate buffer (50 mL, 0.2 M, pH 6.5) was added to 1 g of ground raw or extruded soybean and stirred by a magnetic stirrer for 2 h at room temperature (25 °C). The slurry was then centrifuged at 15 000 rpm at 20 °C for 30 min. The supernatant was filtered and assayed for lipoxygenase activity.

Preparation of Substrate. Linoleic or arachidonic acid stock solution was prepared using 140 mg of acid to which an equal weight of Tween 20 and 8 mL of water were added. Then, 1.1 mL of 0.5 N NaOH was added to clarify the solution, and the volume was brought to 50 mL with water. The stock solutions were diluted (1:40, v/v) with appropriate buffers before use. Linoleic acid stock was diluted with sodium borate buffer, 0.2 M, pH 9.0, for lipoxygenase-1 analysis and with sodium phosphate buffer, 0.2 M, pH 6.5, for lipoxygenase-3. Arachidonic acid stock was diluted with sodium phosphate buffer, 0.2 M, pH 6.1, for lipoxygenase-2 determination.

Determination of Enzyme Activity. Enzymatic activity was measured at room temperature (25 °C) using a Beckman Model DU-6 spectrophotometer. Lipoxygenase-1 activity was measured at 234 nm, lipoxygenase-2 at 238 nm, and lipoxygenase-3 at 280 nm. The cuvette contained 2.5 mL of substrate; 10 μL of the extract was added with rapid mixing for 5 s, and the change in absorbance was recorded for 5 min. If the rate of absorbance increase was larger than 0.05/min, the extract was diluted using the procedure of Tappel (1962). If there was no or very little change in absorbance, the addition of extract was changed to 100 μL. Residual lipoxygenase activity was calculated based on the enzymatic activity of soybeans as 100%.

Statistical Analysis. Data were analyzed using the analysis of variance (ANOVA) procedure of the Statistical Analysis System (SAS, 1993). All significant testing was done at the 5% significance level.

RESULTS AND DISCUSSION

In the first phase of this experiment, the PDI value decreased significantly ($p \leq 0.05$) as extrusion temperature increased, except at 116 °C. However, a 30-min extra hold time after the extrusion did not have a significant ($p \leq 0.05$) additional effect on PDI values of extruded soybeans, except at 96 and 121 °C, where the 30-min, extra hold time significantly ($p \leq 0.05$) reduced the PDI value (Figure 2). The levels of all three lipoxygenase enzyme activities significantly ($p \leq 0.05$) decreased as extrusion temperature increased. Extrusion at 96 °C resulted in a significant ($p \leq 0.05$) decrease in lipoxygenase-3 enzyme activity to 0 activity with a PDI value of 44.2. Lipoxygenase-1 enzyme activity was decreased to 0 with a PDI value of 22.8 when soybeans were extruded at 107 °C. Lipoxygenase-2 enzyme was the most resistant to inactivation during extrusion
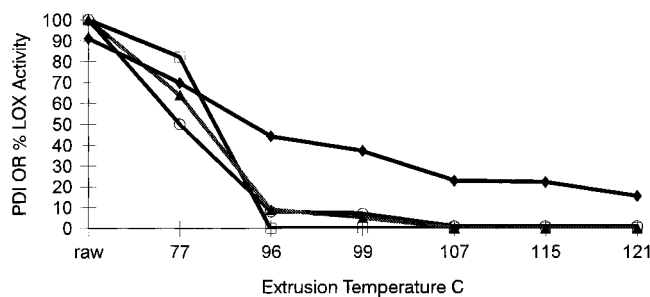

Figure 3. Effect of extrusion temperature on PDI and lipoxygenase enzyme activity (%): (◆) PDI, (▲) lipoxygenase-1, (○) lipoxygenase-2, and (□) lipoxygenase-3.
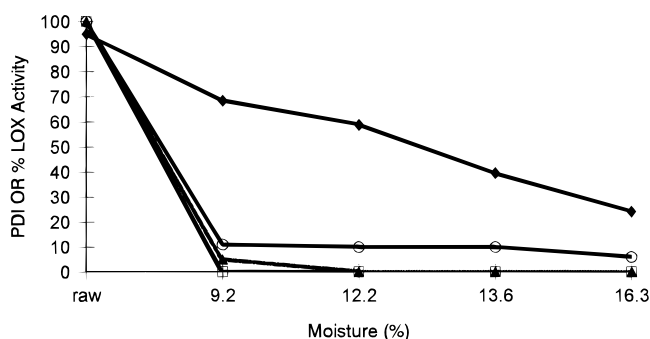

Figure 4. Effect of moisture on PDI and lipoxygenase enzyme activity (%) during extrusion at 99 °C: (◆) PDI, (▲) lipoxygenase-1, (○) lipoxygenase-2, and (□) lipoxygenase-3.

Table 1. Effects of Heat Treatment on Lipoxygenase (L-1, L-2, and L-3) Activity

extrusion temp (°C)	hold time (min)	L-1 (%)	L-2 (%)	L-3 (%)
raw soybeans		100	100	100
76.7	0	64	50	82
	30	56	43	43
96.1	0	9	8	0
	30	9	3	0
98.9	0	5	7	0
	30	4	2	0
107.2	0	0	1	0
	30	0	1	0
115.6	0	0	1	0
	30	0	1	0
121.1	0	0	1	0
	30	0	1	0

among these lipoxygenase enzymes. At 121 °C, lipoxygenase-2 enzyme still had 1% residue activity (Figure 3). Lipoxygenase enzyme activity was in the order of lipoxygenase-2 > lipoxygenase-1 > lipoxygenase-3 enzymes after dry extrusion. The 30-min extra hold time has a significant ($p \leq 0.05$) effect on inactivation of lipoxygenase-3 enzyme at a temperature of 77 °C (Table 1), which was the most heat sensitive isozyme, while the same extra hold time had less of an effect on inactivation of lipoxygenase-1 (Table 1). Lipoxygenase-2 enzyme was significantly ($p \leq 0.05$) sensitive to the 30-min extra hold time when extrusion was performed at 77, 96, and 99 °C. However, at temperatures of 107, 116, and 121 °C, the 30-min. extra hold time had no effect on inactivation of lipoxygenase-2 enzyme (Table 1).

The results of phase 2 experiment are shown in Figure 4. Increasing moisture content from 9.2% to 16.3% in soybeans during extrusion resulted in a rapid and significant ($p \leq 0.05$) decrease in PDI from 68.4 to 24.2. Extrusion at this temperature destroyed the 100% activity of lipoxygenase-3 enzyme at all moisture levels of soybeans, whereas lipoxygenase-1 enzyme activity

was 5% at 9.2% moisture level and 0 at other moisture levels. Lipoxygenase-2 enzyme activity decreased significantly ($p \leq 0.05$) from 11% to 6% as moisture content increased, except at 13.6% moisture level where activity was the same as in the 12.2% moisture level (Figure 4). It was observed that the PDI value was more sensitive during extrusion, than lipoxygenase enzyme activity, to changes with moisture content. Dry extrusion of soybean at 99 °C with lower moisture content yielded higher PDI values than the soybeans with higher moisture. A possible reason for this may be that any process in which moist protein is heated causes denaturation of the protein, evidenced by a loss of protein dispersibility or solubility.

Mustakas et al. (1969), in applying wet heat, direct steam, and dry heat plus wet heat to soybeans, found 96.0%, 99.2%, and 96.0% inactivation of lipoxygenase-1 enzymes, corresponding to 31%, 28%, and 14% NSI, respectively. In our trails dry extrusion of soybeans at 99 and 107 °C plus 30-min hold time achieved 96% and 100% inactivation of lipoxygenase-1 enzyme, corresponding to PDI values of 36.8 (33.45 NSI) and 23.3 (20.84 NSI), respectively. Mustakas et al. (1970) found that heat inactivation of lipoxygenase enzymes before extrusion cooking was essential to achieve a full-fat soybean flour with high flavor quality. According to our experiments, it may be possible to use dry extrusion instead of dry heat before extrusion cooking. Inactivation of essentially 90% of lipoxygenase-1 and lipoxygenase-2 enzymes in soybeans by dry extrusion coincides with an approximately 45 PDI value, while essentially 100% inactivation coincides with a PDI value of about 22. Soy flours with these PDI values are suitable for applications in bakeries and processing of comminuted meat products (Fulmer, 1989). Dehulled soybeans are added to potato starch for protein enrichment in baby foods. Dry extrusion of whole or split dehulled full-fat soybeans may be a means for rapidly arresting development of undesirable flavors by lipoxygenase if the product with the accompanying PDI value is useable for the intended purposes.

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