Factors Promoting the Formation of Nonhydratable Soybean Phosphatides[1]

G.R. List*, T.L. Mounts and A.C. Lanser
Food Quality and Safety Research, National Center for Agricultural Utilization Research, United States Department of Agriculture, Peoria, Illinois 61604

Whole, cracked and flaked soybeans were stored under a variety of conditions. After extraction with hexane, the crude oils were degummed in the laboratory, and the nonhydratable phospholipid (NHP) content was estimated from the phosphorus content of the degummed oil. Results showed that four interrelated factors promote NHP formation. These include (i) moisture content of beans or flakes entering the extraction process; (ii) phospholipase D activity; (iii) heat applied to beans or flakes prior to, and during, extraction; (iv) disruption of the cellular structure by cracking and/or flaking. Results from this study suggest that NHP formation can be minimized by control of the moisture of beans and/or flakes entering the extraction process, inactivation of phospholipase D enzyme, and optimizing temperatures during the conditioning of cracked beans or flakes.

KEY WORDS: Damage, degumming, deterioration, extraction, gums, moisture, oil processing, phospholipase D, phospholipids, steam.

The problem of the so-called nonhydratable phosphatides (NHP) has plagued soybean processors for many years. Unusually early frost, wet harvesting conditions, spontaneous heating while in storage, and oceanic shipment may yield crude oils with abnormally high levels of NHP. Processing of such oils leads to poor quality lecithin, high refining losses and dark-colored salad oils (1-4). Even under normal processing conditions, good quality beans may yield crude oils containing 5-10% NHP (5).

The chemical composition of NHP reportedly consists of phosphatidic acids resulting from the degradation of phosphatidylcholine and phosphatidylethanolamine (6,7). A body of evidence suggests that phospholipase D is responsible for the degradation of soybean phospholipids; however, the mechanism by which NHP are formed is not clearly understood (8,9). Whether NHP are formed in the intact seed or during extraction is open to speculation. Studies performed at the molecular level have shown that phospholipase D rapidly converts phosphatidylcholine and phosphatidylethanolamine to phosphatidic acid (10). Work conducted in the United States and abroad supports the theory that inactivation of phospholipase D plays a key role in preventing the formation of NHP (10-12). Kock (9) showed that treatment of soy flakes with live steam for 10 min yields crude oil virtually free of NHP. Microwave heating has also been shown to inhibit formation of NHP (11). Unfortunately, enzyme-inactivating treatments denature protein as well. The present investigation was undertaken to determine what factors promote the formation of soybean nonhydratable phosphatides.

EXPERIMENTAL PROCEDURES

Williams certified seed soybeans from the 1988 and 1989 crop years were used. The analytical methods, oil processing and radiochemical assays for phospholipase D activity were described previously (11).

Soybeans were dehulled, cracked, and flaked to a thickness of .012-.015 in. Whole beans and flakes were prepared for storage by tempering to the desired moisture levels and then placing them in plastic bags with the necks tied off. Samples were stored at 40°C in a convection oven. Flakes were extracted with hexane in an all-glass Soxhlet apparatus for 5 hr. The miscella was filtered through paper, and the solvent was removed under vacuum in a rotating evaporator.

Crude oils were degummed at 60°C with 2% water for 15 min with vigorous agitation. NHP were determined from the phosphorus content of the crude and degummed oils according to the following equations:

$$\% \text{ phosphatide} = \% \text{ phosphorus} \times 31.7 \quad [1]$$

$$\% \text{ NHP} = \frac{\text{phosphatide in degummed oil}}{\text{phosphatide in crude oil}} \times 100 \quad [2]$$

Soy flakes were treated with steam by placing 1000 g of flakes in a 23″ × 30″ × 1″ pan inside a steam-heated autoclave. Live steam was introduced, and the temperature was rapidly elevated to 112-113°C (235°F). Exposure time was determined after treatment temperature was attained. Steam flow was discontinued and the autoclave was vented. Samples were then removed for further processing.

The assay for phospholipase D activity was described previously (11). Activity is reported here as micromoles of choline liberated per minute per gram of whole beans.

Estimates of error in the NHP analyses were determined from nine replications each of the 10%- and 14%-moisture control beans that received no storage.

At 10% moisture, the average NHP content was calculated to be 8.79% with a standard error of 2.53%; at 14% moisture the average NHP content was 24.48% with a standard error of 2.26%.

RESULTS AND DISCUSSION

Effects of initial bean moisture on NHP content. The effects of initial bean moisture on NHP formation were studied by tempering whole soybeans to moisture contents ranging from 7-18%. The beans were cracked, flaked and extracted with hexane, and the resulting crude oil was degummed. Enzyme activity and NHP content are plotted against the initial moisture content of beans prior to processing in Figure 1a. The results clearly show that

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*To whom correspondence should be addressed at Food Quality and Safety Research, National Center for Agricultural Utilization Research, United States Department of Agriculture, 1815 North University Street, Peoria, IL 61604.

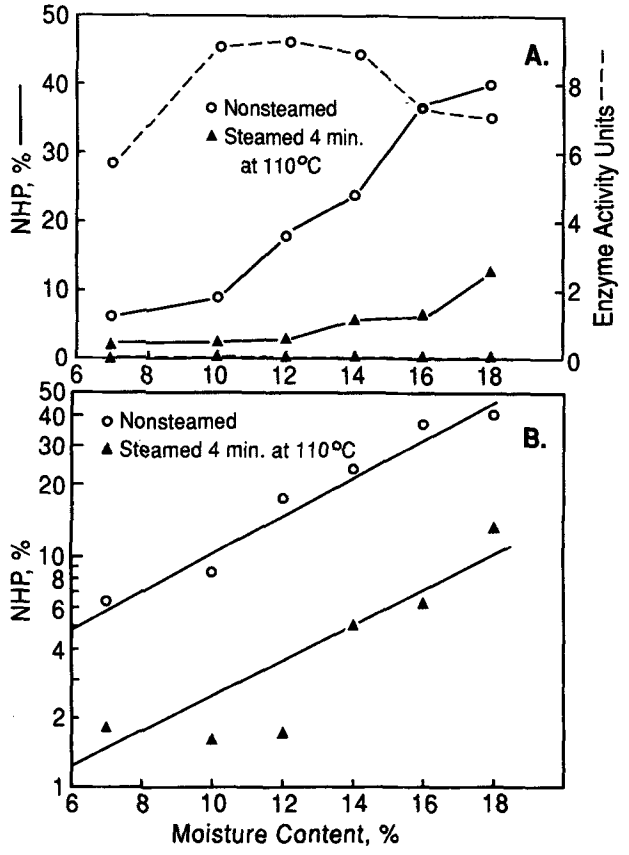


FIG. 1. Effects of moisture content and enzyme inactivation on NHP content of crude oils.

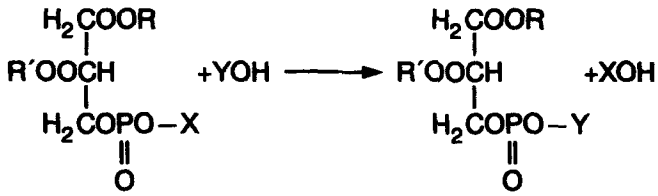
NHP formation increased with increasing moisture content and that enzyme inactivation was a key factor in reducing the NHP content of crude soybean oil. But in steamed flakes where the enzyme was completely inactivated, NHP levels still show small increases at moisture levels higher than 12%. Possibly some nonenzymatic hydrolytic degradation occurred at higher moisture levels.

Phospholipase D activity remains high and fairly constant over the range of 10–18% moisture, yet significant increases in NHP were observed above about 10% moisture (Fig. 1a). The log of the NHP content is a linear function of the initial moisture content of the bean (Fig. 1b). Regression equations are:

$$\text{steam: } \log \%\ \text{NHP} = -1.2114 + .1891 * (\%\ \text{moisture}),\ R^2 = .79 \quad [3]$$

$$\text{nonsteamed: } \log \%\ \text{NHP} = .5564 + .1820 * (\%\ \text{moisture}),\ R^2 = .96 \quad [4]$$

The standard deviation of log % NHP at a given moisture value (root mean square error) was .432 for the steamed and .164 for the nonsteamed. (Converted back from log values, the standard deviation in % NHP was 1.54 percentage points for steamed and 1.18 percentage points for nonsteamed.) These results indicate that both moisture and phospholipase D activity are required for extensive NHP formation. Phospholipase D is known to convert phospholipids to phosphatidic acid (Scheme 1), where R and R′ represent fatty acid acyl residues, X represents choline, ethanolamine, inositol or another moiety in phospholipids (13).



SCHEME 1

With excess water (y = H), the product of the reaction is phosphatidic acid, the main component of the NHP. Apparently, the critical moisture level in soy flakes is 9–10%. Data presented later confirm this observation.

Effects of cellular disruption on NHP formation. Our previous study (11) suggested that cellular disruption is an important factor in promoting NHP formation. Whole beans were shown to be more stable than flakes, and phospholipase D activity increased about two-fold after flaking compared to intact beans. To further study the effects of cellular disruption, whole, cracked and flaked beans were stored at normal (10%) and elevated (14%) moisture contents at 40°C. After extraction, the crude oils were degummed, and the NHP contents are shown in Figure 2a. At 10% moisture, the amount of NHP remained low and little difference was evident between cracked and whole beans. However, as noted previously, moisture content promotes NHP formation as shown in the 14%-moisture whole and cracked beans. The effects of cell disruption are more clearly shown in Figure 2b, where soy flakes were stored at 40°C. Formation of NHP in normal 10%-moisture flakes was more rapid compared to whole and cracked beans. Flakes at 14% moisture undergo rapid deterioration as shown by the marked increase in NHP during storage, *i.e.,* after only 4-hr storage at 40°C, over 60% NHP was formed.

Since the moisture content of flakes appeared to be a critical factor in promoting NHP formation, additional studies were performed in which flakes, ranging in moisture from 7–14%, were stored at 40°C. After extraction, the crude oils were degummed and the results are shown in Figure 3. Moisture levels of 7–9% showed little, if any, increase in NHP after 5 days at 40°C. Moisture contents above 9%, however, were progressively deleterious.

Since 40°C is higher than normally used in soybean processing streams, additional studies were conducted at ambient or room temperature (Fig. 4). Whole beans or flakes at normal 10% moisture, showed little or no deterioration after 8 days storage at room temperature. But flakes at 14% moisture showed rapid deterioration at room temperature. After 24 hr at 23°C, NHP had increased from a normal level of 5% to nearly 30%.

Results from these studies indicate that four interrelated factors promote the formation of NHP: (i) phospholipase D activity; (ii) moisture; (iii) cellular disruption; and (iv) heat applied to beans and/or flakes prior to or during extraction with hexane. Beans that have been exposed to unfavorable harvesting conditions, with a combination of high moisture and enzyme activity, followed by normal

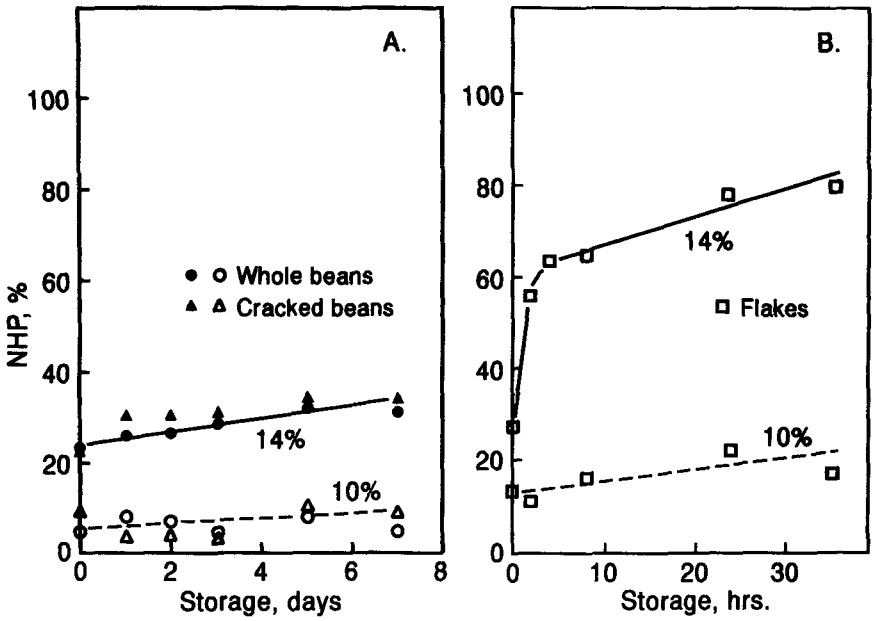


FIG. 2. NHP formation in whole, cracked and flaked beans stored at 40°C.

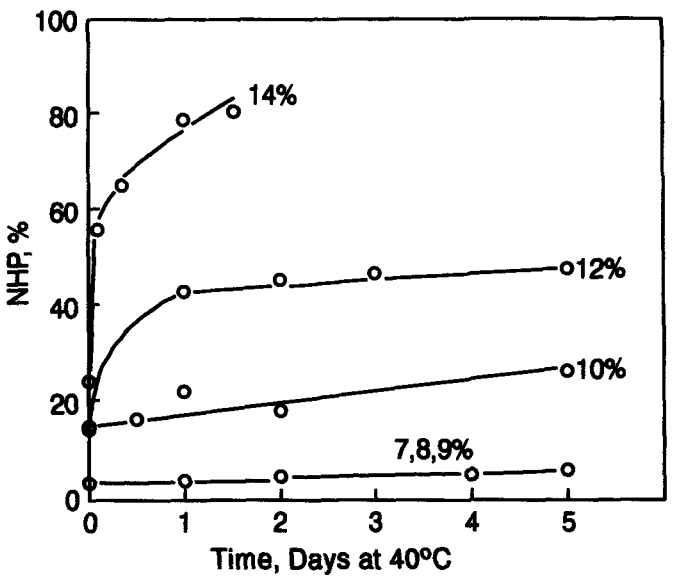


FIG. 3. Effect of moisture on NHP formation in stored flakes.

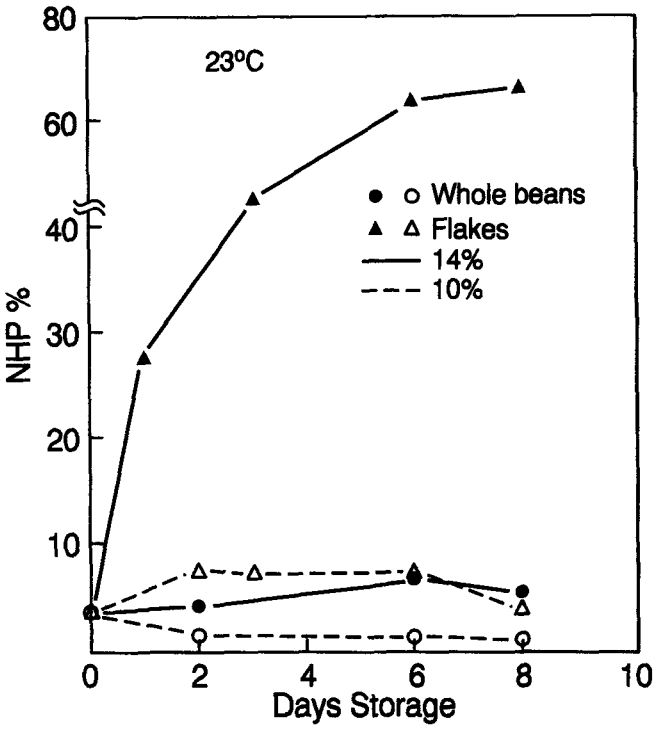


FIG. 4. Formation of NHP in whole beans and flakes stored at ambient (23°C) temperature.

cracking, flaking and extraction are likely to contain high levels of NHP regardless of handling by the processor. However, when processing beans of good quality, with normal 10-12% moisture and low levels of splits, NHP formation can be minimized by controlling the moisture content of the flakes entering the extraction process. Moisture levels of 9% or less appear to be optimum for controlling NHP formation. But, this moisture content may be too low to minimize fines produced in some types of extraction equipment. The inactivation of phospholipase D activity prior to extraction, either by microwave heating or live steam, has been shown to yield crude oils containing low levels of NHP (11). But enzyme-inactivating treatments tend to denature protein as well, thus limiting the use of soybean meal for food use.

Our studies indicated that phospholipase D is active over the entire range of moisture used in commercial

processing. However, by controlling the moisture content, the enzyme activity can be minimized.

Cellular disruption caused by cracking, and more notably by flaking, was shown to be a key factor in NHP formation. Flakes should not be stored and should be handled quickly at the lowest possible temperatures and moisture contents.

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