Origin of the Nonhydratable Soybean Phosphatides: Whole Beans or Extraction?

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Whole soybeans and flakes, tempered to normal (10%) and elevated (14%) moisture levels, were stored and then extracted under a variety of conditions in both the presence or absence of phospholipase activity. Crude oils were degummed, and the resulting nonhydratable phosphatide (NHP) content was determined. Extractions performed on flakes at ambient temperature or at the boiling point of hexane showed that at normal (10%) moisture levels the temperature of extraction had little effect on the magnitude of NHP formation; whereas at 14% moisture, considerably higher levels of NHP were observed at the higher extraction temperature. Studies performed with 10- and 14%-moisture whole beans stored at 40°C for extended periods, with or without inactivation of enzymes, showed that at normal 10% moisture levels little deterioration occurs after one week of storage; however, after four weeks considerable NHP is formed. At 14% moisture, NHP formation was rapid during the first week of storage, and complete destruction of the phospholipid occurred after four weeks' storage at 40°C. The results of these experiments indicate that the adverse effects of storage conditions, excessive moisture levels and elevated temperatures cannot be overcome by inactivation of phospholipase D prior to solvent extraction of the flakes.

KEY WORDS: Crude oils, degumming, extraction, hexane, nonhydratable phosphatides, phospholipase D, phospholipids, soybeans.

Previous work has shown that moisture, phospholipase D activity, cellular disruption and heat are interrelated factors that contribute to the formation of the so-called nonhydratable phosphatides (NHP) (1,2). However, whether these materials are present in whole beans or whether they are formed exclusively during solvent extraction (3) has not been proven unequivocally. Indeed, Nielsen (4), who first characterized these compounds as phosphatidic acids and as derived from soybean phosphatides, believed that the NHP are not formed as results of thermal or enzymatic decomposition during extraction but are present at the time of extraction (1). Other research (1–3) has indicated that inactivation of phospholipase D, either by live steam or microwave heating prior to extraction with hexane, yields crude oils with extremely low levels of NHP. We report some observations concerning the formation of NHP in whole and flaked beans after various enzyme-inactivating treatments and storage conditions.

EXPERIMENTAL PROCEDURES

The materials, methods and experimental details have been described in our previous publications (1,2). Whole soybeans tempered to 10 and 14% moisture were stored for up to one month at 40°C, cracked, flaked and extracted with hexane; the micellas were concentrated in a rotating evaporator; and the crude oils were degummed with water as described previously (1,2).

RESULTS AND DISCUSSION

Flow sheets that show the processing of the beans at 10 and 14% moisture levels and the NHP content of extracted oils are presented in Figures 1 and 2, respectively. Ten percent-moisture beans (no storage), upon cracking, flaking and extracting, yielded a crude oil containing 3.2% NHP. After one week storage at 40°C, the whole beans, after steam inactivation of phospholipase D, were processed by the aforementioned scheme to yield a crude oil containing 1.8% NHP. The stored (one week at 40°C) beans, after cracking, flaking and a 10-min live-steam treatment of the flakes, yielded a crude oil with low NHP content (0.8%). Note that beans or flakes stored for one week and extracted without steam treatment yielded crude oil containing 534 ppm phosphorus (1.69% phosphatide), whereas after steam treatment, phosphorus increased to about 1200 ppm (3.8% phosphatide). This effect also has been reported by others (3). Evidently, moist heat causes more complete cellular disruption, which permits more complete phosphatide extraction than normal cracking and flaking alone. Similarly, the steamed beans or post-steam-treated flakes could be stored an additional three days without excessive buildup of NHP. These results indicate that at moisture levels less than 10%, whole beans can be stored at least a week without excessive NHP formation.

After four weeks' storage, a different picture becomes evident. Storage of 10%-moisture beans resulted in an NHP content of 47.4%. After steaming the whole beans to inactive phospholipase D, the crude oil contained 35.1% NHP. Because, after steaming, there was no enzymatic activity during extraction, most of the NHP can be accounted for as forming during storage of whole beans. Processing the stored beans by the sequence of cracking, flaking and steam-treating resulted in virtually the same result.

Storage (3 d at 40°C) of the flakes, produced either by treating the whole beans (after four weeks) with steam or the steamed flakes, yielded crude oils containing appreciable amounts of NHP (18–27%). Again, as observed with one-week storage, NHP was formed in whole beans, and the value of enzyme inactivation treatment of abused beans appears to be of dubious value.

Storage of 14%-moisture beans (Fig. 2) for one week at 40°C yielded crude oil with NHP content only moderately higher than that of the unstored beans (18.1 *vs.* 23.3%). The increase in phosphorus content and NHP content, between the 10- and 14%-moisture unstored samples, results from a combination of elevated moisture and phospholipase D activity. Steaming of the stored beans to inactivate phospholipase D prior to extraction resulted in a marked lowering of the NHP contents. Flakes made from the steamed beans could be stored for three days

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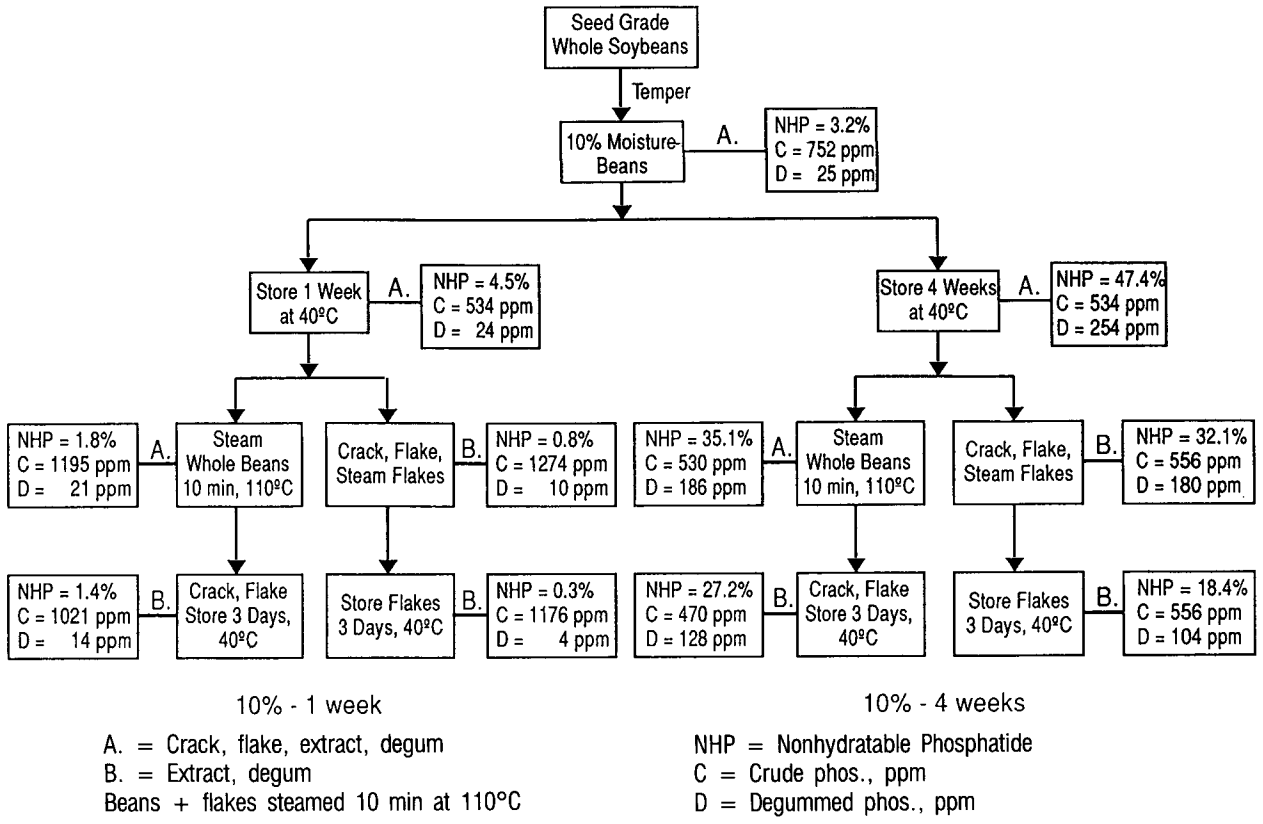

FIG. 1. Effects of bean storage and enzyme inactivation on nonhydratable phosphatide content of crude soybean oil (10% moisture).

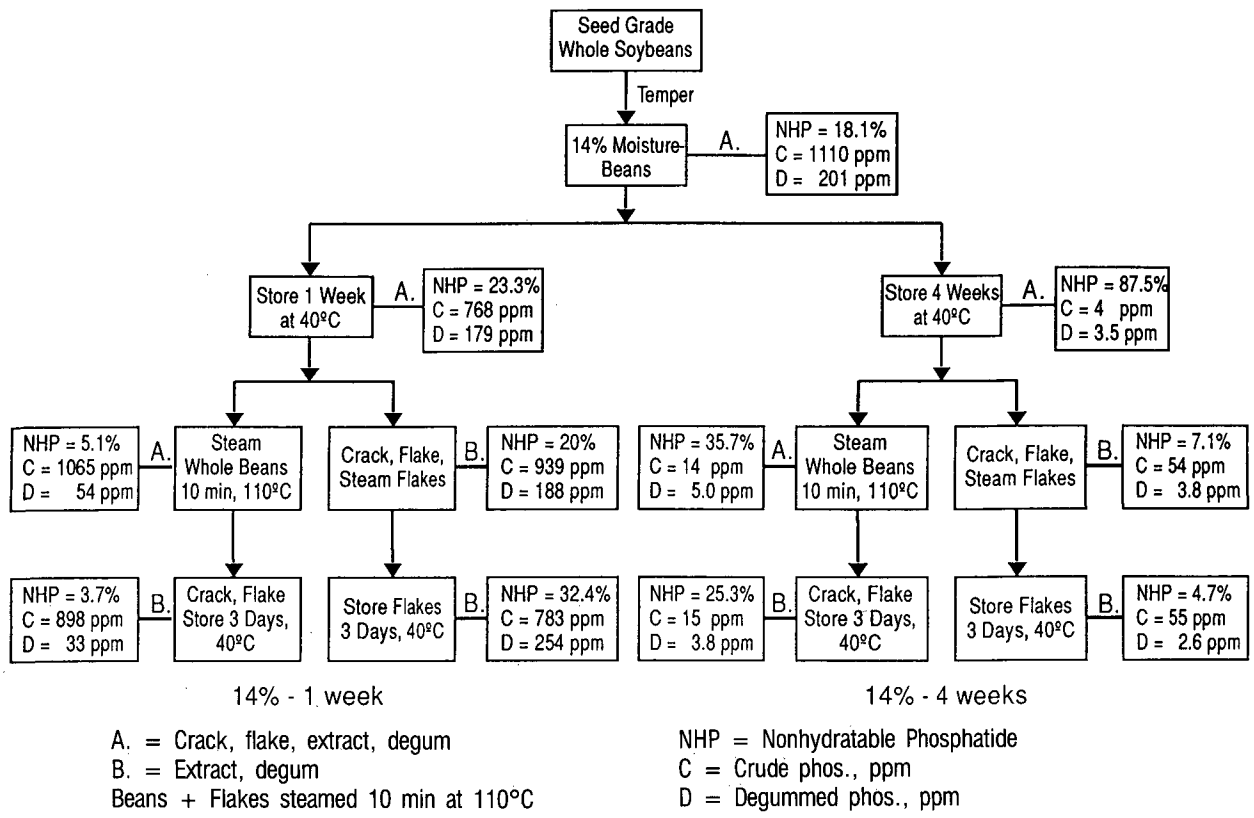

FIG. 2. Effects of bean storage and enzyme inactivation on nonhydratable phosphatide content of crude soybean oil (14% moisture).

TABLE 1

Effect of Extraction Temperature and Post-Steam Treatment on Nonhydratable Phosphatide (NHP) Formation in Soyflakes

Moisture (%)	Extraction temp. (°C)	Flake storage (days @ 40°C)[a]	Phosphorus content		NHP (%)	Phospholipase D activity[b]
			Crude (ppm)	Degummed (ppm)		
10	20	0	204	6	2.9	6.5×10^{-3}
10	60	0	729	67	9.2	5.4×10^{-3}
10	20	3	307	35	11.4	6×10^{-3}
10	60	3	767	112	14.6	7.8×10^{-3}
14	20	0	497	50	10.1	6.4×10^{-3}
14	60	0	1055	238	22.6	6×10^{-3}
14	20	3	496	254	51.2	5.8×10^{-3}
14	60	3	781	487	62.4	5.7×10^{-3}
Post-steam treated flakes[c]						
10	60	0 (4 min)	1240	14	1.1	4×10^{-5}
10	60	0 (10 min)	1281	11	0.9	8×10^{-5}
10	60	3 (4 min)	1200	54	4.5	4×10^{-5}
10	60	3 (10 min)	1245	47	3.8	8×10^{-5}
14	60	0 (4 min)	1235	43	3.5	6×10^{-5}
14	60	0 (10 min)	1180	38	3.2	4.5×10^{-5}
14	60	3 (4 min)	807	616	76.3	5.9×10^{-5}
14	60	3 (10 min)	941	680	72.3	4.6×10^{-5}

[a] Non-steam treated flakes.

[b] Micromoles choline liberated/min/g.

[c] Processing sequence: temper, crack, flake, store at 40°C steam (4 min or 10 min), extract and degum.

without significant formation of NHP. On the other hand, stored beans (one week), processed by cracking, flaking and steaming, yielded crude oils with higher levels of NHP, both with and without three-day storage of the flakes.

After four weeks' storage at 40°C, the 14%-moisture beans showed virtually complete destruction of the phosphatides. This phenomenon has also been reported by Robertson *et al.* (5).

To determine the extent of NHP formation during extraction, 10 and 14% moisture flakes were extracted at ambient temperature and at the boiling point of hexane. Also, to further investigate whether inactivation of phospholipase D prior to solvent extraction would improve the hydratability of the phosphatides, flakes were treated with steam prior to and after storage. The results are shown in Table 1. At 10% moisture, raising the extraction temperature from 20 to 60°C increased the NHP content about fivefold, whereas at 14% moisture, about a twofold increase in NHP content occurred. After storage, however, little difference in NHP could be shown between the extraction temperatures, regardless of moisture levels. These results indicate that moisture of the flakes, in combination with phospholipase D activity, is just as important in minimizing NHP as the extraction temperature. For example, at 10% moisture, flakes yield 9.2% NHP at 60°C, whereas at 14% moisture, the NHP more than doubles to 22.6%. The storage data show that any additional NHP formed during abuse or storage of the flakes is unaffected by extraction temperature. The bottom portion of Table 1 shows that, in the absence of phospholipase D activity, significant amounts of NHP were found only in the 14%-moisture flakes steamed after storage.

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