Soybean Drying in Fluidized Bed. Effect on the Hydratable and Nonhydratable Phosphatide Concentration in Crude and Degummed Crude Oil

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ABSTRACT: The content of nonhydratable phosphatides in soybean crude oil is increased by phospholipase D activity during the oil-making process. Enzyme inhibition would allow to minimize them. Recently harvested soybeans with high moisture levels require adequate drying to store safely. Simultaneous soybean drying and phospholipase D inactivation in a single operation when applying a thermal treatment by the fluidized-bed technique was evaluated. The process conditions for performing the drying and a complete enzyme inhibition on soybeans, with an initial moisture content between 7.4 and 20.6% wet basis, similar to that at the time of the harvest, were fluidizing and drying medium temperature between 110 and 140°C, and a drying time between 1 and 2 min. For the treated soybeans, the phosphorus content increased up to 223% in crude oil and decreased 17% in degummed crude oil with regard to the values of the control sample.

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KEY WORDS: Soybean drying, soybean nonhydratable phosphatides, soybean phosphatides.

The production of refined oil requires phosphatide removal from crude oil (CO). Most soybean CO phosphatides are hydratable (HP) and can be separated by means of aqueous degumming. The increment of nonhydratable phosphatides (NHP) diminishes the degumming efficiency, so it is necessary to use treatments which increase the oil lost (1). Transformation of HP into NHP is carried out during the oil-making process by phospholipase D (PLD) (2), which is freed by cell breakage during cracking and flaking operations (3). Soybean PLD activity increases linearly as temperature increases when the grain moisture content is between 11.3 and 13.6% wet basis (wb), and its greatest activity is at 85°C. NHP content in CO increases at the same time as the extraction temperature (4). List *et al.* (3,5) proved that moisture, cellular disruption, heat, and PLD activity are factors that promote NHP formation; PLD inactivation minimizes it. PLD was fully inactivated in whole soybeans by a 8–9 min microwave treatment at 115–120°C, and by a live steam treatment at about 110°C of soyflakes. In both cases, it was proved that thermal treatment completely destroyed PLD activity and made moisture removal possible.

Kock (4) demostrated by means of a moisture–heat treatment of soyflakes prior to extraction that enzyme activity could be completely eliminated, thereby minimizing NHP formation.

To store soybeans safely when their moisture exceeds 14.2% wb, they must be dried while minimizing grain fissuring, cracking, and dehulling. Florin and Bartesch (6) proposed fluidized-bed (FB) for drying, cooling, dehulling, and conditioning in oil seed processing. This method is advantageous for the following reasons: homogeneous product treatment, additional cleaning, precise control of treatment temperature, low quality losses, and low damage levels of oil and proteins.

Tosi *et al.* (7) dried soybeans in a FB and proposed an empirical equation for the drying kinetics. A 3-min FB treatment at 130°C is enough to achieve soybean drying with 17.5% wb initial moisture content and to destroy urease and the trypsin inhibitor simultaneously. The available lysine lost was less than 2% (8).

The objective of this study was to evaluate simultaneous soybean drying and PLD inhibition by FB short time–high temperature treatment and to determine its influence on the HP and NHP content in both CO and degummed crude oil (DCO), as well as on the oil quality.

MATERIALS AND METHODS

Materials. Coker variety soybeans, air-dried down to 11.3% wb immediately after harvest and kept at 25°C were used. Soybean lecithin and ammonium tetrathiocyanodiammonochromate (ammonium reineckate) were purchased from Sigma Chemical Company (St. Louis, MO); other chemicals used were from Merck (Darmstadt, Germany). All reagents were American Chemical Society reagent grade.

Equipment. The thermal treatment was performed in pilot scale FB. The drying air-mass flow was 3.0 kg dry air/s m^2 and the grain held in the dryer was 29 kg dry solid/m^2. The tests were carried out at different drying temperatures and times on whole soybeans rewetted to current harvest moisture values,

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between 15.4 and 23.0% wb so that, at the end of the treatment, the grain final moisture content (FMC) ranged between 13.5 and 14.2% wb. To obtain this, the grains were rewetted by spraying a water quantity calculated with the drying kinetic empirical equation (7), and they were periodically mixed during 48 h at 23–25°C, according to the rewetting method previously determined. Significant differences in the drying curves, HP and NHP contents, and PLD activity were not found between soybeans with natural moisture and rewetted ones, both at equal initial moisture contents between 14.5 and 22.5% wb.

Rewetted soybeans were submitted to four-stage processing resembling industrial methods: thermal treatment of drying and PLD inhibition, conditioning, flaking, and oil extraction. The control whole soybeans at 11.3% wb storing moisture were submitted to the last three processing stages.

Thermal treatment of drying and PLD inhibition. Rewetted soybeans were treated at each one of the selected drying temperatures, between 50 and 140°C, during the corresponding drying time (Table 1), determined by means of the drying empirical equation (7), until the FMC was reached. For all tested temperatures, the mean transient ranged between 33 and 40 s; within that time, the difference between both drying air and grain temperatures was 0.5 ± 1.2°C. This value decreased as drying time increased.

Conditioning of thermal-treated and control soybean. Grains were conditioned by air drying at 40°C until the moisture content ranged between 11 and 12% wb. After that, they were cracked and heated at 60°C during 3 h in an hermetic spinning container. PLD activity was then determined.

Flaking. Conditioned soybeans were flaked at 0.22 mm mean thickness.

Oil extraction. The extraction was carried out with a Butt extractor (Ricardo Bueloni y Cia, Rosario, Santa Fe, Argentina) on 1000 g of flakes with *n*-hexane. The obtained miscella were desolventized in a rotary evaporator under a nitrogen stream, applying a 3.3 kPa vacuum in the final stage. The obtained oil was degummed by adding 2% distilled water and shaking for 15 min in a 60°C constant-temperature bath. The gums were separated by centrifugation for 15 min at RCF 700 × *g*. Thermal treatment tests and analyses for moisture con-

TABLE 1
Drying Conditions, Phosphorus Content in CO, R_{CO}, Phosphorus Content in DCO, R_{DCO}, and Phospholipase D Activity in Soybeans[a]

	T (°C)	t (min)	M_i (% wb)	M_F (% wb)	P_{CO} (ppm)	R_{CO}	P_{DCO} (ppm)	R_{DCO}	PLD activity $\left(\frac{\text{choline mmol}}{\text{g soybean} \cdot \text{min}}\right)$
Control	—	—	13.6	—	560	—	160	—	6.30×10^{-4}
A	50	30	15.4	13.7	560	1.00	180	1.13	6.00×10^{-4}
	50	60	16.8	13.5	550	0.98	180	1.13	6.10×10^{-4}
	80	10	18.2	14.1	620	1.11	180	1.13	6.10×10^{-4}
	50	120	20.2	13.8	560	1.00	170	1.06	6.10×10^{-4}
	80	20	19.7	13.7	630	1.13	170	1.06	5.80×10^{-4}
	80	30	21.3	13.9	630	1.13	170	1.06	6.00×10^{-4}
	60	60	20.0	13.7	580	1.04	160	1.00	6.20×10^{-4}
	80	40	23.0	13.5	640	1.14	160	1.00	5.70×10^{-4}
	100	4	17.7	13.8	640	1.14	160	1.00	5.80×10^{-4}
B	100	10	19.3	14.1	670	1.20	150	0.94	5.00×10^{-4}
	100	20	22.0	13.7	690	1.23	130	0.81	3.70×10^{-4}
C	110	2	17.4	14.1	710	1.27	120	0.75	ND
	110	4	18.0	13.5	760	1.36	110	0.69	ND
	110	10	20.2	14.0	760	1.36	100	0.63	ND
	120	2	17.7	14.2	750	1.34	100	0.63	ND
	110	15	22.1	13.7	800	1.43	90	0.56	ND
	120	4	18.7	13.6	780	1.39	90	0.56	ND
	120	6	20.1	13.8	800	1.43	85	0.53	ND
	120	10	22.0	13.7	820	1.46	80	0.50	ND
	130	1	17.4	14.1	800	1.43	70	0.44	ND
	130	2	18.2	13.7	840	1.50	70	0.44	ND
	130	4	19.4	13.6	920	1.64	70	0.44	ND
	140	1	17.5	13.7	910	1.63	60	0.38	ND
	140	2	18.5	13.8	950	1.70	60	0.38	ND
	130	6	20.8	14.0	970	1.73	50	0.31	ND
	140	4	20.6	14.0	1100	1.96	40	0.25	ND
	140	5	21.8	13.6	1250	2.23	30	0.19	ND

[a]CO, crude oil; DCO, degummed crude oil; R_{CO}, ratio of phosphorus content in crude oil from treated and untreated soybeans (Eq. 1); R_{DCO}, ratio of phosphorus content in degummed crude oil in treated and untreated soybeans (Eq. 2); M_i, initial moisture content; M_F, final moisture content; P_{CO}, phosphorus content of crude oil; P_{DCO}, phosphorus content of degummed crude oil; PLD, phospholipase D; ND, not detected.

tent, phosphorus in both CO and DCO, and PLD activity were carried out in triplicate.

Moisture content determination. Moisture content was determined according to ISO 665:1977 (9).

Color determination. Color was determined according to British Standard 864 Sec. 1.14 1987, equivalent to AOCS Cc 13c-92, with a Lovibond Tintometer model E [The Tintometer Ltd., Salisbury, England (10)].

Acidity. Acidity was determined according to AOCS Method Ca 5a-40 (11).

Phosphorus determination. Phosphorus determination in CO and DCO was carried out following the method proposed by Tosi *et al.* (12).

PLD activity determination. In previous studies, two methods were compared to choose the PLD activity-determination technique: (i) extraction of the enzyme from ground thermal-treated soybeans with an acetate buffer (10 M, pH 5.0, to 20°C) according to Nakayama *et al.* (13), and (ii) direct PLD action; mixing ground thermal-treated soybeans in the presence of soybean lecithin as substrate, calcium chloride, ethyl ether, and pH 4.8 acetate buffer and shaking for 3 h at 25°C. The remaining solid was separated by centrifugation before quantifying the choline in the supernatant. In both cases the choline obtained was quantified by the Möllering and Bergmeyer method (14).

Some samples that did not show PLD activity by the extraction method showed activity by the direct action method. This difference is attributed to the thermal treatment effects that would deactivate a fraction of the enzyme and at the same time decrease the solubility of the active form, reducing the extracted quantity. The direct action method was chosen, and PLD activity was expressed in choline mmol/g soybean · min. Thermal treatment effects were evaluated by moisture determination, the PLD activity in soybeans, and the phosphorus content in CO and DCO. These were related to those of control soybeans, thus defining the following relations:

$$R_{CO} = \frac{\text{phosphorus content in CO of treated soybean}}{\text{phosphorus content in CO of control soybean}} \quad [1]$$

$$R_{DCO} = \frac{\text{phosphorus content in DCO of treated soybean}}{\text{phosphorus content in DCO of control soybean}} \quad [2]$$

Thermal treatment effect upon quality was evaluated by color and acidity on CO and DCO.

RESULTS AND DISCUSSION

Table 1 shows drying conditions and results. The table can be divided into three parts. Part A shows results of mild treatment conditions without substantial modification of phosphatide content and PLD activity. Part B shows that the treatment effects became noticeable with a reduction of both NHP and PLD activity. Part C shows that PLD activity cannot be measured by the employed method and that NHP decreases gradually as the treatment severity increases. The end of part C represents treatment conditions that ensure both the drying from 21.8 to 13.6% wb and a low level of NHP.

TABLE 2
Mean Value and the Standard Deviation for R_{CO}, R_{DCO}, and PLD Activity[a]

Treatment				
Temperature (°C)	Time (min)	Mean value	Extreme values	Standard deviation
		R_{CO}		
50–100	120–4	1.07	—	0.07
100	10–20	1.22	—	0.02
110–140	15–1	—	0.96–2.23	0.09–0.19
		R_{DCO}		
50–100	120–4	1.06	—	0.06
100	10–20	—	0.94–0.81	
110–140	15–1	—	0.75–0.19	0.07–0.02
		PLD activity[b]		
50–80	120–10	6.1×10^{-4}	—	0.4×10^{-4}
100	4–20	4.35×10^{-4}	—	0.92×10^{-4}
110–140	15–1	Negligible	—	—

[a]See Table 1 for abbreviations.
[b]Choline mmol/g soybean · min.

Standard deviations (Δ) of phosphorus content values for each individual determination were between 2 and 9. The corresponding Δ values for the enzymatic activity were between 0.3×10^{-4} and 0.6×10^{-4}. Table 2 shows the mean value, extreme values, and standard deviation for R_{CO}, R_{DCO}, and PLD activity.

PLD activity. There was an increase of phosphatide extraction when the soybean treatment was increased as previously reported (4). The NHP presence in soybeans treated with zero PLD activity may be explained by: (i) presence in the grain before treatment; (ii) production by nonenzymatic hydrolysis as suggested by List *et al.* (3); or (iii) production during the first part of the treatment, where the grain temperature increases (is transient), until it reaches a level that destroys enzymatic activity by structure alteration. This mechanism would explain the decreasing NHP levels as treatment temperature and heating velocity increase, so that the available period for the enzymatic activity diminishes. Diminishing NHP levels during drying temperatures that exceed those of the enzyme inactivation could also be explained by the covalent combination between the proteins and the peroxidized phosphatides (4), but the latter cannot be produced by lipoxygenase since it is inactivated under the same treatment conditions as PLD. A suggested mechanism would be a phosphatide autoxidation process that produces lipidic free radicals, which covalently link to proteins to form free lipid–protein radicals which produce covalent compounds insoluble in hexane. According to Cheftel *et al.* (15) a covalent link between proteins and lipid oxidation products was established. This has been found in dehydrated or frozen fish, fish flour, and oilseeds.

It is impossible to determine which of these mechanisms prevails from the results of the present work. In the flake-formation process, the behavior of the treated soybean depends on the drying conditions. Differences were not detected for treatments carried out at 110°C up to 10 min and at 120, 130, and 140°C up to 4 min maximal time.

Oil quality decrease was not observed in all the treatments that produced complete PLD inhibition. Color values ranged between 3.4 and 4.2 for the red one and 30 and 40 for the yellow one, and acidity values ranged between 0.54 and 0.82 g oleic acid/100 g oil.

REFERENCES

1. Zhang, S., S.S. Koseoglu, and K.C. Rhee, Effects of Expander Process on the Phospholipids in Soybean Oil, *J. Am. Oil Chem. Soc. 71:*1145–1148 (1994).
2. Haraldsson, G., Degumming, Dewaxing and Refining, *Ibid. 60:*251–256 (1983).
3. List, G.R., T.L. Mounts, and A.C. Lanser, Factors Promoting the Formation of Nonhydratable Soybean Phosphatides, *Ibid. 69:*443–446 (1992).
4. Kock, M., Oilseed Pretreatment in Connection with Physical Refining, *Ibid. 60:*198–202 (1983).
5. List, G.R., T.L. Mounts, A.C. Lanser, and R.K. Holloway, Effect of Moisture, Microwave Heating, and Live Steam Treatment on Phospholipase D Activity in Soybeans and Soy Flakes, *Ibid. 67:*867–871 (1990).
6. Florin, G., and H.R. Bartesch, Processing of Oilseeds Using Fluidbed Technology, *Ibid. 60:*193–199 (1983).
7. Tosi, E., M. Tamburelli, and R. Prado, Cinética del secado de soja *(Glycine max),* Ecuación empírica para el secado en lecho fluidizado, *Rev. Agroquím. Tecnol. Aliment. 30:*130–137 (1990).
8. Osella, O., R. Gordo, R. González, E. Tosi, and E. Ré, Soybean Heat-Treated Using a Fluidized Bed, *Lebensm. Wiss. Technol. 30:*676–680 (1998).
9. International Organization for Standardization, Geneva, Switzerland, 1977.
10. British Standards, Liverpool, England, 1987.
11. AOAC, *Official Methods of Analysis,* 12th edn., Association of Official Analytical Chemists, Washington, DC.
12. Tosi, E.A., A.F. Cazzoli, and L.M. Tapiz, Phosphorus in Oil. Production of Molybdenum Blue Derivative at Ambient Temperature Using Non-Carcinogenic Reagents, *J. Am. Oil Chem. Soc. 75:*41–44 (1998).
13. Nakayama, K., K. Saio, and M. Kito, Decomposition of Phospholipids in Soybean During Storage, *Cereal Chem. 58:*260–264 (1981).
14. Bergmayer, H.U., and K. Gawehn, Enzymes as Biochemical Reagents, *Methods of Enzymatic Analysis*, 2nd edn., Academic Press, New York, 1974, Vol. I, p. 504.
15. Cheftel, J.C., J.L. Cuq, and D. Lorient, Aminoácidos, péptidos y proteínas, in *Química de los Alimentos,* edited by O. Fennema, Acribia SA , Zaragoza, Spain, 1993, p. 388.

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