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Effects of pH and Salt on Yields, Trypsin Inhibitor Content, and Mineral Levels of Soybean Protein Isolates and Wheys

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Protein isolates were prepared from water extracts of defatted soybean flour or flakes by acid precipitation at pH's from 3.5 to 5.2 with or without added 0.1 N NaCl. The resulting wheys and precipitates were analyzed for trypsin inhibitor (TI) activity (TIA), phytic acid, and minerals. TIA varied from 70 mg of trypsin inhibited/g of protein in an isolate precipitated at pH 4.5 from an extract with no added NaCl to 8 mg/g at pH 5.2 with added NaCl. Protein isolate yields varied from 85% of extract proteins at pH 4.5 with no added salt to 59% at pH 5.2 with added salt. The TIA was concentrated to 450 mg/g of freeze-dried permeate by ultrafiltration and dialysis. Retention of the TIA was 50% or more by an ultrafiltration membrane with a 100-kDa cutoff. Since TI's have a molecular weight of 20K or less, their retention indicates they were aggregated to a higher molecular weight. The phytic acid level was highest and zinc level lowest in the pH 3.5 protein precipitate compared to levels in the pH 4.5 and 5.2 precipitates. Levels of Ca, Mg, K, Na, Fe, Mn, and Cu in isolates, wheys, and a TI concentrate were also determined. Extent of removal of minerals and phytic acid from an acid-soluble TI concentrate at pH 2.4 was in the order K > Mn > Zn > Mg > phytic acid > P > Ca > Fe > Cu.

Soybeans are an important source of dietary protein in many parts of the world. High levels of trypsin inhibitor activity (TIA), in raw soybeans, are of nutritional concern in spite of the fact that trypsin inhibitors (TI's) have higher levels of essential amino acids than some storage proteins. Although TI's are found in many food sources, soybeans, potatoes, and eggwhites have relatively high levels of TIA and have received special attention (Liener and Kakade, 1980; Rackis and Gumbmann, 1981; Rackis et al., 1986). Small changes in processing conditions (pH, salt concentration, etc.) have a relatively large influence on the levels of TI's and other proteins in soy protein isolates; processing also affects levels of phytate and minerals and causes changes in functional and nutritional properties (Anderson et al., 1973; de Rham and Jost, 1979). Short-term animal feeding studies have shown that raw soybean meal and purified soybean TI's inhibit growth and enlarge the pancreas in certain monogastric animals (Liener and Kakade, 1980; Rackis and Gumbmann, 1981; Struthers et al., 1982). Other studies showed that the effects of raw soy flour containing diets on the rat pancreas persist and become progressively more marked sometime between 30 and 60 weeks of feeding [see review by McGuiness et al. (1984)]. Therefore, 2-year rat feeding studies were undertaken to study the effects of raw and heated soybean protein products with varying levels of TIA (Rackis et al., 1985; Gumbmann et al., 1985; Liener et al., 1985; Baker and Rackis, 1985).

In support of the long-term feeding studies, we varied precipitation pH and added 0.1 N NaCl to the soybean meal extract to prepare a series of protein isolates with low TIA and corresponding wheys with high TIA. We also concentrated TIA further by combinations of ultrafiltration, dialysis, pH, and diafiltration with salt to separate TI's from other proteins, minerals, and carbohydrates. We determined the effects of acid pH on association of minerals with acid-soluble and acid-insoluble proteins and showed that precipitation pH affects phytate and zinc levels and protein isolate yields.

MATERIALS AND METHODS

Materials. The starting materials for our preparations were a commercial, defatted soy flour, Nutrisoy 7B (Archer Daniels Midland, Decatur, IL), and dehulled, defatted soybean flakes prepared in the laboratory (Sessa et al.,

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Table I. Effects of pH and Salt on Trypsin Inhibitor Activity (TIA) and Yield of Soybean Protein Isolates and Wheys from Nutrisoy 7B Flour Extracts

		protein distribn,[a,c] %		TIA[b]/g meal[c]		TIA[b]/g protein		TIA,[a] %	
pH	sample	no NaCl added	+0.1 N NaCl	no NaCl added	+0.1 N NaCl	no NaCl added	+0.1 N NaCl	no NaCl added	+0.1 N NaCl
5.2	isolate	69	59		2		8		5
5.0	isolate	81 ± 1	63 ± 1	9 ± 2	4 ± 1	26	15	18	9
	whey	18 ± 1	37	41	40	500	270	82	91
4.8	isolate	83	73 ± 2		10		33		24
4.5	isolate	85 ± 1	80 ± 1	25 ± 2	9 ± 1	70	27	46	20
	whey	15 ± 1	20	29	35	450	450	54	80
3.5	isolate	75 ± 1	60 ± 2	3 ± 1	3 ± 1	9	13	7	7
	whey	25 ± 2	40	40	38	350	260	93	93
7.5	orig meal extr	100	100	40	40	89	89	100	100

[a] Distribution of extract or TIA between isolate and whey. [b] Milligrams of trypsin inhibited. [c] Mean ± deviation for two determinations.

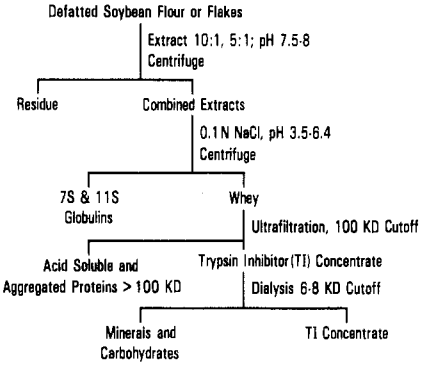

Figure 1. Fractionation of protein extracts of soybean meal for trypsin inhibitor and mineral analysis.

1969) from Amsoy 71 soybeans, 1976 crop. Deionized distilled water was used in all laboratory procedures and sample preparations.

Preparation of Samples for TIA Assay. Samples were prepared by variations of the scheme shown in Figure 1. Precipitates were washed one to three times and the washes added to the wheys. In one series of sample preparations, equal portions of a Nutrisoy 7B flour pH 7.5 extract were acidified to pH's 3.5, 4.5, and 5.0 and centrifuged. The resulting freeze-dried precipitates and wheys were then analyzed for TIA and for protein (Kjeldahl nitrogen times 6.25). For another sample series, the flour extracts were brought to 0.1 N added NaCl. Equal portions of the extract were then acidified to values within the range pH 3.5–5.2 (Figure 1).

Preparation of Samples for Phytic Acid and Mineral Determination. Protein isolates and wheys for phytic acid and mineral determinations were obtained from pH 8 extracts of laboratory-prepared flakes acidified to a selected pH value between 3.5 and 5.2.

Samples of 7S and 11S protein were prepared by the procedure of Thanh and Shibasaki (1976), then brought to pH 7.8 with NaOH, dialyzed for 4 days against water, and freeze-dried.

TI concentrates were prepared by extraction of Nutrisoy 7B flour at pH 4.2 with 0.1 N NaCl (Baker and Rackis, 1985). The extracts were subjected to ultrafiltration, repeated washings at pH 2.4, and freeze-drying, to produce concentrate A. Concentrate B was obtained by a second pH 2.4 ultrafiltration, washing, and freeze-drying of a sample of concentrate A.

Ultrafiltration. Conditions for concentrating TIA by ultrafiltration were examined in two studies on pH 7.5 extracts of 50 g of defatted soybean flakes after adding 0.1 N NaCl (Figure 1). In the first study, 11S proteins were removed from a combined 20:1 and 10:1 extract by acidifying to pH 6.4 and centrifuging at 0–4 °C. The supernatant was acidified to pH 5.0 and centrifuged. The crude 7S precipitate was washed and retained for analysis. The combined pH 5 whey and wash (about 1500 mL) was then brought to 1600 mL. Portions of it were subjected to room-temperature ultrafiltration in an Amicon cell of 200-mL volume through an XM100 membrane (Amicon Corp., Lexington, MA) under N_2 pressure of 10 psi for 16–24 h at a flow rate of 60–80 mL/h. From one to three fractions of permeate were collected. In run 1, retentate and permeate were dialyzed after ultrafiltration. In run 2, a portion of the whey was dialyzed and freeze-dried before redissolving for ultrafiltration. After freeze-drying, the retentate and permeate fractions were analyzed for TIA.

In a second study, proteins were precipitated from a combined 10:1 and 5:1 extract at pH 5.0, and portions of the resulting whey were filtered at 5 °C. After the sample was concentrated by 28%, it was diafiltered with 0.5 N NaCl to enhance solubility and permeability of the proteins. In the first run a whey sample was filtered as is, pH 5.1, while in the second it was first neutralized to pH 7.8. Phosphorus (P) and TIA were determined in retentate and permeate fractions after dialysis and freeze-drying.

Analytical Procedures. TIA was determined by the procedure of Kakade et al. (1974) as modified by Hamerstrand et al. (1981). It is expressed as

$$\text{mg trypsin inhib/g sample} = \frac{A_{std} - A_{sample}}{19} \times \text{dilution factor}$$

based on absorbance at 410 nm of standard trypsin, acting on a benzoylarginine *p*-nitroanilide (BAPA) substrate (A_{std}), minus absorbance with a TI sample added (A_{sample}). Dilution factor is the dilution of sample required to reduce absorbance to 40–60% of the standard trypsin reaction mixture. The factor 19 represents 0.019 absorbance unit/μg of trypsin under assay conditions times 1000 μg/mg. Various aspects of the assay are discussed by Rackis et al. (1986). Protein in whey samples, which were analyzed for minerals, was determined by the procedure of Lowry et al. (1951). Phytic acid, P, and other minerals were determined as in Honig and Wolf (1987).

RESULTS AND DISCUSSION

Effects of pH on TIA and Protein Yield. Varying the precipitation pH from 5.2 to 3.5 affected the distribution of TIA and protein between protein precipitate and whey (Table I). The effect of precipitation in the presence of 0.1 N added NaCl is also shown. At pH 5, 82% of the activity was in the whey and only 18% in the isolate; on a protein basis, TI activity (milligrams of trypsin inhibited/gram of protein) was 500 in whey and 26 in the isolate. At pH 4.5, 46% of the TIA was in the isolate but activity in the whey was 450 mg/g of protein. At pH 3.5, 93% of the activity was in the whey but activity was down to 350

Table II. Ultrafiltration of Soybean Whey To Recover Trypsin Inhibitor[a]

sample	trypsin inhibitor act.[b] mg/g sample	mg/g meal	% total
precipitate (crude 7S)	25	4.8	13
whey	170	32	87
retentate (run 1[c])	375	13.4	48
permeate (run 1[c])	320	14.7	52
retentate (run 2[d])	450	20.3	80
permeate (run 2[d])	300	5.1	20

[a] Water extract was prepared from laboratory-prepared soybean flakes as in Figure 1, and 11S globulins were removed at pH 6.4, 4 °C. Whey and crude 7S were separated at pH 5, and the whey was filtered through 100-kDa cutoff membrane. [b] Milligrams of trypsin inhibited. [c] Filtered at pH 7.2, 10 psi N_2, dialyzed 18 h vs. distilled H_2O. [d] Dialyzed and then filtered at pH 7.5, 7 psi N_2.

mg/g of protein as a result of the higher proportion of non-TI protein remaining in the whey. Activity/gram of protein, however, was only 9 mg in the pH 3.5 isolate. Adding 0.1 N NaCl decreased the TIA in the isolate at all pH's investigated as compared to that at pH 4.5 without added NaCl, although at pH 3.5 TIA was similar with or without added NaCl. The highest activity level with added NaCl was 33 mg/g of protein at pH 4.8, and the lowest was 8 mg/g at pH 5.2, which represented only 5% of the TIA in the starting extract. Anderson et al. (1973) showed that 0.1 N NaCl enhances solubility of TI but higher levels of NaCl also increase solubility of other proteins.

Lower TIA's in the isolates were, however, accompanied by reduced yields of protein (Table I). Addition of 0.1 N NaCl lowered the yield of precipitate at all pH's shown. At pH 4.5 with no NaCl added, the maximum yield of 85% of the extract protein was recovered in the isolate, compared to only 59% at pH 5.2 with added NaCl. Without added NaCl the TIA of the pH 5 precipitate was only 36% of that at pH 4.5 with only a 5% decrease in yield of isolate. A comparable result was obtained by precipitation at pH 4.5 with added NaCl. Vaintraub and Bulmaga (1985) recovered 70% of the extract protein with 10% of the starting TI activity by successive precipitation at pH 5.8 and 4.5, followed by washing the final pH 4.5 precipitate seven times with water acidified to pH 4.5. The combined TIA's of whey and isolate in Table I varied from 41 to 54 mg/g of meal, compared to a value of 40 mg/g for the starting meal and 42 mg/g for raw defatted flour (Rackis et al., 1986). The reason for our high values is unknown. The protein yields were based on N × 6.25 and were uncorrected for nonprotein nitrogen, which would be a higher proportion of the wheys than of the isolates.

Ultrafiltration. Since molecular weights of different classes of TI's vary from 7 to 22K, while major storage proteins range from 180 to 350K (Nielsen, 1985), we investigated purification of TI's by ultrafiltration and dialysis. In the first experiment, 11S globulins were removed from a pH 7.5 aqueous extract by precipitating at pH 6.4. Further acidification to pH 5 precipitated crude 7S, which had a TIA of 25 mg/g, representing 13% of the TIA remaining in the extract after removal of the 11S globulins (Table II). The resulting pH 5 whey had an activity of 170 mg/g of dried whey, 4 times that of the starting meal. The whey sample was brought to pH 7.2 before ultrafiltration to maintain protein solubility. In run 1 (Table II), 88% of the whey TIA was recovered after ultrafiltration but 48% of the recovered activity remained in the retentate. Little additional TIA was recovered in the permeate after the first 2 volumes of diafiltrate were collected. When the whey sample was dialyzed and freeze-dried before resuspending in water and filtering (run 2), only 79% of the TIA was recovered and only 20% of that filtered through. However, the level of activity in the retentate was 11 times that of the starting meal due to concentration in the whey and removal of carbohydrates and minerals.

Table III. Ultrafiltration of Soybean Whey with 0.5 N NaCl To Recover Trypsin Inhibitor[a]

sample	trypsin inhibitor act.[b] mg/g sample	mg/g meal	% total	% P
precipitate (isolate)	22	5.6	13	0.36
whey	110	38.4	87	0.87[e]
retentate (run 1[c])	290	23	66	0.06
permeate (run 1[c])	450	11.9	34	3.45
retentate (run 2[d])	330	23.6	70	0.12
permeate A (run 2[d])	280	6.1	18	0.76
permeate B (run 2[d])	350	4.0	12	6.07

[a] Water extracts prepared from laboratory-prepared soybean flakes as in Figure 1. Whey and protein precipitate were separated at pH 5 in the presence of 0.1 N added NaCl, and whey was then diafiltered with 0.5 N NaCl and a 100-kDa cutoff membrane. [b] Milligrams of trypsin inhibited. [c] Filtered at pH 5.1, 15 psi N_2, dialyzed 18 h. [d] Filtered at pH 7.8, 15 psi, dialyzed 18 h. [e] Calculated value.

In a second experiment (Table III), portions of the whey were diafiltered at two pH values with 0.5 N NaCl to aid in solubilizing the protein. In run 1 at pH 5.1, 91% of the TIA from the starting whey was recovered but only 34% of this was in the permeate. TIA in this permeate had a purity of 450 mg/g, the highest level for any permeate in this investigation. In run 2 at pH 7.8, two permeate fractions were collected: Permeate A represented one volume of ultrafiltrate, while permeate B was the result of diafiltration with 1.4 volumes of 0.5 N NaCl. Only 30% of the recovered TIA was found in the two permeates, and activity was only 350 mg/g of dialyzed, freeze-dried sample in the second permeate. At pH 7.8 there was apparently less loss of minerals or other non-TI material during dialysis, resulting in a lower level of TIA. Retention of most TIA in the retentate may be due to aggregation of protein, which prevented TI from passing through the membrane and also resulted in some loss of activity; alternatively, conformational changes of some TI molecules may have prevented permeation through this membrane.

In experiment two we were also interested in the effects of pH and added NaCl on P and phytate levels associated with whey proteins and in the effect of phytate levels on the permeability of the TI's. Analyses of retentates (runs 1 and 2, Table III) showed that the bulk of the P was removed by ultrafiltration and dialysis. Consequently, failure of TIA to permeate completely cannot be attributed to the presence of P in forms such as phytate. Comparison of P levels in the retentates and the permeates in runs 1 and 2 (Table III) shows that although P was readily removed from the retentate at pH 5.1 or 7.8, about 70% passed through the dialysis membrane (6–8-kDa cutoff); however, it was concentrated 50-fold in the permeate over the level remaining in the retentate. Retention of P by the dialysis membrane suggests that there was still some binding to protein or metal ions. Diafiltration with 0.5 N NaCl increased the level of P in permeate B 8 times over that in permeate A. On the basis of a calculated value of 0.9% P in the whey, 32% of the P was recovered from retentate and permeate after overnight dialysis. de Rham and Jost (1979) found that most phytate was removed by ultrafiltration of a 10% NaCl, pH 8, soyflour extract while TI levels remained high.

pH and Mineral Binding. Some effects of pH on interactions of phytic acid and minerals with acid-soluble

Table IV. Effect of Precipitation pH on Mineral Concentrations of Soybean Protein Samples

	preparation pH[b] (% extract[c])									
	isolate			whey			TI concentrate[a]			
component	3.5 (28.5)	4.5 (53.4)	5.2 (54.2)	3.5 (71.5)	4.5 (46.7)	5.2 (45.8)	A: 4.2	B: 4.2	11S: pH 6.4	7S: pH 4.8
protein,[d] %				45.8	23.6	38.4	77.1	86.3		
				Units: Milligrams/Gram[e]						
P	8.4 ± 1.4	6.0 ± 0.5	3.8 ± 0.05	3.4	4.6	7.3	6.0 ± 0.2	3.6 ± 0.05	0.60	6.1
phytic acid	24.6	15.2	7.0	5.5	4.2	15.2	18.4 ± 1.1	9.0 ± 1.0	0.25	5.4
Ca	0.20 ± 0.05	0.21 ± 0.05	0.32 ± 0.07	2.2	2.7	2.2	0.79 ± 0.05	0.56 ± 0.03	0.23	0.28
Mg	0.10 ± 0.03	0.15 ± 0.005	0.44 ± 0.02	3.0	5.7	5.2	1.1 ± 0.09	0.28 ± 0.01	0.10	0.60
K	0.40 ± 0.04	1.1 ± 0.02	0.60 ± 0.07	28	60	50	4.4 ± 0.32	0.12 ± 0.02	0.10	0.21
Na	0.23 ± 0.06	0.15 ± 0.04	0.18	7.8	13.1	11.8	6.4 ± 0.30	1.5 ± 0.10	1.2	5.3
				Units: Milligrams/Kilogram[e]						
Fe	122 ± 22	110 ± 9	115 ± 10	26	36	36	71 ± 4	81 ± 17	33	129
Mn	9 ± 0.4	14 ± 2	6 ± 1	27	44	37	14 ± 2	2.5 ± 0.8	0	6
Cu	25 ± 1.5	18 ± 1.1	17 ± 0.8	8.4	6.8	9.3	16 ± 0.7	24 ± 1.5	7.3	13.2
Zn	4.8 ± 0.5	16 ± 1.0	22 ± 0.9	60	89	73	20 ± 1.4	4.3 ± 0.1	8.4	24

[a] Prepared as in Baker and Rackis (1985). [b] pH of extract for precipitating acid-insoluble proteins or extraction of TI concentrate. [c] Weight distribution of extract between freeze-dried isolate and whey. [d] Percent of protein in sample of whey solids or final TI concentrate. Protein isolates and 7S and 11S fractions assumed to be over 90%. [e] Mean value and deviation where two or more determinations, otherwise single determination.

and -insoluble proteins are indicated in Table IV. Phytic acid, P, and Cu levels are highest in the pH 3.5 isolate while Zn levels are lowest. In the whey, however, P and phytic acid levels were highest at pH 5.2. Most of the minerals were at much higher levels in whey than in isolate except for P, Fe, and Cu and seemed about equally soluble at the three pH's. Levels of Fe and Cu in the whey may reflect binding to the acid-soluble proteins more than solubility. The Zn to Cu ratio was highest in whey at pH 4.5 and lowest in isolate at pH 3.5. At pH 3.5 more protein is in whey than at other pH values. This affects levels of soluble minerals in the freeze-dried whey, but total amounts are similar over the pH range studied.

Evidence for the relative binding of some minerals and phytic acid to acid-soluble proteins is shown by the values in Table IV for TI concentrates A and B, which were obtained by extraction of Nutrisoy 7B flour at pH 4.2 (Baker and Rackis, 1985) in the presence of 0.1 N added NaCl. The conditions were chosen to selectively remove TI's and other acid-soluble proteins along with associated or acid-soluble minerals. These extracts were subjected to ultrafiltration and repeated washing at pH 2.4 and then freeze-dried to produce concentrate A. Concentrate B was subjected to a second ultrafiltration, washing, and freeze-drying sequence. Levels of P, phytic acid, Fe, and Cu were higher in concentrate A than in whey due to the absence of carbohydrates and other minerals. Their levels probably reflect binding to protein rather than solubility at the acid pH. Levels of other minerals are lower in concentrate A than in the whey. Levels of minerals in concentrate B approached those in the pH 3.5 isolate of acid-insoluble proteins. The ratios of phytic acid and mineral levels in A over those in B are in the order K > Mn > Zn > Mg > phytic acid > P > Ca > Fe > Cu. Minerals were extracted nearly quantitatively with 0.5 N HCl for phytic acid assay. In Table IV, we see also that a sample of crude 11S protein, which was precipitated at pH 6.4, washed, centrifuged at 0 °C and then brought to pH 7.8, and dialyzed 4 days, had minimum levels of P, phytic acid, K, Fe, Mn, Cu, and Zn compared to other fractions. This may reflect lower levels of phytic acid and minerals precipitated at pH 6.4 or lower affinity for 11S protein at that pH. Levels of minerals in the 7S protein precipitated at pH 4.8, however, were more comparable to those in the unfractioned isolates precipitated at pH 4.5 or 5.2. The Na levels shown in Table IV reflect mainly NaOH, added to adjust pH, or NaCl, added to increase protein solubility. No corrections were made for added Na. Dialysis of protein isolate for several days against distilled water brings the pH down to ca. 7. Phytic acid and minerals do not dialyze out readily from unfractionated proteins near pH 8 (Honig and Wolf, 1987).

DISCUSSION

The highest yield of protein isolate was obtained by precipitation at pH 4.5 without added NaCl. However, the isolate with the lowest level of TIA was obtained at pH 5.2 in the presence of 0.1 N NaCl. The whey preparation with the highest TI activity was obtained at pH 5 without added salt (maximum solubilization of TI with minimum solubilization of globulins).

Ultrafiltration and dialysis, under conditions tested, did not effectively separate TI's from other proteins, though they removed some nonprotein components. The highest level of TIA in a permeate was obtained with 0.5 N NaCl at pH 5.0, possibly because more non-TI material subsequently dialyzed out at pH 5. P was removed from the retentate in the presence of 0.5 N NaCl, but only about 70% dialyzed out of the permeate overnight, perhaps because much existed as insoluble phytate mineral complexes. Determining optimum conditions for purifying TI's by ultrafiltration and whether TI's failed to permeate due to molecular configuration or aggregation under these conditions requires further investigation.

These results show that variation of precipitation pH and addition of NaCl affect yield and composition of isolated soybean proteins during processing. These variations in processing conditions affect also the levels of phytic acid and available minerals, and thereby the nutritional value, as shown by de Rham and Jost (1979).

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Registry No. NaCl, 7647-14-5; Ca, 7440-70-2; Mg, 7439-95-4; K, 7440-09-7; Na, 7440-23-5; Fe, 7439-89-6; Mn, 7439-96-5; Cu, 7440-50-8; P, 7723-14-0; Zn, 7440-66-6; soybean trypsin inhibitor, 9078-38-0; phytic acid, 83-86-3.

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Structure of Soyasapogenol B_1

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The structure of soyasapogenol B_1, previously shown to be an artifact of hydrolysis, was elucidated by X-ray crystallography and confirmed by mass spectrometry as 3β,22β,24-trihydroxyolean-13(18)-ene.

We recently reported a method for the analysis of soybean sapogenins by normal-phase, high-performance liquid chromatography (HPLC) and evaporative light-scattering detection (Ireland and Dziedzic, 1985). Our work identified a previously unknown sapogenin, which we tentatively named soyasapogenol B_1 due to its mass spectrum and chromatographic behavior similar to that of soyasapogenol B (Ireland, 1987). Later work showed that soyasapogenol B_1, as well as soyasapogenols C–E, are artifacts of the acid hydrolysis of soybean saponins (Ireland and Dziedzic, 1986). This paper deals with the elucidation of the structure of soyasapogenol B_1 by X-ray crystallography and mass spectrometry.

EXPERIMENTAL SECTION

Isolation of Soyasapogenol B_1. Defatted soy flour was extracted with methanol for 30 h in a Soxhlet apparatus. After removal of the methanol under reduced pressure, the extract was hydrolyzed for 5 h by refluxing in a mixture of concentrated hydrochloric acid, water, and ethanol (2:1:3, v/v/v). The cooled hydrolysate was diluted with water, and the liberated sapogenins were extracted with diethyl ether. The ether extract was washed with 2% potassium hydroxide and water. The soyasapogenols were separated by column chromatography using silica gel and hexane/ethyl acetate (4:1, v/v) and subjected to preparative thin-layer chromatography (Ireland and Dziedzic, 1985). The thin-layer chromatography separation was repeated until pure soyasapogenol B_1 was obtained as determined by HPLC (Ireland and Dziedzic, 1985). Soyasapogenol B_1 was crystallized twice from methanol/water.

Mass Spectra. Mass spectra were recorded on a Kratos MS80RFA mass spectrometer with a Data General Desktop 30 and Kratos DS90 data system. An ionization potential of 70 eV, resolution 3000, 100-μA beam current, 3-s/decade scanning rate, and source temperature 250 °C were used.

X-ray Crystallography. Intensity data were collected on a Stoe Stadi 2 diffractometer using a variable-width ω-scan.

RESULTS AND DISCUSSION

Isolation of Soyasapogenol B_1. Hydrochloric acid in water/ethanol was used as the hydrolysis medium as it had

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