Retention of Isoflavones and Saponins During the Processing of Soy Protein Isolates

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ABSTRACT: The mass balance of saponins during processing of soy protein isolates (SPI) was established, and the effects of precipitating and washing (P/W) temperatures (0, 10, 25, 40, and 50°C) on the retention of isoflavones and saponins were investigated in this study. About 41% of total saponins in soy flour (SF) were found to remain in SPI during processing, whereas 42% remained unextracted in the solid waste. None was detected in the whey or wash water. The study also revealed that only about 27% of total isoflavones from SF remained in the final SPI when P/W was performed at 50°C. As much as 40% of the total isoflavones could be retained in SPI when P/W was conducted at 25, 10, or 0°C. When the P/W temperature was 50°C, the percentages of total isoflavones lost during extraction, precipitation, and washing were 28, 22, and 6%, respectively. When the temperature was changed to 0°C, the percentages of isoflavones lost during extraction, precipitation, and washing were 28, 11, and 5%, respectively. The P/W temperatures did not affect the distribution of saponins in different streams during the processing of SPI. Lowering the P/W temperature did not significantly lower the protein content in SPI unless the temperature was reduced to 0°C.

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Soybeans (*Glycine max*) are an excellent protein source for people throughout the world. Dietary soy proteinsalso have been shown to reduce the risk of coronary heart disease (1) and prevent cancer (2,3). Researchers have found that phytochemicals in soybeans, especially isoflavones (4,5) and saponins (6,7), might be contributing to these health benefits. Isoflavones are a class of phenolic compounds having a multiring structure. The three isoflavones (daidzein, glycitein, and genistein) in soybeans and soy products have four forms, the glucoside, the malonyl glucoside, the acetyl glucoside, and the aglucone (8). Saponins are sterol or triterpene glycosides. Soyasaponins are one of the most important dietary sources of saponins. Three groups of soyasaponins have been found in soybeans: groups A, B, and E (9).

The concentration of isoflavones and saponins in soy foods and soy ingredients varies markedly (10–12). First, the genetics of soybean cultivars and the environment where soybeans are grown have significant impacts on the composition and the amount of isoflavones and saponins in soybeans. Second, processing techniques may affect the concentration of isoflavones and saponins. Systematic studies are needed to investigate the effects of processing conditions on the retention of isoflavones and saponins. New processing technology or modifications of existing processes are needed to minimize the loss of isoflavones and saponins during processing.

Soybeans are generally processed into soybean oil products and soy protein products. Soy proteins are the only plant-based proteins with a protein quality equal to that of meat, milk, and eggs. This high-quality protein comes in three major forms in food applications: soy flour (SF), soy protein concentrate (SPC), and soy protein isolates (SPI). SPI are the most highly refined soy protein products, with a protein content of more than 90% (13). SPI can be used in infant formula, nutritional supplements, meat and dairy products, and meat analogs. They are also used in many food systems for such functional properties as emulsifying, foaming, and fat/water holding agents. Soy processing technologies currently applied in industry were developed many decades ago without considering isoflavone, and saponins. Therefore, no effort was made with these technologies to preserve these health-promoting phytochemicals during processing. Wang *et al.* (8) reported that significant changes occurred to isoflavones during processing of SPI and that the precipitating/washing (P/W) step caused the most isoflavone loss. However, no information exists on the changes in saponins during processing of SPI. Nor do we know the effects of processing conditions on the preservation of isoflavones and saponins. Therefore, the objectives of this study were to establish the mass balance of saponins during processing of SPI (P/W temperature was 50°C) and to investigate the effects of P/W temperatures (0, 10, 25, 40, and 50°C) on the retention of isoflavones and saponins.

MATERIALS AND METHODS

Materials. Defatted SF was purchased from the Archer Daniels Midland Company (ADM) (Decatur, IL). Three isoflavone standards (genistein, daidzein, and genistin) were obtained from LC Laboratories (Woburn, MA). Soyasaponin I standard was isolated and purified as described previously (13). Methanol (HPLC grade), sodium hydroxide (certified A.C.S.), Kjel-Sorb (4% boric acid), and sulfuric acid (certified A.C.S.) were purchased from Fisher Scientific (Fair Lawn, NJ). HCl was purchased from VWR Scientific (Cincinnati, OH). KjelTabs were purchased from Thompson & Capper Ltd. (Runcorn, Cheshire, England).

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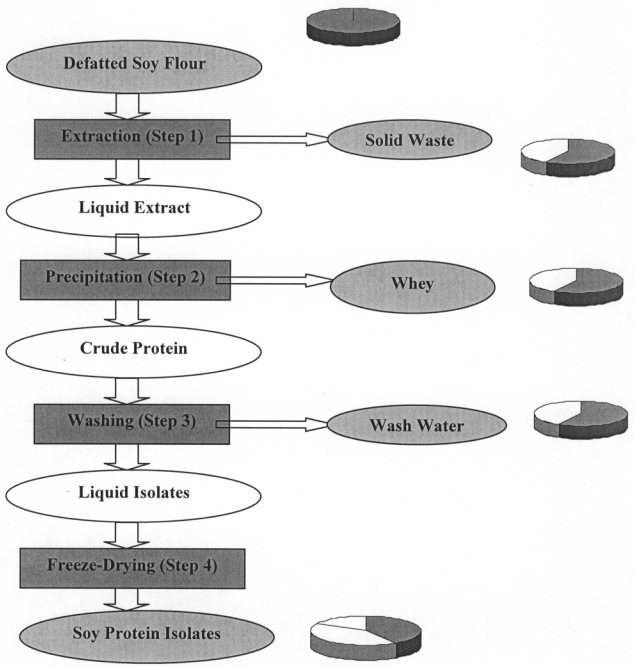
SPI preparation. SPI were prepared from defatted SF in four steps, as shown in Scheme 1: extraction, precipitation, washing, and drying. First, 160 g of SF was extracted with 1440 mL of distilled water at pH 8 (adjusted using 1 N NaOH) for 1 h at 50°C. The slurry was centrifuged for 10 min at 1000 × *g* (RC5C Sorvall Instruments; Dupont Company, Wilmington, DE). Second, the temperature of the supernatant was adjusted to 0, 10, 25, 40, or 50°C, then the pH of the supernatant was adjusted to 4.5 using 0.5 N HCl. The slurry was centrifuged for 10 min at 1000 × *g*, and the precipitates were collected. Third, an equal amount of fresh distilled water, at the same precipitating temperature, was added to the precipitate, and the slurry was centrifuged for 10 min at 1000 × *g*. Finally, the precipitate was collected and freeze-dried. Solid waste (SW), whey (WH), and wash water (WW) were also collected, freeze-dried, and weighed. The experiment was conducted in triplicate.

Isoflavone and saponin extraction. Isoflavone and saponin extractions were conducted as previously described (Lin, J., and C.Y. Wang, unpublished data). A freeze-dried sample (0.2–0.5 g), taken from each processing step, was mixed with 3.0 mL of 80% methanol. The slurry was mixed for 30 min at room temperature using a multitube vortex (VWR Scientific Products, Troemner Inc., Thorofare, NJ). It was then centrifuged for 10 min at 1000 × *g* (Jouan, Winchester, VA). The supernatant was injected into the HPLC system after filtration through a 0.45 mm syringe filter (Titan, Sausalito, CA). Samples were prepared in duplicate.

HPLC analysis. For the quantitative analysis of isoflavones and saponins, an HPLC system consisting of two Waters 510 HPLC pumps (Milford, MA), a Waters 486 UV detector, and an Alltech 2000 ELSD (Deerfield, IL) detector was used. The system was controlled with Waters Millennium 3.2 software.



SCHEME 1

A YMC-pack ODS-AQ S-5 column (5 µm, 120 Å, 250 × 4.6 mm i.d.) protected with an AQ S5 guard column was used (Waters). The HPLC/UV/ELSD instrumentation was operated as previously described (Lin, J., and C.Y. Wang, unpublished data). The concentrations of isoflavones for which we had purified standards (genistin, daidzein, and genistein) were calculated from their corresponding standard curves. The rest of the isoflavones (glycitin, daidzin, malonyl genistin, malonyl glycitin, malonyl daidzin, acetyl genistin, acetyl glycitin, acetyl daidzin, and glycitein) were calculated based on standard curves of the corresponding aglucones. All calculations were conducted on a normalized basis, i.e., all concentrations and mass balances were calculated from the M.W. of the aglucones, without taking the conjugated sugar unit into consideration. In a previous study, Lin and Wang (14) showed that saponins had the same ELSD signal response. Therefore, the concentrations of soyasaponins B were calculated by the standard curve of soyasaponin I.

Mass balance calculation. Isoflavone and saponin concentrations from all processing streams were expressed as µg isoflavone or saponin per g of dry sample. The actual amount of isoflavones or saponins was calculated by multiplying the concentration by the total dry weight of the processing stream collected. Percentages of distribution were also calculated for total isoflavones and saponins.

Soy protein content analysis. Soy protein content of SPI was determined using a Büchi 425 digestor (Laboratoriums-Technik, Flawil, Switzerland) and a Kjeltec (Tecator, Sweden) system 1002 distilling unit according to the AOCS Official Method Ba 4d-90 (15). Protein content was reported as calculated by using the protein/nitrogen ratio of 6.25. The determination was conducted in duplicate.

Statistical analysis. Linear regression was used to obtain standard curves for soyasaponins B [log(peak area Y)] = $a \times$ [log(concentration X) + b] and isoflavones (peak area $Y = a \times$ concentration $X + b$). The data for soy protein and the percentages of distribution for total isoflavones and saponins at different P/W temperatures were statistically analyzed by ANOVA and *t*-test using version 8 of the SAS system (16).

RESULTS AND DISCUSSION

Mass balance of soyasaponins during processing of SPI. Saponin concentrations in SF, SPI, and other processing streams are shown in Table 1. All concentrations are calculated on a dry basis. Eight soyasaponins B were detected and quantified (14): soyasaponin V, I, II, αg, βg, βa, γg, and γa. The total saponin concentration of the SPI was 6177 µg/g, whereas that of SF was 5028 µg/g. The saponin concentration of SW was 6562 µg/g. Saponins were not found in WH and WW. Rickert *et al.* (17) had similar findings. The mass balance of saponins is shown in Scheme 1. About 42% of total saponins in SF remained unextracted in SW. Essentially none was lost during precipitation and washing, and 41% remained in the final product SPI. About 17% of saponins were not accounted for in this mass balance study. The unrecovered saponins were most

TABLE 1
Saponin Concentrations in Processing Streams of SPI with P/W[a,b] at 50°C (μg/g dry weight)

Saponins	SF	SW	SPI
V	50.0	502.4	621.9
I	250.0	2417.2	2315.5
II	93.6	635.1	1244.3
αg	450.2	58.4	37.8
βg	3059.4	2208.0	1479.6
βa	952.9	631.3	360
γg	114.8	74.9	92.0
γa	56.6	34.3	25.4
Total saponins	5027.5	6561.6	6176.5
Weight (g)	160.0	51.6	53.2
Percentage		42.1	40.8

[a]SPI = soy protein isolates; SF = soy flour; SW = solid waste; P/W = precipitating and washing.
[b]No saponins were detected in whey (WH) and wash water (WW).

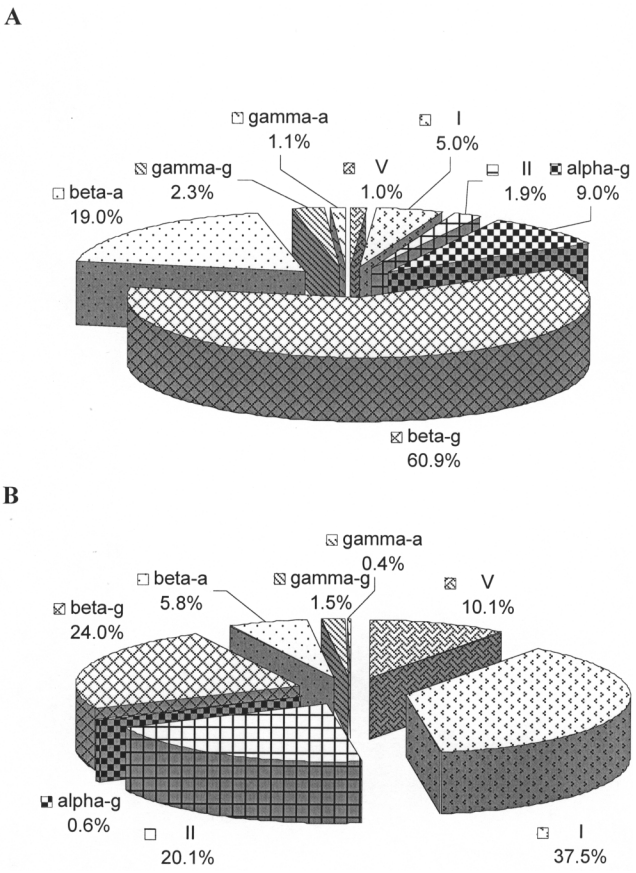


FIG. 1. Comparison of saponin profiles of (A) soy flour and (B) soy protein isolates prepared with precipitating and washing at 50°C.

likely due to the conversion of 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) saponins to non-DDMP saponins. If we assume the conversion is 100%, the amount of saponins would be reduced by about 12% owing to the loss of the DDMP moiety. It was also possible that a minimal amount of microbial degradation occurred during processing. The saponin profiles of SF and SPI are shown in Figure 1, and are dramatically different from each other. The major difference was that the SF contained mainly (92%) DDMP conjugated saponins (soyasaponin αg, βg, βa, γg, and γa) and the SPI contained a high percentage (68%) of non-DDMP saponins (soyasaponin V, I, and II). It was apparent that DDMP saponins were converted to non-DDMP saponins during processing. This result is consistent with the literature that DDMP saponins are thermally unstable. Moderate temperatures would convert αg, βg, βa, γg, and γa into V, I, II, III, and IV, respectively (14). Rickert *et al*. (17) also observed that DDMP-soyasaponins B were easy to convert to soyasaponins B without DDMP when temperature and pH increased.

Effects of P/W temperature on concentrations of isoflavones and saponins. The isoflavone concentrations of SF, SPI, and other processing streams are shown in Table 2. All concentrations are calculated on a dry basis. Twelve isoflavones were detected and quantified (Lin, J., and C.Y. Wang, unpublished data): genistin, glycitin, daidzin, malonyl genistin, malonyl glycitin, malonyl daidzin, acetyl genistin, acetyl glycitin, acetyl daidzin, genistein, glycitein, and daidzein. The total isoflavone contents of the SPI were 1096, 1047, 1504, 1483, and 1571 μg/g when P/W temperatures were 50, 40, 25, 10, and 0°C, respectively. It was apparent that lower P/W temperatures tended to produce SPI with higher isoflavone concentrations. The concentration of isoflavones increased 37% when the P/W temperature was decreased from 50 to 25°C, whereas the concentration of isoflavones increased 43% when the P/W temperature was decreased from 50 to 0°C. It was also found that the concentrations of glucosides, malonyl glucosides, and acetyl glucosides of isoflavones in SPI increased, and the concentrations of the isoflavone aglucones decreased when P/W temperatures decreased from 50 to 0°C. This was probably caused by increased retention of most isoflavones under lower P/W temperatures. However, these low temperatures also produced lower amounts of aglucones. The total isoflavone concentrations of WH ranged from 833 to 1405 μg/g depending on the P/W temperature. Higher P/W temperatures produced WH with higher concentrations of isoflavones. Compared with the total isoflavones in WH, WW had a much higher concentration, ranging from 3800 to 6500 μg/g. As expected, the higher P/W temperatures produced WW with much higher isoflavone concentrations.

Concentrations of genistin, daidzin, glycitin, acetyl genistin, malonyl genistin, malonyl daidzin, and malonyl glycitin in SPI were comparable with those of SF. However, concentrations of daidzein and genistein, the aglucones, were much higher than those of SF. Similar results were also observed by our research group (8), Wang and Murphy (18), and Rickert *et al*. (17).

Table 3 shows the saponin concentrations of processing streams with different P/W temperatures. Overall, the P/W temperature has no impact on the total saponin content in SPI. All SPI processed under different conditions had about 6000 μg/g. However, the temperature did have an impact on the individual saponins. SPI produced with lower P/W temperatures tended to have higher DDMP saponins and lower non-DDMP saponins. This is expected because a higher temperature tends to cause more conversion of DDMP saponins to non-DDMP saponins.

TABLE 2
Isoflavone Concentrations of Processing Streams of SPI Under Different P/W Temperatures[a,b] (μg/g dry weight)

	Glucosides			Malonyglucosides			Acetylglucosides			Aglucone			
	Daid	Gly	Gen	Daid	Gly	Gen	Daid	Gly	Gen	D	Gl	G	Total
SF	140.0	22.1	147.6	281.6	30.6	623.0	14.5	59.6	14.5	14.7	2.6	7.8	1358.6
SW 50°C	99.1^{b}	12.6	144.7	237.2	18.0	434.9	0.9	25.7	4.2	80.0	ND	107.1	1164.4
WH 50°C	92.9^{b}	19.4^{a}	152.5^{a}	425.3^{a}	36.3^{a}	514.8^{a}	4.1^{b}	28.9^{b}	8.0^{a}	69.2^{a}	ND	53.2^{a}	1404.6^{a}
WH 40°C	111.8^{a}	22.1^{a}	114.1^{b}	389.1^{b}	31.4^{b}	501.9^{a}	1.8^{d}	37.7^{a}	4.5^{b}	52.1^{b}	ND	36.8^{b}	1303.3^{b}
WH 25°C	100.6^{a,b}	19.3^{a}	146.1^{a}	376.8^{c}	35.0^{a}	394.2^{b}	3.0^{c}	37.1^{a}	3.4^{b,c}	38.7^{c}	ND	26.1^{c}	1180.3^{c}
WH 10°C	90.1^{b}	19.7^{a}	93.8^{c}	313.2^{d}	22.4^{c}	295.1^{c}	4.8^{a}	17.6^{c}	1.4^{d}	20.3^{d}	ND	12.7^{d}	891.1^{d}
WH 0°C	92.5^{b}	21.9^{a}	86.4^{c}	310.5^{d}	28.8^{b}	244.1^{d}	4.3^{a,b}	13.3^{d}	2.4^{c,d}	18.2^{d}	ND	10.5^{d}	832.9^{d}
WW 50°C	296.5^{e}	61.0^{c}	523.7^{b}	1693.8^{b}	142^{a}	2380.9^{b}	8.0^{b}	140.9^{b}	13.9^{b}	207.6^{a}	ND	128.8^{a}	5597.1^{b}
WW 40°C	476.8^{a}	70.6^{b}	625.7^{a}	2021.1^{a}	149.7^{a}	2642.4^{a}	5.0^{c}	176.7^{a}	19.3^{a}	190.1^{a}	ND	117.2^{a}	6494.6^{a}
WW 25°C	438.7^{b}	88^{a}	515.6^{b}	1602.5^{c}	139.2^{a}	1715.5^{c}	7.3^{b,c}	81.1^{c}	6.9^{c}	136.1^{b}	ND	88.8^{b}	4819.7^{c}
WW 10°C	397.9^{c}	67.2^{b}	436.3^{c}	1341.1^{d}	110.7^{c}	1346.7^{d}	18.9^{a}	78.1^{c,d}	3.6^{d}	92.1^{c}	0.3	58.5^{c}	3951.4^{d}
WW 0°C	356.9^{d}	59.5^{c}	446.2^{c}	1353.0^{d}	126.0^{b}	1226.7^{e}	8.9^{b}	64.1^{d}	2.4^{d}	93.1^{c}	ND	56.7^{c}	3793.5^{d}
SPI 50°C	43.8^{b}	6.0^{c}	97.5^{c}	188.4^{c}	15.9^{c}	346.7^{b}	4.7^{c,b}	27.5^{c}	15.4^{a}	154.3^{a}	5.3^{c}	190.4^{a}	1095.9^{b}
SPI 40°C	49.6^{b}	5.8^{c}	111.0^{c}	182.2^{c}	16.1^{c}	338.0^{b}	4.1^{d}	25.8^{c}	12.0^{c}	133.5^{b}	3.8^{d}	165.4^{a,b}	1047.3^{b}
SPI 25°C	87.5^{a}	12.7^{a,b}	184.4^{a}	288.0^{b}	26.3^{b}	507.7^{a,b}	6.8^{b}	37.7^{a,b}	13.7^{b}	151.0^{a,b}	7.1^{b}	181.3^{a}	1504.2^{a}
SPI 10°C	89.7^{a}	10.5^{b}	160.5^{b}	303.4^{b}	29.8^{b}	486.5^{b}	5.9^{b,c}	34.7^{b}	13.7^{b}	157.3^{a}	5.6^{c}	185.4^{a}	1483.0^{a}
SPI 0°C	97.9^{a}	15.6^{a}	179.0^{a,b}	351.6^{a}	36.9^{a}	535.6^{a}	10.4^{a}	42.0^{a}	13.2^{b,c}	133.1^{b}	8.9^{a}	146.4^{b}	1570.6^{a}

[a]Daid = daidzin; Gen = genistin; Gly = glycitin; D = daidzein; G = genistein; Gl = glycitein; ND = none detected; for other abbreviations see Table 1.
[b]Within a column, means with different superscripts in each section are statistically different ($P < 0.05$); $n = 3$.

Effects of P/W temperature on mass balance of isoflavones and saponins. Table 4 shows the effects of P/W temperature on the mass balance of solids, isoflavones, and saponins among different processing streams. Overall, the P/W temperature does not significantly affect solid distribution. About 27.6%, 11.3–21.8%, 5.1–7.4%, and 26.8–39.5% of total isoflavones ended up in SW, WH, WW, and SPI, respectively. When the precipitating temperature was low, a smaller percentage of total isoflavones was lost in WH, and a higher percentage of the total isoflavones remained in SPI. However, the temperature did not affect the percentage of total isoflavones in WW, except that 40°C yielded a higher percentage. It is not clear why this occurred. Higher washing temperatures were expected to cause higher percentages to be lost in WW owing to higher isoflavone solubility. However, it was possible that at a higher precipitating temperature, more isoflavones had already leached out; therefore, less free isoflavones were available to be lost during washing. About 13.7–20.7% of total isoflavones was not recovered in any fraction; there was no consistent trend in the effect of temperature on these unrecovered percentages. The following could account for the unrecovered isoflavones. First, minor amounts of isoflavones could be lost to the equipment during the experiment. Second, there could be a degradation of isoflavones during processing. However, degradation was more likely to be microbiological than thermal-chemical because a separate experiment showed that isoflavone aglucones were extremely stable to thermal degradation (8). The conversion from malonyl forms to acetyl forms or the conversion from the glucosides to aglucones was not a factor in the unrecovered fraction because normalized calculations were used.

About 42.1% of the total saponins remained unextracted in the SW fraction, and 40.0–42.8% of the total saponins remained in the SPI. No saponins were detected in WH and WW fractions. The P/W temperature did not affect this distribution.

Protein content in SPI. The protein contents of SPI were 91.4, 90.5, 90.5, 90.9, and 85.6% when P/W temperatures were 50, 40, 25, 10, and 0°C, respectively. The P/W temperature did not cause significant differences in protein content when P/W

TABLE 3
Saponin Concentrations in Processing Streams of SPI Under Different P/W Temperatures[a,b,c] (μg/g dry weight)

Saponins	V	I	II	αg	βg	βa	γg	γa	Total
SF	50.0	250.0	93.6	450.2	3059.4	952.9	114.8	56.6	5027.5
SW	502.4	2417.2	635.1	58.4	2208.0	631.3	74.9	34.3	6561.6
SPI 50°C	621.9^{c}	2315.5^{a}	1244.3^{b}	37.8^{e}	1479.6^{b}	360.0^{c}	92.0^{c}	25.4^{c}	6176.5^{a}
SPI 40°C	670.1^{b}	2035.7^{b}	1407.2^{a}	54.0^{d}	1485.6^{b}	363.6^{c}	83.0^{c}	25.4^{c}	6124.6^{a}
SPI 25°C	727.9^{a}	1946.6^{b}	842.8^{c}	75.6^{c}	1656.0^{a,b}	583.2^{b}	144.0^{b}	34.0^{b,c}	6000.1^{a}
SPI 10°C	584.3^{d}	1865.4^{b,c}	842.0^{c}	91.8^{b}	1699.2^{a}	543.6^{b}	153.0^{b}	41.0^{a,b}	5820.3^{a}
SPI 0°C	476.3^{e}	1756.5^{c}	830.4^{c}	102.6^{a}	1818.0^{a}	669.6^{a}	180.0^{a}	44.5^{a}	5877.9^{a}

[a]No saponins were detected in WH and WW.
[b]SF = soy flour; for other abbreviations see Table 1.
[c]Within a column, means with different superscripts in each section are statistically different ($P < 0.05$); $n = 3$.

TABLE 4
Distribution[a,b] of Total Solid, Isoflavones, and Saponins Among Processing Streams at Different P/W Temperatures (%)

P/W temperature (°C)		50	40	25	10	0
Solid	SW	32.3	32.3	32.3	32.3	32.3
	WH	21.1[a]	17.9[b]	18.1[b]	18.1[b]	18.4[b]
	WW	1.4[b]	1.6[a,b]	1.6[a,b]	1.8[a]	1.8[a]
	SPI	33.3[a]	35.1[a]	33.6[a]	35.6[a]	34.2[a]
	Unrecovered	11.9[b]	13.1[a,b]	14.4[a]	12.2[b]	13.3[a,b]
Isoflavones	SW	27.6	27.6	27.6	27.6	27.6
	WH	21.8[a]	17.2[b]	15.7[b]	11.9[c]	11.3[c]
	WW	5.7[b]	7.4[a]	5.8[b]	5.1[b]	5.1[b]
	SPI	26.8[b]	27.1[b]	37.2[a]	38.8[a]	39.5[a]
	Unrecovered	18.1[a,b]	20.7[a]	13.7[c]	16.6[b]	16.5[b]
Saponins	SW	42.1	42.1	42.1	42.1	42.1
	WH	ND	ND	ND	ND	ND
	WW	ND	ND	ND	ND	ND
	SPI	40.9[a]	42.8[a]	40.1[a]	41.1[a]	40.0[a]
	Unrecovered	17.0[a]	15.1[a]	17.8[a]	16.7[a]	17.9[a]

[a]Within a row, numbers with the same letter in their superscripts are not statistically significantly different ($P < 0.05$); $n = 3$.
[b]For abbreviations see Tables 1 and 2.

temperatures were lowered from 50 to 10°C. However, further lowering of the temperature to 0°C caused the protein content to decrease significantly from about 91 to 86%. A lower P/W temperature would be expected to produce SPI with a lower protein content, because less sugar would be washed away. This finding was extremely important because it showed that a temperature range was available for the P/W step at which SPI with high isoflavone and acceptable protein content can be produced.

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