Refunctionalization of Extruded-Expelled Soybean Meals

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ABSTRACT: Soybean meals produced by extruding-expelling (EE) have poor functional properties due to heat denaturation of the proteins, which limits their utilization in foods. Hydrothermal cooking (HTC), a treatment in which steam (150°C) and high shear are applied to a slurry of soybean meal, was used to refunctionalize EE protein meals. Two EE samples with protein dispersibility indexes (PDI) of 35 and 60 were used, along with solvent-extracted white flakes and full-fat whole soy meal as controls. Two HTC methods were explored: One method used a treatment temperature of 154°C and seven different residence times, controlled by varying the holding tube length; the other involved flashing the treated slurry directly into the atmosphere without any back-pressure regulation or holding. Effects of residence time on functional properties of the samples were investigated. The maximum effect of HTC conducted with the use of holding tubes (with-holding-tube HTC) was also compared with that of flash-out HTC. Solid dispersibility, protein dispersibility, and emulsification capacity of both EE meals were significantly improved by both types of HTC treatments. The flash-out HTC showed more benefits than the with-holding-tube HTC in refunctionalizing heat-denatured EE protein. For example, the solid dispersibility, protein dispersibility, and emulsification capacity of EE meal with PDI of 35 were improved 2.0, 4.4, and 2.1 times, respectively, by flash-out HTC treatment. Therefore, the HTC refunctionalization was proved effective in partially restoring the functional properties of the heat-denatured soy proteins.

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KEY WORDS: Dispersibility, emulsification capacity, extruded-expelled soybean meal, foaming property, functional properties, hydrothermal cooking, refunctionalization.

Modern solvent extraction of soybean oil has replaced the traditional mechanical oil extraction, because of its processing scale and efficiency, its high oil recovery, and its yield of fully functional defatted flakes. But recently, interest in mechanical processing has been rekindled, especially after the introduction the extruding-expelling (EE) concept. In 1987, Nelson *et al.* (1) investigated coupling dry extrusion with the mechanical expelling process. When soybeans passed through the extruder, heat was generated, plant tissues and cell structures were disrupted, and oil was released from the seed matrix. The discharged extrudate was conveyed to an expeller where the oil was pressed out. A typical oil recovery of 70% and a press cake with 50% protein, 6% oil, and 90% inactivation of trypsin inhibitors can be obtained from dehulled soybeans (1). Compared with solvent extraction, EE has several advantages, such as low capital cost, relatively simple machinery, no solvent use, and a small production scale. It is ideal for identity-preserved (IP) processing.

With high stability, low phospholipid and FFA contents, and a pleasant nutty flavor, EE oil may be consumed after minimal refining or even without refining. Studies by Wang and Johnson (2,3) showed that with minimal refining, a unique oil product can be obtained. Utilization of EE proteins as a food ingredient has also been studied (4,5). Traditionally, EE meals are used as livestock feed (6,7) because their poor functional properties, caused by protein heat-denaturation, limit food use. If the heat-denatured protein can be refunctionalized, production of various new protein products will be possible and more benefits can be realized by EE technology.

A promising method of protein refunctionalization is hydrothermal cooking (HTC), which involves a system commonly referred to as a jet-cooker, where high-temperature steam and a protein slurry are infused into a holding tube through a restriction orifice. Johnson *et al.* (8) showed that for soymilk preparation, HTC treatment increased soybean protein recovery from the conventional 70% to about 90%. Wang and Johnson (9) employed HTC to refunctionalize ethanol-denatured soy protein concentrate, and their results showed significant increases in the major functional properties, including dispersibility, emulsification, and foaming. But there has been little effort in refunctionalization of heat-denatured proteins, especially those from the EE process. We hypothesize that HTC can improve the functional properties of heat-denatured EE protein as well. The objective of this study was to examine the effect of HTC on the functional properties of EE protein meals having different degrees of heat denaturation.

EXPERIMENTAL PROCEDURES

Soybean meal samples. Two EE flours (EE35 and EE60) were made from cracked and dehulled Stine soybeans, and their protein denaturation and oil content were measured as a protein dispersibility index (PDI) of 35.3 and 62.0 and oil content of 7.6 and 13.6%, respectively. The EE system consisted of an Insta-Pro International extruder Model 2500 and screw press Model 1500. The following extrusion parameters were used to produce the extruded protein meals: 11-11-6-6 shear lock configuration, double flight screws, and a restriction die setting at 3/8 in. (0.94 cm). The temperature in the last segment of the extruder barrel was 132–143°C, and the total residence time was about 20–25 s. One example of solvent-defatted white flakes (ADM Nutrisoy® defatted flakes, PDI

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of 90; ADM, Decatur, IL) and an Iowa-produced Stine soybean were selected as controls. All samples were ground by FITZ®MILL (Model DAS06; The Fitzpatrick Company, Elmhurst, IL) with a 40-mesh screen. To avoid any further protein denaturation, caution was taken to prevent any overheating of the mill. All samples were made into a 20% slurry stirred with a Biomixer™ handheld mixer (ESGE Ltd., Mettlen, Switzerland), then pumped through a Stephan mill (Type MC15; A. Stephan u. Söhne GmbH & Co., Hameln, Germany) to achieve thorough dispersion before HTC treatment.

HTC (jet-cooker) treatment. A Moyno pump (2MI type SSQ; Robin and Myers, Inc., Springfield, OH) was connected with a hydroheater (size 300, type B; Hydrothermal Co., Milwaukee, WI) where culinary-grade steam (~90 psi pressure, 6.5 kg/cm^2) was infused to give the heat and shear treatment (Fig. 1). Two types of HTC treatments were used. One used holding tubes (and was termed "with-holding-tube HTC") and involved combinations of holding tubes (2.54 cm i.d. and 2.66 cm o.d.) of various lengths that gave seven holding times for each sample. All the holding tubes were insulated. The lengths of the seven holding tubes were: 2.58, 4.48, 6.31, 8.22, 10.66, 13.17, and 16.28 m. Owing to viscosity differences among the samples, the residence times of different samples were different even when using the same tube length. The residence time was measured by injecting Brilliant Blue R-250 into the slurry that was about to go into the stabilized or equilibrated HTC system and observing the time needed for the colored material to emerge from the outlet. A back-pressure valve, after which the sample was released into the flash tank, was used to adjust the temperature. Cooking temperature was maintained at 154 ± 0.6°C and monitored with a thermocouple. The second type of HTC treatment was flashing-out HTC. After the slurry had been infused with steam in the hydroheater, the product was discharged directly into the atmosphere without any holding tube or back-pressure control. There was still a short, open tube from the hydroheater to outlet, but the temperature was about 104°C, much lower than 154°C.

The feeding speed for all samples was maintained at a constant rate, 1.5 kg/min, as standardized with water. The released slurry was cooled to below 40°C in a jacketed ice bath. Treated samples were stored in a cold room at 5°C for further analyses.

Characterization of functional properties. All concentrations used were on a dry-matter basis (measured after drying at 130°C for 3 h). Protein content was measured by Kjeldahl method (with a conversion factor of 6.25). Solid dispersibility and protein dispersibility were measures of the total dry matter and protein matter, respectively, in the supernatant of a 10% suspension after centrifugation at 1050 × *g* and 5°C for 5 min, which was modified from a method of Johnson (10). Protein dispersibility as measured in this study is different from the traditional measurement of PDI, for which a set of standard conditions, such as 7% slurry concentration, blending at 8500 rpm for 10 min, and centrifugation at 1400 × *g* for 10 min, has to be followed. Emulsification capacity was measured based on a method of Swift *et al.* (11). Twenty-five milliliters of a 2% dispersion was put into a 400-mL plastic beaker, and a handheld Biomixer™ (BioSpec Products, Bartlesville, OK) was used at high speed (1,200 rpm) to emulsify the protein suspension with a commercially refined soybean oil (Bakers & Chefs™ vegetable oil; North Arkansas Wholesale Co., Bentonville, AR), which was introduced at a rate of about 0.5 g/s. Emulsification capacity was determined as the amount of oil that had been used when an inversion was observed. The inversion point was visually determined as the breakdown of the emulsion, i.e., the creamlike emulsion suddenly broke into separate oil and water phases and viscosity dropped suddenly. A fat-soluble dye, Red Fat 7B, was added at about 4 ppm to the oil to make the end point easy to observe.

To quantify foaming properties, a foaming device, made by fusing a fritted ceramic disk to a graduated glass column, was used (12). Nitrogen gas was purged at 16.7 mL/s to produce 300 mL of foam from 100 mL of 1% sample suspension. Three measurements were made: time of foaming (t_f, in s), volume of sample suspension consumed at the end of foaming (V_{max}, in mL), and time used for half of the incorporated suspension to drain back ($t_{1/2}$, in s). From these measurements, three foaming parameters were calculated: foaming capacity (FC) = $300/(16.7 \cdot t_f)$, which was the number of milliliters of foam formed per milliliter of N_2 purged; K value = $1/(V_{max} \cdot t_{1/2})$, in units of 1/(mL·s), which was used to describe foam stability (a higher value indicates lower stability); and $V_i = V_{max}/t_f$, in units of mL/s, which was the foaming speed, i.e., the speed of liquid being incorporated into foams.

Experimental design and data analysis. HTC treatments were conducted in duplicate. All functional property measurements were in triplicate except for Kjeldahl, which was done in duplicate. Statistical analysis was performed using the General Linear Model procedures of SAS 8.02 (13). For all the functional properties of each sample, the differences among the seven residence times were examined. Comparisons were also made among samples of control, maximum value of the seven with-holding-tube HTC, and flashing-out HTC treatments.

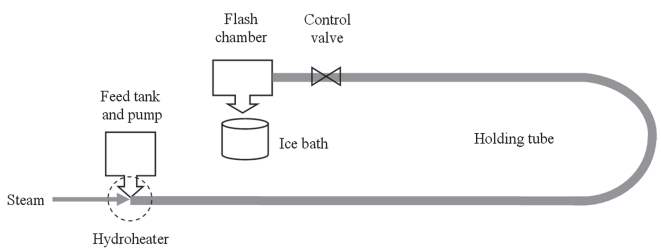

FIG. 1. Hydrothermal cooking system (jet-cooker).

RESULTS AND DISCUSSION

Effect of HTC holding time on EE meal refunctionalization. HTC significantly improved the solid dispersibility of EE35 and whole soy meal (with short residence time), but it reduced that of white flakes. No statistically significant effect was

found on EE60 (Fig. 2A). The LSD values used to compare these differences are presented in Table 1. The two EE meals followed a similar trend: They reached the treatment maximum and then remained relatively unchanged despite the increase in residence time. Whole soy meal achieved the maximum solid dispersibility, from 53 to 70%, at a residence time of 33 s, which then decreased. This result agreed with that of Johnson *et al.* (8), in which solid dispersibility increased from 65 to 86% at 34 s. The different maximal values may be due to different shear forces between two systems. After 33 s the solid dispersibility of the whole soy meal decreased steadily until it became stable at 50% after 60 s. EE35 achieved the highest value of 52% at 42 s; after that, the values remained at nearly 50% except at 100 s (unexplainable drop). The solid dispersibility of white flakes decreased from 61 to 40% at about 16 s, after which it increased to 51% and then decreased to about 40%, which indicates protein denaturation by HTC treatment. All data show that the two EE meals and the whole soy meal benefited from HTC, whereas the white flakes were damaged by such treatment.

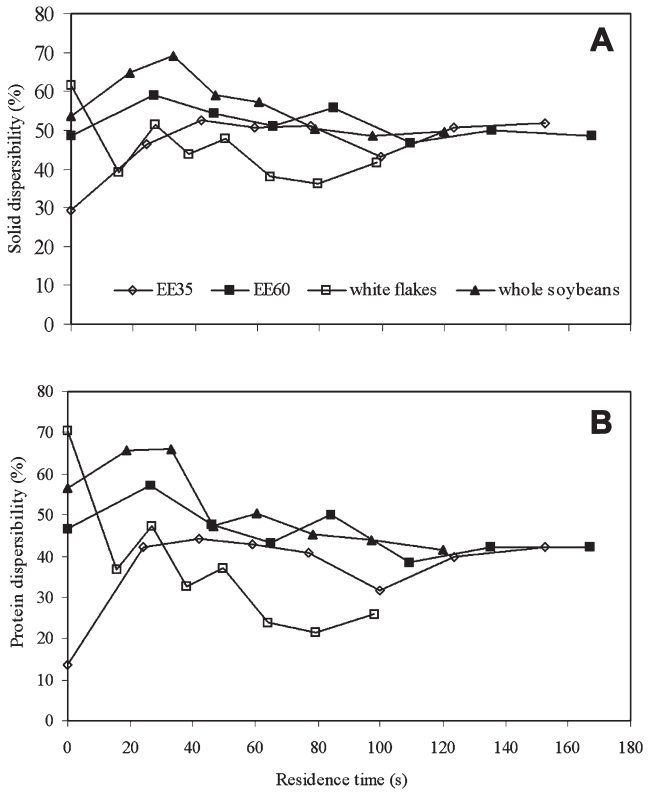

FIG. 2. Effect of residence time during hydrothermal cooking on (A) solid dispersibility and (B) protein dispersibility of various soy protein samples.

TABLE 1
$LSD_{0.05}$ Values for the Functional Properties of Soy Protein Meals Subjected to Hydrothermal Cooking Treatments[a]

	EE35	EE60	White flakes	Whole soybeans
Solid dispersibility	2.50*	6.74	7.46*	7.99*
Protein dispersibility	3.30*	9.20	11.02*	15.96*
Emulsification capacity	6.65	6.77*	16.58*	4.73*
Foaming speed	0.051*	0.054*	0.106*	0.381
Foaming capacity	0.048*	0.039*	0.043*	0.206
Foaming *K* value	0.0002	0.0002	4.7E-5*	0.0003

[a] *,LSD values a with significant treatment (residence time) effect ($P < 0.05$). Units: solid and protein dispersibility, %; emulsification capacity, g soybean oil/0.5 g sample; foaming speed, mL/s; foaming capacity, mL/mL; *K*, 1/(mL·s).

Protein dispersibility followed a trend similar to that of solid dispersibility, but the changes with the increase of residence time were more notable (Fig. 2B) than that of solid dispersibility. EE60 performed more like whole soy meal than EE35. The increases for EE60 and whole soy meal were from 47 to 57% and 57 to 66%, respectively. The maximum protein dispersibility for EE 35 was 43%, an increase of more than three times from the untreated value of 13%. After HTC, the protein dispersibility of white flakes was reduced from 70 to 36%, after which it was increased to 47% then decreased to about 23% with an increase of residence time. It is interesting to observe that after a residence time of 120 s, EE 35, EE60, and whole soy meals had almost the same protein dispersibility of 42%, although the protein denaturation in the starting materials was different (their PDI were 35, 62, and 90, respectively). The white flakes from the solvent extraction process had less than 1% of oil, whereas whole soy meal was full-fat. Their different performance during HTC treatment suggests that the oil in the samples may have played protective roles in preventing soybean protein from forming big, nondispersible aggregates, or in other words, oil on the surface of the particles might have helped smaller aggregates or protein molecules (though mostly denatured) stay dispersible. EE60 had an oil content of about 12% and relatively high PDI, so it is understandable that it showed a response similar to that of whole soy meal during HTC treatment. After HTC, EE35 had a greater quality improvement than EE60. In the EE process, soybean protein experienced high shear, pressure, and heat, and more and larger denatured protein aggregates were probably formed in EE35 than in EE60. During HTC treatment, the soybean slurry was subjected to several forces and conditions: One was the shear force, generated mainly at the point of steam infusion; another was the high-temperature treatment (154°C). Supposedly, shear forces act to break down the large aggregates into smaller ones, whereas high temperature facilitates the aggregation of native proteins. The outcomes of HTC are believed to be the mixed actions of all these major forces.

The emulsification capacities (EC) of untreated materials were very different: Samples having the most native proteins had much higher values (128 and 148 g oil/0.5 g sample for whole soy meal and white flakes, respectively), whereas values for the two EE meals were below 60 g oil/0.5 g sample (Fig. 3). After HTC, both EE meals quickly gained EC to about 100 g oil/0.5 g sample at the shortest residence times of 24 and 26 s, respectively. They remained almost the same even with a longer residence time. EC values of whole soy meal and white flakes dropped abruptly to 90 and 100, respectively. There were some similarities between EC and protein

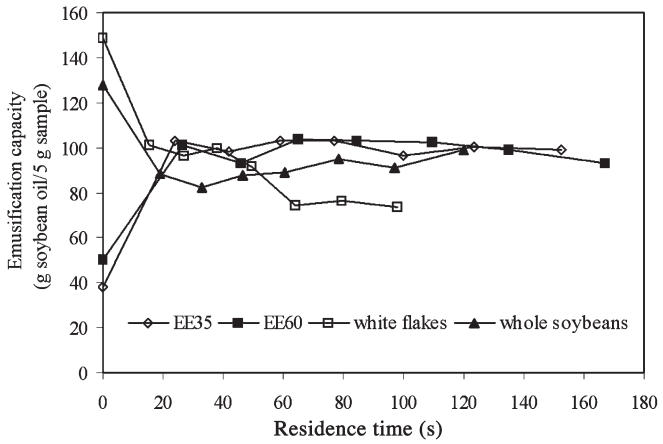

FIG. 3. Effect of residence time during hydrothermal cooking on the emulsification capacity of various soy protein samples.

dispersibility, e.g., for untreated samples, high solid or protein dispersibilities always corresponded to high EC. But the EC curves of HTC-treated samples were quite smooth, with no apparent peaks and fluctuations as shown in protein dispersibilities. This might be because that EC test itself was not as sensitive to aggregate size as protein dispersibility was.

HTC did not significantly affect the foaming capacity of EE60, white flakes, and whole soy meal, but it significantly increased that of EE35 (Fig. 4). HTC improved the foaming speed of EE35 white flakes and whole soy meal. For EE35, the foaming speed increased steadily as residence time increased. For EE60, the value decreased at the beginning but increased slowly with the increasing residence time. Whole soy meal and white flakes showed considerably different curves, and in general, their values slightly increased with time. The two EE samples had very similar performances with longer residence times. *K* value is an indicator of foaming stability, and the lower the value, the higher the stability of the foam. HTC lowered the *K* value of EE35, which indicates significant improvement in its foaming stability. For the other three samples, the values fluctuated, but the overall stability did not change considerably during the various lengths of HTC treatments. In general, HTC improved the foaming properties of EE35, but its effects on EE60, white flakes, and whole soy meal were not significant.

Effect of flashing-out HTC treatment on EE meal refunctionalization. When testing the effect of residence time, we observed that most of the functional property (such as dispersibility and EC) improvements happened at short residence times (Figs. 2–4). Longer residence times had limited (if any) effects on further refunctionalization of EE meals. Therefore, a flash-out HTC experiment was carried out in which all the conditions were the same as with-holding-tube HTC except the treated slurry was flashed out without a valve to maintain temperature at 154°C. Because there was a short tube from the point of steam infusion to flash outlet, a 6-s "residence time" was actually applied. Temperature at the point of infusion was about 160°C, and at the outlet, about 104°C.

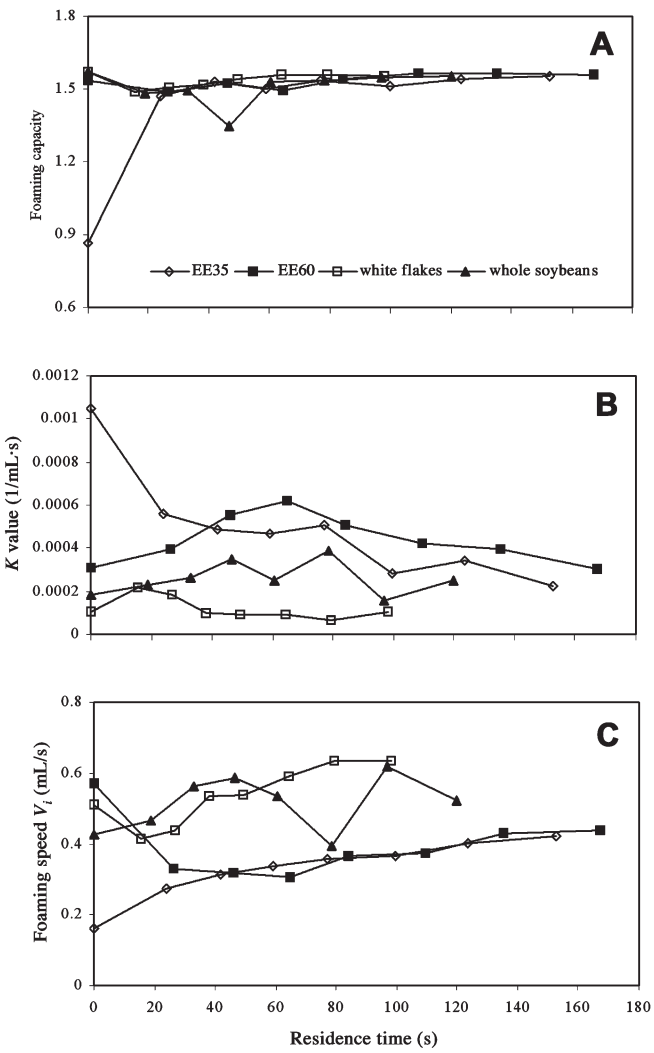

FIG. 4. Effect of residence time during hydrothermal cooking on foaming properties of various protein samples. (A) Foaming capacity; (B) *K* value, or foam stability; (C) foaming speed.

For comparison, the maxima of all quality measurements were selected for each sample from with-holding-tube HTC experiment. Since not all the maxima were achieved at the same residence time (or at the same tube length), these data represent the actual achievable maxima of with-holding-tube HTC. Untreated samples were also included in the comparison.

Flashing-out HTC resulted in a twofold increase in solid dispersibility of EE35, which was also significantly higher than the maximal value in the with-holding-tube HTC treatment (58.4 vs. 52.3%) (Table 2). It also improved the solid dispersibility of whole soy meal, and the increase was the same as the maximal value of with-holding-tube HTC treatment. There was no treatment difference for EE60. For white flakes, flashing-out HTC did not change its solid dispersibility, but with-holding-tube HTC reduced it from 62 to 51%. Similar to solid dispersibility, flashing-out HTC increased protein dispersibility more than the with-holding-tube HTC treatment, except for EE60. For EE35, flashing-out HTC

TABLE 2
Comparison of Solid Dispersibility and Protein Dispersibility Between Flashing-Out Hydrothermal Cooking (HTC) and With-Holding-Tube HTC Treatment[a]

	EE35	EE60	White flakes	Whole soybean meal
Solid dispersibility, %				
Untreated	29.4[c]	48.7[a]	61.6[a]	53.6[b]
Flashing-out HTC	58.4[a]	61.0[a]	62.5[a]	69.8[a]
With-holding-tube HTC	52.3[b]	59.2[a]	51.3[b]	69.3[a]
Protein dispersibility, %				
Untreated	13.5[c]	46.5[a]	70.4[a]	56.5[c]
Flashing-out HTC	58.6[a]	64.2[a]	74.4[a]	78.7[a]
With-holding-tube HTC	44.3[b]	50.0[a]	47.3[b]	66.2[b]

[a]The with-holding-tube HTC treatment values are the highest values obtained with various residence times; means with same superscript roman letters are not significantly different ($P = 0.05$) within the same column and under the same quality parameter.

increased the protein dispersibility by more than four times, compared to two times for solid dispersibility. A similar trend was observed for whole soy meal and white flakes. It indicates that the increase in protein dispersibility was directly related to the improvement in solid dispersibility. Flashing-out HTC resulted in solid and protein dispersibilities that are as good as or better than those of with-holding-tube HTC. This may be explained by less heat treatment in flashing-out HTC (shorter time and lower temperature after steam infusion), resulting in less denaturation or production of smaller protein aggregates.

As with-holding-tube HTC, flashing-out HTC significantly improved the EC of EE35 and EE60 but reduced that of white flakes and whole soybean meal (Table 3). Flashing-out HTC improved EC to a lesser degree than with-holding-tube HTC did for the two EE samples. And flashing-out HTC reduced EC to a greater degree than with-holding-tube HTC did for the whole soybean meal and white flakes. For soy proteins, EC is believed to relate to amphiphilicity, which is the overall ability to interact with both polar and nonpolar molecules to produce a stable emulsion. More heat treatment (higher temperature or longer time) changed soybean protein surface properties in such a way that more amphiphilic ability was produced in with-holding-tube HTC than in flashing-out HTC. But when considering that not all the functional property maxima could be achieved under the same condition in with-holding-tube HTC, and that the operation was not as simple as flashing-out HTC, the flashing-out HTC should be considered as a very useful HTC method to refunctionalize EE soybean meals.

TABLE 3
Comparison of Emulsification Capacity and Foaming *K* Value Between Flashing-Out HTC and With-Holding-Tube HTC Treatment[a]

	EE35	EE60	White flakes	Whole soybean meal
Emulsification capacity				
Untreated	38.1[c]	50.3[c]	148.3[a]	127.8[a]
Flashing-out HTC	80.0[b]	91.8[b]	96.3[b]	51.9[c]
With-holding-tube HTC	103.2[a]	104.1[a]	101.4[b]	99.1[b]
Foaming *K* value				
Untreated	0.0011[a]	0.0003[a]	0.0001[a]	0.0002[b]
Flashing-out HTC	0.0004[b]	0.0003[a]	0.0001[a]	0.0004[a]
With-holding-tube HTC	0.0002[c]	0.0003[a]	0.0001[a]	0.0002[b]

[a]The with-holding-tube HTC treatment values are the highest values obtained with various residence times; means with same superscript roman letters are not significantly different ($P = 0.05$) within the same column and under the same quality parameter. Emulsification capacity is expressed as g soybean oil/0.5 g sample; foaming *K* value is in 1/(mL·s). For abbreviation see Table 2.

Flashing-out HTC increased the foam stability of EE35, but its stability was lower than the maximum in with-holding-tube HTC. For EE60, there was no significant difference among the treatments, and no significant difference was observed between the two HTC treatments for white flakes (Table 3). Flashing-out HTC significantly reduced the foaming stability of whole soy meal. No significant difference was observed between the stability of untreated whole soy meal and that of maximal value of with-holding-tube HTC. Foaming capacity and foaming speed showed similar mixed results (data not shown).

This study demonstrated that HTC can effectively improve most functional properties of heat-denatured soy protein. Most of the maximal treatment effects were achieved in short residence times at temperatures under 154°C. Longer residence times usually decreased the functional properties. The results suggested that solid and protein dispersibilities were improved much more in flashing-out HTC than in with-holding-tube HTC. Emulsification capacities of the two EE samples were also increased by flashing-out HTC treatment, but the values were lower than the maximal increases achieved by with-holding-tube HTC. Foaming properties were somewhat mixed by the two HTC treatments. Flashing-out HTC showed a certain promise, not only for the effective refunctionalization but also for the ease of operation. For both HTC treatments, the more heat-denatured the EE protein is, the more refunctionalization can be achieved. This observation suggests that oil recovery in the EE process need not be sacrificed if HTC treatment is to be used for protein refunctionalization. Since EE meals with PDI of 30 are typical EE processing products, they can be readily hydrothermally cooked to produce highly functional value-added food ingredients. This will further enhance the value and application of EE processing.

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