Effect of Alkali on the Refunctionalization of Soy Protein by Hydrothermal Cooking

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ABSTRACT: The effects of hydrothermal cooking (HTC) at alkaline conditions on refunctionalization of heat-denatured protein of extruded-expelled (EE) soy meals and on preparation of soy protein isolate (SPI) from EE soy meal were determined. Two HTC setups, flashing-out HTC (without holding period) and HTC with holding for 42 s at 154°C, were evaluated. Alkali (NaOH) addition dramatically enhanced the refunctionalization of EE meal having an initial protein dispersibility index of 35. The more alkali added, the more refunctionalization occurred. Extensive refunctionalization was achieved at 0.6 mmol alkali/g EE meal, and additional improvement was small with more alkali. For both HTC setups, the solids and protein yields of SPI from alkali-HTC-treated EE meals were significantly higher than those from HTC without alkali addition. The yield of protein as SPI increased from 40 to 82% after HTC treatment at 0.6 mmol alkali/g EE meal compared with no alkali addition. The emulsification capacities of SPI after alkali-HTC were similar to those from HTC without alkali. SPI from holding-tube HTC-treated EE meals had higher emulsification capacities than those prepared by flashing-out HTC.

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KEY WORDS: Alkali hydrothermal cooking, emulsification capacity, extruded-expelled soybean meal, foaming properties, functional properties, protein, protein dispersibility, refunctionalization, soy protein, soybean meal.

The use of soy protein ingredients by the food industry is increasing rapidly owing to health benefits, low cost, and versatile functionalities. Most soy protein products are manufactured from highly soluble (flash-desolventized) hexane-defatted soybean meal. Solvent extraction is capital intensive and unsuitable for processing small amounts of identity-preserved soybeans. Extrusion-expelling (EE) is a promising technique that presses the oil from the whole or cracked seeds and is suitable for processing small lots of identity-preserved seeds (1,2). EE differs from traditional screw pressing in that a dry extruder replaces steam-heated stack cookers or rotary dryers. Compared with solvent-extracted white flakes, EE protein meals that are processed into EE soy flour have much more heat exposure and protein denaturation [15–60 vs. 80–90 protein dispersibility index (PDI)], contain significantly more residual oil (6–12 vs. ~1%), have lower levels of heat-sensitive antinutritional factors (trypsin inhibitors) and enzymes (such as lipoxygenase), and possess a pleasant nutty flavor. Typical EE meal contains 50% protein and 6% oil, and 90% of its trypsin inhibitors are inactivated (1). The use of protein ingredients prepared from EE meal in foods, however, is limited by their poor functional properties and low yields of soy protein isolate (SPI), a direct consequence of heat denaturation of the protein.

Our previous work showed that SPI could be prepared from EE meals; however, the yields were low (the SPI and protein yields were only 25 and 40%, respectively) (6). Inspired by the work of Johnson (3) and Wang and Johnson (4), we demonstrated that hydrothermal cooking (HTC) could be used to refunctionalize heat-denatured proteins of EE meals (5). We also demonstrated the feasibility of using HTC as a pretreatment in extracting soy protein products, such as soy protein concentrate (SPC) and isolate (SPI), from EE meals; the yields of SPI and protein were 36 and 53%, respectively.

We have observed that the viscosity of a slurry of alcohol-washed SPC, which contains highly denatured protein as a consequence of exposure to alcohol, dramatically increased with mild heating and alkali addition. We also observed similar behavior with EE meals. We believe that the combination of alkali, high shear, and high temperature achieved during HTC can be used to refunctionalize EE soybean meal, increase yields, and improve functional properties of SPI prepared from EE meal.

EXPERIMENTAL PROCEDURES

EE meal and alkali treatments. Typical EE meal, EE35 (with PDI of 35), was prepared from cracked and dehulled commodity soybeans (Stine Seed Co., Adel, IA) by using processing conditions previously described (6). A slurry containing 20% meal solids was prepared by mixing with a Biomixer™ hand-held mixer (ESGE Ltd., Mettlen, Switzerland). Alkali (2 N NaOH) was added at 0.2, 0.4, 0.6, and 0.8 mmol/g EE meal (dry-weight basis) levels immediately before being pumped to a Stephan mill (Type MC15; A Stephan u. Söhne GmbH & Co., Hameln, Germany) for grinding and mixing.

HTC setup and treatment conditions. A Moyno pump (2MI type SSQ; Robin and Myers, Inc., Springfield, OH) was connected to a steam infusion hydroheater (size 300 type B; Hydrothermal Co., Milwaukee, WI) where culinary-grade steam (~90 psi, 6.5 kg/cm^2) was infused into the protein slurry to achieve instantaneous heating and high shear. The slurry feed rate was maintained at 1.5 kg/min for all treatments. Two HTC

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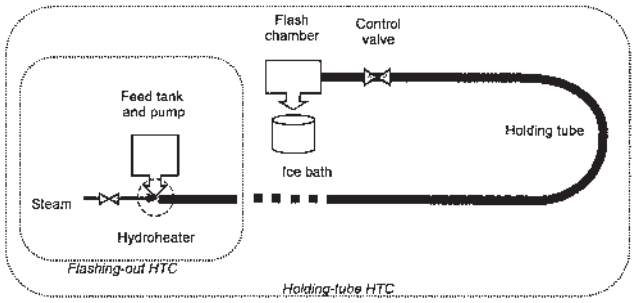

FIG. 1. Schematic diagram of the two hydrothermal cooking (HTC) setups (flashing-out and holding-tube HTC).

setups were used (Fig. 1). For one HTC treatment, the heated slurry was merely flashed out and was designated as flashing-out HTC. In this case, the cooked protein slurry was discharged directly into a flash chamber at atmospheric pressure without any holding tube or backpressure after the steam infusion. The slurry temperature was about 104°C for 2 s. For a more severe heat treatment, we placed a holding tube after the hydroheater. The holding tube was 4.48 m long (2.54 cm i.d. and 2.66 cm o.d.), and provided a 42-s residence time (5). A backpressure valve, after which the sample passed to the flash tank, was used to control the steam pressure and thus temperature. The cooking temperature was maintained at 154 ± 1°C and monitored by using thermocouples and a data logger. This treatment was designated as holding-tube HTC. These conditions were selected based on our previous work (5). The cooked slurry exiting the flash chamber was immediately cooled to <40°C by discharging into a stainless-steel beaker in an ice bath. All treated samples were refrigerated at 5°C until being analyzed.

Only flashing-out HTC was used to evaluate the effects of alkali addition on the refunctionalization of EE meals because of the availability of data on flashing-out refunctionalization of EE meals in our previous work for comparison (5). The alkali-treated samples were neutralized to pH 7 before determining functionality. Both flashing-out and holding-tube HTC with 0.2 and 0.6 mmol alkali/g meal were used to produce refunctionalized materials for SPI preparation.

SPI preparation. SPI was prepared using methods described by Lusas and Rhee (7) with minor modifications (Fig. 2). Protein extraction was carried out at the pH obtained after HTC with 0.0, 0.2 and 0.6 mmol alkali/g meal addition, diluting to a 1:10 solids/water ratio and stirring for 30 min at 60°C as in our previous work (6). The pH values of the protein slurries under various conditions are shown in Table 1.

Characterization of functional properties. All compositional data are reported on dry-weight basis. Moisture was determined by using oven-drying at 130°C for 3 h. Protein content was measured by using AOAC method 993.13 (8) with a Rapid N III analyzer (Elementar Analysesysteme GmbH, Germany) and 6.25 as the N conversion factor. Solids and protein dispersibilities were the dry matter and protein, respectively, in the supernatant relative to the total dry and protein matter after centrifuging a 10% suspension at 1050 × *g* at 5°C for 5 min (3).

Emulsification capacity was measured by a method based

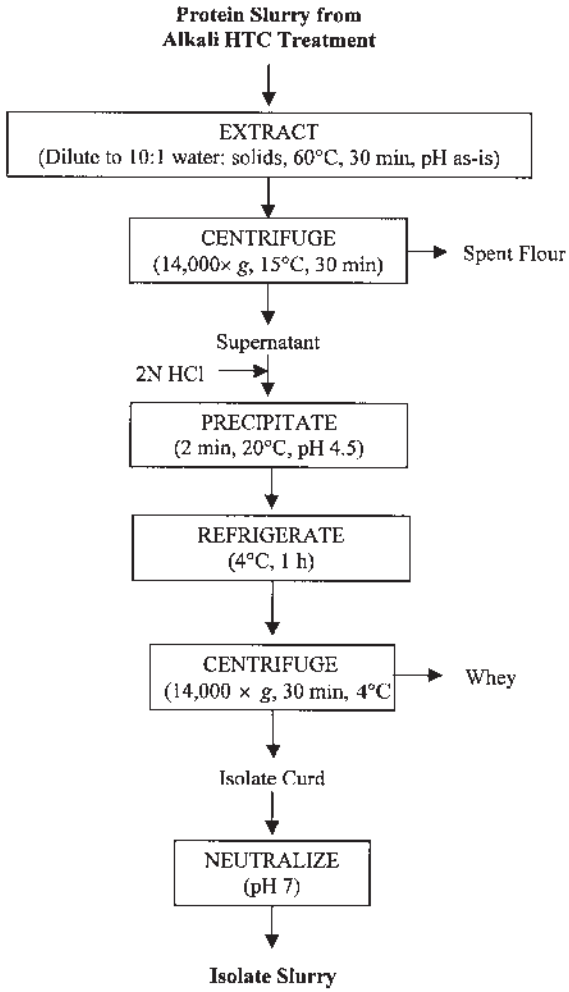

FIG. 2. Procedure for producing soy protein isolate (SPI) from alkali-HTC-treated extruded-expelled (EE) soy meal. For other abbreviation see Figure 1.

on Swift *et al.* (9) where 25 mL of a 2% solids dispersion was placed into a 400-mL plastic beaker, and a hand-held Biomixer™ was used at high speed (1,200 rpm) to emulsify the protein suspension with a commercially refined soybean oil, which was introduced at a rate of about 0.5 g/s. Emulsification capacity was the amount of oil that caused phase inversion. The inversion point was detected by observing a sudden separation of oil and water phases, an abrupt decline in viscosity, and a change in pitch of the noise from the mixer. A fat-soluble dye, Red Fat 7B, was added to the oil at about 4 ppm to make the end point more easily observed.

To quantify foaming properties, a foaming column made by fusing a fritted ceramic disk into a graduated glass column was used (10). Nitrogen gas was purged at 16.7 mL/s to produce 300 mL of foam from 100 mL of 1% solids sample suspension. Three measurements were recorded: time to produce 300 mL of foam (t_f, in s), volume of sample suspension incorporated into foam at the end of the foaming period ($V_{max,}$ in mL), and time used for one-half of the foamed suspension to drain back ($t_{1/2}$, in s). From these measurements, three foaming parameters were calculated:

TABLE 1
pH of Protein Slurries (9.1% solids) at Different Alkali Concentrations Before and After Hydrothermal Treatment Cooking (HTC)

	Alkali concentration (mmol/g meal)		
Location of alkali addition	0	0.2	0.6
Before HTC, 25°C	6.7	9.4	11.4
Before HTC, 60°C	6.4	8.3	10.0
After HTC, 60°C	6.4	7.9	9.6

foaming capacity (FC) = 300/(16.7·t_f), which was expressed as mL of foam formed per mL of N_2 purged; K value = 1/(V_{max}·$t_{1/2}$), in units of 1/(mL·s), which was used to describe foaming stability (higher value indicates lower stability); and foaming rate, $V_i = V_{max}/t_f$, in unit of mL/s, i.e., the rate of liquid incorporation into foam.

SDS-PAGE. SDS-PAGE was performed by using a method similar to that of Jung *et al*. (11). Soy protein samples and standards (glycinin and β-conglycinin) were dissolved in a 2× sample buffer (125 mM THAM at pH 6.8, 0.2% SDS, 20% glycerol, 5.0 M urea, and 0.01% bromophenol blue) to achieve 1 mg/mL protein concentration. Storage and resolve gels contained 4 and 13% polyacrylamide, respectively. The loading volumes for samples and standards were 5 and 15 µL, respectively. Electrophoresis was conducted at 200 V for 45 min by using a Mini-PROTEIN® 3 Cell electrophoresis device (Bio-Rad Laboratories, Inc., Hercules, CA). Gels were stained for 1 h with a solution of methanol/acetic acid/water (50:10:18, by vol) and 0.22% Coomassie blue and destained for 4–5 h in methanol/acetic acid/water (50:10:40, by vol) solution. After washing with water, the gels were packed and dried overnight in a fume hood.

Experimental design and data analysis. A two-factor factorial design was used to evaluate the effects of the two HTC setups (flashing-out HTC and holding-tube HTC) at three alkali addition levels (0, 0.2, 0.6 mmol alkali/g meal) on protein refunctionalization and SPI preparation. Each treatment was replicated two times. All SPI preparations and functional property measurements were performed in triplicate. Statistical analysis was performed using General Linear Model procedures of SAS 8.02 (12).

RESULTS AND DISCUSSION

pH of protein dispersion at different alkali additions. To understand pH effects, all pH values were determined at the same solids concentration (1:10 solids/water ratio or 9.1% dry solids) at 25 and 60°C after stirring for 30 min as was used for extracting SPI (Table 1). After HTC, the pH dropped about 0.4 units compared with those without HTC treatment. The reduction in pH probably occurs because alkali-HTC dispersed or dissolved some of the heat-denatured protein, exposing more ionizable amino acid side chains of the protein to the aqueous environment, thus increasing the buffering capacity of the protein.

One potential concern with alkali HTC is the high pH environment and possible protein degradation. It was reported that at pH values higher than 9, cystine could be degraded to form dehydroalanine, which may further react with lysine to produce lysinoalanine, whose toxicological property is not yet fully understood (13,14). Kidney cell enlargement was observed in rats due to lysinoalanine, but at the consumption dose of 1.5 mg/kg of body weight per day, no adverse effect was observed. When free lysinoalanine was fed to quail, mice, hamsters, rabbits, dogs, and monkeys, no kidney effects were observed (14). Research has so far failed to provide a definitive conclusion on its toxicity. Lysinoalanine is present in most proteinaceous foods or ingredients, including heated milk, cooked chicken, simulated cheese, cooked egg whites, hydrolyzed vegetable protein, milk powder, and casein (14).

Effect of alkali concentration on HTC refunctionalization of EE meals. Alkali-HTC improved solids and protein dispersibilities, emulsification capacity, and foaming stability (lower K value indicates better stability) of EE35 meals over the control that was not HTC treated nor exposed to alkali (Fig. 3). Maximum solids and protein dispersibilities were achieved by carrying out alkali-HTC at 0.6 mmol alkali/g meal. Protein dispersibility may have accounted for most of the solids dispersibility since the two curves and the relative improvement were similar, and the other components may not have responded to alkali treatment.

Although there were some significant differences in emulsification capacities among different alkali concentrations, the differences were probably not sufficient to be practically significant. There were no differences in foaming capacities among different alkali concentrations during HTC treatment; however, these foaming capacities were lower than that of EE meal without any treatment (Fig. 3), and we could not explain this outcome. For foaming stability, the maximum effect was achieved at the alkali addition of 0.6 mmol/g meal, where the K value was the lowest.

Preparation of SPI after alkali HTC refunctionalization of EE meal. For both flashing-out and holding-tube HTC, alkali addition significantly improved the solids and protein yields of SPI compared with HTC without alkali (Fig. 4). The improvements were more significant when compared to the initial meal without HTC. Higher alkali addition resulted in higher SPI yields. The protein concentrations in SPI prepared from flashing-out HTC and holding-tube HTC were 75–76% without significant differences among them. Although these protein concentrations were significantly lower than for the SPI from EE meals without HTC treatment (80%), the difference was only about 4–5%. Because these SPI products do not meet the minimum concentration requirement of 90% protein, they cannot be marketed as "soy isolate." We believe that the lower protein purity (than SPI requires) was the result of the presence of significant levels of residual oils and carbohydrates in SPI, similar to the SPI prepared from EE meals without HTC treatment (6).

There were no statistically significant differences in SPI yield, protein yield, and protein content for the two HTC setups (Table 2). The maximum SPI protein yield (82%) was achieved with holding-tube HTC at 0.6 mmol alkali/g meal. This protein yield was more than twice as much as from the untreated EE meal (~40%) and 1.7 times more than from HTC-treated EE meals without alkali (~48%).

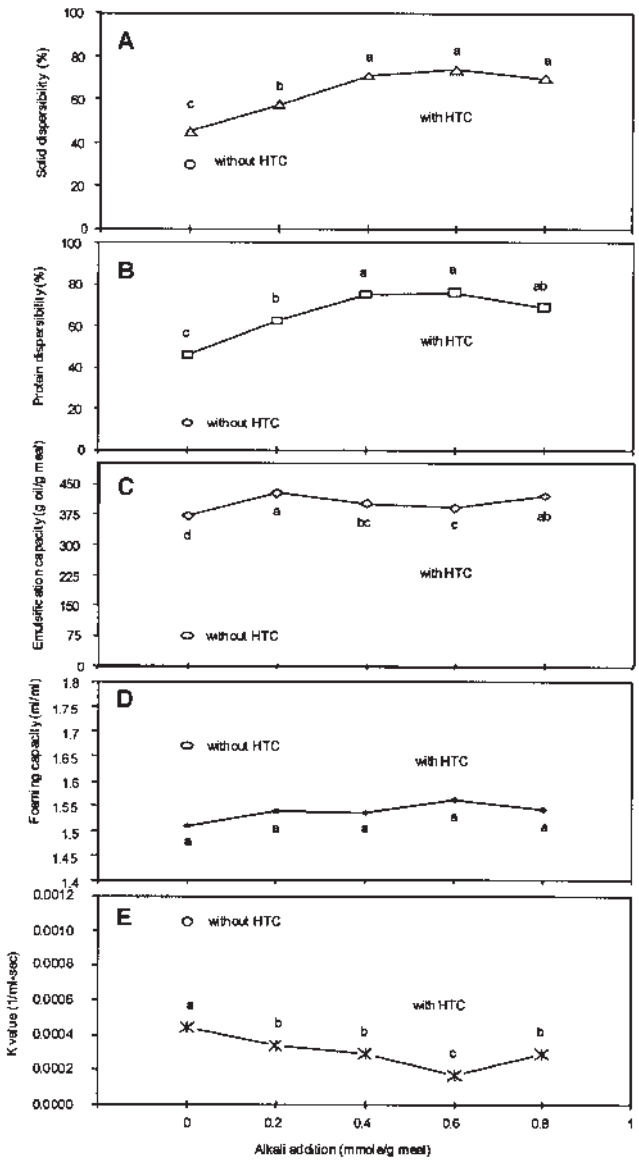

FIG. 3. Effects of alkali (NaOH) addition and flashing-out-HTC on the major functional properties [solids dispersibility (A), protein dispersibility (B), emulsification capacity (C), foaming capacity (D), and *K* value (E)] of EE35 meal, which had a protein dispersibility index of 35. Points with the same letters within the same chart are not significantly different ($P = 0.05$). For other abbreviations see Figures 1 and 2.

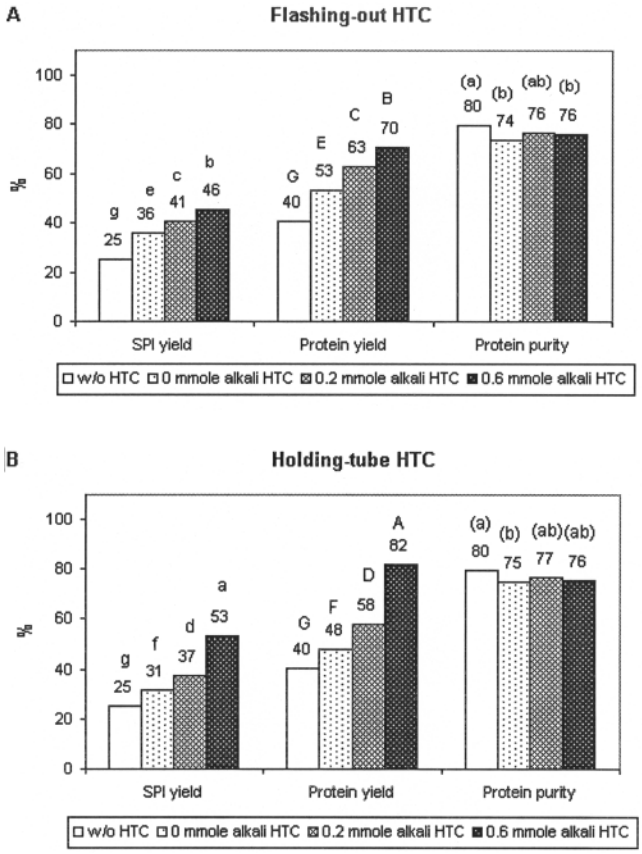

FIG. 4. Comparisons of solids yields, protein yields, and protein purities of SPI from flashing-out (A) and holding-tube (B) HTC-treated EE meals at different alkali (NaOH) concentrations. Points with the same letters within and across charts are not significantly different ($P = 0.05$). For abbreviations see Figures 1 and 2.

Functionalities of SPI produced from alkali-HTC refunctionalized EE flour. HTC setup had a significant effect on emulsification capacity (Table 2). SPI from holding-tube HTC had higher emulsification capacity than did SPI from flashing-out HTC, and the difference was about 100 g oil/g SPI, which was 25% higher than flashing-out HTC. The level of alkali addition had little effect on emulsification capacity within the flashing-out HTC or the holding-tube HTC treatment groups (Fig. 5). The emulsification capacities of SPI from holding-tube alkali-HTC-treated EE meal were lower than that from EE meal without any treatment (633 g oil/g). One possible explanation is that SPI recovered from the original EE meal (with very low yield) consisted of mainly native proteins and thus gave high emulsification capacity. HTC treatment most likely had denatured all the remaining native proteins. It was not clear why SPI from holding-tube HTC had much higher emulsification capacity than did the SPI prepared by flashing-out HTC under both neutral and alkaline conditions.

We speculate that since EE meals contain both native and heat-denatured proteins, HTC decreased the emulsification capacity of the native proteins and at the same time increased the emulsification capacity of the denatured protein by disintegrating the large heat-denatured protein aggregates. The overall emulsification capacity of SPI was the combination of the two opposing actions. Compared with flashing-out HTC, holding-tube HTC exposed the protein to a much higher temperature for a longer period. This may have been more effective in breaking up the aggregates and thereby resulting in a protein product with an improved balance of hydrophobic and hydrophilic surfaces. Although the emulsification capacity was the same for SPI from HTC without alkali, alkali-HTC resulted in much higher SPI yield.

HTC setup and alkali addition significantly affected both foaming capacity and foaming stability (*K* value) of the SPI

TABLE 2
P-Values for Treatment Effects on SPI Preparation and Major Functional Properties[a]

Treatment	d.f.	SPI yield	SPI protein content	SPI protein yield	EC[a]	FC[a]	*K* value[a]
HTC setup	1	0.257	0.659	0.480	<0.0001	0.0027	0.016
Alkali addition	2	<0.0001	0.074	<0.0001	0.620	0.0003	0.0005
HTC alkali addition (interaction)	2	<0.0001	0.761	0.0002	0.694	0.378	0.038

[a]EC, emulsification capacity; FC, foaming capacity; *K* value, foaming stability; SPI, soy protein isolate; for other abbreviation see Table 1.

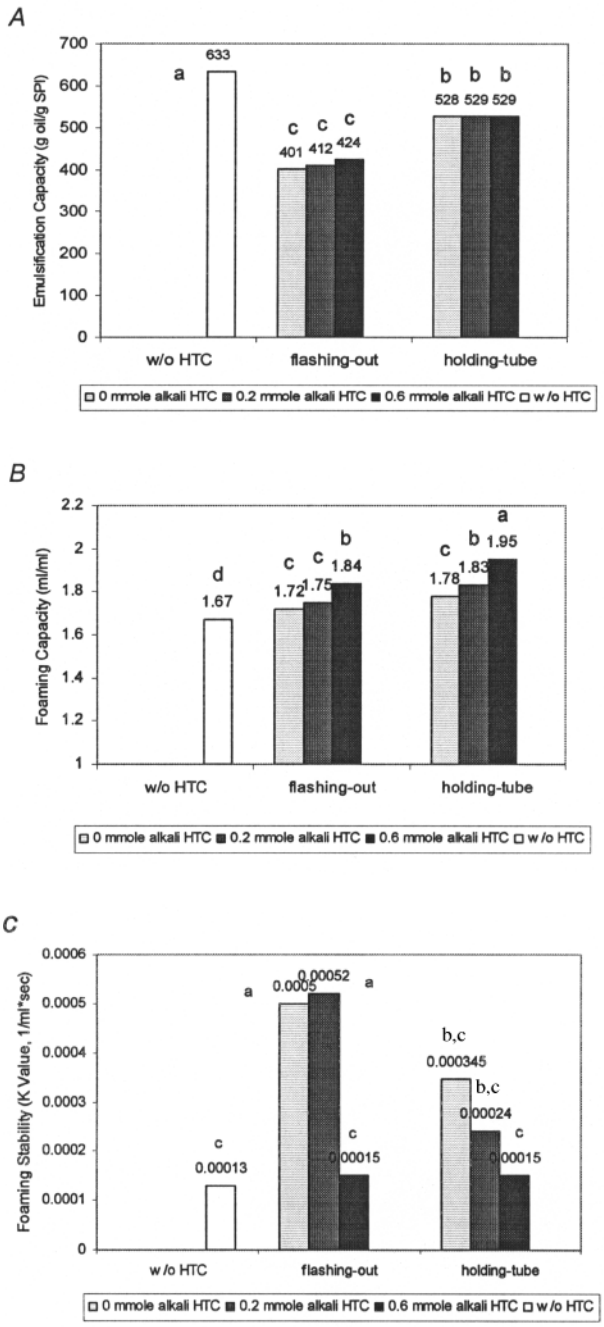

FIG. 5. Effects of alkali-HTC on emulsification capacities (A), foaming capacities (B, and foaming stabilities (C) of SPI. Points with the same letters within the same chart are not significantly different ($P = 0.05$). For abbreviations see Figures 1 and 2.

(Table 2). The more alkali was added, the higher was the foaming capacity, especially for holding-tube HTC. The foaming capacities of SPI prepared from HTC-treated EE meals were higher than for the SPI from original EE meal. Holding-tube HTC increased foaming capacity more than flashing-out HTC at the same alkali concentration, but the increases were not great (Fig. 5). Alkali addition of 0.2 mmol/g meal significantly decreased foaming stability of SPI compared with that of original EE meals, but when alkali addition was increased to 0.6 mmol/g meal, foaming stability was improved and was similar to SPI from original EE meal (Fig. 5). Since the foaming properties are related to the surface behavior of the proteins at the interface between the solution and air of the foam bubbles, the exact mechanism is difficult to understand except that alkali-HTC probably changed the conformations of the soy protein molecules and resulted in modified surface properties. It is worth noting that foaming stability of SPI from HTC-treated EE meal without alkali decreased (*K* value increased) compared with that of SPI from original EE meal, similar to the trend in emulsification capacity (Figs. 5A, 5C). This may be explained by the denaturation of the residual native protein in the original EE meal during HTC without alkali treatment.

Characterization of SPI by SDS-PAGE. The initial objective in determining the SDS-PAGE profile of the SPI was to see whether protein decomposition or hydrolysis occurred during alkali-HTC. No major peptide decomposition was found in SDS-PAGE profiles (Fig. 6). The difference in protein band density of SPI between flashing-out and holding-tube HTC at the same alkali concentration was not great. Alkali-HTC treatment decreased the band densities of major protein subunits. At higher alkali addition (especially 0.6 mmol alkali/g meal), the subunit bands became lighter and at the same time, a darker residual band remained on the top of the gel, indicating more protein was not dissolved even in the presence of strong denaturation agents (SDS, urea) and reducing agent (2-mercaptoethanol). The accumulation of proteins at the gel top suggested that strong interactions between protein and protein, or protein and nonprotein components (possibly carbohydrates) were formed. These interactions, however, did not affect the extraction of the protein or protein functionalities. There were considerable nonprotein components, including carbohydrates, in SPI from EE meals. The SPI prepared from original EE35 meal contained 80% protein, 8% carbohydrates (by phenol–sulfuric acid method), 9% crude fat (by acid hydrolysis method), and no crude fiber (6). Typical SPI from alkali-HTC contained 76% protein.

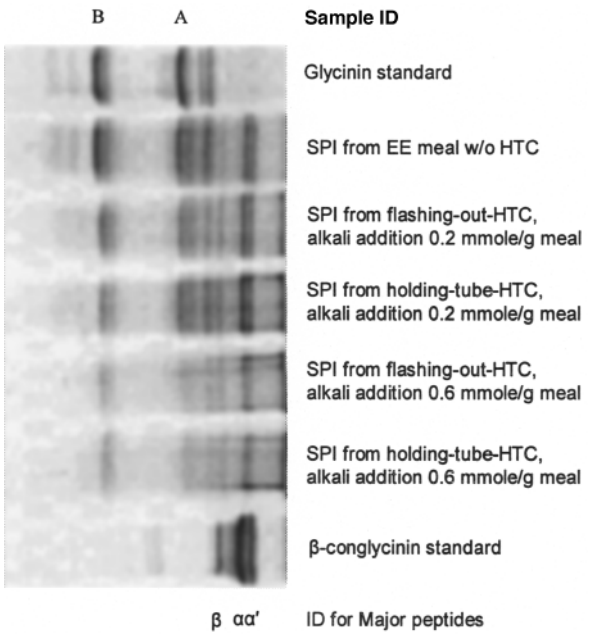

FIG. 6. SDS-PAGE profiles of SPI from alkali-HTC treatments (α′, α, and β, are the major subunits of β-conglycinin, acidic (A), and basic (B) subunits are the major components of glycinin).

Overall, alkali addition enhanced the refunctionalization of EE meal and dramatically increased the protein yield of SPI, which had similar or improved functionalities compared with SPI from HTC without alkali addition. Holding-tube HTC resulted in SPI with better functional properties than flashing-out HTC.

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