Supporting Information

A comparative study on protein digestion of four different soy beverages: Effects of composition, microstructure, and protein digestibility evaluation method

Junna Zhao[a, b], Xiangzhen Kong[a, b]*, Caimeng Zhang[a, b], Yufei Hua[a, b], Yeming Chen[a,b], Xingfei Li[a,b]

[a]State Key Laboratory of Food Science and Resources, Jiangnan University, Wuxi, P. R. China

[b]School of Food Science and Technology, Jiangnan University, Wuxi, P. R. China

*Corresponding author: Xiangzhen Kong

State Key Laboratory of Food Science and Resources

Jiangnan University

1800 Lihu Avenue, Wuxi, 214122

Jiangsu Province, PR China

Phone: 86-13915299381

Fax: 86-510-85329091

Email: xzkong@jiangnan.edu.cn

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Details of the AOAC methods for composition analysis

(1) AOAC 930.29 method (Kjeldahl Method) for the determination of protein.

(2) AOAC 925.10 is a method for the determination of solids in a sample (air oven method).

(3) AOAC 985.29 is a method for the determination of dietary fiber (including soluble, insoluble, and total fiber).

Table S1 Amino acid composition (g/100 g protein) for the precipitate of digesta for WS, DO-WS, DL-WS and HSPI at different phases of in vitro digestion.

	WS-P0	WS-P120	WS-P240	DO-WS-P0	DO-WS-P120	DO-WS-P240	DL-WS-P0	DL-WS-P120	DL-WS-P240	HSPI-P120	HSPI-P240
Asp	12.09±0.08^{ab}	12.50±0.08^{a}	12.41±0.38^{a}	11.60±0.28^{a}	12.27±0.54^{ab}	11.59±0.27^{b}	12.23±0.18^{ab}	12.61±0.07^{a}	12.26±0.14^{ab}	12.25±0.57^{ab}	12.48±0.12^{a}
Glu	17.64±0.34^{cd}	19.24±0.59^{ab}	18.34±0.06^{bcd}	16.16±0.48^{ef}	17.16±1.05^{cde}	15.42±0.33^{f}	19.25±0.24^{ab}	19.88±0.77^{a}	16.37±0.52^{def}	17.64±1.12^{c}	15.92±0.65^{ef}
Ser	4.34±0.23^{a}	4.45±0.04^{a}	4.55±0.06^{a}	4.34±0.16^{a}	4.54±0.16^{a}	4.65±0.35^{a}	4.44±0.06^{a}	4.54±0.13^{a}	4.68±0.25^{a}	4.28±0.11^{a}	4.42±0.20^{a}
His	2.74±0.12^{a}	2.56±0.06^{ab}	2.52±0.06^{ab}	2.54±0.33^{ab}	2.57±0.03^{ab}	2.61±0.12^{ab}	2.53±0.15^{ab}	2.41±0.27^{ab}	2.55±0.19^{ab}	2.47±0.23^{ab}	2.13±0.10b
Gly	5.23±0.03^{a}	5.23±0.04^{a}	5.12±0.08^{a}	4.98±0.14^{a}	5.06±0.08^{a}	5.14±0.06^{a}	4.84±0.11^{a}	4.92±0.45^{a}	5.48±0.59^{a}	4.98±0.25^{a}	5.12±0.28^{a}
Thr	3.99±0.16^{a}	3.70±0.01^{a}	3.76±0.17^{a}	4.13±0.07^{a}	3.87±0.18^{a}	3.96±0.15^{a}	3.77±0.33^{a}	3.69±0.09^{a}	3.95±0.38^{a}	3.58±0.53^{a}	3.75±0.35^{a}
Arg	6.48±0.14^{c}	6.64±0.37^{c}	7.48±0.12^{ab}	6.83±0.13^{bc}	6.62±0.11^{c}	7.92±0.20^{a}	6.79±0.12^{bc}	6.99±0.61^{bc}	6.82±0.14^{bc}	6.60±0.35^{c}	6.98±0.59^{bc}
Lys	7.24±0.04^{a}	5.75±0.06^{cd}	5.91±0.27^{bc}	7.12±0.05^{a}	5.20±0.08^{de}	5.68±0.15^{cd}	6.35±0.06^{b}	6.02±0.18^{bc}	6.14±0.16^{bc}	5.67±0.16^{cd}	5.05±0.83^{e}
Tyr	2.70±0.08^{f}	3.19±0.27^{cdef}	3.36±0.13^{bcde}	3.77±0.03^{abc}	3.65±0.15^{abcd}	3.35±0.15^{bcde}	2.80±0.11^{ef}	3.27±0.18^{cdef}	3.07±0.18^{def}	3.89±0.16^{ab}	4.08±0.71^{a}
Cys	0.20±0.02^{b}	0.31±0.07^{ab}	0.33±0.04^{ab}	0.32±0.11^{ab}	0.50±0.06^{a}	0.52±0.08^{a}	0.25±0.04^{ab}	0.38±0.08^{ab}	0.36±0.23^{ab}	0.45±0.04^{ab}	0.38±0.14^{ab}
Ala *	5.27±0.10^{ab}	4.81±0.07^{bcd}	4.81±0.35^{bcd}	5.43±0.10^{a}	5.06±0.06^{abcd}	5.18±0.11^{abc}	4.88±0.11^{bcd}	4.60±0.34^{d}	5.04±0.34^{abcd}	4.69±0.13^{cd}	5.09±0.13^{abcd}

	WS-P0	WS-P120	WS-P240	DO-WS-P0	DO-WS-P120	DO-WS-P240	DL-WS-P0	DL-WS-P120	DL-WS-P240	HSPI-P120	HSPI-P240
Val *	6.69±0.04^{a}	6.20±0.03^{a}	6.21±0.64^{a}	6.79±0.05^{a}	6.60±0.11^{a}	6.77±0.33^{a}	6.42±0.17^{a}	6.07±0.25^{a}	6.98±1.27^{a}	6.65±0.49^{a}	7.13±0.76^{a}
Met *	0.63±0.09^{c}	0.76±0.06^{bc}	0.69±0.09^{c}	1.38±0.02^{a}	1.23±0.07^{a}	1.26±0.03^{a}	0.72±0.04^{c}	0.84±0.08^{bc}	0.62±0.27^{c}	1.22±0.06^{a}	0.96±0.11^{b}
Phe *	5.80±0.04^{a}	6.23±0.04^{abc}	6.07±0.09^{abc}	5.96±0.08^{bc}	6.65±0.04^{a}	6.37±0.03^{abc}	6.08±0.06^{abc}	5.89±0.25^{bc}	6.16±0.33^{abc}	6.40±0.11^{abc}	6.52±0.68^{ab}
Ile *	5.17±0.09^{ab}	5.25±0.07^{ab}	5.16±0.11^{ab}	5.25±0.04^{ab}	5.74±0.08^{a}	5.82±0.14^{a}	5.15±0.15^{ab}	4.98±0.26^{b}	5.49±0.44^{ab}	5.48±0.14^{ab}	5.65±0.66^{ab}
Leu *	8.78±0.45^{ab}	8.66±0.49^{ab}	8.63±0.53^{ab}	8.91±0.64^{ab}	9.51±0.44^{ab}	9.53±0.66^{ab}	8.65±0.49^{ab}	8.22±0.53^{b}	9.10±0.28^{ab}	9.24±0.06^{ab}	9.73±0.66^{a}
Pro *	5.02±0.09^{a}	4.47±0.16^{ab}	4.65±0.18^{a}	4.49±0.13^{ab}	3.73±0.18^{b}	4.26±0.43^{ab}	4.85±0.07^{a}	4.67±0.47^{a}	4.89±0.24^{a}	4.47±0.75^{ab}	4.61±0.35^{ab}
HAA	37.34±0.91^{ab}	36.37±0.92^{ab}	36.20±1.99^{b}	38.20±1.06^{ab}	38.51±0.98^{ab}	39.18±1.73^{a}	36.75±1.10^{ab}	35.25±2.17^{b}	38.28±3.17^{ab}	38.15±1.74^{ab}	39.68±3.35^{a}
EAA	33.13±0.82^{ab}	31.30±0.69^{ab}	31.27±1.79^{ab}	34.29±0.91^{a}	33.07±0.92^{a}	33.56±1.35^{a}	32.00±1.12^{ab}	30.74±1.38^{b}	32.96±2.69^{ab}	32.76±1.41^{ab}	33.14±3.39^{ab}

Results were presented as the mean ± SD (n = 3).

HAA (Hydrophobic amino acid) = Ala + Val + Met + Phe + Ile + Leu + Pro.

"*" indicated hydrophobic amino acids; tryptophan was not measured.

“P” - the precipitate of digesta. P0: gastrointestinal digestion for 0min; P120: gastrointestinal digestion for 120min; P240: gastrointestinal digestion for 240min.

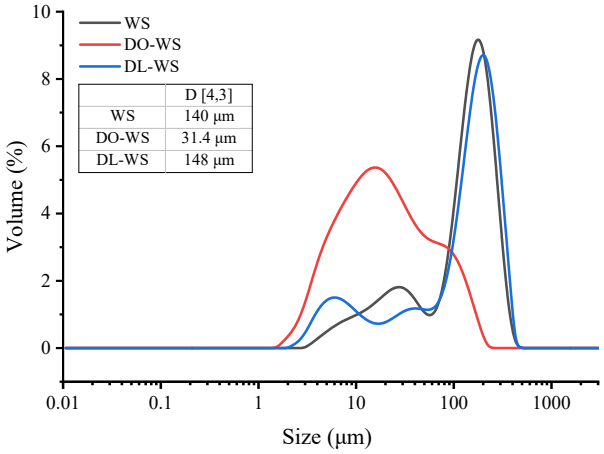
Table S2 Sensory evaluation[1] of WS, DO-WS, DL-WS and HSPI soy beverages.

	Brightness	Beany flavor	Smoothness	Global impression
WS	5.26±0.76^{ab}	5.42±1.17^{ab}	3.85±1.13^{bc}	7.69±0.61^{a}
DO-WS	5.78±0.81^{ab}	7.03±1.66^{a}	7.09±1.46^{ab}	8.37±1.10^{a}
DL-WS	6.78±1.10^{a}	5.82±1.22^{ab}	3.74±1.02^{c}	6.09±1.10^{b}
HSPI	3.83±1.03^{b}	3.19±0.71^{b}	7.88±0.91^{a}	6.87±1.06^{ab}

Each value represented the mean of three replications per panellist. Different letters indicated a significant difference ($p < 0.05$).

[1] Attributes evaluated using non-structured sensory scales which varied from 1 to 9.

Figure S1. Particle size distribution of soy beverages.



Details of the AOAC methods for composition analysis

(1) AOAC 930.29 method (Kjeldahl Method) for the determination of protein.

Samples of 0.5 to 1 g (For samples with higher protein content such as soybean and soy protein isolate powder, 0.1 - 0.5 g was weighed) were accurately weighed out (accurate to four decimal places) and then digested with 10 mL of concentrated sulfuric acid in a Kjeldahl flask. The catalyst was a mixture of K_2SO_4 (3.0 g) and $CuSO_4 \cdot 5H_2O$ (0.2 g).

The Kjeldahl flask was placed on the digestion furnace and heating was started at 200 °C. After the foam disappeared, the temperature was gradually increased (to 420 °C) to keep the digestion liquid slightly boiling. It was digested until the solution was clear and blue-green, and digestion was continued for 1 h. A blank (including filter paper and no soybean powder, as the products were analyzed) was digested and distilled. The blank values were recorded. When the digestion was complete, the Kjeldahl flask was removed and cooled to room temperature.

A Kjeldahl nitrogen analyzer (K9840, Hanon, China) was used for further analysis. After cooling, the sample was connected to the distillation unit and 10 M NaOH was added, followed by distillation. The ammonia released from the ammonium sulfate after adding NaOH to the test tube was separated, and the ammonia separated by distillation was trapped in 4% boric acid. At the last stage, the ammonia obtained by distillation was titrated with an acid (0.05 M HCl). Until the color of the solution changed from blue-green to gray-red, it was the end point of titration. The consumption of standard acid solution (V) was recorded.

Calculate the nitrogen content of the sample according to the following formula:

$$N\,(\%) = \frac{(C \times V) \times 0.014}{m} \times 100$$

Where C was the concentration of the standard acid solution (mol/L), V was the titration volume of the standard acid solution (mL), 0.014 was the molar mass of nitrogen (g/mmol), and m was the mass of the sample (g).

Protein content calculation:

Protein content (%) = Nitrogen content (%) × conversion factor (for soy beverages, 6.25)

(2) AOAC 925.10 method for the determination of solids in a sample (air oven method).

In a cooled and weighed empty crucible (provided with a cover), which had been previously heated to 130±3 °C, 2 g of a well-mixed sample was accurately weighed. The test portion was uncovered, and the dish, cover, and contents were dried for 1 h in an oven that had an opening for ventilation and was maintained at 130±3 °C (the 1 h drying period began when the oven temperature actually reached 130 °C). While still in the oven, the dish was covered, transferred to a desiccator, and weighed soon after reaching room temperature. The residue was reported as total solids and the loss in weight was reported as moisture (indirect method). The total solids were calculated using the following formula:

$$Total\ solids\,(\%) = \frac{W_3}{W_1 + W_2} \times 100$$

where W_1 was the weight of the empty crucible, W_2 was the weight of the sample, and W_3 was the weight of the crucible with dried sample.

(3) AOAC 985.29 method for the determination of dietary fiber (including soluble, insoluble, and total fiber).

In short, fifty grams of dried powder was dissolved in 500 mL phosphate buffer (pH 6.0, 0.08 M) and adjusted to pH 6.0. Afterward, α-amylase (3.75 mL) was added and hydrolyzed at 37 °C for 1 h in a water bath. The beakers were shaken gently at 5 min intervals throughout incubation. The pH of the α-amylase treated suspensions was adjusted to 7.5 before the incubation of the resultant mixture with alcalase (1.5 mL) at 60 °C for 1 h. Amyloglucosidase (1.75 mL, 50 mg/mL phosphate buffer, pH 6) was finally added to each mixture after the pH was adjusted to 4.5 and held at 60 °C for another 1 h. After centrifugation at 4000g for 10 min, the supernatant was discarded. The sediment was washed 2 times with 70 °C hot water (removing soluble dietary fiber), 2 times with 95% ethanol, 2 times with acetone, and 1 time with water and then it was freeze-dried to obtain the insoluble dietary fiber (IDF).

The water washings and the supernatant were pooled together for isolation of the soluble dietary fiber (SDF) by precipitating with 4 volumes of 80% (v/v) ethanol at room temperature for 1 h and was centrifuged (4000*g*, 20 min). The residue was washed twice with 78% (v/v) ethanol, 95% (v/v) ethanol and acetone.

For the total dietary fiber (TDF), the other enzymatically treated suspension was precipitated with 4 volumes of 80% (v/v) ethanol (preheated at 60 °C), followed by centrifugation, and washing of the residual mass as described above.