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ORIGINAL PAPER

Changes of heat-treated soymilks in bioactive compounds and their antioxidant activities under in vitro gastrointestinal digestion

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Abstract To investigate the influence of in vitro gastrointestinal digestion on contents of bioactive compounds and antioxidant activities of heat-treated soymilks, changes of total phenolics content (TPC), total flavonoids content (TFC), content and profile of isoflavones, oxygen radical absorbance capacity (ORAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric-reducing antioxidant power (FRAP) were assayed after different heat treatments (at 95 °C for 20, 40, 60 min; 121 °C for 3, 6, 9 min and 143 °C for 20, 40, 60 s respectively) and gastrointestinal digestion. Results showed that digestion significantly influenced the contents of bioactive compounds and antioxidant activities of soymilks. Increases of DPPH radical scavenging activity, FRAP and ORAC of the heat-treated soymilks after gastric digestion were consistent with the increases of TPC (110.68–152.60 %) and TFC (4.48–31.10 %). In the dialysate fractions as the absorbable and utilized part, it was found that TPC, TFC, isoflavones and antioxidant activities (DPPH radical scavenging activity, FRAP and ORAC) were significantly decreased as compared with the gastric digestion fractions and duodenal fractions. Analysis showed that the bioaccessibility of TPC reached 107.17–125.14 %, TFC reached 34.63–67.19 % and total isoflavones reached 34.40–41.22 %, respectively, indicating the rich bioaccessible compounds in soymilk. Daidzein and its derivates were proven as the most bioaccessible isoflavones (about 36.99–44.14 %). Glucoside isoflavones showed the highest bioaccessibilities followed by malonylglucosides, acetylglucosides and aglycones. Overall, the soymilks treated at 95 °C and 60 min and 121 °C and 9 min had higher bioactive compounds contents, antioxidant activities and bioaccessibilities in the dialysate fractions.

Keywords Heat-treated soymilk · In vitro gastrointestinal digestion · Bioactive compounds · Antioxidant activity · Bioaccessibility

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Introduction

As one of the most popular processed foods consumed in Asian countries, soymilk provides high-quality proteins and essential fatty acids and accordingly is thought to be an excellent alternative for people who suffer from the lactose intolerance and milk allergy due to its free of cholesterol, gluten and lactose [1]. During the recent years, there is growing evidence that soy-based foods have potential health-promoting effects. Known as the phytoestrogen, isoflavones are important bioactive compounds in soybean and processed soy products. It was reported that soy isoflavones were effective in promoting bone health, reducing the risk of various cancers, and reducing symptoms of diabetes [2–5]. Besides, soybean isoflavones showed significant antioxidant activities such as scavenging free radicals, inhibiting lipid oxidation [6, 7]. In addition to isoflavones, a number of phenolic acids, for example, gallic acid, 4-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid, naringenin, rutin, quercetin and (+)-catechin in soybean and soy products, are also involved in antioxidant activities [8].

However, the availability of most of the bioactive compounds and their antioxidant activities in soymilk were easily affected by thermal processing. The isoflavones in conjugated forms, especially the malonylglucoside and acetylglucoside isoflavones, are thermolabile and prone to convert to their relatively stable forms. Moreover, Xu and Chang [1] found that thermal treatment significantly reduced the total phenolics content (TPC), while increased the total flavonoids content (TFC) and antioxidant activities [1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric-reducing antioxidant power (FRAP)].

It is considered that prior to be absorbed, the bioactive compounds must be released from the food matrix and modified in the gastrointestinal tract [8]. Due to the difficulty in studying the in vivo changes of food constituents during the process of digestion and absorption, in vitro methodology has been developed as an alternative approach to in vivo study [9]. Changes in the profiles of phenolic compounds and antioxidant activities of foods as affected by in vitro gastrointestinal digestion have been reported [10–13]. However, as far as we know, there are only few reports about the changes of phenolic compounds, isoflavones and antioxidant activity of the heat-treated soymilk during the in vitro gastrointestinal digestion [8]. Moreover, little information is available in the literature about the effect of in vitro gastrointestinal digestion on the changes of bioactive compounds and antioxidant activity of soymilk treated by various temperatures and times. Only one commercial soymilk was reported in the work conducted by Rodríguez-Roque et al. [8]. In the present paper, various combinations of thermal processing temperatures such as 95, 121 and 143 °C and different treatment time lengths, corresponding to pasteurization, in-container sterilization and ultra-high-temperature (UHT) heat treatment, were applied respectively to process soymilks, and the effect of in vitro gastrointestinal digestion on the contents and antioxidant activities of bioactive compounds in thermal-processed soymilks was studied. Results of this study would be beneficial to understand changes of the bioactive compounds in soymilks during digestion and provide meaningful information for the production of high-quality soymilk.

Materials and methods

Materials

Soybean cultivar HX9 used in the present study was grown in the farm of South China Agricultural University (Zengcheng, Guangdong, China), and the seeds were harvested in October 2011. The broken and damaged seeds together with foreign materials were removed from samples.

The main chemicals, such as the isoflavone standards (including daidzein, daidzin, genistein, genistin, glycitein and glycitin), were purchased from the International Laboratory, USA (South San Francisco, CA., USA). DPPH was purchased from the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Trypsin (from porcine pancreas, 14,476 units/mg protein) was purchased from Sigma-Aldrich Co. (St. Louis, MO., USA).

Preparation of raw soymilk

Soybean was soaked in distilled water at the ratio of 1:10 (soybean/water, g/mL) for overnight (about 15 h). Then, the soaked soybean was blended along with the water with a soymilk maker (DJ12B-A10, Joyoung, China) in an unheating model. The blended slurry was filtered through a 200-mesh nylon film, and the residue was discarded. The soymilk was subjected to heat process by a self-made capillary tube as reported by Huang et al. [14]. The heating temperature and time used in the present study were 95 °C for 20, 40 and 60 min, 121 °C for 3, 6 and 9 min and 143 °C for 20, 40 and 60 s, simulating pasteurization, in-container sterilization and UHT heat treatment as used in commercial soymilk production, respectively. The raw and heated soymilks were freeze-dried with a vacuum freeze-drier for further use.

In vitro gastrointestinal digestion

The in vitro gastrointestinal digestion consisted of a gastric digestion (pepsin-HCl digestion) and an intestinal digestion (pancreatin digestion) as previously reported by Miller et al. [15] and Gil-Izquierdo et al. [16] with a few modifications.

Gastric digestion

First of all, 234 mL concentrated HCl (36–37 %, w/v) was diluted to 1,000 mL with distilled water and marked as solution A. Then, 16.4 mL of solution A was diluted to 1,000 mL and marked as solution B. Pepsin solution was prepared by dissolving 100 mg pepsin (from porcine gastric mucosa, 35,000 unites/mg) in solution B at the final concentration 4 g/L.

Five grams of the freeze-dried soymilk was dissolved in 100 mL distilled water in a beaker and was adjusted to pH 2.0 by solution A. After 1 mL pepsin solution was pipetted in, the mixture was stir-incubated at 37 °C for 2 h in a shaker at 120 rpm. At the end of the incubation, 15 mL of this gastric digest solution was sampled and stored at −20 °C for analysis. The rest of gastric digest solution was available for simulating intestinal digestion.

Intestinal digestion

Intestinal digestion was performed according to the method reported by Miller et al. [15] and Gil-Izquierdo et al. [16]. For pancreatin digestion, segments of tubing dialysis bags (30 cm in length; made from cellulose membrane with 14,000 Da cutoff molecular weight) loaded 25 mL distilled water–$NaHCO_3$ were completely immersed in the gastric digest solution in the beaker. $NaHCO_3$ amount in the dialysis bag was equivalent to the amount of $NaHCO_3$ consumed to neutralize the gastric digestion. The beaker with the gastric digest solution and dialysis bag was stir-incubated at 37 °C, 150 rpm until the pH value reached approximately 5.0 (about 40 min) in a shaker. Then, 10-mL pancreatin (4 g/L)-bile extract (25 g/L) mixture (dissolved in 0.1 mol/L $NaHCO_3$) was added to the beaker, and the beaker was stir-incubated at 37 °C for another 2 h at 120 rpm. After incubation, the dialysis bag was removed and rinsed with distilled water. The dialysate (inside the dialysis bag) corresponded to the bioactive compounds that available for absorption and the duodenal fraction (outside the dialysis bag) corresponded to the unabsorbed bioactive compounds. At the end of intestinal digestive phase, a 15-mL aliquot of the dialysate and duodenal fraction was sampled and stored at −20 °C for further analysis.

Preparation of extracts

The undigested freeze-dried soymilk was re-dissolved in distilled water at a ratio of 1:20. Digested or undigested soymilk solution was mixed with aceton at the ratio of 1:1, and the mixture was extracted in an orbital shaker at 250 rpm at room temperature for overnight. After centrifuging at 10,000 rpm for 10 min, the supernatant was pipetted into tube and stored at 4 °C and available for analysis.

Total phenolics content determination

TPC was determined according to the method previously described by Singleton and Rossi [17] and Singleton et al. [18] with slight modification. Briefly, 100 μL extract, 2.95 mL distilled water, 250 μL Folin–Ciocalteu's solution and 750 μL Na_2CO_3 (7 %) were mixed and reacted at room temperature for 8 min. Then, the mixture was made up to 5 mL by distilled water and incubated in dark for 2 h at ambient temperature. The absorbance of the mixture was read at 765 nm. The reducing powers of the samples were compared with standard phenolic acid (gallic acid). The results were reported as milligrams of gallic acid equivalents per litre of undigested or digested soymilk (mg GAE/L).

Total flavonoids content determination

TFC was determined by a colorimetric method described previously by Dewanto et al. [19] with modification. The mixture of 1 mL sample extract and 150 μL $NaNO_2$ solution (5 %) was incubated at room temperature for 6 min. Then, 300 μL $AlCl_3{\cdot}6H_2O$ solution (10 %) was pipetted to the mixture and incubated for another 5 min. After the addition of 1 mL NaOH (1 mol/L), the mixture was finally fixed to 3.5 mL with distilled water. The absorbance was measured immediately at 510 nm. (+)-Catechin was used as standard, and the results were expressed as milligrams of catechin equivalents per litre of undigested or digested soymilk (mg CE/L).

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity assay

DPPH radical scavenging activity assay was carried out according to the procedure of Chen and Ho [20] with slight modification. One millilitre sample extract and 3 mL DPPH radical solution (0.1 mmol/L) were mixed and reacted in dark for 30 min. Then, the absorbance at 517 nm was determined immediately with Trolox as the standard. Blank (A_{blank}) and control ($A_{control}$) were obtained by adding 3 mL ethanol to 1 mL extract and by adding 3 mL DPPH solution to 1 mL extract solution (50 % aceton), respectively. The DPPH radical scavenging activity was expressed as micromoles of Trolox equivalents per litre of undigested or digested soymilk (μmol TE/L).

Ferric-reducing antioxidant power assay

FRAP assay was performed according to method reported by Benzie and Strain [21] previously with modification. FRAP reagent was prepared by mixing the solutions of acetate buffer (300 μmol/L, pH 3.6), TPTZ (10 mmol/L in 40 mmol/L HCl) and $FeCl_3{\cdot}6H_2O$ (20 mmol/L) in proportion of 10:1:1 and incubated at 37 °C in a water bath prior to be used. Three millilitres freshly prepared FRAP reagent, 100 μL extract and 300 μL distilled water were mixed and reacted at 37 °C for 4 min. The absorbance of the mixture at 593 nm was determined immediately with Fe^{2+} ($FeSO_4{\cdot}7H_2O$) as the standard. FRAP value was expressed as millimoles of Fe^{2+} equivalent (FE) per litre undigested or digested soymilk (mmol FE/L).

Oxygen radical absorbance capacity assay

ORAC assay was conducted according to Prior et al. [22] and Wu et al. [23] with slightly modification. The extracts were diluted (150 times) with phosphate buffer (75 mmol/L, pH 7.4) to an appropriate concentration range so as to fit

for the standard curve. Briefly, 20 μL of the blank, Trolox standard (6.25–50 μmol/L) or the sample extract and fluorescein solution (0.956 μmol/L) were loaded into the wells of a black, clear-bottom 96-well microplate in duplicate based on a randomized layout. After shaking for 1 min, the plate was incubated at 37 °C for 20 min. At the end of incubation, 20 μL freshly prepared AAPH (119.4 mmol/L) was added to each well. The plate was shaken for 15 s prior to the first reading. After shaking 15 s, the fluorescence was recorded by a Varioskan Flash Spectral Scan Multimode Plate Reader (Thermo Fisher Scientific, Waltham, MA) every 2 min during a 120 min period at 37 °C, with the excitation and emission wavelengths at 485 and 535 nm respectively. The final ORAC values were calculated using a linear equation between the Trolox concentrations and net areas under the fluorescence decay curve. Data were expressed as millimoles of Trolox equivalents (TE) per litre undigested or digested soymilk (mmol TE/L). Fluorescence measurements were normalized to the curve of blank. From the normalized curve, the area under curve (AUC) was calculated as follows:

$$AUC = (0.5 + f_1/f_0 + f_2/f_0 + \cdots + f_i/f_0 + \cdots + f_{60}/f_0) \times CT$$

where f_0 is the initial fluorescence reading at 0 min, f_i is the fluorescence reading at time i and CT is the cycle time in minutes. The net AUC was calculated as follows:

$AUC_{net} = AUC_{sample/Trolox} - AUC_{blank}$ [22].

Analysis of soybean isoflavones by HPLC

Isoflavones was extracted and analysed according to the method reported by Murphy et al. [24] and Xu and Chang [25] with slight modification. HPLC analysis of isoflavone was conducted on a Dionex UltiMate 3000 system equipped with an UV–Vis detector and controlled by Chromeleon software. A Venusil MP-C_{18} column (5 μm, 250 × 4.6 mm i.d., Agela Technologies Inc., Newark, DE, USA) was used for the separation. Daidzein, daidzin, genistein, genistin, glycitein and glycitin were used as external standards. For isoflavones without commercial standards, the standard curves were calculated from the standard curves of respective glucoside isoflavones based on the difference in molecular weight [25]. Content of isoflavone was expressed as milligrams of isoflavone per litre undigested or digested soymilk (mg/L). Calculation of the subtotal isoflavones (total daidzeins, total glyciteins and total genisteins) and total isoflavones was according to the work reported previously by Murphy et al. [24].

Analysis of bioaccessibility

Bioaccessibility is defined as the amount of bioactive compounds released from the food matrix by in vitro gastrointestinal digestion and available for absorption [26], calculated based on the following:

$$Bioaccessibility\% = (BC_{dialysed}/BC_{non-digested}) * 100\ \%$$

where $BC_{dialysed}$ and $BC_{non\text{-}digested}$ corresponded to the concentration of bioactive compounds (mg/mL) in dialysate fraction and non-digested soymilk, respectively [8].

Statistical analysis

All the above experiments and determinations were carried out in triplicate. Data were expressed as the mean ± standard deviation and analysed by one-way analysis of variance using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The differences between group means were performed by Duncan's multiple range tests. Statistically significant was considered as a $p < 0.05$. A two-tailed Pearson's correlation test was conducted to determine the correlations.

Results and discussion

Changes of total phenolics content and total flavonoids content under in vitro gastrointestinal digestion

The effect of in vitro gastrointestinal on TPC and TFC of the thermal-processed soymilks is shown in Tables 1 and 2. In Table 1, TPC in the raw-undigested soymilk was 245.35 mg GE/L. Except for 121 °C and 6 min and 143 °C and 40 s treatments, by which TPC was significantly increased and decreased, respectively ($p < 0.05$), other heat treatments showed no significant effect on TPC basically. However, undigested heat-treated soymilks showed significantly higher TFC (11.20–14.06 mg CE/L) than raw soymilk (8.97 mg CE/L) (Table 2). Research conducted by Xu and Chang [1] found that TPC of the soymilks treated by traditional stove-cooking (100 °C and 20 min), steam injection processing (100 °C and 20 min) and direct and indirect UHT processing (143 °C and 60 s) was decreased by 15–18, 8.5–11, 15.5–42.5 and 7.5–16 %, respectively, whereas TFC was increased by 20–23, 28–65, 48–90 and 74–113 %, respectively. The different results between these different researches may be resulted from the differences in soybean variety and heat treatment. Heating mainly results in degradation and conversion of polyphenols and lignin [25]. Previous report showed that breakdown of lignins increased TFC in thermal-processed soybeans due to the release of the free phenolic compounds [25]. In the present research, the minor influence of heating on the most treated soymilks in TPC and TFC probably is due to the equal decrease and increase among the different polyphenol components, but further detailed research should be performed.

Table 1 Effect of in vitro gastrointestinal digestion on TPC of the heat-treated soymilks (mg GAE/L)

	Gastric digestion	Intestinal digestion		Undigested	Bioaccessibility (%)
		Duodenal fraction	Dialysed fraction		
95 °C					
20 min	535.61 ± 4.01 b	622.55 ± 28.54 d	268.43 ± 9.1 bc	242.01 ± 4.05 c	110.92
40 min	550.99 ± 17.67 ab	635.26 ± 18.67 cd	286.32 ± 4.51 b	246.02 ± 15.09 bc	116.38
60 min	549.32 ± 31.63 ab	629.24 ± 8.76 cd	312.90 ± 8.03 a	260.74 ± 3.48 ab	120.01
121 °C					
3 min	558.68 ± 21.86 ab	680.40 ± 16.92 ab	290.16 ± 10.15 b	245.69 ± 5.59 bc	118.10
6 min	596.13 ± 41.32 a	688.09 ± 18.74 a	313.40 ± 1.50 a	263.74 ± 17.63 a	118.83
9 min	582.42 ± 28.84 ab	689.76 ± 9.32 a	321.26 ± 17.84 a	256.72 ± 3.62 abc	125.14
143 °C					
20 s	545.30 ± 34.05 b	654.32 ± 7.39 bc	276.12 ± 24.19 bc	241.67 ± 6.26 c	114.25
40 s	549.65 ± 19.99 ab	693.44 ± 23.36 a	255.38 ± 17.95 cd	217.60 ± 11.17 d	117.37
60 s	554.67 ± 25.62 ab	697.79 ± 10.25 a	268.43 ± 4.05 bc	250.70 ± 3.48 abc	107.07
Raw	581.75 ± 4.37 ab	621.88 ± 5.31 d	238.67 ± 6.02 d	245.35 ± 2.90 bc	97.27

Data were expressed as mean ± SD ($n = 3$)

Values in a column marked by the same letters were not statistically different ($p > 0.05$)

Table 2 Effect of in vitro gastrointestinal digestion on TFC of the heat-treated soymilks (mg CE/L)

	Gastric digestion	Intestinal digestion		Undigested	Bioaccessibility (%)
		duodenal fraction	dialysed fraction		
95 °C					
20 min	16.03 ± 0.31 a	13.44 ± 0.00 a	6.38 ± 0.15 c	13.35 ± 0.31 bc	47.79
40 min	14.60 ± 0.31 bc	13.35 ± 0.15 a	6.38 ± 0.41 c	13.97 ± 0.00 a	45.65
60 min	15.49 ± 0.56 ab	13.08 ± 0.41 a	9.15 ± 0.27 a	13.61 ± 0.56 ab	67.19
121 °C					
3 min	16.03 ± 0.56 a	12.10 ± 0.54 b	5.22 ± 0.15 de	12.90 ± 0.00 c	40.44
6 min	15.94 ± 0.15 a	11.83 ± 0.46 b	7.14 ± 0.94 b	13.61 ± 0.15 ab	52.42
9 min	15.49 ± 0.41 ab	12.36 ± 0.27 b	5.53 ± 0.40 d	14.06 ± 0.15 a	39.32
143 °C					
20 s	14.69 ± 1.27 bc	10.58 ± 0.31 c	4.50 ± 0.31 f	11.20 ± 0.31 e	40.18
40 s	15.40 ± 0.15 ab	11.69 ± 0.67 b	4.19 ± 0.13 f	12.10 ± 0.00 d	34.63
60 s	15.85 ± 0.93 a	11.74 ± 0.31 b	4.59 ± 0.27 ef	12.18 ± 0.15 d	37.68
Raw	13.61 ± 0.41 c	10.09 ± 0.40 c	3.25 ± 0.27 g	8.97 ± 0.41 f	36.25

Data were expressed as mean ± SD ($n = 3$)

Values in a column marked by the same letters were not statistically different ($p > 0.05$)

It was worth noting that gastric digestion treatment led to an increase in the release of phenolic compounds and flavonoids from food matrix. Regardless of the temperature and time used in the experiments, TPC in all tested samples was increased 1.1- to 1.5-folds as compared with that in undigested soymilk, as shown in Table 1. Compared with the undigested heat-treated soymilks, the increasing percentage of TFC in gastric fractions of the heat-treated soymilks was 4.48–31.10 %, with the lowest in 95 °C and 40 min treatment and the highest in 143 °C and 20 s treatment (Table 2). However, raw-gastric-digested soymilk showed the highest increase by 51.80 % in TFC. Rodríguez-Roque et al. [8] also found that TPC (analysed by HPLC) and TFC of soymilk were increased by 70 and 33 % after gastric digestion, respectively. Since few researches were focused on in vitro digestion of soymilk, it was impossible to compare with more literatures. However, in other food materials, Tagliazucchi et al. [12] observed

an increase of approximately 21.93 and 77.16 % of TPC and TFC respectively in grape after the simulated stomach digestion. Liyana-Pathirana and Shahidi [27] reported that the crude phenolic extracts of soft and hard wheat were increased 1.25- to 2.5- and 1.7- to 2.3-folds after digestion. Similar increase of TPC and TFC after gastric digestion was also reported by Bouayed et al. [28]. The increase of phenolic compounds content was mainly attributed to the acidic pH and enzymatic activity of gastric digestion, which induced the hydrolysis of phenoic compounds. Moreover, the extraction of free and esterified phenolic compounds may be enhanced under the acidic condition. Besides, it is believable that polyphenols are available to complex with proteins. However, these polyphenol–protein complexes are subjected to the influence of digestion and consequently release polyphenols [10]. In this way, the polyphenols in gastric digestion fractions were reasonably increased.

TPC in the duodenal fraction was found to vary from 622.55 to 697.79 mg GE/L, as shown in Table 1, significantly higher than those in the gastric digestion fraction and undigested soymilks. On the other hand, TPC in the dialysed fraction (238.67–321.26 mg GE/L) was significantly lower than those in the gastric digestion fraction and the duodenal fraction, whereas higher or equal to those in undigested soymilk. However, compared with gastric digestion, pancreatin digestion increased the total polyphenols and flavonoids in grapes by 20.24 and 43.17 %, respectively [12]. In Table 2, TFC in the dialysed fraction was 2.35–9.15 mg CE/L, dramatically decreased in comparison with the undigested or other digested solutions. Similarly, Argyri et al. [29] revealed that phenolic compounds in dialysates of all the tested red wine were dramatically lower than those in the respective undigested samples. Loss of flavonols in chokeberry juice after pancreatic digestion varied between 15.5–29.6 % [11]. Decreases of TPC and TFC in the dialysates of apples were also reported [28]. The decrease of polyphenols in the dialysates may due to the low solubility or large molecular weight of polyphenols. According to previous report, polyphenols may interact with polyphenols and other compounds forming complexes with low solubility or large molecular weight. Since these complexes cannot cross the dialysis membrane, the absorption of phenols was reduced [8]. Furthermore, Scalbert and Williamson [30] reported that the absorption of phenolic compounds in the gut was related to their molecular weight.

In the present research, after the heat treatments (such as 95 °C and 40 min and 95 °C and 60 min, 121 °C and 3 min and 121 °C and 9 min, 121 °C and 9 min and 143 °C and 60 s), there were no significant differences in TPC among samples when tested the undigested heat-treated samples, gastric digestion fractions and duodenal fractions; however, there were significant differences among the dialysed fractions. This suggests that the influence of temperatures on the degradation and conversion of polyphenols or polyphenol–protein complexes is possibly temperature-dependent. The formerly existed polyphenols or their complexes with other compounds were disrupted at various levels depending on the temperature applied. However, the newly formed polyphenols or complexes were different from the existed ones in solubilities or molecular weights which determine their permeability across the dialysis membrane.

The bioaccessibilities of total phenolics and total flavonoids are displayed in Tables 1 and 2. The term bioaccessibility of TPC and TFC was used to represent the amount of total phenolics and total flavonoids released from soymilk and then pass through the intestinal barrier and consequently to be metabolized. In the present study, the bioaccessibilities of TPC ranged between 97.27 and 125.14 % with the lowest in the raw soymilk and the highest in 121 °C and 9 min treated soymilk, indicating that thermal treatment led to an increase of bioaccessibility of TPC. On the other hand, total flavonoids showed various bioaccessibilities from 34.63 to 67.19 % depending on treatments. Except for 143 °C and 40 s, all the other heat treatments increased the bioaccessibility of TFC. The bioaccessibilities of TPC and TFC in a commercial soymilk were 20 and 16 %, respectively [8], whereas Akillioglu and Karakaya [10] reported the bioaccessibilities from 19.76–39.52 % for TPC and 1.26–4.39 % for TFC, depending on the treatments and beans used. The higher bioaccessibilities of TPC and TFC in the present study may attribute to the soybean variety. Compared with 95 and 121 °C treatments, the 143 °C treated soymilks have lower TPC and TFC in the dialysates in most cases even though not significant. In the present study, temperature did not show regular effect on TPC and TFC; however, the 95 °C and 60 min and 121 °C and 6/9 min treated soymilks showed relatively higher TPC and TFC in dialysed fractions as well as the acceptable bioaccessibilities of TPC and TFC, and thereby, these treatments could be the reasonable perfect choice for the production of soymilk with higher quality.

Soybean isoflavones

As an important process of production of various soy-based foods, thermal treatment affects both the content and profile of isoflavones [1, 14, 25, 31, 32]. Results of the present study showed that the total soybean isoflavones of the undigested soymilks varied from 128.11 to 140.09 mg/L (Table 3). Significant increase in total isoflavones content was found in the 95 °C and 40/60 min and 121 °C and 3/6/9 min treated undigested soymilks. In raw and heat-treated soymilks, daidzein derivates (daidzein, daidzin, acetyldaidzin and malonyldaidzin) were proven as the dominant isoflavones, followed by genistein and glycitein derivates. Besides the change of total isoflavones, the profile of

Table 3 Contents of isoflavones of the heat-treated-undigested soymilks (mg/L)

	Aglycones	Glucosides	AceGlu	MalGlu	T-daidzeins	T-genisteins	T-glyciteins	Total isoflavones
95 °C								
20 min	26.57 ± 1.06 e	48.70 ± 0.72 de	3.93 ± 0.05 a	48.03 ± 0.67 b	66.99 ± 0.46 d	45.02 ± 0.06 c	15.21 ± 0.25 d	127.22 ± 0.48 c
40 min	28.31 ± 1.10 e	64.82 ± 0.99 c	3.57 ± 0.04 b	37.74 ± 0.01 f	70.83 ± 1.13 b	47.81 ± 0.69 ab	15.80 ± 0.25 c	134.44 ± 2.04 b
60 min	33.73 ± 1.62 d	75.19 ± 1.36 b	2.86 ± 0.07 d	28.31 ± 0.77 h	73.35 ± 1.03 a	49.61 ± 1.03 a	17.13 ± 0.26 b	140.09 ± 2.06 a
121 °C								
3 min	41.42 ± 0.18 b	47.49 ± 0.95 e	3.43 ± 0.03 b	43.75 ± 0.49 cd	72.24 ± 0.47 ab	47.94 ± 0.80 ab	15.92 ± 0.27 c	136.09 ± 1.48 ab
6 min	51.10 ± 2.63 a	49.41 ± 0.09 d	3.25 ± 0.04 c	31.07 ± 0.17 g	70.17 ± 0.81 bc	47.55 ± 1.41 ab	17.11 ± 0.20 b	134.83 ± 2.35 b
9 min	27.86 ± 0.67 e	77.01 ± 1.30 a	3.51 ± 0.08 b	31.39 ± 0.43 g	71.23 ± 1.09 b	48.93 ± 0.61 a	19.60 ± 0.33 a	139.76 ± 1.97 a
143 °C								
20 s	51.68 ± 1.57 a	31.71 ± 0.67 h	2.15 ± 0.08 f	42.83 ± 0.41 d	68.65 ± 1.23 cd	46.11 ± 1.19 bc	13.61 ± 0.23 e	128.38 ± 2.56 c
40 s	52.93 ± 0.30 a	32.51 ± 0.65 h	2.58 ± 0.11 e	40.75 ± 0.63 e	68.38 ± 0.57 cd	46.28 ± 0.91 bc	14.12 ± 0.11 e	128.77 ± 1.54 c
60 s	35.97 ± 2.11 cd	44.35 ± 1.69 f	3.49 ± 0.17 b	44.43 ± 1.49 c	67.49 ± 2.63 d	45.18 ± 2.19 c	15.57 ± 0.65 cd	128.24 ± 5.44 c
Raw	38.52 ± 1.78 c	35.65 ± 0.20 g	2.03 ± 0.11 f	51.91 ± 0.56 a	68.31 ± 0.64 cd	46.21 ± 1.15 bc	13.59 ± 0.22 e	128.11 ± 1.75 c

Data were expressed as mean ± SD ($n = 3$)

Values in a column marked by the same letters were not statistically different ($p > 0.05$)

For the calculation of total content of glucosides, acetylglucosides and malonylglucosides, content of glucosides, acetylglucosides and malonylglucosides was conversed to their corresponding aglycones based on the differences in molecular weights. Glucosides = (daidzin*254)/416 + (glycitin*284)/446 + (genistin*270)/432; acetylglucosides = (acetyldaidzin*254)/458 + (acetylglycitin*284)/489 + (acetylgenistin*270)/474; malonylglucosides = (malonyldaidzin*254) /502 + (malonylglycitin*284)/532 + (malonylgenistin*270)/518

AceGlu acetylglucosides, *MalGlu* malonylglucosides, *T-daidzeins* total daidzeins, *T-genisteins* total genisteins, *T-glyciteins* total glyciteins

Table 4 Changes in isoflavone contents of the gastric digestion fractions of the heat-treated and in vitro-digested soymilks (mg/L)

	Aglycones	Glucosides	AceGlu	MalGlu	T-daidzeins	T-genisteins	T-glyciteins	Total isoflavones
95 °C								
20 min	20.00 ± 0.23 g	53.77 ± 1.08 c	3.01 ± 0.11 bc	43.32 ± 1.33 b	63.63 ± 0.45 cd	41.93 ± 0.55 c	14.54 ± 0.16 cd	120.10 ± 1.13 b
40 min	26.83 ± 1.98 e	65.03 ± 3.44 b	3.15 ± 0.16 b	38.36 ± 0.97 c	69.74 ± 1.69 a	47.41 ± 0.64 a	16.21 ± 0.56 b	133.36 ± 2.80 a
60 min	23.36 ± 0.59 f	76.95 ± 2.88 a	2.77 ± 0.09 d	28.64 ± 1.69 d	68.44 ± 2.63 ab	47.12 ± 1.77 a	16.16 ± 0.07 b	131.72 ± 4.40 a
121 °C								
3 min	32.76 ± 0.01 c	43.12 ± 1.46 d	3.11 ± 0.02 b	39.63 ± 0.06 c	62.50 ± 0.11 cd	42.03 ± 1.27 c	14.10 ± 0.23 de	118.63 ± 1.47 b
6 min	36.35 ± 1.03 b	51.17 ± 1.10 c	2.88 ± 0.05 cd	28.26 ± 0.63 d	61.54 ± 1.45 d	41.88 ± 0.95 c	15.23 ± 0.26 c	118.65 ± 2.64 b
9 min	19.87 ± 0.08 g	75.78 ± 0.83 a	3.56 ± 0.01 a	30.46 ± 0.36 d	65.18 ± 0.61 bc	46.10 ± 0.60 ab	18.39 ± 0.07 a	129.67 ± 1.28 a
143 °C								
20 s	30.06 ± 0.58 d	37.02 ± 1.02 e	2.42 ± 0.25 e	50.08 ± 1.65 a	62.83 ± 1.39 cd	43.48 ± 0.69 bc	13.27 ± 0.56 f	119.58 ± 2.60 b
40 s	31.24 ± 2.46 cd	43.49 ± 1.85 d	3.07 ± 0.17 bc	51.57 ± 2.24 a	67.89 ± 2.52 ab	46.64 ± 2.36 a	14.83 ± 0.61 cd	129.37 ± 5.49 a
60 s	37.12 ± 2.77 b	45.59 ± 0.22 d	3.50 ± 0.07 a	43.64 ± 0.31 b	67.62 ± 1.72 ab	46.12 ± 1.73 ab	16.11 ± 0.53 b	129.85 ± 2.94 a
Raw	41.31 ± 1.30 a	36.25 ± 2.40 e	0.49 ± 0.01 f	42.68 ± 3.32 b	63.66 ± 3.03 cd	43.67 ± 2.39 bc	13.40 ± 0.71 ef	120.73 ± 6.00 b

Data were expressed as mean ± SD ($n = 3$)

Values in a column marked by the same letters were not statistically different ($p > 0.05$)

For the calculation of total content of glucosides, acetylglucosides and malonylglucosides, content of glucosides, acetylglucosides and malonylglucosides was conversed to their corresponding aglycones based on the differences in molecular weights. Glucosides = (daidzin*254)/416 + (glycitin*284)/446 + (genistin*270)/432; acetylglucosides = (acetyldaidzin*254)/458 + (acetylglycitin*284)/489 + (acetylgenistin*270)/474; malonylglucosides = (malonyldaidzin*254) /502 + (malonylglycitin*284)/532 + (malonylgenistin*270)/518

AceGlu acetylglucosides, *MalGlu* malonylglucosides, *T-daidzeins* total daidzeins, *T-genisteins* total genisteins, *T-glyciteins* total glyciteins

isoflavones was also significantly influenced by the thermal treatment. The major isoflavones in raw soymilk existed in the form of malonylglucoside isoflavones, whereas the dominant isoflavone existed in the forms of glucosides in the 95 °C and 40/60 min and 121 °C and 3/9 min treated samples, aglycones in the 121 °C and 6 min and 143 °C and 20/40 s treated soymilks, glucosides and malonylglucosides in the 95 °C and 20 min and 143 °C and 60 s treated soymilks (Table 3). Previous research revealed that hot aqueous extraction of soymilk resulted in formation of glucosides [33]. Overall, decrease of malonylglucosides was accompanied by increase of aglycones, glucosides or acetylglucosides. Decrease of malonylglucosides mainly led to increase of glucosides in the heat-treated soymilks except for the 143 °C and 20/40 s treated soymilk which led to increase of aglycones (Table 3). Consistent results were also reported by Xu and Chang [25], who found that thermal process resulted in decrease of maloylglucosides and increase of aglycones and glucosides in yellow and black soybeans. Though contents of individual isoflavones in the heat-treated soymilks were significantly influenced by thermal treatment in the most samples, contents of total and subtotal isoflavones were only slightly changed, indicating that the effect of heat on isoflavones was mainly on conversion instead of degradation. For the pasteurization (95 °C and 40/60 min)-treated and in-container sterilization (121 °C and 3/6/9 min)-treated soymilks, subtotal and total isoflavones were increased to a certain content. But for the UHT (143 °C and 20/40/60 s)-treated samples, slight or no change was found basically. This demonstrated that UHT treatment took no effect on release of isoflavones from food matrix or conversion from other compounds.

The concentration and profile of soybean isoflavones of the thermal-treated soymilks were remarkably different after in vitro gastrointestinal digestion. After gastric digestion, total soybean isoflavone content of all the tested samples was decreased by 0.80–12.83 %, except for the 143 °C and 40 s and 143 °C and 60 s treated soymilks, which were slightly increased by 0.46 and 1.26 %, respectively (Table 4). Comparing with the references reported by different authors, there are controversial results about the effect of gastric digestion on isoflavones. Sanz and Luyten [34] found that the acidic stomach incubation decreased the total amount of soybean isoflavones. However, Rodríguez-Roque et al. [8] found that the concentration of total soybean isoflavones was significantly increased by 22 % after gastric digestion and proposed that the acidic conditions of gastric digestion improved release of isoflavones from food matrix. The controversial results possibly because Rodríguez-Roque et al. [8] only determined the aglycone and glucoside isoflavones whereas Sanz and Luyten [34] and present study also determined acetylglucoside or/and malonylglucoside isoflavones. Since aglycone and glucoside isoflavones could be increased easily by hydrolysis of acetylglucoside and malonylglucoside isoflavones under mild acidic conditions [35]. Results in the present study confirmed that glucoside isoflavones were increased after gastric digestion (Tables 3, 4). Regardless of the temperature and time applied, the decrease of aglycones was accompanied with the increase of glucosides after gastric digestion, indicating that effect of gastric digestion on individual isoflavones was dependent on forms of isoflavones. Similarly, Sanz and Luyten [34] also reported that aglycones were much sensitive to gastric digestion, who also found that the concentration and profile of isoflavones showed no significant difference under the both enzymatic and non-enzymatic conditions in vitro gastric digestion [34]. Furthermore, Piskula et al. [36] believed that although as the necessary enzyme for isoflavone hydrolysis, there was no glucosidase in stomach. Based on these findings, it is reasonably believed that the effect of gastric digestion on isoflavone was mainly dependent on pH and food matrix instead of enzyme.

The amounts of the total soybean isoflavones in the duodenal fraction were found significantly lower than the undigested soymilks or gastric digestion fractions. Except for 121 °C and 3 min treatment, heat treatment significantly increased content of total isoflavones in duodenal fractions (Table 5). Total and individual isoflavones in the dialysed fractions were remarkably decreased as compared with the gastric digestion and duodenal fractions (Tables 4, 5 and 6). The concentrations of total isoflavones in dialysed fractions varied between 46.38 and 57.60 mg/L, 59.16–65.60, 55.57–65.15 and 43.78–57.16 % lower than the undigested soymilks, gastric digestion fractions and duodenal fractions, respectively. Analysis revealed that the bioaccessibility of the total soybean isoflavones of the heat-treated soymilks reached 34.40–41.22 %, depending on the temperature and time applied (Table 7). To the best of our knowledge, this result was the first report on the bioaccessibility of total isoflavones (12 isoflavones), so it is difficult to compare with literatures. However, Sanz and Luyten [34] reported that in soy germ extract, total isoflavones after intestinal digestion were increased by 16.6 % than the undigested treatment. The same study also showed that the increase of glucosides in the intestinal digestion samples was companied with the decrease of acetylglucosides [34]. The higher bioaccessibilities of isoflavones reported by Sanz and Luyten [34] probably were due to the difference in food matrix, since the same authors found that isoflavones in the CMC custards showed lower bioaccessibilities than starch custards. For the subtotal individual isoflavones, daidzein derivates showed the highest bioaccessibilities from 36.99 to 44.14 % (Table 7). In other words, daidzein and its derivates were more available for body absorption than other forms of isoflavones. Similar research also revealed that daidzein was more accessible

Table 5 Changes in isoflavone contents of the duodenal fractions of the heat-treated and in vitro-digested soymilks (mg/L)

	Aglycones	Glucosides	AceGlu	MalGlu	T-daidzeins	T-genisteins	T-glyciteins	Total isoflavones
95 °C								
20 min	20.55 ± 1.01 ef	43.51 ± 0.48 c	2.82 ± 0.05 a	34.94 ± 1.11 b	53.52 ± 0.68 abc	36.63 ± 0.48 bc	11.67 ± 0.22 b	101.83 ± 1.31 b
40 min	19.36 ± 1.62 f	60.70 ± 4.27 a	1.98 ± 0.11 c	21.13 ± 1.93 e	53.71 ± 2.64 abc	37.58 ± 1.77 ab	11.87 ± 0.44 b	103.17 ± 4.82 ab
60 min	21.68 ± 1.04 e	51.26 ± 1.76 b	2.49 ± 0.17 b	26.21 ± 2.25 d	53.11 ± 2.40 abc	36.85 ± 1.69 abc	11.67 ± 0.36 b	101.64 ± 4.43 b
121 °C								
3 min	28.58 ± 0.97 c	35.30 ± 2.03 d	2.37 ± 0.13 b	29.51 ± 1.04 c	50.73 ± 1.76 c	34.35 ± 1.59 d	10.68 ± 0.43 c	95.76 ± 3.72 c
6 min	36.71 ± 0.58 a	44.16 ± 0.83 c	2.37 ± 0.07 b	25.05 ± 0.39 d	55.92 ± 0.47 a	39.09 ± 0.45 a	13.28 ± 0.32 a	108.28 ± 1.15 a
9 min	17.29 ± 0.80 g	58.87 ± 0.41 a	2.41 ± 0.08 b	23.88 ± 0.15 de	52.05 ± 0.59 bc	36.82 ± 0.55 bc	13.58 ± 0.30 a	102.45 ± 1.44 b
143 °C								
20 s	26.99 ± 0.53 cd	31.53 ± 1.61 ef	2.34 ± 0.06 b	41.25 ± 3.29 a	54.13 ± 2.78 ab	37.24 ± 1.79 abc	10.73 ± 0.44 c	102.11 ± 5.02 b
40 s	26.45 ± 1.79 d	33.98 ± 1.25 de	2.68 ± 0.08 a	39.34 ± 0.75 a	53.99 ± 0.7 abc	37.55 ± 0.18 ab	10.90 ± 0.26 c	102.44 ± 0.72 b
60 s	31.56 ± 1.03 b	35.84 ± 0.82 d	2.51 ± 0.06 b	32.42 ± 1.00 bc	53.38 ± 1.27 abc	37.18 ± 0.73 abc	11.77 ± 0.35 b	102.33 ± 1.81 b
Raw	35.14 ± 0.86 a	29.04 ± 1.04 f	1.76 ± 0.08 d	30.25 ± 1.74 c	51.54 ± 1.41 bc	35.05 ± 0.8 cd	9.60 ± 0.16 d	96.19 ± 2.21 c

Data were expressed as mean ± SD ($n = 3$)

Values in a column marked by the same letters were not statistically different ($p > 0.05$)

For the calculation of total content of glucosides, acetylglucosides and malonylglucosides, content of glucosides, acetylglucosides and malonylglucosides was conversed to their corresponding aglycones based on the differences in molecular weights. Glucosides = (daidzin*254)/416 + (glycitin*284)/446 + (genistin*270)/432; acetylglucosides = (acetyldaidzin*254)/458 + (acetylglycitin*284)/489 + (acetylgenistin*270)/474; malonylglucosides = (malonyldaidzin*254) /502 + (malonylglycitin*284)/532 + (malonylgenistin*270)/518

AceGlu acetylglucosides, *MalGlu* malonylglucosides, *T-daidzeins* total daidzeins, *T-genisteins* total genisteins, *T-glyciteins* total glyciteins

Table 6 Changes in isoflavone contents of the dialysate fractions of the heat-treated and in vitro-digested soymilks (mg/L)

	Aglycones	Glucosides	AceGlu	MalGlu	T-daidzeins	T-genisteins	T-glyciteins	Total isoflavones
95 °C								
20 min	3.98 ± 0.07 g	26.62 ± 0.43 b	1.30 ± 0.02 a	20.07 ± 0.58 b	29.28 ± 0.53 b	16.59 ± 0.25 b	6.08 ± 0.10 cd	51.95 ± 0.86 b
40 min	4.54 ± 0.08 f	28.46 ± 2.24 b	0.73 ± 0.05 e	12.74 ± 0.28 g	26.20 ± 1.45 d	14.59 ± 0.74 cd	5.68 ± 0.03 ef	46.47 ± 2.23 d
60 min	4.06 ± 0.10 g	37.26 ± 0.99 a	0.89 ± 0.04 cd	14.04 ± 0.76 ef	31.61 ± 1.09 a	17.83 ± 0.53 a	6.82 ± 0.09 b	56.25 ± 1.70 a
121 °C								
3 min	6.65 ± 0.05 d	20.72 ± 1.56 e	1.22 ± 0.05 a	20.05 ± 0.56 b	27.21 ± 1.26 cd	15.35 ± 0.69 c	6.08 ± 0.28 cd	48.64 ± 2.23 cd
6 min	8.47 ± 0.46 b	23.94 ± 2.06 c	0.93 ± 0.01 cd	13.05 ± 0.78 fg	26.27 ± 1.48 d	14.33 ± 0.91 d	5.79 ± 0.35 de	46.38 ± 2.69 d
9 min	4.14 ± 0.25 fg	37.12 ± 0.90 a	1.25 ± 0.08 a	15.09 ± 0.30 de	31.44 ± 0.52 a	18.43 ± 0.35 a	7.74 ± 0.17 a	57.60 ± 1.04 a
143 °C								
20 s	7.50 ± 0.24 c	21.70 ± 0.90 de	1.21 ± 0.02 a	21.48 ± 1.08 a	29.26 ± 0.24 a	16.38 ± 0.20 b	6.24 ± 0.22 c	51.88 ± 0.54 b
40 s	6.05 ± 0.44 e	21.60 ± 0.46 de	1.05 ± 0.06 b	19.46 ± 0.95 b	27.42 ± 0.40 b	15.33 ± 0.32 c	5.41 ± 0.05 f	48.16 ± 0.76 cd
60 s	7.54 ± 0.28 c	23.08 ± 0.23 cd	0.98 ± 0.05 bc	16.39 ± 0.30 c	27.31 ± 0.43 cd	15.13 ± 0.27 cd	5.54 ± 0.17 ef	47.99 ± 0.84 cd
Raw	11.4 ± 0.09 a	21.53 ± 0.20 de	0.88 ± 0.06 d	15.70 ± 0.17 cd	28.33 ± 0.02 cd	15.31 ± 0.11 c	5.86 ± 0.07 de	49.50 ± 0.06 bc

Data were expressed as mean ± SD ($n = 3$)

Values in a column marked by the same letters were not statistically different ($p > 0.05$)

For the calculation of total content of glucosides, acetylglucosides and malonylglucosides, content of glucosides, acetylglucosides and malonylglucosides was conversed to their corresponding aglycones based on the differences in molecular weights. Glucosides = (daidzin*254)/416 + (glycitin*284)/446 + (genistin*270)/432; acetylglucosides = (acetyldaidzin*254)/458 + (acetylglycitin*284)/489 + (acetylgenistin*270)/474; malonylglucosides = (malonyldaidzin*254) /502 + (malonylglycitin*284)/532 + (malonylgenistin*270)/518

AceGlu acetylglucosides, *MalGlu* malonylglucosides, *T-daidzeins* total daidzeins, *T-genisteins* total genisteins, *T-glyciteins* total glyciteins

than genistin for potential uptake [39]. For the individual isoflavones, aglycone isoflavones in the dialysate fractions of heat-treated soymilks showed the maximum decrease in content as compared with the undigested soymilks, by over 70 %; especially, genistein showed the highest decrease by 87 % (Table 6). These results indicated that aglycones were much difficult to be absorbed than other forms of isoflavones. In addition, calculation of bioaccessibility showed that glucosides had the highest bioaccessibility, while aglycones had the lowest, consistent with the previous researches. Rodríguez-Roque et al. [8] also reported that the bioaccessibilities of daidzin and genistin (27 and 53 %) were higher than daidzein and genistein (17 and 17 %). However, Zubik and Meydani [37] did not find difference in the body absorption when daidzein and genistein were consumed either in forms of aglycone or glucoside. Izumi et al. [38] believed that aglycones had higher bioaccessibilities. The higher percentage of daidzein and genistein after intestinal digestion than daidzin and genistin indicates the higher bioaccessibilities of daidzein and genistein [34]. These differences might be resulted from the in vitro test (in the present research and the work of Rodríguez-Roque et al. [8]) and in vivo test (in the work of Zubik and Meydani [37] and Izumi et al. [38]). The in vivo bioaccessibility of soybean

Table 7 Bioaccessibilities of isoflavones of the heat-treated soymilks after gastrointestinal digestion (%)

	Aglycones	Glucosides	AceGlu	MalGlu	T-daidzeins	T-genisteins	T-glyciteins	Total isoflavones
95 °C								
20 min	14.97	54.66	32.99	41.78	43.71	39.96	36.86	40.84
40 min	16.05	43.91	20.39	33.75	36.99	35.95	30.51	34.57
60 min	12.04	49.56	31.14	49.60	43.10	39.80	35.93	40.16
121 °C								
3 min	16.06	43.64	35.43	45.83	37.67	38.19	32.03	35.74
6 min	16.57	48.45	28.56	42.01	37.43	33.83	30.14	34.40
9 min	14.87	48.20	35.68	48.07	44.14	39.49	37.66	41.22
143 °C								
20 s	14.50	68.43	56.13	50.14	42.63	45.83	35.52	40.41
40 s	11.42	66.45	40.76	47.75	40.10	38.32	33.13	37.40
60 s	20.96	52.04	28.07	36.89	40.47	35.61	33.49	37.42
Raw	29.60	60.38	43.14	30.24	41.48	43.09	33.13	38.64

For the calculation of total content of glucosides, acetylglucosides and malonylglucosides, content of glucosides, acetylglucosides and malonylglucosides was conversed to their corresponding aglycones based on the differences in molecular weights. Glucosides = (daidzin*254)/416 + (glycitin*284)/446 + (genistin*270)/432; acetylglucosides = (acetyldaidzin*254)/458 + (acetylglycitin*284)/489 + (acetylgenistin*270)/474; malonylglucosides = (malonyldaidzin*254) /502 + (malonylglycitin*284)/532 + (malonylgenistin*270)/518

AceGlu acetylglucosides, *MalGlu* malonylglucosides, *T-daidzeins* total daidzeins, *T-genisteins* total genisteins, *T-glycitein* total glyciteins

Table 8 Effect of in vitro gastrointestinal digestion on DPPH radical scavenging activity of the heat-treated soymilks (μmol TE/L)

	Gastric digestion	Intestinal digestion		Undigested
		Duodenal fraction	Dialysed fraction	
95 °C				
20 min	161.18 ± 2.31 d	155.43 ± 3.26 e	27.99 ± 0.25 cd	123.34 ± 3.40 cde
40 min	171.37 ± 3.26 c	157.71 ± 3.02 de	27.39 ± 1.81 d	120.09 ± 1.95 e
60 min	170.61 ± 3.60 c	153.92 ± 1.42 ef	30.53 ± 1.23 c	129.09 ± 2.46 ab
121 °C				
3 min	173.76 ± 0.56 c	165.73 ± 7.20 cd	28.91 ± 1.91 cd	131.91 ± 4.93 ab
6 min	175.06 ± 2.28 bc	174.95 ± 4.42 ab	33.68 ± 1.60 ab	130.39 ± 4.87 ab
9 min	176.90 ± 5.29 abc	176.36 ± 8.80 a	35.09 ± 2.61 a	134.18 ± 0.99 a
143 °C				
20 s	171.70 ± 0.99 c	146.00 ± 6.31 fg	31.02 ± 0.98 bc	121.17 ± 3.29 e
40 s	180.59 ± 2.28 ab	162.59 ± 3.74 cde	29.67 ± 0.86 cd	127.68 ± 2.48 bcd
60 s	181.02 ± 7.81 ab	166.93 ± 1.17 bc	30.53 ± 1.31 c	128.65 ± 1.79 abc
Raw	182.00 ± 2.17 a	139.17 ± 1.67 g	31.08 ± 2.31 bc	122.37 ± 0.33 de

Data were expressed as mean ± SD ($n = 3$)

Values in a column marked by the same letters were not statistically different ($p > 0.05$)

Table 9 Effect of in vitro gastrointestinal digestion on FRAP of the heat-treated soymilks (mmol FE/L)

	Gastric digestion	Intestinal digestion		Undigested
		Duodenal fraction	Dialysed fraction	
95 °C				
20 min	0.97 ± 0.03 c	1.27 ± 0.11 b	0.57 ± 0.01 b	0.89 ± 0.01 c
40 min	1.00 ± 0.02 bc	1.18 ± 0.03 bcd	0.54 ± 0.04 bc	0.90 ± 0.01 bc
60 min	1.06 ± 0.07 bc	1.14 ± 0.07 bcd	0.61 ± 0.05 a	0.90 ± 0.01 bc
121 °C				
3 min	0.98 ± 0.07 c	1.21 ± 0.08 bc	0.62 ± 0.04 a	0.90 ± 0.01 bc
6 min	1.15 ± 0.04 a	1.07 ± 0.05 d	0.47 ± 0.01 de	0.92 ± 0.02 b
9 min	1.22 ± 0.04 a	1.26 ± 0.07 bc	0.51 ± 0.01 cd	0.95 ± 0.03 a
143 °C				
20 s	1.16 ± 0.05 a	1.12 ± 0.05 cd	0.38 ± 0.02 f	0.77 ± 0.01 f
40 s	1.22 ± 0.02 a	1.40 ± 0.12 a	0.44 ± 0.03 e	0.82 ± 0.00 e
60 s	1.15 ± 0.01 a	1.22 ± 0.08 bc	0.46 ± 0.01 e	0.85 ± 0.03 d
Raw	1.06 ± 0.03 b	1.20 ± 0.02 bcd	0.45 ± 0.01 e	0.80 ± 0.01 e

Data were expressed as mean ± SD ($n = 3$)

Values in a column marked by the same letters were not statistically different ($p > 0.05$)

isoflavones is affected by many factors such as food matrix and the gut microbiota. Food constituents such as fats and proteins might form micelles with isoflavones and subsequently enhances the bioaccessibility of aglyones especially genistein [39]. Besides, food constituents play important roles in composition of gut microbiota which in turn has a strong effect on the bioaccessibility of isoflavones.

Antioxidant activities

It was well known that soymilk is a rich source of antioxidants such as phenolic compounds and flavonoids. In the present study, the antioxidant activities of the heat-treated soymilks after the simulate stomach and intestinal digestion were determined by DPPH radical scavenging activity, FRAP and ORAC methods. Results were displayed in Tables 8, 9 and 10. DPPH radical scavenging activity in the undigested heat-treated soymilks ranged between 120.09 and 134.18 μmol/L (Table 8). As compared to raw soymilk, a significant increase on DPPH radical scavenging activity was found in 95 °C and 60 min, 121 °C and 3/6/9 min and 143 °C and 60 s treated soymilks. In the case of 95 and 143 °C, extended heating time induced a significant increase on DPPH radical scavenging activity. FRAP of the most heat-treated soymilks was significantly increased except for 143 °C and 20 s and 143 °C and 40 s treatments (Table 9). No difference was found in FRAP among the 95 °C treated soymilks, whereas FRAP increased with prolonged treat time in 121 and 143 °C treated soymilks. As for ORAC, the soymilks of 95 °C and 20 min treatment and 121 °C and 6 min treatment showed an increase in ORAC; however, 95 °C and 40/60 min, 121 °C and 3/9 min and

Table 10 Effect of in vitro gastrointestinal digestion on ORAC of the heat-treated soymilks (mmol TE/L)

	Gastric digestion	Intestinal digestion		Undigested
		Duodenal fraction	Dialysed fraction	
95 °C				
20 min	16.24 ± 0.12 cd	14.67 ± 0.21 a	7.01 ± 0.28 bc	9.55 ± 0.27 a
40 min	16.85 ± 0.29 abc	10.80 ± 0.14 f	8.09 ± 0.47 a	4.15 ± 0.25 e
60 min	15.61 ± 0.31 d	12.73 ± 0.16 e	7.48 ± 0.03 b	5.76 ± 0.45 d
121 °C				
3 min	17.18 ± 0.40 ab	9.49 ± 0.20 g	4.12 ± 0.12 e	4.19 ± 0.11 e
6 min	13.62 ± 0.62 f	11.05 ± 0.40 f	6.96 ± 0.23 c	8.69 ± 0.31 b
9 min	14.78 ± 0.29 e	13.00 ± 0.32 de	8.35 ± 0.21 a	6.05 ± 0.01 d
143 °C				
20 s	15.61 ± 0.43 d	13.96 ± 0.22 b	7.15 ± 0.32 bc	7.56 ± 0.19 c
40 s	17.40 ± 0.32 a	13.53 ± 0.30 bc	5.76 ± 0.02 d	5.83 ± 0.57 d
60 s	17.17 ± 0.55 ab	13.30 ± 0.43 cd	7.03 ± 0.26 bc	7.51 ± 0.14 c
Raw	16.52 ± 0.13 bc	13.88 ± 0.42 b	6.17 ± 0.27 d	7.72 ± 0.12 c

Data were expressed as mean ± SD ($n = 3$)

Values in a column marked by the same letters were not statistically different ($p > 0.05$)

143 °C and 20 s treatment showed decreases in ORAC (Table 10). Xu and Chang [1] reported that traditional (100 °C 20 min) heat-treated soymilks showed significantly increase on DPPH radical scavenging activity and FRAP but decrease in ORAC, whereas the UHT (143 °C and 60 s)-treated soymilks showed significant increase on DPPH radical scavenging activity, FRAP and ORAC. The differences in results may due to the difference in soybean variety, temperature and assay method applied. As hydrogen donor, the content of isoflavones determines the antioxidant activities of foods to some extent. It was found that DPPH radical scavenging activity was significantly correlated with TFC, subtotal daidzein, subtotal genistein, subtotal glycitein and total isoflavones ($p < 0.05$, $r = 0.363$, 0.412, 0.372, 0.630 and 0.516, respectively), and FRAP was significantly correlated with TPC, TFC, daidzin, genistin, glycitin, subtotal daidzein, subtotal genistein, subtotal glycitein and total isoflavone ($p < 0.01$, $r = 0.480$, 0.806, 803, 0.810, 0.808, 0.475, 0.464, 0.864 and 0.654, respectively). The correlation of DPPH and FRAP with bioactive compounds revealed that heat treatment plays a role on antioxidant activities (DPPH radical scavenging activity and FRAP) of soymilks via influencing the content of bioactive compounds. Research conducted by Malenčić et al. [40] also indicated that soybeans poor in phenolic compounds had low levels of DPPH radical scavenging activity. Furthermore, the increase in antioxidant activities of heat-treated soymilks may result from the formation and conversion of new compounds such as Maillard reaction products which possess antioxidant activity, or the increase of antioxidant capacity of the existent compounds already.

After gastric digestion, DPPH, FRAP and ORAC determinations proved that the antioxidant activities of the heat-treated soymilks were increased by 30.68–42.70, 9.24–51.44 and 56.75–310 %, respectively, further confirming that the acidic condition during gastric digestion enhanced the release of antioxidants. The DPPH inhibition of soymilk, gooseberry, soft and hard wheat was also enhanced after gastric digestion [8, 27, 41]. Significant decrease of FRAP in three apple varieties after gastric digestion was reported [28]. The increment of DPPH, FRAP and ORAC might be attributed to the increase of the total phenolics and total flavonoids during the gastric digestion which enhanced the release of polyphenols from high molecular weight complexes and food matrix. Moreover, antioxidant compounds such as Millard reaction products (not determined in the present work) might also attribute to the increment of antioxidant activities.

The antioxidant activities measured via DPPH, FRAP and ORAC in the duodenal fractions were much higher than the undigested soymilks. However, the antioxidant activities of bioactive compounds in dialysed fractions were significantly decreased, as shown in Tables 8, 9 and 10. In dialysed fractions, 95 °C and 20/40/60 min treated and 121 °C and 3/6/9 min treated soymilks showed relatively higher antioxidant activities (DPPH, FRAP or ORAC) than raw soymilk, whereas 143 °C has little influence on antioxidant activities. Compared with the undigested soymilks, the DPPH radical scavenging activity and FRAP in the dialysed fractions decreased by 73.85–78.08 and 30.93–50.93 %, respectively. Similarly, Rodríguez-Roque et al. [8] reported that a decrease of 96 and 99 % in DPPH radical scavenging activity inhibition for hydrophilic and lipophilic compounds was observed in the soymilks processed by intestinal digestion. However, Akillioglu and Karakaya [10] found that significantly increase of DPPH inhibition was observed in the dialysed fractions of common beans, whereas decrease was observed in pinto beans. The controversial results indicated the impact of food matrix on the digestion of food. Overall, the decrease of antioxidant capacity of present study was resulted from the markable decrease of flavonoids and isoflavones. Moreover, due to the sensitivity to alkaline pH, polyphenols may be transformed into different structural forms with chemical property changed [11].

Conclusion

Thermal process affects on TPC, TFC and antioxidant activities (DPPH, FRAP and ORAC) as well as the contents and profiles of soybean isoflavones. In comparison with the irregular influence of temperatures on bioactive compounds and antioxidant activities, in vitro gastrointestinal digestion showed significant influence on the contents of bioactive compounds and antioxidant activities. The acidic pH in gastric digestion enhanced the release of phenols, flavonoids and other compounds with antioxidant activity from food matrix and thereby increased the contents of bioactive compounds after simulated stomach digestion. Significant decreases were observed for flavonoids and isoflavones in dialysed fractions which represent the part that can be absorbed and utilized by human. However, total phenolics showed relatively higher bioaccessibility, while daidzein and its derivates were the most bioaccessible isoflavones in soymilk. Glucoside isoflavones showed the highest bioaccessibility followed by malonylglucosides, acetylglucosides and aglycones. The antioxidant activities measured via DPPH, FRAP and ORAC were also significantly influenced by in vitro gastrointestinal digestion. The lowest antioxidant activities were found in dialysed fractions. Overall, heat-treated soymilks at different temperatures and times resulted in significant changes of bioactive compounds and antioxidant activities. Besides the higher bioaccessibility, 95 °C and 60 min treated and 121 °C and 9 min treated soymilks also showed the higher bioactive

compounds contents and antioxidant activities in the dialysed fractions. Though temperatures have no regular effect on the contents of bioactive compounds, heat treatment improved the bioaccessibility of TPC and TFC.

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Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

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