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A value-added cooking process to improve the quality of soybean: Protecting its isoflavones and antioxidant activity

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ABSTRACT

This study investigated the effects of domestic cooking process on the variations of soybean isoflavones, aiming at understanding the conversion of β-glucosides and aglycones isoflavones during the process and the relation with antioxidant activity. It was found that β-glucosides isoflavones was significantly increased from 223.01 (raw) to 727.29 mg/g (frying at 160 °C for 2 min), but boiling showed only a slight increase to 258.14 mg/g. The process for the mixed cooking of soybeans with vegetables was also evaluated, which is quite common in home cuisine. The results showed all bioactive ingredients were aggressively destroyed by over processing, but interestingly, green pepper and kelp exhibited isoflavones generation potentials for soybean. In addition, cooking from 60 to 160 °C for 2 or 5 min, showed a significantly decrease on FRAP. However, in the case of fried soybeans which treated at 120 °C or 160 °C, when extending the heating time to 5 min, their FRAP activity got a significant increase. The present study may provide a practical guidance for healthy soybean cooking, by using frying around 120 °C for 5 min and mixed with some vegetables such as green peppers or kelp.

1. Introduction

Soybeans are rich in dietary isoflavones, which can reduce the incidence of chronic diseases including breast cancer, osteoporosis, and coronary heart disease. Three aglycones, namely genistein, glycitein and daidzein, are mainly known for isoflavone, which are often represented in acetylglucoside, β-glucosides, and malonylglucosides in natural soy foods. Among them, malonylglucosides and β-glucosides account for 68.0–93.0% of total isoflavones content. And various processing techniques are critical for isoflavone composition in soy foods. For instance, under the production of soymilk and tofu, hot water extraction could lead to malonylglucosides hydrolysis and generated more isoflavone β-glucosides; while the fermentation could increase the yield of isoflavone aglycones. In humans, isoflavone glycosides can be metabolized by β-glucosidase in the small intestine to aglycones. In general, isoflavone aglycones are more easily absorbed compared with their β-glucoside forms, and the bioavailability of isoflavone aglycones is higher than its glycosidic forms.

Soybeans are required to multiple processes such as soaking, boiling, roasting, steaming, and/or fermentation before tabled. Such pretreatment can effectively enhance their flavor and palatability, and also improve the bioavailability of bioactives by inactivating anti-nutritional components, such as trypsin inhibitors [1]. But these physicochemical processes with heat, pressure, soaking and fermentation can affect the stability of the nutrients and bioactives in soybeans as well [2]. For instance, cooking can cause 30–60% elimination of polyphenols in soybeans. Especially, the boiling process lost more phenolic compounds than the steaming process [3], while microwave heating compromises approximately 40% of the tocopherol content in soybeans [4]. Besides, soyfood manufacturing process is able to alter the constitution of bioactive compounds as well. For instance, the thermal processing of soybeans can transform its bound phenolic into corresponding soluble/free forms [5]. During the manufacture of tofu or soymilk, the thermal processing transferred malonylglucoside isoflavone conjugates into β-glucoside form, and the fermentation of soy foods can generate more aglycone form than β-glucoside conjugates [2]. Up to now, soybeans are usually cooked with some vegetables (green peppers, celery, kelp and etc.), but whether or not the vegetables mixing affect bioactive compounds in soybeans are still unknown. The

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present work aimed at the investigation on the effects of thermal processing and pressure processing on the bioactive compounds in soybean-dishes mixtures. Particularly, the profile of isoflavone and antioxidant capacity are determined during several cooking stages respecting the raw soybean.

2. Materials and methods

2.1. Chemicals

Daidzein, daidzin, genistein glycitein, and glycitin (>98.0%) were purchased from Beijing Solarbio Science and Technology co. (Beijing, China). 1,3,5-Tri(2-pyridyl)-2,4,6-triazine (TPTZ), rutin, gallic acid, Trolox and Folin-Ciocalteu's phenol reagent were purchased from Aladin Co. Ltd. (Shanghai, China). HPLC grade solvents including acetic acid, acetonitrile, and methanol were provided from Sigma-Aldrich Co. (Shanghai, China). And other reagents and chemicals used were analytical grade. Millipore water was used throughout the experiment.

2.2. Foodstuffs and cooking conditions

Fresh green pepper (*Piper nigrum* L.), celery (*Apium graveolens* L.), kelp (*Saccharina japonica*) and soybean were obtained from local supermarkets in Fuzhou, China. The vegetables were washed and dried, and the soybean and kelp were soaked at room temperature for 24 h; the leaves were removed from the celery, and the seed were removed from the pepper. Each vegetable was cut into small cubes to reduce cooking time. Then, each vegetable is separately mixed with soybean with a ratio of 1:2. The edible blend oil from Dongguan (Guangdong, China) was kept under 4 °C. Two domestic cooking methods (deep frying and boiling) were selected to study the chemical changes of soybean during thermal processing. The parameters such as the ratio (w/w) of heat transfer medium and foodstuff were determined by traditional Chinese recipes. Raw soybean was used as a control. Each group has 60 g soybean. Deep-frying processing employed the soybeans and vegetable cubes by 5:1 proportion at 60 °C, 120 °C, 160 °C for 2 min or 5 min, respectively, while boiling was processed at 100 °C for 5 min. After that, all samples were homogenized immediately using a high speed blender JYL-Y99 (Shangdong, China), and freeze-dried by a freeze-drier, and finally stored in 4 °C refrigerator for further studies.

2.3. Extraction

Samples were extracted following our previous method [6]. Two gram of the dried sample were separately weighed, mixed with 10 ml of acidified methanol (pH 2 with HCl 2 mol/L), and extracted by vibration at 200 rpm for 2 h. The extract was cooled and centrifuged at 8000 *g* for 10 min. The supernatant was collected, and the residues were extracted again using the same conditions. All supernatants were collected, and the solvent was removed with a rotary vacuum evaporator. After that, the residue was dissolved in 2 ml methanol, filtered through a 0.45 μm membrane and 10 μL of filtered extract was injected into chromatographic system for flavonoids quantification.

2.4. Total polyphenol content (TPC) and total flavonoid content (TFC)

Folin-Ciocalteu method was used to determine the TPC content in the sample [6] and the results were expressed as mg Gallic acid equivalent per g dry weight (mg GAE/g DW). TFC analyses were preformed according to a previous study (Chen & Kang, 2014) and the results were expressed as mg rutin equivalent per g dry weight (mg RE/g DW).

2.5. Antioxidant activity

The extract/standards of 20 ml were reacted with 180 μL of ferric-TPTZ reagent, which was made by blending 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$, and 300 mM acetate buffer (pH 3.6) at 10:1:1 (v/v/v), and heated to 37 °C before use. After 20 min of reaction, the absorbance of the mixture was recorded at 593 nm. The standard curve was prepared using $FeSO_4$ ranging from 0.3 mM to 2.4 mM. All experiments were tested in triplicate. Results were expressed as micromole $FeSO_4$ equivalents (FE) antioxidant capacity per gram of dry weight (mmol FE/g DW).

2.6. HPLC analysis of flavonoid content

The HPLC analysis was employed using a Agilent 1260 LC with a diode array detector, and an ODS HYPERSIL column was used for flavonoids separation (5 μm, 250 × 4 mm, Thermo Fisher Scientific Inc., Shanghai, China). HPLC conditions were as follows: Solvent A, 0.1% acetic acid; solvent B, acetonitrile; linear gradient, 5% in B in A (v/v) linearly to 15% B at 22 min, 22% at 28 min, 28% at 35 min, 31% at 38 min, 35% at 45 min, 5% at 50 min. Flow rate was set at 1.0 mL/min; injection volume was 10 μL, and DAD was monitored at 262 nm. Daidzin, glycitin, daidzein, glycitein, and genistein were dissolved in methanol to make 1.0 mg/ml stock solutions. The identification works were accomplished by comparing the retention time with the known standards. Isoflavone content was presented as micrograms of isoflavone per gram of dry weight basis.

2.7. Statistical analysis

All samples obtained from various cooking conditions were extracted and analyzed in triplicate. The data are expressed as mean ± standard deviation. The statistically significant ($P < 0.05$) differences were conducted by analysis of variance (ANOVA) using SPSS software.

3. Results and discussion

3.1. HPLC analysis of isoflavones of selected soybean

Fig. 1 shows the HPLC profile of isoflavones separated from the soybean samples. Although neither acetyl nor malonyl-glycosides of isoflavones were available in this study. Since many authors have used the standard curves for glycoside isoflavones to quantify malonyl and acetyl isoflavones, and the similarities of extinction coefficients can be referred [7–10]. Some of isoflavones including daidzin, daidzein, glycitin, glycitein, genistein, and genistin were authenticated by comparing absorption spectra with standards (Fig. 1A) and others were identified by comparing the retention time according to previous study [7]. Isoflavone standards gave good linear responses at 262 nm for calibration concentrations. Campos et al. [11] evidenced that the derivatization and *O*-linked glycosylation of flavonoids, such as flavonoid 7-O-glucose, did not affect the absorption feature, but increased the retention time. Based on their hydrophobicity, the chromatographic behavior of different isoflavones to protonate the malonyl forms on a reversed-phase column when acid was presented in the mobile phase was as following order: aglycone > acetylglycoside > malonylglycoside > β-glycoside [12,13]. The precisions were determined, and the results are shown in Fig. 1B.

3.2. Effects of heating treatment on TPC, TFC, and isoflavone content

The influence of thermal processing on the flavonoid and phenolic contents in soybean was elucidated in Table 1, and non-thermal

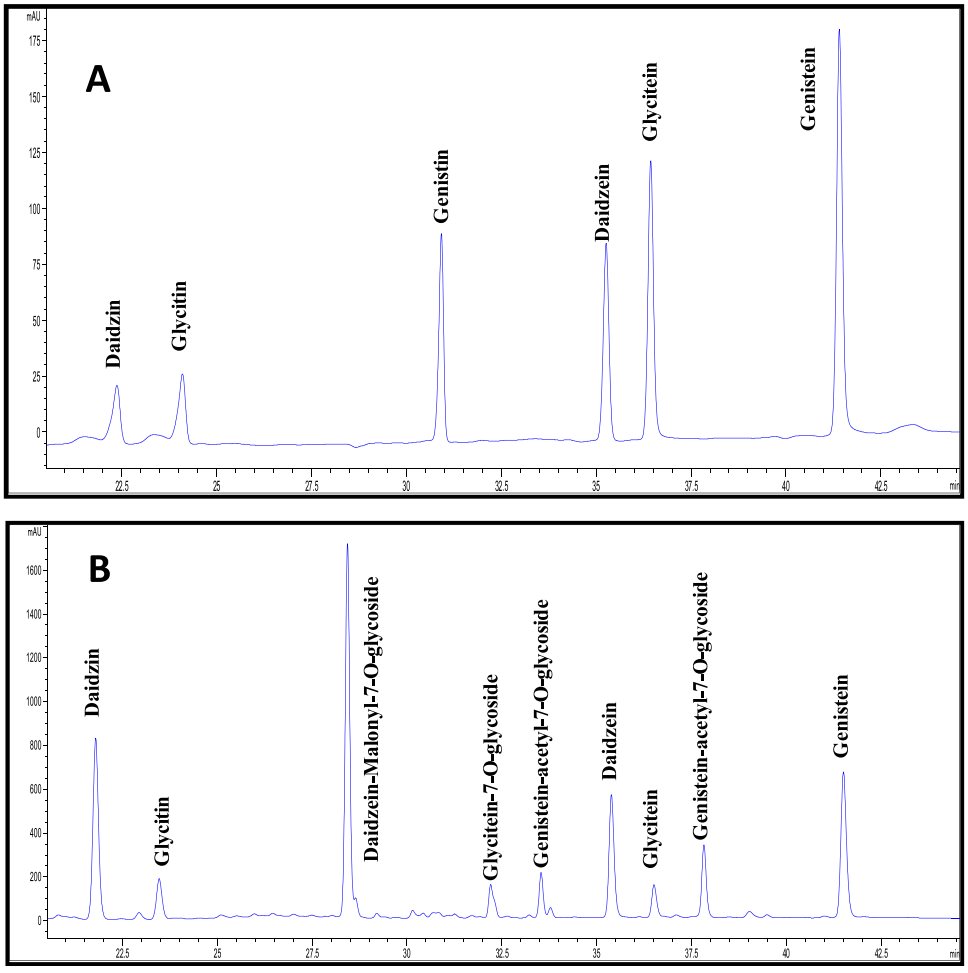

Fig. 1. HPLC-UV chromatograms of (A) standard, (B) sample at 262 nm.

Table 1
Variations for total polyphenol content (TPC), total flavonoid content (TFC), β-glycoside and aglycones isoflavone and total isoflavones in soybeans under thermal processing.

Samples	TPC	TFC	β-glycoside (mg/g)	Aglycones (mg/g)	total-isoflavones (mg/g)
Raw	0.193^{b}	0.325^{b}	223.01^{g}	205.15^{a}	428.16^{e}
Boiled	$0.145^{d}\downarrow$	0.325^{b}-	$258.14^{f}\uparrow$	$111.05^{b}\downarrow$	$369.20^{f}\downarrow$
60 °C 2 min Fried	$0.192^{b}\uparrow$	$0.312^{c}\uparrow$	$512.72^{e}\uparrow$	$52.62^{e}\downarrow$	$565.34^{d}\uparrow$
60 °C 5 min Fried	$0.180^{b}\downarrow$	$0.298^{d}\downarrow$	$524.63^{e}\uparrow$	$52.98^{e}\downarrow$	$573.62^{d}\uparrow$
120 °C 2 min Fried	$0.126^{e}\downarrow$	$0.228^{e}\downarrow$	$599.83^{c}\uparrow$	$57.09^{d}\downarrow$	$656.92^{c}\uparrow$
120 °C 5 min Fried	$0.172^{c}\downarrow$	$0.311^{c}\downarrow$	$565.80^{d}\uparrow$	$222.3^{a}\uparrow$	$788.10^{a}\uparrow$
160 °C 2 min Fried	$0.121^{e}\downarrow$	$0.172^{f}\downarrow$	$727.29^{a}\uparrow$	$57.65^{d}\downarrow$	$784.95^{a}\uparrow$
160 °C 5 min Fried	$0.249^{a}\uparrow$	$0.368^{a}\uparrow$	$642.80^{b}\uparrow$	$76.52^{c}\downarrow$	$719.32^{b}\uparrow$

Data are expressed as means of triplicate experiments on dry weight basis.
Values marked by the same letter with in each soybean in each column are not significantlydifferent ($p < 0.05$).

processed samples were used as a control. The amount of TPC determined in the raw soybean extract (0.193 mg/ g D.W) was comparatively lower than that found in other yellow (2.15 mg/ g D.W) or black (6.96 mg/ g D.W) soybeans [5]. The reason for that difference may mainly be explained as different species, cultivation locations, season and climate, and even affected by the discrepancy of analysis method for phenolic and flavonoids quantification. Comparing with the original raw soybeans, the TPC and TFC values of all fried soybean samples showed significant differences ($p < 0.05$), and heating at either 120 or 160 °C caused significant ($p < 0.05$) reduce in most of the measured phenolic indexes, excepting for the treatments at 60 °C for 2 min retained greater TPC and TFC values. The lowest TPC was found in the sample fried at 160 °C for 2 min (0.121 mg GAE/g d.w) with the weight loss of 37.3%, while its TFC level was registered as 0.172 mg RE/g d.w ($p < 0.05$). These results revealed that thermal processing may cause intricate alterations on chemical constituents, and it might induce the polyphenols to degrade and bound phenolics to release. Beside of this, the changing of soybean phenolic profiles after thermal processing may also be related to the variations in content and distribution of individual phenolics in seed cotyledon and coat.

In fact, data on TPC and TFC changes in cooked soybeans are relative limited. Bressani and Elias (1980) summarized the results of cooking common bean samples and TPC without removing the cooking water. It can be seen that polyphenols content decreased by 30–49% from common beans by cooking processing. In this work, approximately 7–37% of TPC and 8–47% of TFC were decreased in fried soybeans. A good agreement was confirmed in previous studies, Xu and Chang [5] found that heating processing decreased the TFC and TPC in all experimental soybeans, and Ismail et al. [14]

Table 2
Changes of five isoflavones during mixed cooking process of vegetable and soybean.

Sample	Isoflavones (mg/g)							
	Daidzin	Glycitin	Total β-glycosides	Daidzein	Glycitein	Genistein	Total aglycones	Total isoflavones
Raw soybean	179.75 ± 5.41	43.26 ± 1.13	223.01	83.32 ± 1.08	22.95 ± 2.01	98.88 ± 3.71	205.15	428.16f
60 °C, 2 min								
FB	444.94 ± 15	67.78 ± 2.74	512.72	14.23 ± 0.15	17.20 ± 0.10	21.19 ± 0.10	52.62	565.34e
CFB	468.85 ± 8.05	76.66 ± 1.09	545.51	15.52 ± 0.10	10.24 ± 1.46	21.64 ± 0.37	47.4	592.92d
KFB	492.70 ± 7.98	79.95 ± 1.04	572.65	9.06 ± 0.08	26.04 ± 0.09	13.09 ± 0.09	48.19	620.84c
GPFB	516.74 ± 27.78	96.08 ± 6.43	612.82	13.88 ± 0.84	18.18 ± 0.72	19.43 ± 1.04	51.49	664.31c
60 °C, 5 min								
FB	448.86 ± 21.82	75.77 ± 1.18	524.63	12.31 ± 0.11	22.17 ± 0.18	18.50 ± 0.13	52.98	573.62d
CFB	383.33 ± 21.47	69.97 ± 0.36	453.3	11.79 ± 0.17	19.52 ± 0.31	16.55 ± 0.31	47.86	501.16e
KFB	631.37 ± 46.98	96.41 ± 5.84	727.78	12.10 ± 0.86	18.45 ± 1.36	17.71 ± 1.17	48.26	776.04a
GPFB	557.98 ± 17.75	86.55 ± 0.80	644.53	19.41 ± 0.08	5.21 ± 0.62	28.29 ± 0.36	52.91	697.45ab
120 °C, 2 min								
FB	511.28 ± 13.24	88.55 ± 0.66	599.83	11.79 ± 0.11	27.42 ± 0.20	17.88 ± 0.19	57.09	656.92c
CFB	502.36 ± 10.33	85.10 ± 1.24	587.46	12.76 ± 0.16	12.28 ± 0.08	17.39 ± 0.19	42.43	629.89c
KFB	516.83 ± 2.43	78.80 ± 1.15	595.63	8.51 ± 0.02	18.46 ± 0.32	13.52 ± 0.09	40.49	636.12c
GPFB	579.85 ± 5.09	98.78 ± 0.68	678.63	12.39 ± 0.10	19.71 ± 0.2	17.99 ± 0.22	50.09	728.71a
120 °C, 5 min								
FB	502.72 ± 54.79	63.08 ± 6.37	565.8	30.02 ± 2.87	137.53 ± 40.06	54.75 ± 2.04	222.3	788.10a
CFB	631.13 ± 19.03	96.98 ± 1.77	728.11	16.41 ± 0.20	14.16 ± 0.26	23.01 ± 0.51	53.58	781.69a
KFB	651.15 ± 43.86	99.83 ± 6.57	750.98	9.86 ± 1.26	8.76 ± 0.99	16.71 ± 1.18	35.33	786.31a
GPFB	559.19 ± 2.30	88.16 ± 1.70	647.35	12.01 ± 0.25	11.93 ± 0.08	20.01 ± 0.24	43.95	691.29bc
160 °C, 2 min								
FB	628.25 ± 6.81	99.04 ± 1.55	727.29	15.42 ± 0.09	19.49 ± 0.09	22.74 ± 0.19	57.65	784.95a
CFB	356.61 ± 6.87	67.05 ± 0.94	423.66	10.56 ± 0.30	11.51 ± 0.83	14.21 ± 0.28	36.28	459.95f
KFB	566.61 ± 7.08	91.32 ± 0.70	657.93	12.62 ± 0.29	9.99 ± 0.28	18.40 ± 0.10	41.01	698.95b
GPFB	546.98 ± 14.06	90.46 ± 2.24	637.44	12.80 ± 0.30	11.07 ± 0.53	19.27 ± 0.40	43.14	680.58bc
160 °C, 5 min								
FB	571.71 ± 8.3	71.09 ± 0.74	642.8	22.53 ± 0.05	17.13 ± 2.08	36.86 ± 0.44	76.52	719.32b
CFB	579.70 ± 6.32	93.67 ± 0.90	673.37	17.07 ± 0.03	12.46 ± 0.35	24.58 ± 0.34	54.11	727.48b
KFB	525.94 ± 0.52	73.76 ± 1.41	599.7	16.65 ± 0.36	4.50 ± 0.13	25.08 ± 0.07	46.23	645.93c
GPFB	532.78 ± 2.91	89.46 ± 1.61	622.24	13.23 ± 0.08	10.97 ± 0.11	21.02 ± 0.04	45.22	667.46bc

[1] n = 3, mean ± standard deriation.
[2] FB = fried beans, CFB = celery fried beans, KFB = kelp fried beans, GPFB = green pepper fried beans.
[3] Values followed by the same letter in the same row are not significantly different ($p < 0.05$).

exhibited similar trends that thermal processing caused about 40% of TPC and 60% of TFC losses in black common beans.

As shown in Table 2, all of the domestic cooked samples show higher TPC and TFC losses than simple heat treatments, especially, in celery fried bean, TPC was decreased by 33% and TFC was decreased by 26%, respectively. These significant losses could be attributed to water soluble phenolics leaching into cooking water before and during thermal processing as well as breakdown of phenolics during processing. In the case of kelp fried bean, about 24% of TPC and 10% of TFC losses were found in 120 °C, 2 min treatments, while in sample of green pepper fried bean, TPC and TFC losses were found to be 26% and 20%, respectively, in 160 °C, 2 min treatments. It is widely believed that much food composites can be significantly lost as a consequence of industrial sterilization, pasteurization, and dehydration, as well as home-cooking [15]. However, cooking processing does not always promises the changes and destruction of the photochemical components. Sometimes, processing factors such as the medium, methods and temperature of cooking could induce the formation of new compounds [16,17]. The thermal treatment during cooking could increase the content and biological activity of antioxidant compounds of eggplants, and to give rise to an increase in phenolics in purple corn [18], pepper [19], and broccoli [20]. In addition, thermal treatments by steam increased the TPC of sweet potatoes by 2–13 times as compared to raw sweet potatoes [21]. In the present study, domestic cooking based on the thermal treatment of soybean dishes, increased TPC about 30.8% and increased TFC of 45.5% in kelp fried bean at 60 °C for 5 min. The increased total phenolic values might be related to the release of phenolic or phenolic analogue substances with reactivity toward phenolic detection reagents (such as Folin-Ciocalteu reagent) from polymerized structural substances (such as lignin) in cell walls upon thermal processing. Lignin is covalently bound to cellulose in the cell walls.

3.3. *Effects of heating on isoflavones compositions*

Thermal treatment is an important process for various soybean-based products, which can affect not only the content but also the profile of isoflavones [22]. Data obtained from the present study indicated that the total β-glycosides of isoflavones in the soybeans ranged from 223 to 727 μg/g d.w. (Table 1). The total isoflavones content was significantly improved in the samples after being fried at 160 °C for 2 min. Daidzein derivatives, including acetyldaidzin, daidzin, daidzein, and malonyldaidzin, were confirmed as the dominant isoflavones in all soybeans samples, followed by genistein and glycitein derivates.

Besides of the variation of total isoflavones content, thermal processing significantly affected the profile of isoflavones as well (Fig. 1). The major isoflavones in raw soybeans were malonylglucosidic forms (Fig. 1B), whereas the dominant isoflavones in the boiled sample were β-glycosidic forms (258 μg/g d.w.) and total aglycones content was decreased by about 25% after boiling (Table 1). An early published work [23] reported that hot water extraction of soybean products led to glucosides generation. In general, Table 1 displays the decrease of aglycones (aglycones, daidzein, glycitein or acetylglucosides) mainly led to the increase of glucosides (daidzin or glycitin) in the heat-treated soybeans. A good agreement was found in Xu and Chang [5], who indicated that thermal processing resulted

Table 3
Comparisons of total polyphenol content (TPC), total flavonoid content (TFC), β-glycoside and aglycones isoflavone and total isoflavones in vegetable mixed fried soybeans under different conditions.

Samples	TPC	TFC	β-glycoside (mg/g)	Aglycones (mg/g)	total-isoflavones (mg/g)
RAW	0.193^{e}	0.325^{e}	223.01^{m}	205.15^{a}	428.16^{h}
60° 2min					
FB	0.22^{c}	0.41^{b}	512.72^{j}	52.62^{c}	565.34^{f}
CFB	0.192^{e}↓	0.297^{g}↓	545.51^{i}↑	47.40^{d}↓	592.92^{g}↑
KFB	0.165^{g}↓	0.289^{g}↓	572.65^{h}↑	48.19^{d}↓	620.84^{f}↑
GPFB	0.244^{b}↑	0.358^{c}↑	612.82^{f}↑	51.49^{c}-	664.31^{d}↑
60° 5min					
FB	0.18^{f}	0.298^{g}	524.63^{i}	52.98^{c}	573.62^{f}
CFB	0.138^{i}↓	0.273^{h}↓	453.30^{k}↓	47.86^{d}↓	501.16^{g}↓
KFB	0.279^{a}↑	0.473^{a}↑	727.78^{b}↑	48.26^{d}↓	776.04a↑
GPFB	0.284^{a}↑	0.378^{c}↑	644.53^{d}↑	52.91^{c}-	697.45c↑
120° 2min					
FB	0.126^{j}	0.228^{j}	599.83^{g}	57.09^{c}	656.92^{d}
CFB	0.178^{f}↑	0.316^{f}↑	587.46^{g}↓	42.43^{d}↓	629.89^{e}↓
KFB	0.147^{i}↑	0.292^{g}↑	595.63^{g}-	40.49^{d}↓	636.12^{e}↓
GPFB	0.184^{e}↑	0.342^{d}↑	678.63^{c}↑	50.09^{c}↓	728.71^{b}↑
120° 5min					
FB	0.172^{f}	0.311^{f}	565.8^{h}	222.3^{a}	788.1^{a}
CFB	0.159^{h}↓	0.267^{i}↓	728.11^{b}↑	53.58^{c}↓	781.69^{a}-
KFB	0.202^{d}↑	0.296^{g}↓	750.98^{a}↑	35.33^{e}↓	786.31^{a}-
GPFB	0.165^{g}↓	0.212^{k}↓	647.35^{d}↑	43.95^{d}↓	691.29^{c}↓
160° 2min					
FB	0.121^{j}	0.172^{l}	727.29^{b}	57.65^{c}	784.95^{a}
CFB	0.168^{g}↑	0.225^{j}↑	423.66^{l}↓	36.28^{e}↓	459.95^{g}↓
KFB	0.228^{c}↑	0.298g↑	657.93^{d}↓	41.01^{d}↓	698.95^{c}↓
GPFB	0.144^{i}↑	0.260 ^{i}↑	637.44^{e}↓	43.14^{d}↓	680.58^{c}↓
160° 5min					
FB	0.249^{b}	0.368^{c}	642.8^{d}	76.52^{b}	719.32^{b}
CFB	0.129^{j}↓	0.240^{j}↓	673.37^{c}-	54.11^{c}↓	727.48^{b}-
KFB	0.174^{f}↓	0.340^{d}↓	599.70^{g}↓	46.23^{d}↓	645.93^{e}↓
GPFB	0.145^{i}↓	0.325^{e}↓	622.24^{e}↓	45.22^{d}↓	667.46^{d}↓

1) Data are expressed as means of triplicate experiments on dry weight basis.
2) FB = fried beans, CFB = celery fried beans, KFB = kelp fried beans, GPFB = green pepper fried beans.
3) Values marked by the same letter with in each soybean in each column are not significantlydifferent ($p < 0.05$).

in decreasing aglycones and increasing their glycosides in common soybeans. Despite thermal treatment prominently affected the contents of individual isoflavones in most of the heat-treated soybean samples, the contents of total and subtotal isoflavones were not significantly influenced when the temperature increased to 120 °C, indicating that variation of isoflavones from soybeans was mainly depend on conversion instead of degradation during thermal processing.

3.4. Isoflavones content in cooked soybeans

The retention time for daidzin, glycitin, genistin, daidzein, glycitein and genistein standards were about 23.040, 24.687, 35.770, 36.269, 42.430 min, respectively (Fig. 1A). According to the HPLC analysis, the contents for individual isoflavones from the raw and home cooked soybeans were summarized in Table 3. The total isoflavones were the addition of total aglycones and β-glucosides. Compared with the raw soybean, increased total isoflavones contents were noticed in the fried beans, as well as in the mix cooked samples with celery, kelp and green pepper. The highest total isoflavones content was 664.31 μg/g d. m in green pepper mix fried bean (60 °C, 2 min), followed by kelp mix fried bean (620.84 μg/g d. m.), celery mix fried bean (592.92 μg/g d. m.) and fried bean (565.34 μg/g d. m.). The total isoflavone contents underwent a significantly increase and then decreased during cooking process. While the highest total isoflavone content (788.10 μg/g d. m.) was found in the fried beans through 120 °C and 5 min treatment. Interestingly, higher total isoflavone contents were also found in vegetable mix cooked samples at the same heating temperature, which celery and kelp mix fried beans got the highest values of 781.69 μg/g d. m. and 786.31 μg/g d. m. under 120 °C for 5 min, respectively; while green pepper mix fried bean quickly reached the summit level (728.71 μg/g d. m.) at 120 °C for 2 min. However, a decline trend for the total isoflavones was noted when heating temperature increased, reaching the lowest values of 719.32 μg/g (160 °C, 5 min), 459.95 μg/g (160 °C, 2 min), 645.93 μg/g (160 °C, 5 min), 667.46 μg/g (160 °C, 5 min) for fried beans, celery fried beans, kelp fried beans, and green pepper fried beans, respectively. Compared with the raw soybean, there were increases in total β-glycosides content (sum of daidzin and genistin) in the four fried vegetables. Non-significant ($p > 0.05$) increase was found in the boiled soybean. In contrast, the total aglycones content decreased in mix cooked soybean samples with celery, kelp, and green pepper, while the decrease was smaller in the boiled soybean. Aglycones accounts for the smallest proportion of the total isoflavones, and the variations during cooking process tend to reduce the amount of aglycones. Specially, in comparison to the raw soybean, the total aglycones content in fried beans experienced a sharp fall when being heated at 60 °C, and then gradually increased when heating temperature increased to 120 °C, and attained the highest level of 222.30 μg/g for 5 min (Table 3). This differs from the vegetable mix cooked samples (Table 3), which also significantly decreased but maintained stable with the temperature elevating. Total aglycones content in kelp fried beans (120 °C, 5 min) was 4.8-fold lower than that in raw soybean. The changes of the major isoflavones and its isomers in each soy ingredient are shown in Fig. 2. These results also revealed that the major isoflavones in whole soybeans or other domestic cooking products were in the esterified, glycoside, malonylglycoside, or acetylglucoside forms (as described in Section 3.1, Fig. 1). However, the distribution of

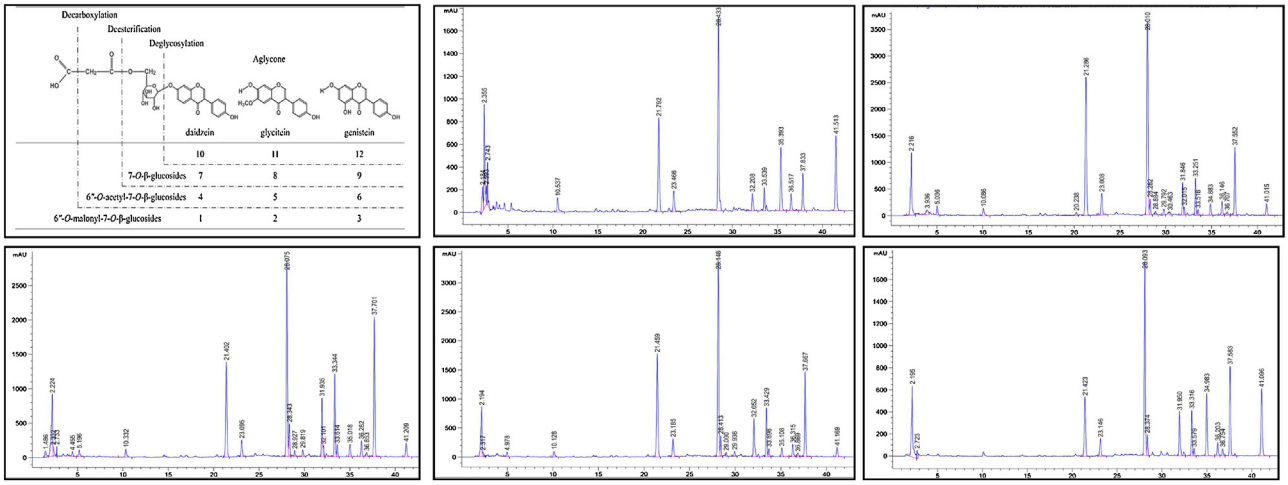

Fig. 2. Chemical constrictuions for isoflavones from soybean (A), and their HPLC profiles for fried soybeans (B), celery mix fried beans (C), kelp mix fried bean (D), green pepper mix fried bean (E), and boiled soybean (F) during the domestic procedures.

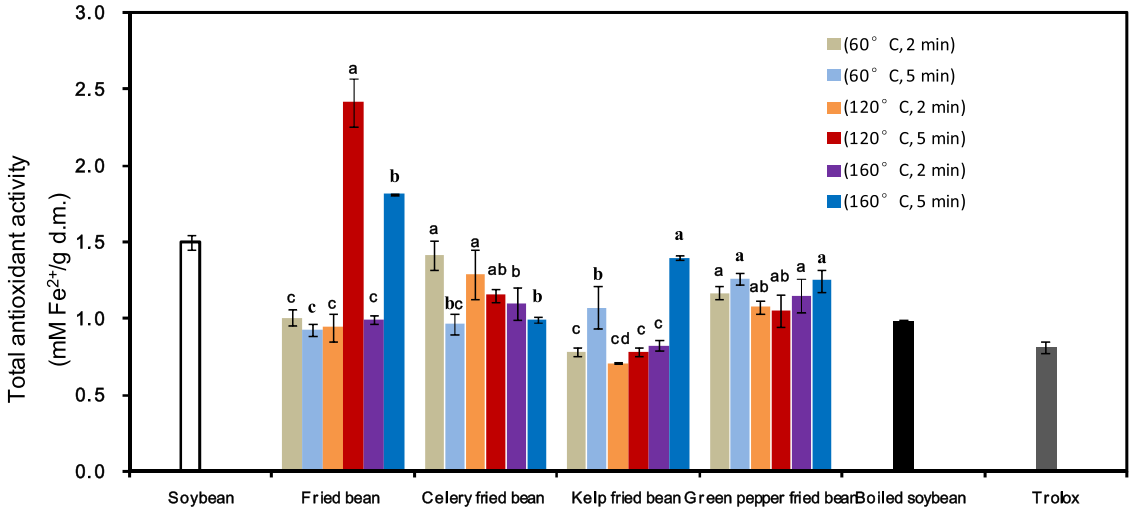

Fig. 3. Changes in antioxidant activities in fried bean, celery fried bean, kelp fried bean, green pepper fried bean, and boiled soybean during the domestic procedures. Each bar represents the mean of three independent experiments (n = 3) with error bar showing the standard deviation, $P < 0.05$.

individual glycosides varied according to the different types of soy ingredients.

3.5. *Effects of cooking conditions on antioxidant activity*

As displayed in Fig. 3, FRAP activity in the un-treated soybeans around 1.5 mM Fe^{2+}/g d.m. As compared to raw soybeans, a decrease on antioxidant activity was found in the boiled soybeans. In general, domestic cooking by traditional (from 60 to 160 °C for 2 or 5 min) heat-treated showed a significantly decrease on FRAP. However, in the case of fried soybeans which treated at 120 °C or 160 °C, when extend the heating time to 5 min, their FRAP activity got a significant increase. Possible interpretation may be the production and conversion of new compounds from Maillard reaction which have antioxidant ability, or the enhanced antioxidant capacity of the formerly known compounds [24]. In addition, as hydrogen donor, the isoflavones content decides the FRAP activity of cooking products [25]. A research conducted paper [26] also suggested that soybeans by-product was poor in phenolics and isoflavones and had lower levels of antioxidant activity. As shown earlier, not only can food processing lead to alterations in the pehnolics' quantity, but it also can alter the phenolics' composition [27]. Modulation of the quality of the phenolic compounds could affect the antioxidant capacity, since different chemical structures have distinct radical scavenging properties. The formation of novel substances, such as products of the Maillard reaction, could also increase the antioxidant capacity, especially in samples like potatoes [28]. In addition, each polyphenol has a higher or lower affinity to the FRAP activity, which could influence the variations observed in the antioxidant capacity between vegetables and between the cooking methods used in this study (Heim et al., 2002). Furthermore, the correlation of FRAP with bioactive compounds indicated that heat treatment plays a role on antioxidant activity of domestic cooking soybean products by affecting the chemical construction and content of bioactive compounds.

Conflict of interest

All authors read and approved the final manuscript of "A value-added cooking process to improve the quality of soybean: protecting its isoflavones and antioxidant activtiy" All authors declare that there are no conflicts of interest.

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