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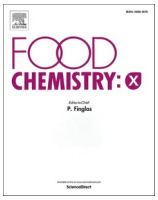

Changes in nutritional compositions and digestive enzyme inhibitions of isoflavone-enriched soybean leaves at different stages (drying, steaming, and fermentation) of food processing

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ABSTRACT

Isoflavone-enriched soybean leaves (IESLs) were processed for drying, steaming, and fermentation, and bioactive compounds and biological activities were analyzed. During food processing, the content of fatty acids, water-soluble vitamins, total phenolics, total flavonoids, and isoflavone-aglycones increased from dried IESLs (DrIESLs) to fermented IESLs (FeIESLs). Especially, oleic acid (53.4 → 113.1 mg/100 g, 2.1-folds), γ-aminobutyric acid (357.36 → 435.48 mg/100 g, 1.2-folds), niacin (19.0 → 130.6 mg/100 g, 6.9-folds), folic acid (9.7 → 25.5 mg/100 g, 2.6-folds), daidzein (270.02 → 3735.10 μg/100 g, 13.8-folds), and genistein (121.18 → 1386.01 μg/100 g, 11.4-folds) dramatically increased. Correspondingly, the antioxidant and digestive enzyme inhibitory activities increased. Therefore, solid-state lactic acid fermentation (SLAF) was suggested as a suitable technique for mass-processing IESLs. FeIESLs with SLAF have the potential to be utilized as a functional food.

1. Introduction

In the contemporary food market, soybeans come in various types and have become a globally popular food known for its versatility and functional benefits. However, in this situation, we have overlooked soybean leaves. Unlike the common soybeans and soybean-based products that we commonly encounter in our routine lives, the utilization of soybean leaves is rare, mostly because of the lack of knowledge about soybean leaves. Among the key substances well known to be abundant in soybeans are vegetable proteins and functional substances such as isoflavones. Interestingly, isoflavones are also present in soybean leaves (Lee et al., 2023; Yuk et al., 2016). It was especially reported that soybean leaves treated with ethephon in the field have more isoflavone content compared to normal soybean leaves and were named isoflavone-enriched soybean leaves (IESLs) because of these results. In the case of IESLs, isoflavones exist that glycosides (daidzin, genistin, malonyldaidzin, and malonylgenistin) and aglycones (daidzein and genistein)(Lee et al., 2023; Xie et al., 2021; Yuk et al., 2016). However, there are limits to obtaining isoflavones by consuming soybean leaves as a general food. A processing method is needed to effectively obtain isoflavones from IESLs. Additionally, for commercial use, it is important to identify processing methods suitable for mass production (Yuk et al., 2016).

Current trends in the global isoflavone market include a focus on the extraction, analysis methods, and biological activities of these compounds (Campos, 2021). Especially the numerous biological attributes of soy isoflavone were reported in several studies. Isoflavone was reported also known as phytoestrogens, are recognized as beneficial components for middle-aged woman owing to their potential to relieve symptoms associated with menopause (Hirose et al., 2016; Lee et al., 2017). Additionally, research has expanded to support their preventative effects on adult diseases such as anticancer, osteoporosis, and heart diseases (Basu & Maier, 2018; Xie et al., 2021). These trends highlight the diverse applications and potential of isoflavones in various industries, driving innovation and research in this field.

Fermentation is extremely important and widespread in our daily lives, and each country has its representative fermented foods. A diversity of microorganisms, including fungi, yeast, and lactic acid

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bacteria (LAB), are used for fermentation. According to previous reports, LAB fermentation can convert to fatty acids, amino acids, and isoflavones in fermentation substrates, which from linolenic acid, glutamic acid, and glycoside isoflavones to conjugated linoleic acid, γ-aminobutyric acid (GABA), and aglycone isoflavones (Lee et al., 2018; Lee et al., 2022). These converted compounds have health benefits such as anti-obesity, reduced blood pressure, and anti-diabetes (Chen et al., 2018; Lee et al., 2018; Lee et al., 2022). Especially, aglycones thus produced are easily digestible, easily absorbed in the body, and have higher bioavailability than glycosides (Kim et al., 2022; Liu et al., 2023; Lu et al., 2024). Also, the reported increase of functional components was higher in combination fermentation than in single LAB (Lee et al., 2018).

Therefore, in this study, optimal mass processing methods were confirmed by comparing dried IESLs (DrIESLs), steamed IESLs (StIESLs), and fermented IESLs (FeIESLs). At that time, two LAB strains with excellent probiotic activity, *Lactiplantibacillus plantarum* P1201 and *Levilactobacillus brevis* BMK184, were used for solid-state lactic acid fermentation (SLAF) of IESLs. For this, we investigated nutritional compositions and digestive enzyme inhibitions.

2. Materials and methods

2.1. Preparation of IESLs and starters

The IESLs were provided in the dried state by JC & Farm Corporation (Namhae-gun, Gyeongsangnam-do, Republic of Korea), and they were named DrIESLs. At that time, the IESLs were prepared with the following methods. First, the soybean leaves were grown until the growth stage R3 (the beginning of pod development) at a time with a growth period of about 60 days after planting, and the height of the plant was 120 cm on average. Then, an ethephon of 200 μg/mL concentration was sprayed on soybean leaves of the R3 growth stage until the solution dripped every 24 h and 2 times. That is, the ethephon solution (200 μg/mL) was treated at an amount of 30 ± 5 mL per plant. Finally, the treated soybean leaves were harvested and called IESLs (Yuk et al., 2016). Provided DrIESLs were ground and stored at −40 °C in a freezer (MDF—U5412, Panasonic Co., Osaka, Japan); they were used as needed. Highly active probiotic strains *L. plantarum* P1201 and *L. brevis* BMK184 were used for fermentation. These microorganisms were previously isolated from the traditional Kimchi and bitter melon fermentation (Lee et al., 2018; Lee et al., 2022).

2.2. Culture medium, chemical regents, and instruments

The microbial culture medium and chemical reagents for analysis of viable cell numbers, isoflavones, and biological activities (including antioxidant and digestive enzyme inhibitory activities) were used in the same manner as previously described by our research team. The same instruments, such as gas chromatography (GC, Nexis GC-2030, Shimadzu Corp., Kyoto, Japan), automatic amino acid analyzer (L-8900, Hitachi High-Technologies Co., Tokyo, Japan), high-performance liquid chromatography (HPLC, Agilent 1200 system, Agilent Technologies Inc., Waldbronn, Germany), and spectrophotometer (UV-1800 240 V, Shimadzu Corp., Kyoto, Japan) were used for measuring the content of fatty acids, amino acids, isoflavones, total phenolics (TP), and total flavonoids (TF), as described previously (Lee et al., 2022; Lee et al., 2023).

2.3. Processing methods of IESLs

Processing of IESLs was performed as Lee et al. (2022) reports. Before this study was performed, we had already studied optimizing fermentation time for IESLs with *L. plantarum* P1201 and *L. brevis* BMK184. IESLs were fermented at 25, 30, and 35 °C, respectively. As a result of that, the optimal fermentation time was 35 °C. For the fermentation process, DrIESLs were mixed with sucrose (2 % of DrIESLs weight) and tap water (4-fold of DrIESLs weight, *v/w*) and sterilized at 121 °C for 30 min; it was named StIESLs. And then cooled to prepare the FeIESLs, the starter cultures (with a mixed ratio of *L. plantarum* P1201: *L. brevis* BMK184 = 1:1) at 5 % (*v/v*) were inoculated and then fermented at 35 °C for 72 h. Then, StIESLs and FeIESLs were collected, dried (for 48 h at 55 °C), and ground (100 mesh size) for use in the experiments.

2.4. Determination of physicochemical properties, viable cell numbers, and proximate compositions

The pH measurement was performed by using a pH meter (Orion STAR A211, Thermo Fisher Scientific, Waltham, MA, USA), and 1 g of the sample was mixed with 50 mL of distilled water via neutralization titration, and the neutralized titration was performed to pH 8.2 ± 0.1 with 0.1 N NaOH. Thereafter, an appropriate amount of 0.1 N NaOH was calculated and converted into lactic acid equivalents. The viable cell counts were determined by serial dilution using sterile water, followed by plating on MRS agar plates and incubating at 37 °C for 48 h for colony enumeration. The analysis of proximate compositions was conducted in accordance with AOAC (Latimer, 2012): The moisture content was measured using the air oven method, while ash content was determined with a muffle furnace. Protein and fat content were analyzed through the Kjeldahl (using Kjeldahl apparatus, DNP-1500, Raypa, Seoul, Korea) and the Soxhlet (using Soxtec, ST243, Foss Analytical Co., Luoyang, China) method, respectively. Carbohydrate content was calculated using the difference method. These results were presented as percentages of dry weight (DW).

2.5. Preparation of extracts

The extracts were prepared by modifying the method described previously by Lee et al. (2023). The extraction was performed by adding 40-fold 80 % ethanol to 1 g of each powder sample and allowing it to stand at room temperature (20 ± 5 °C) for 12 h. The extract was then centrifuged for 30 min, and the supernatant was filtered through a 0.45-μm membrane filter (Dismic-25CS, Toyoroshikaisha, Ltd., Tokyo, Japan). This filtered extract was used for the determination of TP, TF, and isoflavone contents. For the measurements of the biological activities, filtered extracts were evaporated using a rotary evaporator (N-1300, SHANGHAI EYELA Co., Ltd., Shanghai, China) and freeze-dried after being maintained at −40 °C.

2.6. Determination of fatty acid contents

For fatty acids analysis, the sample preparation and preprocessing were performed using the modified method of Lee et al. (2018). Briefly, the course was performed as per the fatty acid esterification process. First, 25 mg of sample powder, 0.5 mL of 0.5 N NaOH dissolved in methanol, and 0.5 mL of triundecanoin as an internal standard ($C_{11:0}$, 2 mg/mL), were mixed with vortex. The mixture heated at 100 °C for 5 min. After, 2 mL of 14 % BF_3 (Supelco, Bellefonte, PA, USA) was mixed, and heated at 100 °C for 30 min. Then, 1 mL of isooctane was added and vigorously stirred for 30 s. Immediately, add 5 mL of saturated NaCl solution and shake. After cooling to 25 °C, separate the isooctane layer from the aqueous layer and dehydrate it with sodium sulfite anhydrous. The pretreated samples were filtered with a 0.45 μm-membrane for analysis with GC. Finally, the fatty acid amount of each sample was analyzed using a GC equipped with an SP-2560 capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness, Supelco, St. Louis, MO, USA) and a flame ionization detector. The injection volume and the injector temperature were set at 1 μL and 225 °C, operating in split mode with a split ratio of 200:1. The carrier gas (Helium) was flown at a rate of 0.75 mL/min. The initial oven temperature was held at 100 °C for 4 min, then increased to 240 °C at a rate of 3 °C/min, and held at 240 °C for 15 min.

2.7. Determination of free amino acid contents

To analyze the free amino acids, the sample preparation and pre-processing were performed in accordance with the amino acid analysis method a previously described method by Khan et al. (2018). Briefly, the program was prosecuted according to the amino acid derivatization process. The treated samples were centrifuged at 15,000 ×*g* for 3 min and filtered through a syringe filter. The filtrates were concentrated by using a rotary evaporator (N-1300, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) at 50 °C. Subsequently, 2 mL of lithium buffer (pH 2.2) was added to dissolve the concentrate, and the solution was filtered through a 0.45-μm membrane filter. The final filtered sample was quantitatively analyzed by using an automatic amino acid analyzer.

2.8. Determination of water-soluble vitamin contents

The analysis of water-soluble vitamins was conducted in accordance with the vitamin analysis a slight modification of the method described by Lee et al. (2023). First, 10 mL of 50 % MeOH was added to 1 g of each sample, followed by extraction for 12 h, and centrifugation at 4000 ×*g* to separate the supernatant. The supernatant was then filtered through a 0.45-μm membrane filter. Subsequently, the analysis was performed using HPLC. The analysis was conducted by using the LiChrospher® Si 60 column (LichroCART 250–4, 5 μm, 125 mm × 4 mm, Merck KGaA, Darmstadt, Germany). Mobile phase solvent A consisted of HPLC water composed of 0.1 % folic acid, while mobile phase solvent B was composed of HPLC acetonitrile. The analysis conditions included a sample injection volume of 20 μL, a flow rate of 1 mL/min, a column temperature of 30 °C, and a detection wavelength of 256 nm. The linear gradient for solvent B was as follows: 0 % (0–5 min), 0–75 % (5–15 min), and 75 % (15–25 min).

2.9. Determination of isoflavones

The analysis of isoflavones was conducted using HPLC following the method described by Lee et al. (2022). The analysis was performed using Lichrospher 100 RP C18 columns (LichroCART 125–4, 5 μm, 125 mm × 4 mm, Merck KGaA, Darmstadt, Germany) as the analysis column. Mobile phase solvent A was composed of HPLC water containing 0.2 % acetic acid, while mobile phase solvent B was composed of HPLC acetonitrile containing 0.2 % acetic acid. The gradient for the mobile phase was set based on the mobile phase solvent B as follows: 0 % (0 min), 10 % (15 min), 20 % (25 min), 25 % (35 min), and 35 % (45–50 min). The analysis conditions included a sample injection volume of 20 μL, a flow rate of 1 mL/min, a column temperature of 30 °C, and a detection wavelength of 254 nm using a diode array detector. The experiments were conducted in pentaplicate and the HPLC chromatograms of the six isoflavone derivatives were detected. The isoflavones in the sample were identified by matching the retention time of respective standards and then quantified using linear calibration curves for each standard, applying external standardization. The calibration curves were developed using seven concentration points (1.0, 0.8, 0.6, 0.4, 0.2, 0.1, and 0.05 mg/mL) derived from a stock solution with a concentration of 1 mg/mL for each standard, correlation coefficient (r^2) higher than 0.998.

2.10. Determination of TP and TF contents

TP and TF contents were determined according to the Folin-Denis and Dvis's methods, respectively, with Lee et al. (2022). The TP and TF contents were calculated with a standard curve using gallic acid and rutin (concentrations: 1, 5, 10, 20, 50, 100, 200, and 500 μg/mL), respectively.

2.11. Determination of antioxidant activities

For determining the antioxidant activities, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydroxyl radical activities, and ferric reducing/antioxidant power (FRAP), the reactions were performed according to a previously described method by Zhang et al. (2015) and Lee et al. (2023). The three radical scavenging activities were determined by measuring the absorbance at 525, 732, and 520 nm, respectively. In all cases, the absorbance of the test sample and the negative control were measured, and the percentage (%) was calculated as follows (1). FRAP measurements were performed by determining the absorbance at 525 nm.

$$\text{Radical scavenging activity } (\%) = \left[1 - \left(A_{sample}/A_{control}\right)\right] \times 100 \quad (1)$$

A_{sample} = absorbance in sample, $A_{control}$ = absorbance of control.

2.12. Determination of digestive enzyme inhibitory activities

To determine the digestive enzyme inhibitory activities, including α-glucosidase and pancreatic lipase inhibitory activities, the reactions were performed according to the previously described method by Zhang et al. (2015) and Lee et al. (2023). The reactions were then terminated by adding 750 μL of 100 mM Na_2CO_3, and the absorbance was measured at 420 nm, respectively. For the negative control, the sample solvent was used instead of the sample, and the absorbance of both the experimental and negative control groups was measured and expressed as a percentage (%), calculated by the following formula (2).

$$\text{Digestive enzyme inhibitory activity } (\%) = \left[1 - \left(A_{sample}/A_{control}\right)\right] \times 100 \quad (2)$$

A_{sample} = absorbance of sample, $A_{control}$ = absorbance of control.

2.13. Statistical analysis and data processing

All experiments were performed in pentaplicate, and the results were expressed as the mean values. The results of all experiments were presented as mean ± standard deviation using analysis of variance (ANOVA) conducted with the Statistical Analysis System (SAS 9.4, SAS Institute, Cary, NC, USA). For assessing the significance of the ANOVA results, Duncan's multiple range tests was performed at a significance level of $p < 0.05$. We performed principal component analysis (PCA) and clustering heatmap analyses in R software version 4.3.3 (R Project for Statistical Computing, Vienna, Austria).

3. Results and discussion

3.1. Comparison of physicochemical properties, viable cell counts, and proximate compositions in the DrIESLs, StIESLs, and FeIESLs

The analysis results of physicochemical properties and proximate composition are shown in supplementary Table S1. In terms of the pH, acidity, and viable cell numbers, the FeIESLs indicated lactic acid fermentation. The pH decreased from 6.19 to 4.89, and the acidity increased from 0.39 to 1.10 % in an inverse proportion to pH. The viable cell numbers measured 10.77 log CFU/g at the fermentation sample (FeIESLs). In the proximate composition analysis, the moisture rate slightly increased (11.4 → 12.2 and 11.9 %), and the protein rate was not significantly different from 30.5 to 29.0 %. The carbohydrate rate slightly increased (45.0 → 46.9 and 46.0 %), and the ash and fat contents significantly increased from 6.9 and 5.2 % to 7.3 and 5.8 %, respectively.

LAB primarily utilizes carbohydrates as carbon sources and small peptides and amino acids as nitrogen sources, thereby exhibiting varied auxotrophism depending on the species and strains (Khan et al., 2018).

Consequently, the substances generated during fermentation can vary with the species, strains, and environmental conditions of the LAB. Thus, selecting appropriate substrates, environmental conditions, and strains is crucial for obtaining the desired compounds (Okabe et al., 2011). In our study, based on the literature data, IESLs were cocktail-fermented using *L. plantarum* P1201 and *L. brevis* BMK184 and then categorized into DrIESLs, StIESLs, and FeIESLs (Lee et al., 2018; Lee et al., 2022). Our fermentation process was effectively conducted, as indicated by the analysis of physicochemical properties. A decrease in pH and an increase in the titratable acidity observed were attributed to the accumulation of organic acids produced through lactic acid fermentation. Furthermore, the concurrent increase in viable cell counts and a decrease in the pH value can be explained by the proliferation of LAB. Both in the fermentation of soy milk using *L. plantarum* WCFS1 and in the single and mixed fermentation of soybean leaves using *L. plantarum* P1201 and *L. brevis* BMK184, the pH value decreased along with an increase in the viable cell count, thereby exhibiting different changes depending on the fermentation strains and duration (Lee et al., 2022; Papadia et al., 2018). In addition, the decrease in pH can induce acid stress, potentially leading to various chemical transformations. Thus, the acidic environment resulting from lactic acid fermentation may serve as a crucial factor (Saubade et al., 2017). The mass FeIESLs were shown a similar pattern of small scare fermentation soybean leaves in previous research.

3.2. Comparison of fatty acid contents in the DrIESLs, StIESLs, and FeIESLs

The results of fatty acid analysis according to the processing stages are shown in Table 1 and Fig. 1. The relatively pronounced changes were observed in γ-linolenic acid, docosadienoic acid, eicosenoic acid, nervonic acid, arachidic acid, and behenic acid. These components showed a low overall proportion, and they were not detected in FeIESLs. Oleic acid, the major unsaturated fatty acid, exhibited an increasing trend from 53.4 to 113.1 mg/100 g, while linoleic acid exhibited a slight increase after a decrease from 144.5 to 136.9 mg/100 g. In the case of saturated fatty acids, palmitic acid (254.6–337.3 mg/100 g) and stearic acid (68.3–90.2 mg/100 g) showed a high proportion, and both of these components increased equally in FeIESLs. The total fatty acid contents increased after a slight decrease to 601.3, 587.4, and 741.5 mg/100 g of DrIESLs, StIESLs, and FeIESLs, respectively. The analysis of PCA and heatmap in Fig. 1 demonstrate specific differences between each sample at the processing stages. The variability of each of these samples was significantly different using the 2D score, with 97.99 % (PC1, 78.35 %; PC2, 19.64 %) (Fig. 1A) and 95.78 % (PC1, 70.49 %; PC2, 25.29 %) (Fig. 2B) for saturated and unsaturated fatty acids, respectively. In the heatmap data shown in Fig. 1C, there were respectively seven components that increased or decreased in FeIESLs compared to that in DrIESLs.

Table 1
Comparison of fatty acid contents in the DrIESLs, StIESLs, and FeIESLs.

Contents[1] (mg/100 g)	Processing stages[2]		
	DrIESLs	StIESLs	FeIESLs
Saturated fatty acids (SFA)			
Palmitic acid (C16:0)	254.6 ± 12.3b	257.8 ± 8.3b	337.3 ± 16.9a
Stearic acid (C18:0)	68.3 ± 1.4b	65.3 ± 1.1c	90.2 ± 0.9a
Arachidic acid (C20:0)	5.8 ± 0.1b	6.5 ± 0.1a	nd[3]
Behenic acid (C22:0)	11.1 ± 0.6a	9.8 ± 0.2b	nd
Lignoceric acid (C24:0)	14.2 ± 0.9a	11.8 ± 1.0b	12.4 ± 1.0ab
Total	354.0	351.2	439.9
Unsaturated fatty acids (USFA)			
Palmitoleic acid (C16:1)	30.9 ± 0.9b	36.0 ± 1.6a	36.5 ± 1.6a
Oleic acid (C18:1n9c)	53.4 ± 2.3c	66.3 ± 2.2b	113.1 ± 7.9a
Linoleic acid (C18:2n6c)	144.5 ± 4.6a	112.9 ± 4.2b	136.9 ± 3.4a
γ-linolenic acid (C18:3n6)	nd	2.9 ± 0.1a	nd
α-linolenic acid (C18:3n3)	4.2 ± 0.3b	3.7 ± 0.3b	8.8 ± 0.5a
Eicosenoic acid (C20:1)	3.0 ± 0.1a	3.0 ± 0.2a	nd
Eicosatrienoic acid (C20:3n3)	4.5 ± 0.2b	4.7 ± 0.3b	6.3 ± 0.4a
Docosadienoic acid (C22:2)	3.3 ± 0.1a	3.1 ± 0.1a	nd
Nervonic acid (C24:1)	3.5 ± 0.1a	3.6 ± 0.2a	nd
Total	247.3	236.2	301.6
Total fatty acids	**601.3**	**587.4**	**741.5**

[1] All values are presented as the mean ± SD of pentaplicate determination. Different letters correspond to the significant differences relating to samples using Duncan's multiple range tests ($p < 0.05$).

[2] Processing stages: DrIESLs, fresh isoflavone-enriched soybean leaves; StIESLs, steamed isoflavone-enriched soybean leaves; and FeIESLs, fermented isoflavone-enriched soybean leaves.

[3] nd: not detected.

Through fermentation using LAB, lipids can be decomposed, although not all strains exhibit this capability. Among them, *L. brevis*, used in the present study, is known as a hetero fermentative LAB strain with a lipid-degrading activity (Ziarno et al., 2020). The major fatty acids identified in DrIESLs were palmitic acid, stearic acid, oleic acid, and linoleic acid. The fatty acid profile of medium and small soybean powder also indicated a high proportion of palmitic acid, linoleic acid, and oleic acid (Lee et al., 2022). In addition, it was confirmed that the ratio of palmitic acid, stearic acid, and oleic acid was increased in the fermentation process of white bean soymilk compared to raw bean seeds (Ziarno et al., 2020). Particularly, linoleic acid decreased during sterilization, but increased during fermentation, possibly due to decomposition under heat stress (Wang et al., 2021). In comparison, arachidic acid, behenic acid, γ-linolenic acid, eicosenic acid, docosadienoic acid, and nervonic acid were not detected post-fermentation. This finding may be attributed to their bioconversion into hydroxy fatty acids by *Lactobacillus* spp. generated hydratase or decomposition into hydrocarbons, alcohols, aldehydes, ketones, and aromatic compounds through enzymatic action and oxidation (Wang et al., 2021). While saturated fatty acids are associated with the risks of coronary heart disease, cardiovascular diseases, and type 2 diabetes, stearic acid is inversely related and can increase the content of fatty acid β-oxidation by promoting mitochondrial fusion and reducing long-chain acylcarnitine (Senyilmaz-Tiebe et al., 2018). Oleic acid, which exhibited more than a 2.1-fold increase, exerted beneficial effects such as reducing low-density lipoprotein (LDL) cholesterol, lowering the risk of cardiovascular diseases, improving glucose regulation and insulin sensitivity, and decreasing lymphocyte proliferation (Calder, 2015; De Carvalho & Caramujo, 2018). In addition, essential fatty acids such as linoleic acid can lower serum cholesterol and LDL cholesterol levels (Calder, 2015). Our outcomes confirmed that fermentation processing of IESLs can obtain the positive effect of such as oleic acid.

3.3. Comparison of free amino acid contents in the DrIESLs, StIESLs, and FeIESLs

The results of free amino acid analysis are shown in Table 2 and Fig. 2. In the free amino acid, except for the non-essential amino acids citrulline, cystine, β-alanine, and GABA, all other components decreased in FeIESLs compared to StIESLs. Among them, citrulline significantly increased by approximately 6-fold from 7.16 (StIESLs) to 59.50 (FeIESLs) mg/100 g, and β-alanine increased from 47.86 (StIESLs) to 70.39 (FeIESLs) mg/100 g. Furthermore, GABA increased from 308.88 (StIESLs) to 435.48 (FeIESLs) mg/100 g, which accounted for a large proportion. Although most free amino acids decreased during fermentation, the non-essential free amino acids that accounted for a large proportion of all amino acids included aspartic acid (from 1169.37 to 993.13 mg/100 g), glutamic acid (from 286.74 to 156.93 mg/100 g), alanine (from 163.56 to 135.02 mg/100 g), tyrosine (from 105.39 to 75.74 mg/100 g), and arginine (from 246.64 to 177.00 mg/100 g), and the essential free amino acids included valine (from 219.96 to 181.00

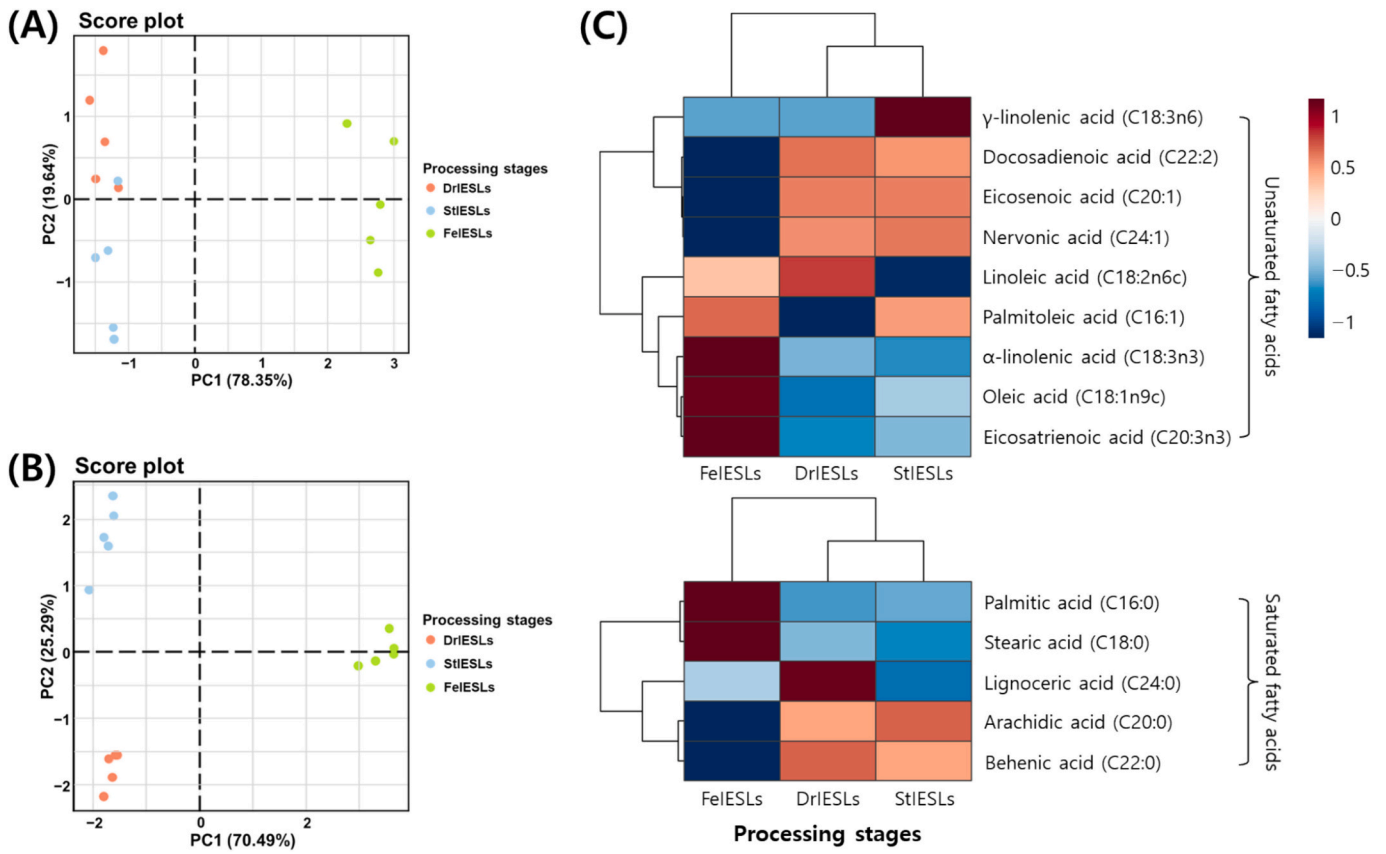

Fig. 1. Comparison of fatty acids in the DrIESLs, StIESLs, and FeIESLs. (A) and (B) 2D score plot with principal component analysis, and (C) heatmap analysis. Processing stages: DrIESLs, dried isoflavone-enriched soybean leaves; StIESLs, steamed isoflavone-enriched soybean leaves; FeIESLs, fermented isoflavone-enriched soybean leaves.

mg/100 g), isoleucine (from 135.41 to 97.75 mg/100 g), leucine (from 133.71 to 91.13 mg/100 g), and phenylalanine (from 178.52 to 124.34 mg/100 g). The total free amino acid contents decreased in the following order: DrIESLs (3938.98 mg/100 g), StIESLs (3704.55 mg/100 g), and FeIESLs (3053.41 mg/100 g) (Table 2). The results of the PCA for non-essential and essential amino acids demonstrate a considerable degree of variability among the samples during the processing stages. The PC variability, as indicated by the 2D score plot, revealed that the PC variability for non-essential amino acids was 92.78 % (PC1, 74.42 %; PC2, 18.36 %) (Fig. 2A) and that for essential amino acids was 99.10 % (PC1, 92.41 %; PC2, 6.69 %) (Fig. 2B). In the free amino acid heatmap, except for the non-essential amino acids citrulline, cystine, β-alanine, and GABA, all other components decreased in FeIESLs (Fig. 2C).

In our study, most free amino acids tended to decrease after fermentation, which may be attributed to the proteolytic system for the growth of LAB and the action of various enzymes such as protease, peptidase, and amino acid decarboxylase in response to pH stress (Saubade et al., 2017; Wang et al., 2021; Zhang, Xia, et al., 2023). Furthermore, LAB exhibited varying auxotrophism across strains, with some strains necessitating amino acids and peptides as nitrogen sources, which may elucidate the reduction in free amino acids post-fermentation (Kieliszek et al., 2021). Even in the dried longan (*Dimocarpus longan*) fermentation, the total free amino acid contents decreased with a tendency to decrease in most free amino acids, and it was confirmed that the free amino acid contents decreased in the process of sterilizing vegetable baby foods (Khan et al., 2018; Kieliszek et al., 2021). This trend was also observed in mass processing stages in this study. Since amino acids can also be converted into various flavor compounds, such as aldehydes, alcohols, and esters, analyzing these compounds may provide insights into the metabolic pathways of amino acids (Wang et al., 2021). A notable finding in our study was increased citrulline, β-alanine, and GABA during fermentation. Citrulline is a non-essential amino acid that serves as the end product of glutamine metabolism as well as a metabolite of arginine. The inverse proportion between arginine and citrulline observed in DrIESLs and FeIESLs suggested a potential metabolic interplay. Citrulline offers various health benefits, including enhanced exercise performance, reduced muscle fatigue, blood pressure regulation, and cholesterol reduction (Papadia et al., 2018). Carnosine is a combination of β-alanine and histidine, and anserine is another carnosine derivative, which is a combination of β-alanine and 1-methyl-histidine. The increase in β-alanine through lactic acid fermentation may be attributed to uracil or anserine and carnosine degradation due to the action of enzymes produced during the fermentation (Hoffman et al., 2015). GABA, a non-protein amino acid, acts as an inhibitory neurotransmitter in mammals and is synthesized through the irreversible decarboxylation of L-glutamic acid by glutamate decarboxylase (GAD), with the amino group positioned on the gamma carbon, comprising four carbons (Lee et al., 2022; Wang et al., 2021). LAB served as a significant producer of GABA in IESLs containing glutamic acid; it could be facilitated by the presence of pyridoxal-5′-phosphate as a cofactor (Lee et al., 2022; Wang et al., 2021). Although the characteristics of GAD vary across different strains and lineages of LAB, which mostly indicates the optimal activity of *Lactobacillus* GAD, is pH 4.0–5.0, and it is known that the C-terminal region is also involved in the pH dependence of the catalysis (Pannerchelvan et al., 2023). Particularly, *L. brevis* exhibited a higher yield of GABA when compared to other LABs (Wang et al., 2021). In this study, *L. brevis* BMK184 may also have played an important role in producing GABA than L. *plantarum* P1201. FeIESLs with GABA-enriched are expected to provide various health benefits, including hypertension prevention, sedative and

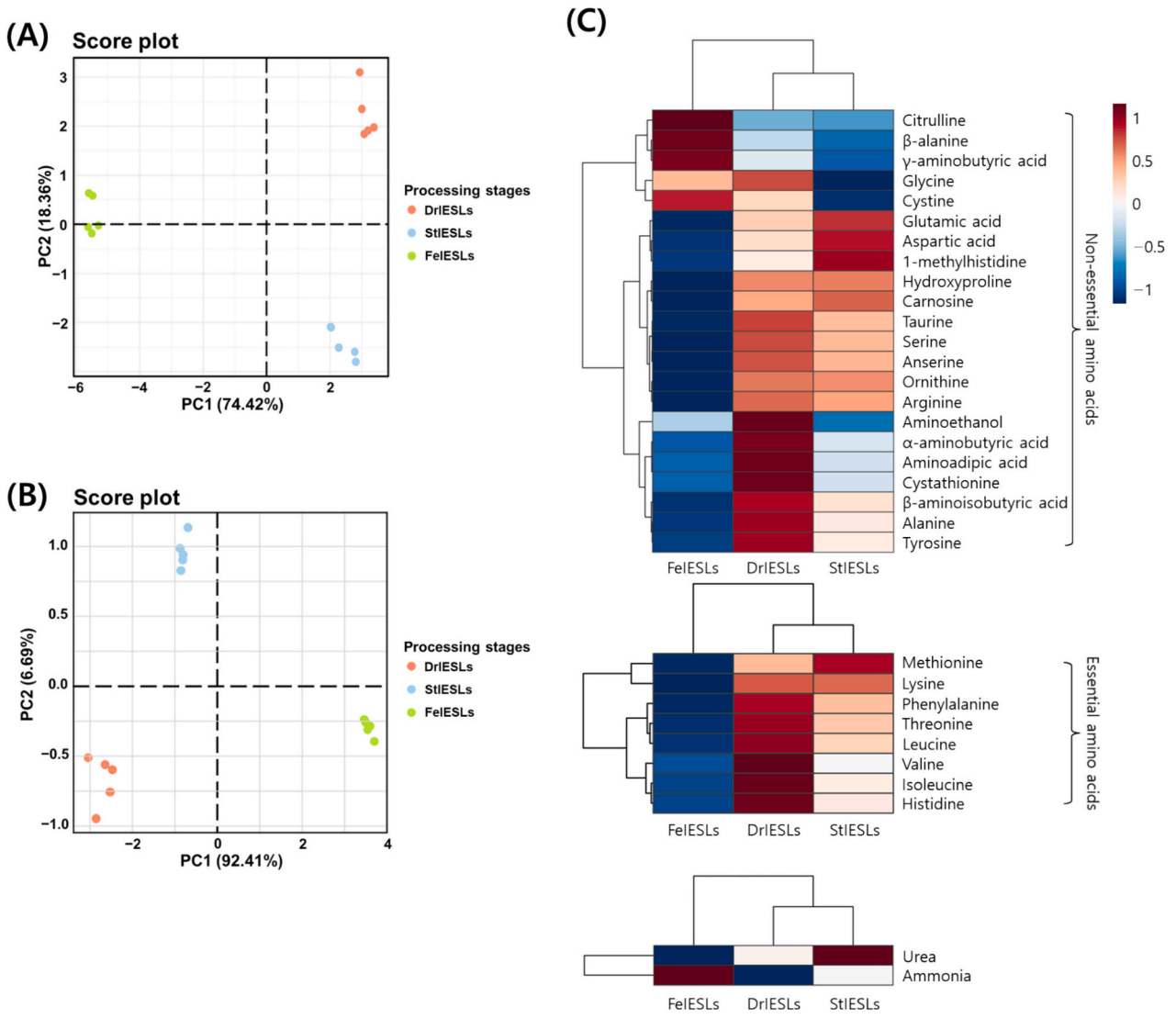

Fig. 2. Comparison of free amino acids in the DrIESLs, StIESLs, and FeIESLs. (A) and (B) 2D score plot with principal component analysis, and (C) heatmap analysis. Processing stages: DrIESLs, dried isoflavone-enriched soybean leaves; StIESLs, steamed isoflavone-enriched soybean leaves; FeIESLs, fermented isoflavone-enriched soybean leaves.

diuretic effects, anxiety, and depression relief (Pannerchelvan et al., 2023).

3.4. Comparison of water-soluble vitamin contents in the DrIESLs, StIESLs, and FeIESLs

The results of water-soluble vitamin analysis are shown in Fig. 3. Comparing DrIESLs and FeIESLs for each ingredient, B5 (175.9–131.2 mg/100 g) decreased by approximately 25 % during fermentation, while C (12.0–0.6 mg/100 g) decreased by 95 %. In contrast, B3 (19.00–130.6 mg/100 g) increased by approximately 6.9-fold, representing a significant proportion, and B9 (9.7–25.5 mg/100 g) increased by around 2.5-fold. The total water-soluble vitamin contents were increased after slightly decreasing to 216.7, 214.3, and 288.0 mg/100 g in the DrIESLs, StIESLs, and FeIESLs, respectively (Fig. 3A). Fig. 3B provides clear evidence of a stepwise distinction during the processing stages, as indicated by the PCA. Furthermore, the variability of each axis was 71.78 % for PC1 and 20.06 % for PC2, demonstrating a significant change. In the heatmap depicting changes in the content of water-soluble vitamins, FeIESLs exhibited a notable pattern of decrease in B5 and C, except for B2, while B3 and B9 displayed significant increases (Fig. 3C).

Vitamins are organic compounds that are not completely or substantially synthesized in the human body, and they are essential micronutrients for humans as they maintain major cell biochemical reactions (LeBlanc et al., 2015; Walther & Schmid, 2017). In our study, we confirmed the tendency of water-soluble vitamins to increase and decrease through SLAF of IESLs. Vitamins are known to be produced through fermentation, and the vitamins generated by various factors such as fermentation strains, substrates, and conditions are diverse (LeBlanc et al., 2015; LeBlanc et al., 2020; Walther & Schmid, 2017). In other words, when a precursor of vitamin is present in the fermentation substrate, it can be biosynthesized through LAB (Wang et al., 2021). The precursor of niacin is tryptophan, and, when looking at the change in the content of free amino acids, it was mostly decreased after fermentation. It has been reported that niacin is biosynthesized through tryptophan present in IESLs. Some LAB strains possess genes involved in folate biosynthesis (Walther & Schmid, 2017; Wang et al., 2021). This process includes the step of converting the precursor guanosine triphosphate to tetrahydrofolic acid, and this step could have progressed due to an increase in the folate content in our study (Zhao & Shah, 2016). Some vitamins increase in content due to the influence of LAB, and vitamins are sometimes required for the growth of LAB (LeBlanc et al., 2020).

Table 2
Comparison of free amino acid contents in the DrIESLs, StIESLs, and FeIESLs.

Contents[1] (mg/100 g)	Processing stages[2]		
	DrIESLs	StIESLs	FeIESLs
Non-essential amino acids			
Taurine	37.27 ± 0.88a	36.32 ± 1.44a	32.78 ± 1.06b
Aspartic acid	1107.24 ± 40.04a	1169.37 ± 65.81a	993.13 ± 37.85b
Serine	160.40 ± 5.73a	138.71 ± 2.25b	45.44 ± 2.12c
Glutamic acid	251.77 ± 9.06b	286.74 ± 20.89a	156.93 ± 3.10c
Aminoadipic acid	64.99 ± 0.97a	59.20 ± 0.97b	56.41 ± 1.74c
Glycine	45.30 ± 1.46a	42.74 ± 0.90b	44.78 ± 1.03ab
Alanine	184.82 ± 4.85a	163.56 ± 3.97b	135.02 ± 3.25c
Citrulline	9.87 ± 0.17b	7.16 ± 0.13c	59.50 ± 0.90a
α-aminobutyric acid	17.29 ± 0.57a	15.31 ± 0.31b	14.12 ± 0.72c
Cystine	10.34 ± 0.66b	nd[3]	15.06 ± 0.63a
Cystathionine	15.43 ± 0.44a	13.12 ± 0.48b	12.01 ± 0.48c
Tyrosine	129.47 ± 4.28a	105.39 ± 3.45b	75.74 ± 2.09c
β-alanine	54.62 ± 2.69b	47.86 ± 1.23c	70.39 ± 0.82a
β-aminoisobutyric acid	84.57 ± 1.04a	65.26 ± 2.26b	31.96 ± 1.33c
γ-aminobutyric acid	357.36 ± 6.01b	308.88 ± 4.56c	435.48 ± 4.12a
Aminoethanol	38.77 ± 0.91a	33.49 ± 0.81b	34.74 ± 0.35b
Hydroxyproline	13.71 ± 0.54a	13.96 ± 0.39a	2.68 ± 0.26b
Ornithine	3.83 ± 0.09a	3.68 ± 0.12a	0.42 ± 0.01b
1-Methylhistidine	3.53 ± 0.13b	5.08 ± 0.04a	1.50 ± 0.05c
Anserine	27.86 ± 0.14a	25.54 ± 0.45b	14.92 ± 0.28c
Carnosine	8.07 ± 0.09b	8.98 ± 0.15a	1.59 ± 0.05c
Arginine	254.60 ± 6.24a	246.64 ± 4.66a	177.00 ± 2.70b
Total	2881.11	2796.98	2411.59
Essential amino acids			
Threonine	90.41 ± 2.22a	76.86 ± 0.70b	48.62 ± 0.50c
Valine	267.87 ± 2.96a	219.96 ± 4.68b	181.00 ± 2.47c
Methionine	18.34 ± 0.40b	22.37 ± 0.52a	7.77 ± 0.40c
Isoleucine	171.50 ± 7.17a	135.41 ± 4.34b	97.75 ± 2.26c
Leucine	157.86 ± 2.61a	133.71 ± 3.90b	91.13 ± 2.01c
Phenylalanine	199.59 ± 2.73a	178.52 ± 2.35b	124.34 ± 3.17c
Lysine	80.58 ± 3.54a	79.50 ± 3.11a	41.88 ± 0.68b
Histidine	71.71 ± 1.72a	61.23 ± 0.94b	49.34 ± 0.44c
Total	1057.87	907.57	641.82
Total amino acids	**3938.98**	**3704.55**	**3053.41**
Ammonia	113.08 ± 4.72b	118.50 ± 3.85ab	124.10 ± 3.75a
Urea	867.15 ± 52.44b	1012.49 ± 78.07a	712.49 ± 53.43c

[1] All values are presented as the mean ± SD of pentaplicate determination. Different letters correspond to the significant differences relating to samples using Duncan's multiple range tests ($p < 0.05$).
[2] Processing stages: DrIESLs, fresh isoflavone-enriched soybean leaves; StIESLs, steamed isoflavone-enriched soybean leaves; and FeIESLs, fermented isoflavone-enriched soybean leaves.
[3] nd: not detected.

This effect varies by species and lineage; for instance, in our study, the ascorbic acid content was decreased. In addition, some vitamins may be reduced due to heat processing steps involved in the processing and cooking of food (Senyilmaz-Tiebe et al., 2018). In our fermentation process, the contents of pantothenic acid and ascorbic acid were also decreased after the sterilization process. The deficiency in increased folate through lactic acid fermentation can lead to potential risks such as megaloblastic anemia, neural tube defects seen in congenital malformations, and low birth weight. The positive effects can reduce the risk of colon cancer in patients with anti-inflammatory and inflammatory bowel disease, and folic acid supplementation is especially recommended for pregnant women (LeBlanc et al., 2020; Walther & Schmid, 2017). In addition, niacin is known to exert benefits such as cardiovascular disease prevention and stroke reduction, albeit it has not been evaluated to have any significant effect in recent clinical trials (D'Andrea et al., 2019). Our results demonstrated that the fermentation process of IESLs is particularly associated with increasing niacin and folic acid levels.

3.5. Comparison of isoflavone contents in the DrIESLs, StIESLs, and FeIESLs

The isoflavone chromatogram analysis revealed significant differences among the samples, as depicted in Fig. 4. The glycoside isoflavones increased and then decreased, and malonylglycoside isoflavones continuously decreased. In contrast, the aglycone isoflavones consistently increased. In Fig. 5A, the contents of glycoside, malonylglycoside, and aglycone forms for each sample are as follows: DrIESLs (3053.84, 5478.05, and 391.20 μg/g, respectively), StIESLs (5027.14, 2678.79, and 2271.41 μg/g, respectively), and FeIESLs (735.03, 561.47, and 5121.12 μg/g, respectively). The sterilization process led to a decrease in the malonylglycosides and an increase in the glycosides and aglycones. Conversely, during fermentation, the content of malonyl and glycosides decreased, while that of aglycones increased. The total isoflavone contents increased slightly to 8923.09, 9977.34, and 6417.62 μg/g in DrIESLs, StIESLs, and FeIESLs, respectively, and then decreased. This difference was clearly evident in the PCA and heatmap. The PCA demonstrated that PC1 and PC2 explained 79.00 % and 20.81 % of the variation in the data, respectively, thereby highlighting the significant differences between the samples (Fig. 5B). Particularly, the heatmap results highlighted changes in isoflavone derivatives at each processing stage (Fig. 5C). The changes in the bioconversion mechanism of isoflavone compounds according to the overall process are depicted in Fig. 6.

Isoflavones are the flavonoid compounds mainly contained in soybean crops and are also called phytoestrogen owing to their chemical structure, which is similar to that of estrogen (Lee et al., 2018). Generally, sugars form glucoside bonds and exist in the form of glycosides, and when ingested, they are decomposed into aglycones due to the enzymatic action of intestinal microorganisms, and are only partially absorbed (Zhang, Zhang, et al., 2023). In other words, it is important not only to produce DrIESLs by treating ethylene and ethephon, as shown previously, but also to efficiently utilize them, and it is important to increase their bioavailability through bioconversion to the non-glycoside form of aglycones (Lee et al., 2023; Yuk et al., 2016). We found the answer in the SLAF process at a small scale from several previous research data (Lee et al., 2018; Lee et al., 2022; Zhang, Zhang, et al., 2023). In our study, during the sterilization step of fermentation, the isoflavones content in malonylglycosides decreased, and that in glucosides and aglycones increased. Thus, isoflavones in the form of malonyl glucosides are relatively heat-labile, deglycosylated, and deesterified during the sterilization process (Andrade et al., 2016; Kuligowski et al., 2022). The increase in total isoflavone contents may be seen as a de-esterification of acetyl glycoside isoflavones as well as malonylglycosides (Lee et al., 2018). In FeIESLs, both malonyl and glycoside bound forms of isoflavones decreased significantly, while the aglycone form of isoflavones increased markedly. These results confirmed that the conversion of isoflavones was superior in the acid and β-glucosidase enzymes produced by LAB, even during mass fermentation processing (Cho et al., 2011;Lee et al., 2018; Lee et al., 2022). Also, mass fermentation processing suggests that the isoflavone compounds in IESLs may have transformed into other phenolic compounds, such as flavonols and phenolic acids (Cho et al., 2011). Mass processing also, it also improves the overall antioxidant capacity. Furthermore, it is expected that may have various health benefits such as anti-inflammatory, anti-cancer, anti-obesity, anti-osteoporosis, and menopausal symptom relief (Andrade et al., 2016; Hirose et al., 2016; Lee et al., 2017; Xie et al., 2021; Zhang, Zhang, et al., 2023).

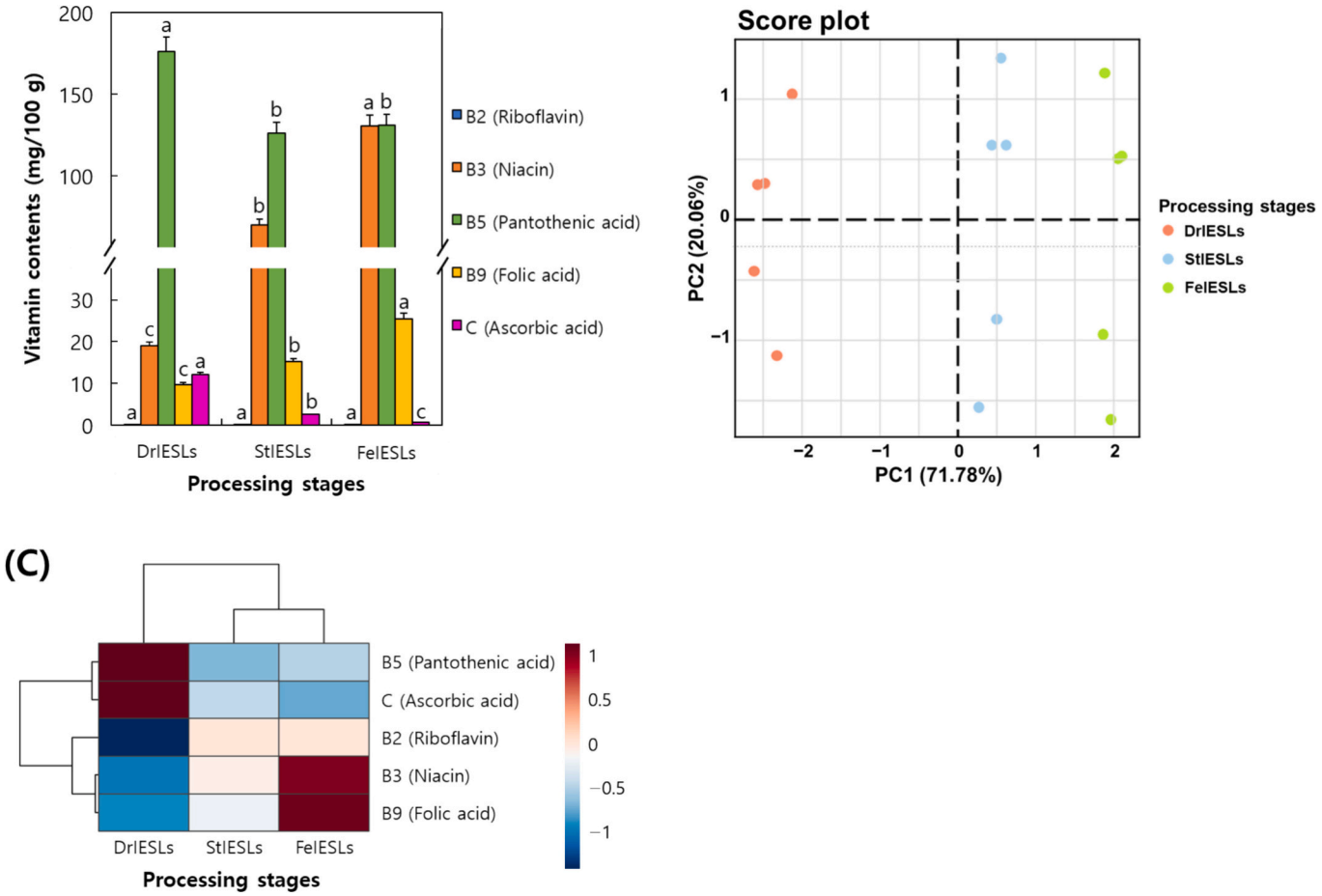

Fig. 3. Comparison of water-soluble vitamin contents in DrIESLs, StIESLs, and FeIESLs. (A) Water-soluble vitamin contents, (B) 2D scores plot with principal component analysis, and (C) heatmap analysis. Processing stages: DrIESLs, dried isoflavone-enriched soybean leaves; StIESLs, steamed isoflavone-enriched soybean leaves; FeIESLs, fermented isoflavone-enriched soybean leaves. All values are presented as the mean ± SD of pentaplicate determination, and different small letters correspond to the significant differences relating to the fermentation time and starter using Duncan's multiple tests ($p < 0.05$).

3.6. Comparison of TP and TF contents in the DrIESLs, StIESLs, and FeIESLs

The changes in the TP and TF contents are depicted in Fig. 7. For DrIESLs, StIESLs, and FeIESLs, the TP contents were 8.38, 8.44, and 10.37 GAE mg/g, respectively, while the TF contents were 14.37, 15.90, and 18.35 RE mg/g, respectively (Fig. 7A). These contents commonly increased slightly after sterilization and increased further during fermentation. Regarding the alterations in TP and TF, as indicated by PCA, the difference was minimal for DrIESLs and StIESLs. However, specific differences were observed for FeIESLs (Fig. 7B).

The phenolic compounds are secondary metabolites commonly present in plants and are produced by various factors occurring during plant growth (Cho et al., 2011). Fermentation is known to increase these phenolic compounds (Lee et al., 2018; Lee et al., 2022). Particularly, through the action of hydrolases such as β-glycosidase and decarboxylase, which are produced by bacteria in fermentation using LAB, the binding phenolic compounds bound to the plant cell wall can be decomposed, and the glycosylation flavonoid compounds can be decomposed into aglycone forms to form compounds with increased bioavailability (Cho et al., 2011; Khan et al., 2018; Zhang, Zhang, et al., 2023). Lactic acid fermentation of dried longan, soybean milk, jujube–wolfberry, and African nightshade displayed the same trend of increasing total phenolic and flavonoid contents as in our study (Cho et al., 2011; Khan et al., 2018; Septembre-Malaterre et al., 2018). It was also converted into other phenolic compounds via thermal decomposition during heat processing for sterilization during the fermentation process, as in the morphological change of isoflavones. In our study, the TP and TF contents changed slightly during the sterilization process and were significantly increased during fermentation, suggesting that the enzyme and metabolism of the used LAB are suitable strains for producing and degrading phenolic compounds to produce compounds with increased bioavailability on mass fermentation processing.

3.7. Comparison of biological activities in the DrIESLs, StIESLs, and FeIESLs

The results of antioxidant activities (such as DPPH, ABTS, hydroxyl radical scavenging activities, and FRAP) and digestive enzyme inhibitory activities (including α-glucosidase and pancreatic lipase inhibitory activities) are depicted in Fig. 8. The results measured at a concentration of 1 mg/mL exhibited that, for DrIESLs, StIESLs, and FeIESLs, respectively, the DPPH radical scavenging activity values were 64.08, 64.58, and 82.66 % (Fig. 8A), the ABTS radical scavenging activity values were 68.74, 73.52, and 90.21 % (Fig. 8B), the hydroxyl radical scavenging activity values were 40.12, 44.42, and 54.79 % (Fig. 8C), and the FRAP values were 0.53, 0.54, and 0.68 $OD_{525\ nm}$ (Fig. 8D). In addition, similar antioxidant activities patterns were demonstrated at concentrations of 0.5 and 0.25 mg/mL. Similar to the TP and TF contents, a slight increase upon sterilization and a more significant increase upon fermentation were noted. At a concentration of 1 mg/mL, the α-glucosidase inhibitory activity for DrIESLs, StIESLs, and FeIESLs was 35.13, 38.44, and 50.46 %, respectively (Fig. 8E), while that for pancreatic lipase inhibitory activity for DrIESLs, StIESLs, and FeIESLs was 43.25, 44.50, and 64.56

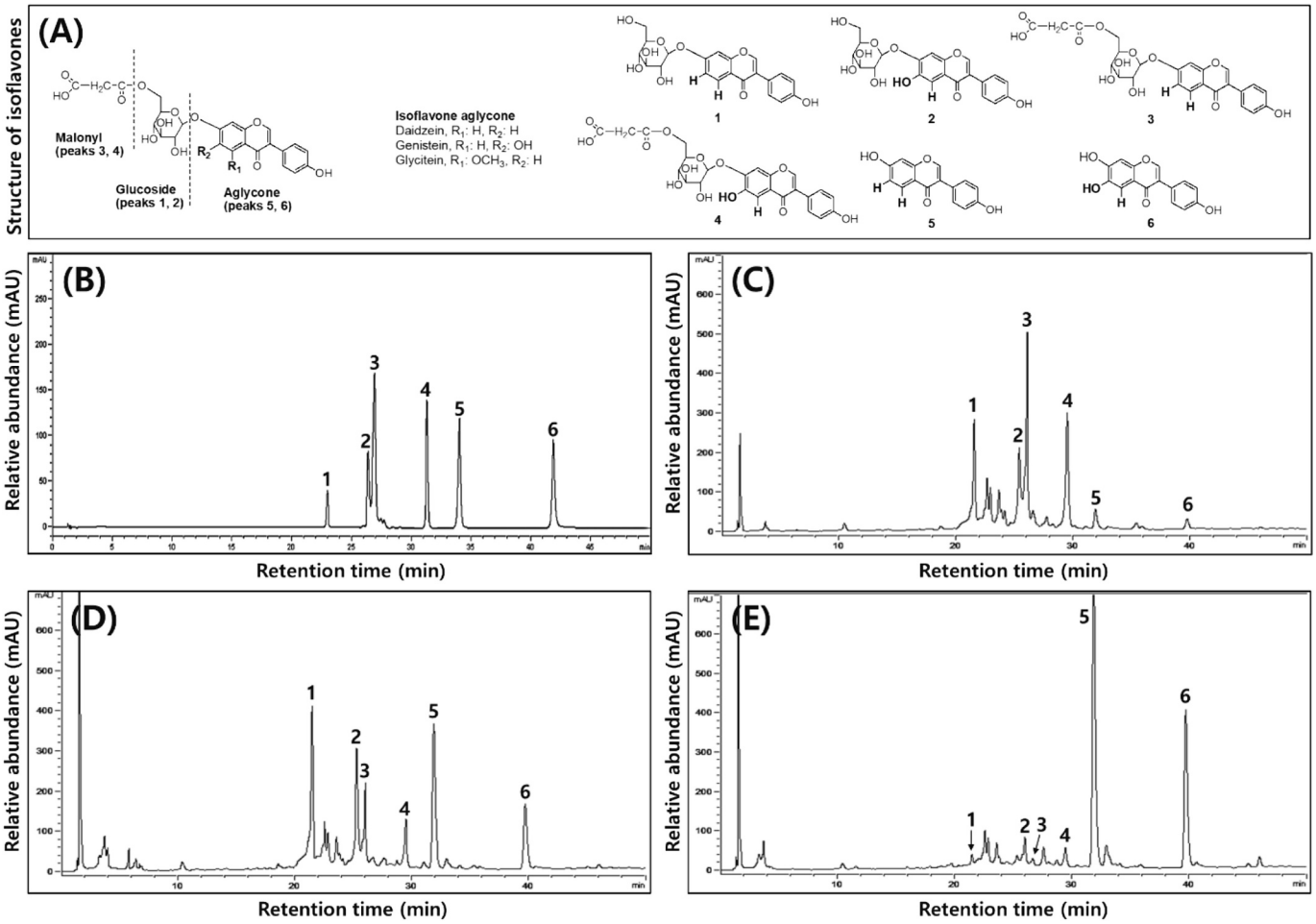

Fig. 4. Typical isoflavones HPLC chromatogram of the 50 % methanol extracts in the DrIESLs, StIESLs, and FeIESLs. (A) Chemical structure of isoflavones, (B) standards, (C) 50 % methanol extracts of DrIESLs, (D) 50 % methanol extracts of StIESLs, and (E) 50 % methanol extracts of FeIESLs. Processing stages: DrIESLs, dried isoflavone-enriched soybean leaves; StIESLs, steamed isoflavone-enriched soybean leaves; FeIESLs, fermented isoflavone-enriched soybean leaves. 1, daidzin; 2, genistin; 3, malonyldiadzin; 4, malonylgenistin; 5, daidzein; and 6, genistein.

%, respectively (Fig. 8F). In addition, similar digestive enzyme inhibitory activities patterns were noted at concentrations of 0.5 and 0.25 mg/mL. Both the digestive enzyme inhibitory activities followed the pattern of slightly increasing after sterilization and then further increasing upon fermentation, similar to that for the TP, TF contents, and the antioxidant activities.

We confirmed that the phenolic and flavonoid contents increased through fermentation, indicating a positive correlation with antioxidant activities that are related to the structure of the phenolic compounds. The structural characteristic of phenolic compounds is that they have hydroxyl groups, which prevent free radicals from attacking other cells by reducing the numbers of hydrogen and electrons to free radicals and creating a stable state (Sun et al., 2022; Zeb, 2020). Particularly, the deglycosylated isoflavone by β-glucosidase may have increased active hydroxyl groups, which increases the antioxidant potential (Lee et al., 2022). As with previous studies, LAB contributed to increasing the antioxidant activity of IESLs during mass fermentation (Lee et al., 2018; Papadia et al., 2018; Sun et al., 2022; Zhao & Shah, 2016). The increase in phenolic compound content and antioxidant activity of soybean leaves through lactic acid fermentation in a mass process suggests their commercial potential (Wang et al., 2021; Zeb, 2020). When we consume foods, some enzymes are activated and involved in the digestion in the human body. Among them, α-glucosidase is an enzyme that acts in the final stage of carbohydrate digestion in the mucosa of the small intestine by hydrolyzing carbohydrates into monosaccharides (Lee et al., 2022; Nurhayati et al., 2017). Pancreatic lipase is a fat-digesting enzyme that aids in the absorption of dietary triglycerides (Lee et al., 2022). The inhibition of such digestive enzymes plays an important role in type 2 diabetes management and anti-obesity activities (Lee et al., 2018; Nurhayati et al., 2017). In our study, an increase in digestive enzyme inhibitory activities was confirmed through the mass fermentation processing of IESL. The structure of phenolic compounds varies according to their type, and the inhibition of digestive enzymes may occur through irreversible or reversible inhibition (competitive and non-competitive inhibition) among the compounds of various structures (Tan et al., 2017). In lactic acid fermentation on mass, increasing the content of phenolic compounds caused an increase in the digestive enzyme inhibitory activities (Lee et al., 2018; Sun et al., 2022). Therefore, mass fermentation processing IESLs can be a good alternative to drug therapy and synthetic compounds that induce side effects such as gastrointestinal infection and loss of appetite (Nurhayati et al., 2017).

4. Conclusions

In our study, we observed changes in the nutritional compositions (including fatty acid, free amino acid, and water-soluble vitamin) and isoflavones of IESLs through mass SLAF using *L. plantarum* P1201 and *L. brevis* BMK184. The content of bioactive compounds, such as oleic acid, γ-aminobutyric acid, niacin, folic acid, daidzein, and genistein, increased dramatically after the SLAF of DrIESLs. Particularly, it can be

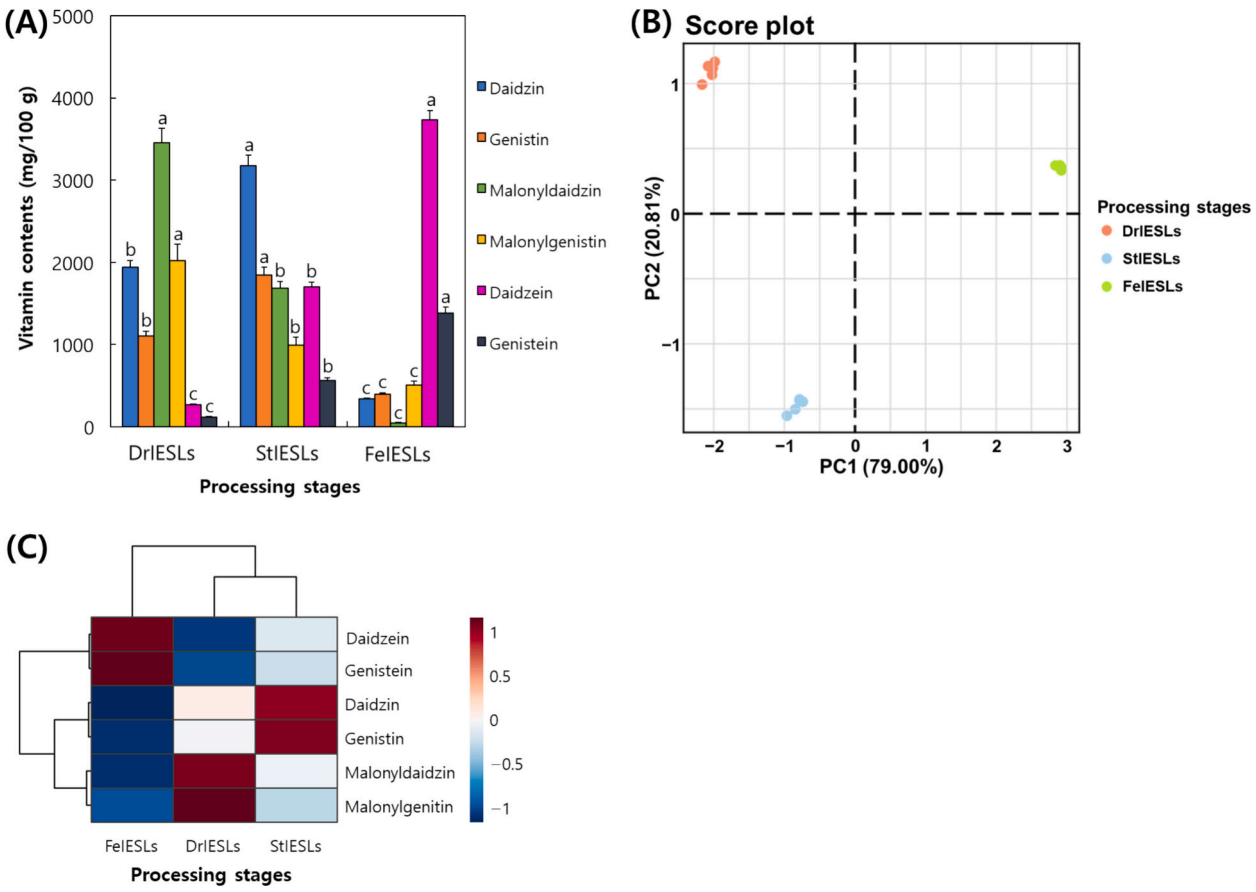

Fig. 5. Comparison of isoflavones in the DrIESLs, StIESLs, and FeIESLs. (A) Isoflavone contents, (B) 2D scores plot with principal component analysis, and (C) heatmap analysis. Processing stages: DrIESLs, dried isoflavone-enriched soybean leaves; StIESLs, steamed isoflavone-enriched soybean leaves; FeIESLs, fermented isoflavone-enriched soybean leaves. All values are presented as the mean ± SD of pentaplicate determination, and different small letters correspond to the significant differences relating to the fermentation time and starter using Duncan's multiple tests ($p < 0.05$).

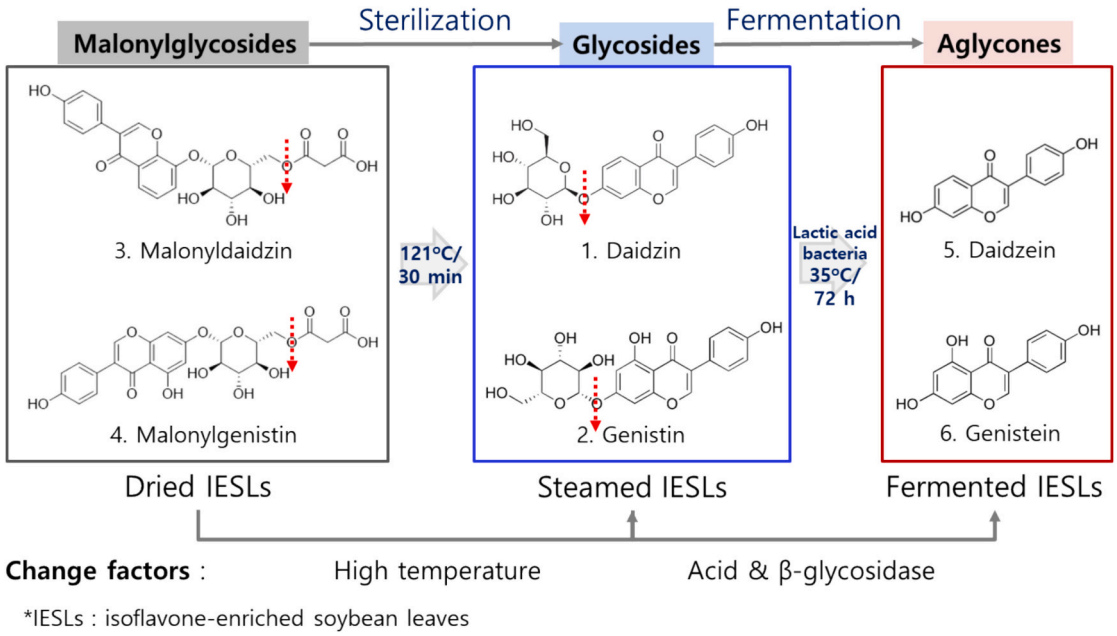

Fig. 6. Bioconversion mechanism of isoflavone compounds by the steaming and fermenting process of IESLs. Processing stages: DrIESLs, dried isoflavone-enriched soybean leaves; StIESLs, steamed isoflavone-enriched soybean leaves; FeIESLs, fermented isoflavone-enriched soybean leaves.

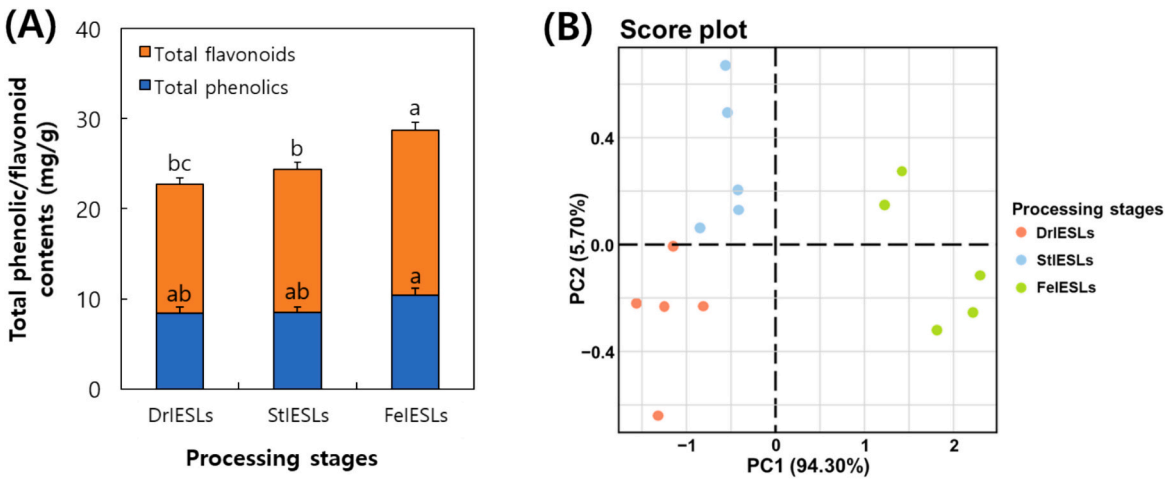

Fig. 7. Comparison of total phenolic and total flavonoid contents in the DrIESLs, StIESLs, and FeIESLs. (A) Total phenolic and flavonoid contents, and (B) 2D scores plot with principal component analysis. Processing stages: DrIESLs, dried isoflavone-enriched soybean leaves; StIESLs, steamed isoflavone-enriched soybean leaves; FeIESLs, fermented isoflavone-enriched soybean leaves. All values are presented as the mean ± SD of pentaplicate determination, and different small letters correspond to the significant differences relating to the fermentation time and starter using Duncan's multiple tests ($p < 0.05$).

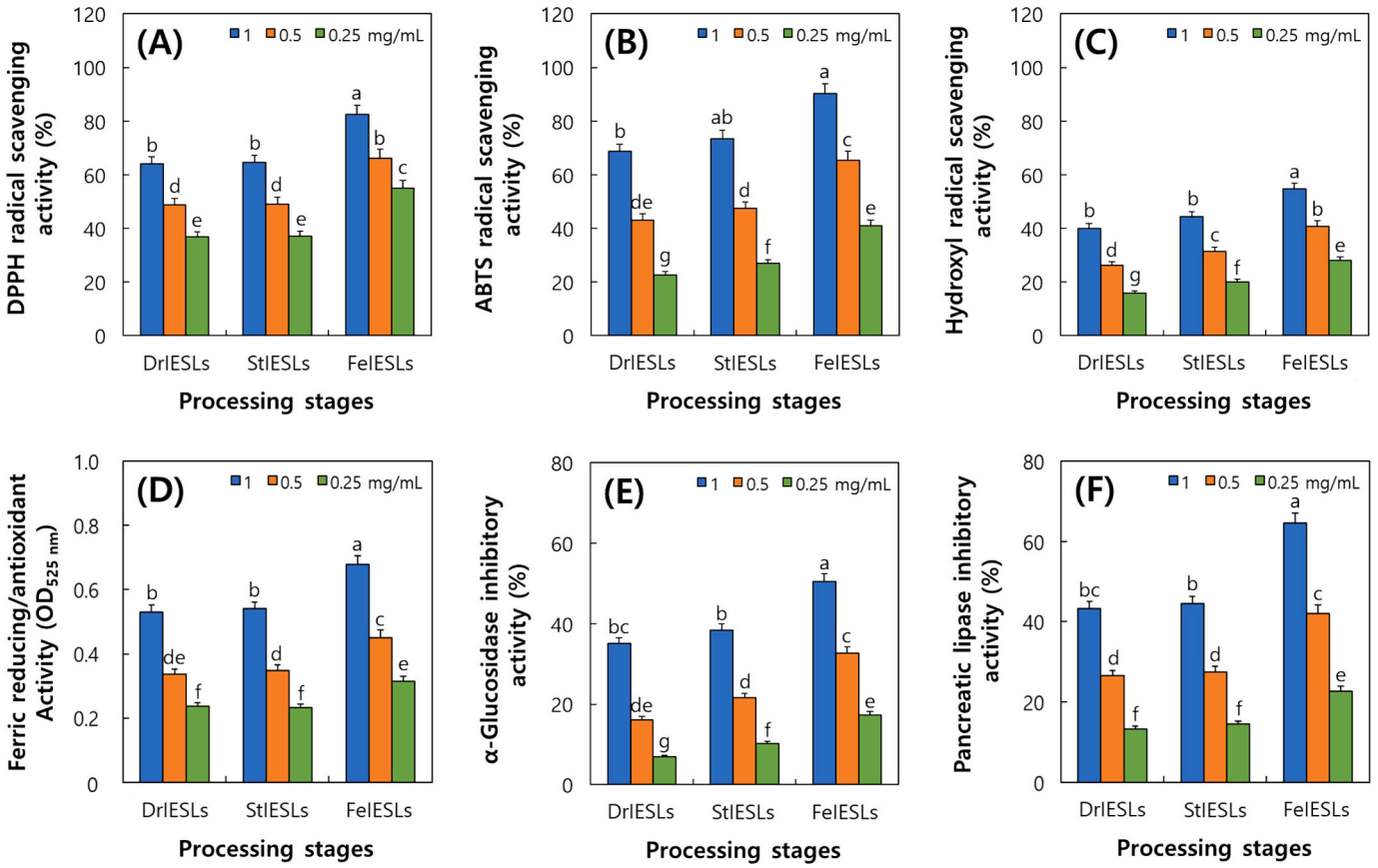

Fig. 8. Comparison of antioxidant and digestive enzyme inhibitory activities in the DrIESLs, StIESLs, and FeIESLs. (A) DPPH radical-scavenging activity, (B) ABTS radical-scavenging activity, (C) hydroxyl radical-scavenging activity, (D) ferric reducing/antioxidant power, (E) α-glucosidase inhibitory activity, and (F) pancreatic lipase inhibitory activity. Processing stages: DrIESLs, dried isoflavone-enriched soybean leaves; StIESLs, steamed isoflavone-enriched soybean leaves; FeIESLs, fermented isoflavone-enriched soybean leaves. All values are presented as the mean ± SD of pentaplicate determination, and different small letters correspond to the significant differences relating to the fermentation time and starter using Duncan's multiple tests ($p < 0.05$).

assumed that these changes are promoted by the action of enzymes such as GAD and β-glycosidase produced by *L. plantarum* P1201 and *L. brevis* BMK184. Consequently, positive aspects such as the bioconversion of glycoside isoflavones to aglycone isoflavones and glutamic acid to GABA, and increased antioxidant and digestive enzyme inhibitory activities due to increased phenolic and flavonoid contents were observed. The SLAF system facilitates the effective utilization of IESLs through artificial processing methods. Therefore, by setting the desired target substance, understanding and appropriately applying the properties of the fermentation substrate, assessing the impact of processing

methods and environmental factors, and analyzing the positive aspects that can be obtained therefrom, materials with enhanced nutritional value can be secured and utilized efficiently. Finally, *L. plantarum* P1201 and *L. brevis* BMK184, along with mass IESL, demonstrated positive synergistic effects, which suggest their potential utility as functional food material in commercial market.

CRediT authorship contribution statement

Hee Yul Lee: Writing – review & editing, Writing – original draft, Visualization, Project administration. **Ji Ho Lee:** Writing – original draft, Methodology, Investigation, Data curation. **Du Yong Cho:** Validation, Software, Resources. **Kyeong Jin Jang:** Investigation, Formal analysis, Data curation. **Jong Bin Jeong:** Investigation, Formal analysis. **Min Ju Kim:** Formal analysis, Data curation. **Ga Young Lee:** Formal analysis, Data curation. **Mu Yeun Jang:** Formal analysis, Data curation. **Jin Hwan Lee:** Writing – review & editing, Funding acquisition, Conceptualization. **Kye Man Cho:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Abbreviation used

The Abbreviation used in the study are DrIESLs, dried isoflavone-enriched soybean leaves; StIESLs, steamed isoflavone-enriched soybean leaves; and FeIESLs, fermented isoflavone-enriched soybean leaves.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101999.

Data availability

Data will be made available on request.

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