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Impact of Pulsed Electric Field Pre-Treatment on the Isoflavone Profile of Soymilk

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Abstract: In this study, pulsed electric fields (PEFs) were evaluated as extraction-aiding technology during soymilk manufacturing to improve its isoflavone profile. Low-intensity PEFs were applied at different processing conditions in two stages of the soymilk extraction process, hydrated soybeans (HSB) and soybean slurry (SBS), with the soymilk extracted from the conventional process as control (CSM). Overall, resultant soymilk samples from PEF-HSB and PEF-SBS presented lower concentrations of glucosides isoflavones and greater aglycone content than those in CSM. In contrast to genistin (Gin) and daidzin (Din), which decreased around 18.5–52.6% and 10.9–54.6%, respectively, an increase in genistein (Ge, 12.3–64.4%) and daidzein (Da, 9–55.8%) was observed. The total isoflavone content (TIC) of most soymilk samples prepared from PEF-HSB was lower than that of the CSM. Conversely, when PEF-SBS was used, the TIC of resultant soymilk was not significantly affected or slightly decreased. However, PEF treated HSB at 10 $kVcm^{-1}$/100 pulses and SBS at 6 $kVcm^{-1}$/10 pulses led to a significant augment in TIC, of up to 109 ± 2.39 and 110 ± 1.26 μg/g, respectively, in the extracted soymilk samples. These results indicated that low-intensity PEF is a potential technology that could be implemented during soymilk manufacturing processing to modify the isoflavone profile and content of soymilk, mainly increasing its aglycone concentration.

Keywords: low-intensity pulsed electric fields; extraction-aiding technology; soymilk; isoflavones; aglycones

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1. Introduction

Isoflavones are naturally occurring phenolic secondary metabolites usually present in soybeans and their derivative products [1]. Their consumption has been associated with a low risk of chronic and degenerative diseases such as cardiovascular diseases, osteoporosis, menopausal symptoms, hormone-dependent cancers, obesity, diabetes, etc. [2]. Furthermore, isoflavones have other biological functions such as anti-inflammatory and antioxidant activities [3,4]. According to their chemical structure, isoflavones are classified into four groups: aglycones, β-glucosides, acetyl-conjugated β-glucosides, and malonyl-conjugated β-glucosides [1,5]. Depending on their chemical structure, they present different biological activities; among them, aglycones have the highest bioactivity and bioavailability [6,7]. However, the isoflavone profile and concentration in soy derivative products are influenced by several factors, including soybean variety, crop and harvesting season, and storage time. In addition, processing conditions such as temperature, pH, microbial fermentation, and enzymatic or acid hydrolysis affect the content and distribution of the different isoflavones forms in soy-based products, changing their absorption and metabolism rate [8,9].

Among soybean derivative products, soymilk is one of the most popular consumed worldwide. It is a colloidal dispersion resulting from the aqueous extraction of soybean

grains [5], which is characterized by a significant content of high-quality protein, vitamins, and minerals, with low concentrations of carbohydrates and fats [10]. This plant-based beverage is appreciated by consumers with lactose intolerance and milk protein allergy; it is an affordable substitute for cow's milk [11,12]. Its manufacturing process consists of five main stages: selection and dehulling of soybeans, water soaking, grinding to obtain the slurry, filtering to separate the liquid from the okara (solid residue), and thermal pasteurization to inactivate antinutritional factors, enzymes, and pathogenic microorganisms [5,13]. Other processing steps such as formulation, fortification, and packaging are also carried out, depending on the producer [14].

It has been reported that processing operations and conditions applied during soymilk extraction significantly impact its isoflavone content and profile, modifying their structure, especially in the conversion to nonconjugated forms [15–17]. Hence, one of the key research challenges for scientists and technologists is to develop effective processes capable of increasing the isoflavone aglycone content in processed soymilk, obtaining soy-based beverages with enhanced bioavailability, and bioactivity [1].

Alternative extraction-aiding technologies, such as microwave, ultrasound, enzymes, and high-pressure homogenization, have been used to improve the isoflavone profile of soymilk with interesting results [5,18–20]. Among novel extraction-aiding technologies, pulsed electric fields (PEFs) at low field strength (1–10 $kVcm^{-1}$) induce cell membrane permeability, which can allow the recovery and an increase in the number of high-added-value compounds from different matrices, resulting in products with potential functionality and health-related benefits [21].

PEF technology consists of the application of pulsed electric field strengths of 1 to 80 $kVcm^{-1}$ during short periods of time (microseconds to milliseconds) into a food matrix placed between two electrodes [21,22]. Depending on the intensity of the treatment, it could be classified in low- or moderate-intensity PEF (1–10 $kVcm^{-1}$), medium-intensity PEF (10–20 $kVcm^{-1}$), or high-intensity PEF (20–40 $kVcm^{-1}$). Low or moderate PEF has been successfully applied to enhance the extraction of bioactive compounds from different food matrices [21]. However, to the best of the authors' knowledge, there is no information about the use of low-intensity PEF processing to enhance the isoflavone profile in soymilk. Hence, the aim of this research was to apply low-intensity PEF as an extraction-aiding procedure in two different stages of soymilk processing—namely, (a) hydrated soybeans (HSB) and (b) soybean slurry (SBS)—to evaluate its effect on the isoflavone profile and total isoflavone content of resultant soymilk.

2. Materials and Methods

2.1. Soymilk Preparation Procedure

Soybeans (Glycine max) were purchased at Lleida, Spain, in a local market, and stored at room temperature until they were used. The soymilk (SM) was prepared according to the procedure established by Yeo and Liong [23] and Morales-de la Peña et al. [5]. Soybeans (100 g) were washed with tap water and soaked in distilled water (300 mL) at room temperature (16 h). Hydrated soybeans (HSB) were drained and rinsed. Then, they were milled with distilled water (400 mL) for 3 min in a blender (Explorian Series E310, Vitamix, Olmsted Falls, OH, USA). After milling, a slurry (SMS) was obtained and filtered through a four-layer cheesecloth to separate the liquid from the okara and obtain raw soymilk (SM). A batch pasteurization process (60 °C, 30 min) was applied to the SM to mainly inactivate the lipoxygenase enzyme. Immediately after processing, the SM was cooled down (5 ± 1 °C), freeze-dried (0.024 mm Hg and −52 °C, Virtis FM 25 EL-85, SP Scientific freeze dryer group, Warminster, PA, USA), and stored until isoflavone analysis.

2.2. Low-Intensity PEF Processing

A batch system equipped with a 0.1 μF capacitator (Physics International, San Leandro, CA, USA), a TG-70 gas control unit, a pulse generator (PT-55, Pacific Atlantic Electronics Inc., Incline Village, NV, USA), and a parallelepiped methacrylate treatment chamber

(20 × 8 cm), with two stainless-steel parallel electrodes, was used for low-intensity PEF treatments. Low-intensity PEF processing was applied in two steps of the SM preparation procedure—HSB and SBS (Figure 1), resulting in two different SM systems for the study, i.e., PEF-HSB and PEF-SBS, respectively, using the control system (CSM) as a reference.

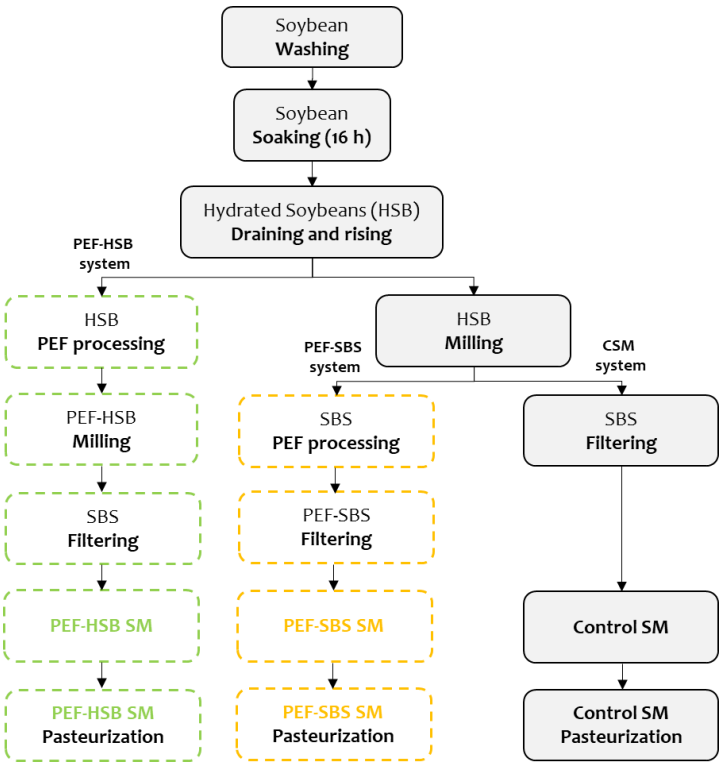

Figure 1. General soymilk (SM) preparation procedure with the application of low-intensity pulsed electric fields (PEFs) in hydrated soybeans (HSB) and soybean slurry (SBS).

Low-intensity PEF processing was applied as follows: First, 100 g of HSB immersed in tap water (1:4, HSB: water) and 100 mL of SBS were placed in the PEF treatment chamber, applying 10, 55, and 100 monopolar exponential pulses (n) of 4 µs width at different electric field strength (E) of 2, 6, and 10 kVcm^{-1}, according to the experimental design described below. Processing conditions were selected based on preliminary studies; data are not shown. Initial (15–18 °C) and final (27–36 °C) temperatures were monitored with a thermometer before and immediately after PEF processing, respectively. Immediately after processing, the SM was obtained following the procedure described in Figure 1, freeze-dried (0.024 mm Hg and −52 °C, Virtis FM 25 EL-85, SP Scientific freeze dryer group, Warminster, PA, USA), and stored until isoflavone analysis.

2.3. Experimental Design

Minitab® software (version 19, Minitab Inc., State College, PA, USA) was used to generate the experimental design. Low-intensity PEF parameters—n (10, 55, 100 pulses) and E (2, 6, and 10 kVcm^{-1})—were selected as independent variables for the central composite design, which consisted of 13 experimental runs (Table 1). All trials were conducted in duplicate in each system. PEF-HSB and PEF-SBS were used to produce SM samples following the procedure mentioned above (Figure 1). The total isoflavone content (TIC) of the obtained SM samples, calculated by the sum of the individual isoflavone concentrations, was selected as the response variable.

Table 1. Experimental design for the application of low-intensity pulsed electric field processing.

Std Order	Run Order	Blocks	E (kVcm^{-1})	n
1 (**A**)	1	1	2	10
2 (**B**)	2	1	10	10
3 (**C**)	3	1	2	100
4 (**D**)	4	1	10	100
5 (**E**)	5	1	2	55
6 (**F**)	6	1	10	55
7 (**G**)	7	1	6	10
8 (**H**)	8	1	6	100
9 (**I**)	9	1	6	55
10 (**J**)	10	1	6	55
11 (**K**)	11	1	6	55
12 (**L**)	12	1	6	55
13 (**M**)	13	1	6	55

2.4. Isoflavone Extraction, Analysis, and Quantification

Isoflavones were extracted and analyzed following the procedure carried out by Morales-de la Peña et al. [2], which quantified β-glucoside and aglycone forms. A portion of 1 g of the freeze-dried SM was weighed into a set of 20 mL screw-top centrifuge tubes. Then, 15 milliliters of 80% ethanol acidified with hydrochloric acid (1 M) was added to each tube and slightly shaken. The tubes were incubated at 80 °C for 1 h. Then, samples were cooled, shaken for 2 min, and centrifuged (12,000× g/4 °C/10 min). The supernatant was separated into a 25 mL volumetric flask, and the residue was re-extracted with 7.5 mL of ethanol (80%). The supernatant of the second extraction was combined with the previous one into the volumetric flask and filled up with ethanol (80%). Then, the samples were filtered using a 0.2 µm Millipore filter and kept at 4 °C prior to chromatographic analysis.

Isoflavone identification and quantification were conducted in an HPLC system (Waters, Milford, MA, USA) equipped with a 600 Controller, a 486 absorbance detector (200 to 350 nm), a thermostatic column compartment, and a 717 plus autosampler, with a cooling system. An aliquot of 20 µL of the extract was injected into the HPLC system, and the isoflavones separation was achieved in a C18 SunFire™ (3.5 µm) stainless steel analytical column (150 × 3 mm) (Waters, Milford, MA, USA) connected with a C18 SunFire™ (5 µm) guard column. The mobile phase (0.3 mL/min) consisted of two eluents: (A) water–methanol (80:20) and (B) water–methanol–acetonitrile (40:40:20). Gradient elution was conducted to achieve the separation as follows: 0–25 min: 20% B; 25–52 min: 100% B; 52–70 min: 20% B. Column temperature was controlled at 37 °C, while sample vials were preserved at 4 °C on the autosampler. Each isoflavone was identified by comparison of its retention time and UV–Vis spectra with those of the reference standards—daidzein (Da), genistein (Ge), glycitein (Gly), daidzin (Din), genistin (Gin), and glycitin (Glyn), from Sigma Aldrich, St. Louis, MO, USA. Their quantification was performed by integration of the peak areas. Data were compared to calibration curves of each isoflavone, and results are expressed as µg/g of SM (dry base).

2.5. Statistical Analysis

Treatments were carried out in duplicate and three replicates, and analyses were conducted for each soymilk system: CSM, PEF-HSB, and PEF-SBS. The mean and standard deviation values (mean ± SD) were estimated based on the obtained results. The statistical difference between treatments was analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's test. The confidence interval was set at 0.95. Statistical analysis was performed using Minitab® software (version 19, Minitab Inc., State College, PA, USA).

3. Results

3.1. Total Isoflavone Content and Profile of SM from Conventional Extraction Process

TIC and individual isoflavones of the soymilk obtained by the conventional extraction process (CSM) are presented in Table 2. TIC of CSM (104 ± 1.22 μg/g) was within the range of total isoflavone concentration reported for different soy products (50–20,000 μg/g) [24].

Table 2. Isoflavone content of control soymilk (CSM) obtained from conventional extraction process.

Isoflavone		Concentration (μg/g)
Aglycone	Da	14.0 ± 0.31
	Ge	26.1 ± 1.44
Glucoside	Din	19.6 ± 0.39
	Gin	44.3 ± 2.75
Total	TAC	40.1 ± 0.87
	TGC	63.9 ± 1.89
	TIC	104 ± 1.22

Da: daidzein, Ge: genistein, Din: daidzin, Gin: genistin, TAC: total aglycones content, TGC: total glucosides content, TIC: total isoflavones content.

As can be inferred from the results, the glucoside forms Gin and Din represented 61.4% of the TIC, while their respective aglycones, Ge and Da, corresponded to 38.6%. Further, it can be observed that Gin was the most abundant isoflavone in the CSM, representing 42.5% of the TIC, followed by Ge (25.1%), Din (18.9%), and Da (13.5%). Glyn, and the concentrations of its respective aglycone form, Gly, were below the detection limit (≈0.2%) and considered negligible; therefore, these data are not shown.

3.2. Individual Isoflavone Content of SM from PEF-HSB and PEF-SBS

The effects of low-intensity PEF applied in two stages of the soymilk extraction process (Figure 1) were evaluated in the individual isoflavone profile and TIC of the resultant SM samples from the different extraction systems, PEF-HSB and PEF-SBS, using CSM as reference. As seen in Figure 2, the application of low-intensity PEF at different treatment conditions to the HSB caused a significant decrease (13.9–39.9%) in the concentration levels of glucoside forms (Din and Gin) in the resultant SM samples, modifying the isoflavone profile toward the aglycone forms (Da and Ge), which increased around 14.4–49.4%, compared with the aglycone content of the CSM sample. Specifically, the concentration values of Din and Gin diminished up to 49.8% and 40.1%, respectively, compared with the CSM sample. The clearest effects were observed in the SM obtained from the HSB sample treated at 2 $kVcm^{-1}$/55 pulses (Treatment E, Din: 9.86 ± 0.27 μg/g) and 6 $kVcm^{-1}$/100 pulses (Treatment H, Gin: 26.5 ± 0.32 μg/g). On the other hand, the Ge content of the resultant SM from PEF-HSB at the highest intensity level (D: 10 $kVcm^{-1}$/100 pulses) was 64.4% higher than that of the CSM sample (26.22 ± 1.44 μg/g). Interestingly, the concentration of aglycone Da in the SM obtained from the HSB sample at different PEF processing conditions did not present a clear trend. While in some treatments (E, F, I, J, K, L, M), Da content significantly decreased around 9.4% and 21.7%, in other treatments, such as the D treatment (10 $kVcm^{-1}$/100 pulses), it increased in a significant way (21.5%), compared with the initial value of the SM obtained by the conventional procedure (CSM: 14.02 ± 0.31 μg/g).

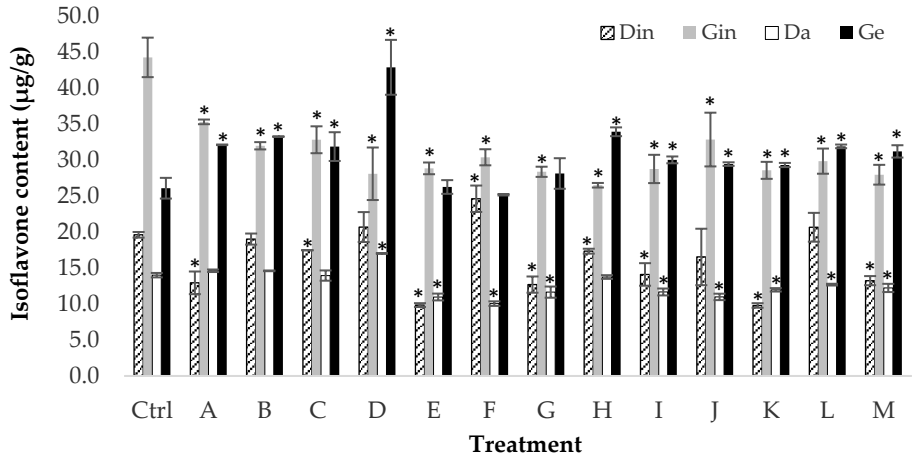

Figure 2. Individual isoflavone content (μg/g) of soymilk prepared from PEF-HSB. * Indicates significant difference ($p < 0.05$) between soymilk obtained by PEF-HSB at specific treatment conditions and control soymilk. **Ctrl**: Soymilk obtained from conventional extraction process. **A**: $E = 2$ kV/cm, $n = 10$; **B**: $E = 10$ kV/cm, $n = 10$; **C**: $E = 2$ kV/cm, $n = 100$; **D**: $E = 10$ kV/cm, $n = 100$; **E**: $E = 2$ kV/cm, $n = 55$; **F**: $E = 10$ kV/cm, $n = 55$; **G**: $E = 6$ kV/cm, $n = 10$; **H**: $E = 6$ kV/cm, $n = 100$; **I–M**: $E = 6$ kV/cm, $n = 55$.

Regarding the isoflavone profile of the SM samples prepared with PEF-SBS systems (Figure 3), a possible interconversion from glucoside isoflavones to aglycones was observed at all treatment conditions. While the concentration of glucosides Gin and Din in the resultant SM sample from PEF-SBS significantly diminished between 18.5–52.6% and 37.2–54.6%, respectively, the content of their respective aglycones increased up to 28.2–60.5% (Ge) and 9.0–55.8% (Da). Furthermore, from the results, it can be elucidated that PEF processing at 6 $kVcm^{-1}$ with 10 and 55 pulses applied in the SBS caused the highest increase in the concentration of both aglycones in the obtained SM samples, reaching values of 20.2 ± 0.95–21.9 ± 0.06 μg/g for Da and 40.0 ± 0.21–41.8 ± 0.66 μg/g for Ge. These values are significantly higher than those observed in the CSM sample (Da: 14.02 ± 0.31 μg/g, Ge: 26.11 ± 1.44 μg/g), i.e., they are 0.5- and 0.6-fold more than the values in the conventional SM.

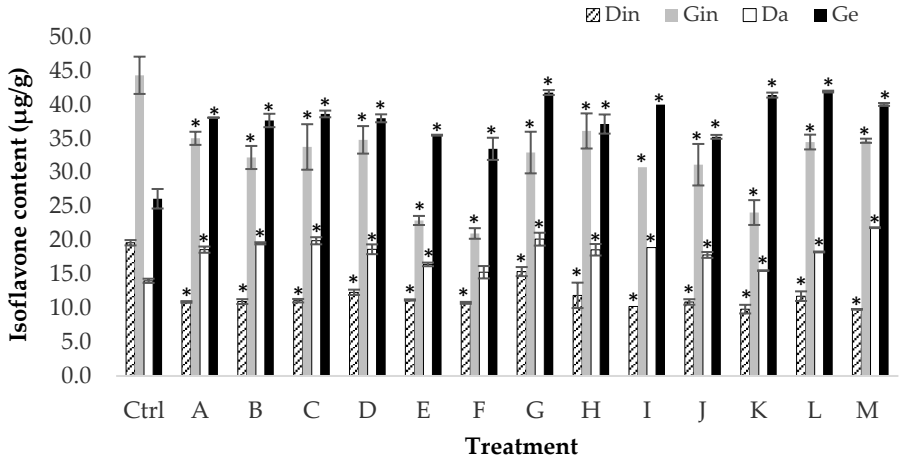

Figure 3. Individual isoflavone content (μg/g) of soymilk prepared from PEF-SBS. * Indicates significant difference ($p < 0.05$) between soymilk obtained by PEF-SBS at specific conditions and control soymilk. **Ctrl**: Soymilk obtained from conventional extraction process. **A**: $E = 2$ kV/cm, $n = 10$; **B**: $E = 10$ kV/cm, $n = 10$; **C**: $E = 2$ kV/cm, $n = 100$; **D**: $E = 10$ kV/cm, $n = 100$; **E**: $E = 2$ kV/cm, $n = 55$; **F**: $E = 10$ kV/cm, $n = 55$; **G**: $E = 6$ kV/cm, $n = 10$; **H**: $E = 6$ kV/cm, $n = 100$; **I–M**: $E = 6$ kV/cm, $n = 55$.

3.3. Total Isoflavone Content of SM from PEF-HSB and PEF-SBS

As a result of the changes in the content amount of individual isoflavones present in soymilk samples prepared from PEF-processed HSB and SBS, their TIC (calculated by the sum of Din, Gin, Da, and Ge content) also varied, compared with that of the CSM sample (Table 3). The resultant SM prepared from PEF-HSB systems contained lower TIC, regardless of the PEF treatment conditions, except in the SM prepared from PEF-HSB at the highest intensity of 10 kVcm^{-1}/100 pulses (109 ± 2.39 μg/g). In comparison, no significant changes were observed in the TIC of SM prepared from PEF-SBS, or it slightly decreased. Interestingly, when SBS was treated at 6 kVcm^{-1}/10 pulses, the TIC of the resultant SM was higher (110 ± 1.26 μg/g) than that observed in the CSM sample.

Table 3. Total isoflavone content (μg/g) of soymilk obtained from conventional extraction process and hydrated soybean and soybean slurry processing with low-intensity pulsed electric field.

Treatment	PEF-HSB	PEF-SBS
Ctrl	104 ± 1.22 [a]	
A	95.1 ± 0.53 [b]	103 ± 0.39 [a]
B	99.0 ± 0.33 [b]	100 ± 0.80 [b]
C	96.2 ± 1.15 [b]	103 ± 1.16 [a]
D	109 ± 2.39 [c]	104 ± 0.93 [a]
E	76.0 ± 0.63 [d]	86.0 ± 0.27 [c]
F	90.3 ± 0.84 [e]	80.5 ± 0.87 [d]
G	80.9 ± 1.18 [f]	110 ± 1.26 [e]
H	91.6 ± 0.38 [g]	104 ± 1.60 [a]
I	84.6 ± 1.12 [h]	101 ± 1.58 [b]
J	89.9 ± 2.09 [g]	95.1 ± 1.04 [f]
K	79.7 ± 0.51 [f]	90.8 ± 0.72 [g]
L	95.2 ± 1.03 [b]	106 ± 0.50 [a]
M	84.7 ± 0.86 [h]	106 ± 0.55 [a]

Values in the same column with different letter were significantly different ($p < 0.05$). PEF-HSB: low-intensity pulsed electric field treated hydrated soybean system; PEF-SBS: low-intensity pulsed electric field treated soybean slurry system; **Ctrl**: soymilk obtained from conventional extraction process; **A**: E = 2 kV/cm, n = 10; **B**: E = 10 kV/cm, n = 10; **C**: E = 2 kV/cm, n = 100; **D**: E = 10 kV/cm, n = 100; **E**: E = 2 kV/cm, n = 55; **F**: E = 10 kV/cm, n = 55; **G**: E = 6 kV/cm, n = 10; **H**: E = 6 kV/cm, n = 100; **I**–**M**: E = 6 kV/cm, n = 55.

4. Discussion

The TIC (76.0 ± 0.63–110 ± 1.26 μg/g) of the SM samples obtained from conventional extraction method, as well as those from PEF-treated HSB and SBS at different processing conditions, was within the range reported in previous studies for different soy products (50–20,000 μg/g) [24]. It has been corroborated that soy-based food products have a wide variation in their total isoflavone content, mainly depending on different factors such as the soybean cultivar used for the formulation, the extraction method, preparation, and preservation procedures, as well as storage time and conditions [14,25–27].

Among the content distribution of individual isoflavones of the CSM sample, it was observed that glucoside forms were the most abundant, with Gin being the isoflavone present at the highest concentration (Table 2). Kao et al. [17] and Xu and Chang [28] reported that malonyl-glucoside forms are the main isoflavones in raw soymilk; however, after thermal processing, they are transformed to their glucoside forms. In this sense, the pasteurization step carried out at the end of the SM production process may cause the hydrolysis of ester bonds of the malonyl-glucoside forms, resulting in a higher content of the glucosides, especially Gin. In addition, other investigations have also demonstrated that soymilk had a higher concentration of glucosides than aglycone isoflavones, with Gin as the most abundant compound [25,28,29]. Consistently, in a previous study conducted by our research group [5], it was proved that glucoside conjugated forms represented approximately 87% of the total isoflavone content of the SM sample obtained by the same procedure used in this research.

The application of low-intensity PEF to HSB or SBS to prepare soymilk induced significant changes in the isoflavone profile of the resultant SM samples, regardless of the treatment conditions (Figures 2 and 3). Nonetheless, SM samples prepared from PEF-SBS presented higher concentration levels of aglycones than those extracted from PEF-HSB. To the best of the authors' knowledge, this is the first study evaluating the effects of low-intensity PEF processing applied as an extraction-aiding treatment to modify the isoflavone profile of SM. Usually, PEF technology has been only applied at high intensities (20–40 $kVcm^{-1}$) as a nonthermal preservation method of soymilk or soymilk-mixed beverages. Moreover, only a few studies have evaluated the impact of high-intensity pulsed electric fields (HIPEFs) on the isoflavone profile and content of soy-based products. Specifically, Rodríguez-Roque et al. [30] observed that HIPEF processing (4 µs square-bipolar pulses at 35 $kVcm^{-1}$, 200 Hz and 2000 µs) applied to a soymilk-mixed beverage led to a significant increase in its content of Gin and Din isoflavones (9–25%), and only a slight increase in Da concentration (2%). According to the authors [30], this increase could be due to the electroporation effects that occurred during HIPEF processing, which facilitates the extractability of isoflavones from the food matrix. Contrarily to our results, the observed increase in aglycones concentration in the SM extracted from PEF-treated HSB or SBS might not be related to the electroporation effects of PEF at low intensities (2–10 $kVcm^{-1}$) but to the interconversion reactions of glucosides forms to aglycones. In this regard, different authors have stated that some reactions such as decarboxylation, de-esterification, or de-glycosylation of isoflavones could occur in soy-based products depending on processing and storage conditions, as well as the molecular configuration present in the raw material [17,31–33].

Although no application of low-intensity PEF has been evaluated before for isoflavone profile modification in soymilk, other extraction-aiding technologies such as ultrasound and high hydrostatic pressures have been investigated with the same purpose [13]. Specifically, Morales-de la Peña et al. [5] applied ultrasound processing to hydrated soybeans and soybean slurry at different conditions, reporting that the aglycones isoflavones concentration of the SM samples obtained from sonicated hydrated soybeans or soybean slurry significantly increased to 90–131% and 19.6–59%, respectively. Likewise, Fahmi et al. [34] observed that soymilk extracted from ultrasound processed soybean slurry at 35 and 150 kHz had a higher concentration of glycosides and aglycone isoflavones than that obtained from a traditional extraction process. The modification in the isoflavone profile of SM prepared from sonicated soybeans or soybean slurry was attributed to the cavitation phenomena, which might induce the release of isoflavones from intracellular tissues or the interconversion of malonyl-Din to Din, and of β-glucosides to aglycones [5]. Interestingly, Jung et al. [16] applied high hydrostatic pressure processing to hydrated soybeans (100–750 MPa) and soymilk (400–750 MPa; 25 and 75 °C) and evaluated the treatment effects on isoflavone profile. The authors observed that the isoflavone profile of the SM prepared from pressurized hydrated-soybeans remained with no significant changes compared to the standard soymilk. However, when soymilk was processed by a combination of high pressure (750 MPa) and moderate-temperature thermal treatment (75 °C), its isoflavone distribution changed, shifting the malonyl-glucosides structures toward β-glucosides. These results suggest that high-pressure levels combined with moderate temperatures had a synergistic effect and promoted the interconversion of the malonyl isoflavones to glucosides forms due to adiabatic heating.

Interconversion reactions among glucoside isoflavones to aglycones have been mainly attributed to the action of the β-glucosidase (β-Glu) enzyme, which is known to convert glucosides to free aglycones [35]. Xu et al. [36] and Otieno et al. [37] reported that the presence of β-Glu enzymes in different soy derivatives was the main cause for the reduction in glucoside content. Interestingly, Aguiló-Aguayo et al. [38] demonstrated that PEF processing with monopolar pulses at 35 $kVcm^{-1}$ induced a rise above the initial values in β-Glu activity present in raw strawberry juices. Therefore, it may be possible that low-intensity PEF processing at the PEF assayed conditions in the soybean systems (HSB or

SBS) enhanced the endogenous β-Glu enzyme activity, which led to hydrolysis reactions and breakdown of the conjugated isoflavones (Gin and Din), thus obtaining aglycone-rich SM samples with better functionality. Nevertheless, there is a need for more in-depth research to provide evidence of enzyme activity of the food matrix after low-intensity PEF processing.

Regarding the analysis of TIC of SM samples prepared from different soybean systems, it was observed that SM samples extracted from PEF-HSB presented significantly lower total isoflavone concentration than that of CSM. This effect could be associated with the electroporation effect that occurred during the processing of HSB, leading to cell membrane permeabilization and a release of intracellular polyphenolic compounds, including isoflavones, into the soaking water in which the HSB samples were treated. It has been reported that plant cell permeabilization improves the mass transfer, facilitating the extraction of plant metabolites [22,39]. Since soaking water was discarded after PEF processing, some isoflavones molecules could be transferred there, resulting in SM samples with lower concentrations. These results confirm the observations of other authors that reported a higher extraction of polyphenols in PEF-treated grape skins at 5 and 10 $kVcm^{-1}$ used during Tempranillo grape vinification [40]. On the other hand, the application of PEF in the SBS system caused no significant changes or a decrease in the TIC of the SM samples at most treatment conditions, except in the SM prepared from PEF-treated SBS with 10 pulses at 6 $kVcm^{-1}$. At these processing conditions, the obtained SM had the highest level of TIC (110 ± 1.26 μg/g), compared with CSM and the SM sample obtained from PEF-HSB. This increase might be associated with the β-Glu enzyme activity, which could be more affected by these processing conditions, inducing interconversion reactions between malonyl- and acetyl-β-glucosides to glucosides and aglycones.

5. Conclusions

The application of low-intensity PEF as extraction-aiding technology during the soymilk manufacturing process induced interconversion reactions of glucosides forms to aglycones, regardless of whether the system in which the PEF processing was applied was HSB or SBS. Furthermore, β-glucosidase enzyme activity might be increased during PEF processing of HSB and SBS causing hydrolysis of malonyl- and acetyl structures and increasing the aglycones and total isoflavone content. Low-intensity PEF treatment applied in SBS caused a higher content of Da and Ge in the resultant soymilk than its application in the SBS. Hence, the food matrix has a significant impact on the low-intensity PEF processing effects. The integration of novel treatments such as low-intensity PEF during the soymilk extraction process seems to be an interesting way to increase the aglycone content, enhancing its functionality and potential health benefits. Further research is recommended to better understand the interconversion reaction among isoflavones and related enzymes' activity.

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References

1. De Queirós, L.D.; De Ávila, A.R.A.; Botaro, A.V.; Chirotto, D.B.L.; Macedo, J.A.; Macedo, G.A. Combined isoflavones biotransformation increases the bioactive and antioxidant capacity of soymilk. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 10019–10031. [CrossRef] [PubMed]
2. Morales-De La Peña, M.; Salvia-Trujillo, L.; Rojas-Graü, M.; Martin-Belloso, O. Isoflavone profile of a high intensity pulsed electric field or thermally treated fruit juice-soymilk beverage stored under refrigeration. *Innov. Food Sci. Emerg. Technol.* **2010**, *11*, 604–610. [CrossRef]
3. Hiramatsu, E.Y.; De Ávila, A.R.A.; Gênova, V.M.; de Queiros, L.D.; Macedo, G.A.; Martins, I.M.; Macedo, J.A. Biotransformation processes in soymilk isoflavones to enhance anti-inflammatory potential in intestinal cellular model. *J. Food Biochem.* **2020**, *44*, e13149. [CrossRef] [PubMed]
4. De Ávila, A.R.A.; de Queiros, L.D.; Ueta, T.M.; Macedo, G.A.; Macedo, J.A. Exploring in vitro effects of biotransformed isoflavones extracts: Antioxidant, antiinflammatory, and antilipogenic. *J. Food Biochem.* **2019**, *43*, e12850. [CrossRef]
5. Morales-de la Peña, M.; Martín-Belloso, O.; Welti-Chanes, J. High-power ultrasound as pre-treatment in different stages of soymilk manufacturing process to increase the isoflavone content. *Ultrason. Sonochemistry* **2018**, *49*, 154–160. [CrossRef]
6. Setchell, K.D.; Brown, N.M.; Zhao, X.; Lindley, S.L.; Heubi, E.J.; King, E.C.; Messina, M.J. Soy isoflavone phase II metabolism differs between rodents and humans: Implications for the effect on breast cancer risk. *Am. J. Clin. Nutr.* **2011**, *94*, 1284–1294. [CrossRef]
7. Islam, M.A.; Bekele, R.; Vanden Berg, J.H.J.; Kuswanti, Y.; Thapa, O.; Soltani, S.; van Leeuwen, F.X.R.; Rietjens, I.M.C.M.; Murk, A.J. Deconjugation of soy isoflavone glucuronides needed for estrogenic activity. *Toxicol. In Vitro* **2015**, *29*, 706–715. [CrossRef]
8. Nufer, K.R.; Ismail, B.; Hayes, K.D. The Effects of Processing and Extraction Conditions on Content, Profile, and Stability of Isoflavones in a Soymilk System. *J. Agric. Food Chem.* **2009**, *57*, 1213–1218. [CrossRef]
9. Andrade, J.; Mandarino, J.; Kurozawa, L.; Ida, E. The effect of thermal treatment of whole soybean flour on the conversion of isoflavones and inactivation of trypsin inhibitors. *Food Chem.* **2016**, *194*, 1095–1101. [CrossRef]
10. Jinapong, N.; Suphantharika, M.; Jamnong, P. Production of instant soymilk powders by ultrafiltration, spray drying and fluidized bed agglomeration. *J. Food Eng.* **2008**, *84*, 194–205. [CrossRef]
11. Reilly, J.K.; Lanou, A.J.; Barnard, N.D.; Seidl, K.; Green, A.A. Acceptability of Soymilk as a Calcium-Rich Beverage in Elementary School Children. *J. Am. Diet. Assoc.* **2006**, *106*, 590–593. [CrossRef]
12. Xu, B.; Chang, S.K.C. Phytochemical Profiles and Health-Promoting Effects of Cool-Season Food Legumes as Influenced by Thermal Processing. *J. Agric. Food Chem.* **2009**, *57*, 10718–10731. [CrossRef] [PubMed]
13. Han, H.; Choi, J.K.; Park, J.; Im, H.C.; Han, J.H.; Huh, M.H.; Lee, Y.B. Recent innovations in processing technologies for improvement of nutritional quality of soymilk. *CyTA J. Food* **2021**, *19*, 287–303. [CrossRef]
14. Giri, S.K.; Mangaraj, S. Processing Influences on Composition and Quality Attributes of Soymilk and its Powder. *Food Eng. Rev.* **2012**, *4*, 149–164. [CrossRef]
15. Huang, H.; Liang, H.; Kwok, K.C. Effect of thermal processing on genistein, daidzein and glycitein content in soymilk. *J. Sci. Food Agric.* **2006**, *86*, 1110–1114. [CrossRef]
16. Jung, S.; Murphy, P.A.; Sala, I. Isoflavone profiles of soymilk as affected by high-pressure treatments of soymilk and soybeans. *Food Chem.* **2008**, *111*, 592–598. [CrossRef]
17. Kao, T.; Lu, Y.; Hsieh, H.; Chen, B. Stability of isoflavone glucosides during processing of soymilk and tofu. *Food Res. Int.* **2004**, *37*, 891–900. [CrossRef]
18. Penha, C.B.; Falcão, H.G.; Ida, E.I.; Speranza, P.; Kurozawa, L.E. Enzymatic pretreatment in the extraction process of soybean to improve protein and isoflavone recovery and to favor aglycone formation. *Food Res. Int.* **2020**, *137*, 109624. [CrossRef]
19. Preece, K.; Hooshyar, N.; Zuidam, N. Whole soybean protein extraction processes: A review. *Innov. Food Sci. Emerg. Technol.* **2017**, *43*, 163–172. [CrossRef]
20. Varghese, T.; Pare, A. Effect of microwave assisted extraction on yield and protein characteristics of soymilk. *J. Food Eng.* **2019**, *262*, 92–99. [CrossRef]
21. Barba, F.J.; Parniakov, O.; Pereira, S.A.; Wiktor, A.; Grimi, N.; Boussetta, N.; Saraiva, J.A.; Raso, J.; Martin-Belloso, O.; Witrowa-Rajchert, D.; et al. Current applications and new opportunities for the use of pulsed electric fields in food science and industry. *Food Res. Int.* **2015**, *77*, 773–798. [CrossRef]
22. Vorobiev, E.; Lebovka, N.I. Extraction of intercellular components by pulsed electric fields. In *Pulsed Electric Fields Technology for the Food Industry: Fundamentals and Applications*, 1st ed.; Raso, V.H.J., Ed.; Springer: New York, NY, USA, 2006; pp. 153–194.
23. Yeo, S.K.; Liong, M.T. Effect of ultrasound on bioconversion of isoflavones and probiotic properties of parent organisms and subsequent passages of Lactobacillus. *LWT-Food Sci. Technol.* **2013**, *51*, 289–295. [CrossRef]
24. Song, T.; Barua, K.; Buseman, G.; Murphy, A.P. Soy isoflavone analysis: Quality control and a new internal standard. *Am. J. Clin. Nutr.* **1998**, *68*, 1474S–1479S. [CrossRef]
25. Eisen, B.; Ungar, Y.; Shimoni, E. Stability of Isoflavones in Soy Milk Stored at Elevated and Ambient Temperatures. *J. Agric. Food Chem.* **2003**, *51*, 2212–2215. [CrossRef]
26. Chun, J.; Kim, J.S.; Kim, J.H. Enrichment of isoflavone aglycones in soymilk by fermentation with single and mixed cultures of Streptococcus infantarius 12 and *Weissella* sp. 4. *Food Chem.* **2008**, *109*, 278–284. [CrossRef]

27. Callou, K.R.D.A.; Sadigov, S.; Lajolo, F.M.; Genovese, M.I. Isoflavones and Antioxidant Capacity of Commercial Soy-Based Beverages: Effect of Storage. *J. Agric. Food Chem.* **2010**, *58*, 4284–4291. [CrossRef] [PubMed]
28. Xu, B.; Chang, S.K.C. Isoflavones, Flavan-3-ols, Phenolic Acids, Total Phenolic Profiles, and Antioxidant Capacities of Soy Milk as Affected by Ultrahigh-Temperature and Traditional Processing Methods. *J. Agric. Food Chem.* **2009**, *57*, 4706–4717. [CrossRef]
29. Ishihara, M.; Singh, H.; Chung, G.; Tam, C. Content composition and antioxidant activity of isoflavones in commercial and homemade soymilk and tofu. *J. Sci. Food Agric.* **2007**, *87*, 2844–2852. [CrossRef]
30. Rodríguez-Roque, M.J.; De Ancos, B.; Sánchez-Vega, R.; Sánchez-Moreno, C.; Elez-Martínez, P.; Martín-Belloso, O. In vitro bioaccessibility of isoflavones from a soymilk-based beverage as affected by thermal and non-thermal processing. *Innov. Food Sci. Emerg. Technol.* **2020**, *66*, 102504. [CrossRef]
31. Coward, L.; Smith, M.; Kirk, M.; Barnes, S. Chemical modification of isoflavones in soyfoods during cooking and processing. *Am. J. Clin. Nutr.* **1998**, *68*, 1486S–1491S. [CrossRef] [PubMed]
32. Grün, I.U.; Adhikari, K.; Li, C.; Li, Y.; Bin Lin, B.; Zhang, A.J.; Fernando, L.N. Changes in the Profile of Genistein, Daidzein, and Their Conjugates during Thermal Processing of Tofu. *J. Agric. Food Chem.* **2001**, *49*, 2839–2843. [CrossRef] [PubMed]
33. Otieno, D.; Ashton, J.; Shah, N. Isoflavone phytoestrogen degradation in fermented soymilk with selected β-glucosidase producing L. acidophilus strains during storage at different temperatures. *Int. J. Food Microbiol.* **2007**, *115*, 79–88. [CrossRef]
34. Fahmi, R.; Khodaiyan, F.; Pourahmad, R.; Emam-Djomeh, Z. Effect of ultrasound assisted extraction upon the Genistin and Daidzin contents of resultant soymilk. *J. Food Sci. Technol.* **2014**, *51*, 2857–2861. [CrossRef] [PubMed]
35. Matsuura, M.; Obata, A.; Fukushima, D. Objectionable Flavor of Soy Milk Developed during the Soaking of Soybeans and its Control. *J. Food Sci.* **1989**, *54*, 602–605. [CrossRef]
36. Xu, Z.; Wu, Q.; Godber, J.S. Stabilities of Daidzin, Glycitin, Genistin, and Generation of Derivatives during Heating. *J. Agric. Food Chem.* **2002**, *50*, 7402–7406. [CrossRef] [PubMed]
37. Otieno, D.; Ashton, J.; Shah, E.N. Stability of β-glucosidase Activity Produced by Bifidobacterium and *Lactobacillus* spp. in Fermented Soymilk During Processing and Storage. *J. Food Sci.* **2006**, *70*, M236–M241. [CrossRef]
38. Aguiló-Aguayo, I.; Sobrino-López, Á.; Soliva-Fortuny, R.; Martín-Belloso, O. Influence of high-intensity pulsed electric field processing on lipoxygenase and β-glucosidase activities in strawberry juice. *Innov. Food Sci. Emerg. Technol.* **2008**, *9*, 455–462. [CrossRef]
39. Toepfl, S.; Heinz, V.; Knorr, D. Applications of pulsed electric fields technology for the food industry. In *Pulsed Electric Fields Technology for the Food Industry: Fundamentals and Applications*, 1st ed.; Raso, V.H.J., Ed.; Springer: New York, NY, USA, 2006; pp. 197–222.
40. López, N.; Puértolas, E.; Condón, S.; Alvarez, I.; Raso, J. Effects of pulsed electric fields on the extraction of phenolic compounds during the fermentation of must of Tempranillo grapes. *Innov. Food Sci. Emerg. Technol.* **2008**, *9*, 477–482. [CrossRef]