Scilight

Food Science and Processing

https://www.sciltp.com/journals/fsp

Review

Check for updates

From Conjugates to Aglycones: How Food Processing Technologies Reshape Soy Isoflavone Profiles

Marcela Fernanda Geton Guelfi [1], Fernando Sanches de Lima [2], Cíntia Ladeira Handa [3], Tahis Regina Baú [4], Heloísa Gabriel Falcão [5], Claudimara da Silva Portela [6] and Tatiana Colombo Pimentel [1,2,*]

[1] State University of Maringá, Campus Maringá, Maringá 87020-900, PR, Brazil
[2] Federal Institute of Paraná, Campus Paranavaí, Paranavaí 87703-536, PR, Brazil
[3] Federal Institute of Paraná, Campus Pitanga, Pitanga 85201-106, PR, Brazil
[4] Federal Institute of Santa Catarina, Campus São Miguel do Oeste, São Miguel do Oeste 89900-000, SC, Brazil
[5] Federal Institute of Goiás, Campus Inhumas, Inhumas 76600-000, GO, Brazil
[6] State University of Londrina, Londrina 86057-970, PR, Brazil
* Correspondence: tatiana.pimentel@ifpr.edu.br

How To Cite: Guelfi, M.F.G.; de Lima, F.S.; Handa, C.L.; et al. From Conjugates to Aglycones: How Food Processing Technologies Reshape Soy Isoflavone Profiles. *Food Science and Processing* **2026**, *2*(3), 11. https://doi.org/10.53941/fsp.2026.100011

Received: 8 March 2026
Revised: 14 July 2026
Accepted: 15 July 2026
Published: 31 July 2026

Abstract: Soybean is the primary dietary source of isoflavones, bioactive phenolic compounds whose physiological effects depend strongly on their chemical form. In raw grains, isoflavones occur predominantly as β-glucosides and malonyl- and acetyl-glucosides, which exhibit lower bioavailability than their aglycone counterparts. This mini-review examines how food processing technologies reshape soy isoflavone profiles, focusing on technological strategies that promote the conversion of conjugated forms into aglycones. The chemical and biochemical mechanisms underlying isoflavone interconversion are discussed, emphasizing the roles of β-glucosidase activity, temperature, pH, water availability, and matrix structure in modulating conversion efficiency. Solid-state and submerged fermentations consistently emerge as the most effective approaches for aglycone enrichment, although outcomes remain strain- and substrate-dependent. In contrast, thermal processing primarily drives interconversion among conjugated forms through decarboxylation and deesterification, with significant aglycone formation occurring only when mild pre-heating conditions transiently preserve endogenous enzymatic activity. Emerging non-thermal technologies, including ultrasound, pulsed electric fields, and high hydrostatic pressure, enhance matrix permeability and enzyme–substrate interactions, enabling controlled modulation of isoflavone profiles while minimizing nutritional and sensory losses. Overall, integrating fermentation, controlled hydration, and process-intensification strategies represents a rational pathway to design soy-based products with enhanced aglycone content and improved bioaccessibility, supporting the development of functional foods with greater health-promoting potential.

Keywords: daidzein; genistein; glycitein; phytochemicals; nutraceuticals; functional foods

1. Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most widely produced and consumed legumes worldwide, playing a central role in global food systems and industrial processing chains. Beyond its high protein content, soybeans are the primary dietary source of isoflavones, bioactive phenolic compounds that have been extensively investigated for their physiological and health-related effects. In raw soybeans, isoflavones occur predominantly

as β-glucosides and malonyl- and acetyl-glucosides, chemical forms that directly influence their stability, bioaccessibility, and intestinal absorption [1–3].

Among these compounds, the aglycones (genistein, daidzein, and glycitein) share structural similarity with 17β-estradiol, enabling their interaction with estrogen receptors and modulation of estrogen-responsive pathways. Experimental and epidemiological studies associate isoflavone intake with antioxidant and anti-inflammatory effects, regulation of lipid and glucose metabolism, and reduced risk of chronic conditions such as cardiovascular diseases, type 2 diabetes, osteoporosis, and hormone-related disorders [4–6]. Importantly, these biological outcomes depend not only on total intake but also on the chemical form consumed, as aglycones generally display higher intestinal bioavailability than their glycosylated counterparts. Therefore, aglycone enrichment is not only relevant from a nutritional standpoint but also represents an important technological objective in the development of soy-based foods and ingredients. Processing strategies that can modulate isoflavone profiles offer opportunities to enhance the functional value of soybean-derived products through the controlled bioconversion of conjugated forms [3,7].

Conversion of conjugated isoflavones into aglycones may occur in vivo through intestinal microbiota activity; however, technological interventions applied during food processing can preemptively modulate this conversion [8,9]. Processing operations such as soaking, fermentation, thermal treatments, and emerging non-thermal technologies induce chemical and enzymatic transformations that reshape isoflavone profiles in soy-based products [10–14]. While previous reviews have addressed nutritional and clinical aspects of soy isoflavones, fewer studies have systematically examined how specific processing parameters govern structural interconversion among conjugated and aglycone forms. This review aims to examine food processing technologies applied to soy-based foods and ingredients and their effects on modifying the isoflavone profile. The approach focuses primarily on the conversion mechanisms and technological factors governing aglycone formation, while bioaccessibility outcomes are discussed when they are directly associated with processing-induced changes.

In this context, this mini-review critically evaluates how food processing technologies drive the conversion of conjugated isoflavones to aglycones in soybean matrices. By integrating mechanistic insights with technological considerations, this work aims to clarify process–isoflavone profile relationships and to support the design of soy-based foods with optimized isoflavone profiles and enhanced functional potential.

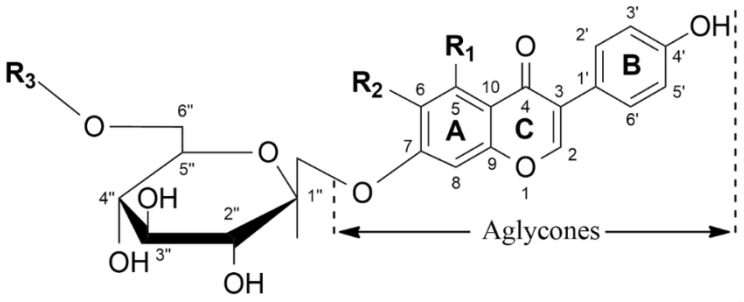
2. Chemical and Biochemical Bases of the Conversion of Glycosylated Isoflavones into Aglycones

Isoflavones constitute one of the major classes of secondary metabolites, specifically phenolic compounds, found in soybeans and their derivatives (*Glycine max* L.), chemically classified as flavonoid polyphenols. Their core structure is based on the 3-phenylchromone skeleton (Figure 1), which supports structural diversification through glycosylation and acylation reactions [15,16]. In soy matrices, isoflavones occur in twelve principal chemical forms grouped into four categories: aglycones (daidzein, genistein, glycitein), β-glucosides (daidzin, genistin, glycitin), acetylglucosides (6″-*O*-acetyldaidzin, 6″-*O*-acetylgenistin, and 6″-*O*-acetylglycitin), and malonylglucosides (6″-O-malonyldaidzin, 6″-O-malonylgenistin, and 6″-*O*-malonylglycitin) [3,16–18].

In raw soybeans, malonylglucosides and β-glucosides predominate, whereas aglycones typically account for less than 5% of total isoflavones. This distribution is technologically relevant because aglycones exhibit greater membrane permeability and intestinal absorption, largely explaining their higher bioavailability and biological activity [17,19,20]. Consequently, processing-induced structural conversion becomes a key strategy for modulating functional value [3,18,20].

Isoflavone interconversion occurs through both chemical and enzymatic pathways. Specifically, chemical interconversion refers to non-enzymatic transformations among conjugated species, such as the thermal deesterification of malonylglucosides into β-glucosides [1,17,21]. Enzymatic deglycosylation involves the hydrolytic cleavage of the β-glycosidic linkage by β-glucosidases, thereby liberating free bioactive aglycones [1,22–24]. Lastly, secondary transformations and degradation encompass the irreversible alteration, ring-cleavage, or environmental leaching of the core flavone skeleton under excessive thermal severity or high fluid volumes, leading to a net reduction in total isoflavone content [1,21,25]. Thermal processing primarily drives non-enzymatic transformations among conjugated forms, following the general sequence malonylglucosides → acetylglucosides → β-glucosides via decarboxylation and deesterification reactions (Figure 2) [13,16–18]. Direct hydrolysis of β-glucosides into aglycones, however, is mainly catalyzed by β-glucosidase (EC 3.2.1.21), which cleaves β-D-glycosidic bonds and releases free aglycones and glucose [22,26]. Severe thermal or chemical conditions may promote non-specific glycosidic cleavage, but such treatments often compromise aglycone stability and generate undesirable by-products. Consequently, desirable conversion pathways and undesirable degradation or leaching reactions take place

competitively, with the kinetic balance shifting dynamically as a function of process severity, exposure time, and solvent volume [1,16,17,25,27].



Group	Isoflavone	R_1	R_2	R_3
Aglycones	Daidzein	-H	-H	-
	Genistein	-OH	-H	-
	Glycitein	-H	$-OCH_3$	-
β-glucosides	Daidzin	-H	-H	-H
	Genistin	-OH	-H	-H
	Glycitin	-H	$-OCH_3$	-H
Acetylglucosides	6''-*O*-acetyldaidzin	-H	-H	$-COCH_3$
	6''-*O*-acetylgenistin	-OH	-H	$-COCH_3$
	6''-*O*-acetylglycitin	-H	$-OCH_3$	$-COCH_3$
Malonylglucosides	6''-*O*-malonyldaidzin	-H	-H	$-COCH_2COOH$
	6''-*O*-malonylgenistin	-OH	-H	$-COCH_2COOH$
	6''-*O*-malonylglycitin	-H	$-OCH_3$	$-COCH_2COOH$

Figure 1. Chemical structures of the twelve main soy isoflavones, comparing their four structural groups based on specific substituent positions (R_1, R_2, R_3): free aglycones, 7-O-β-glucosides, 7-O-(6″-O-acetyl-β-glucosides), and 7-O-(6″-O-malonyl-β-glucosides).

Both endogenous soybean enzymes and microbial β-glucosidases contribute to deglycosylation during processing [22,26]. Microbial enzymes generally provide greater operational robustness and broader pH–temperature tolerance, explaining the high conversion efficiency observed during fermentation [26,28,29]. Beyond isoflavones, β-glucosidase activity may also liberate other bound phenolics, further influencing antioxidant potential [30,31].

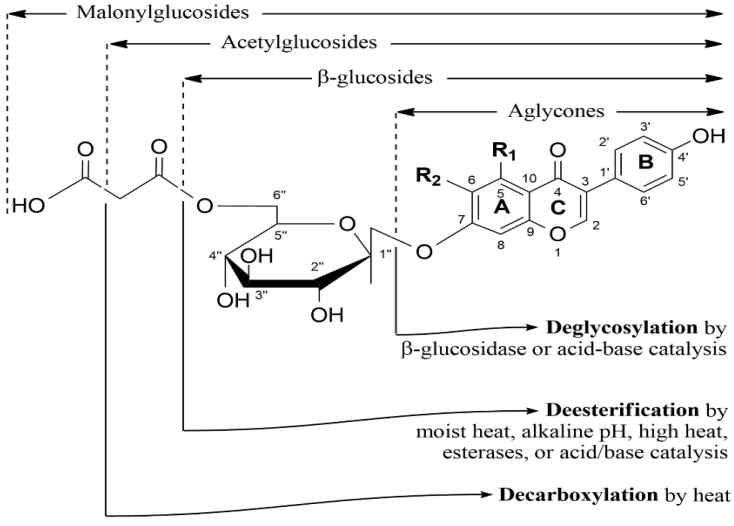


Figure 2. Chemical structures of the major isoflavone conjugates (malonyl glucosides, acetyl glucosides, and β-glucosides) and their enzymatic and non-enzymatic conversion pathways to aglycones.

Processing parameters modulate the balance between chemical interconversion and enzymatic hydrolysis. The primary factors governing this equilibrium include temperature, pH, water availability (including the seed-to-water ratio), and substrate accessibility within the food matrix, as these variables collectively influence reaction thermodynamics, mass transfer, substrate hydration, and enzyme activity [1,13,22,23]. Temperature governs reaction kinetics and enzyme stability, with mild hydrothermal treatments and fermentation favoring aglycone formation, whereas high-temperature, low-moisture operations such as extrusion, predominantly promote

conjugate rearrangement due to rapid enzyme inactivation [13,18]. Similarly, pH affects both ester hydrolysis and β-glucosidase activity, shaping conversion efficiency [23,29].

Matrix characteristics further determine the accessibility and conversion efficiency of conjugated isoflavone substrates [24,31]. Liquid systems such as soymilk facilitate mass transfer and enzyme–substrate contact, while solid matrices require controlled moisture to sustain enzymatic mobility [9,22]. In solid matrices and by-products such as okara and defatted soybean flour, biotransformation strategies enable the valorization of industrial residues into aglycone-rich functional ingredients, contributing to circular economy approaches in soybean processing [18,30,32]. Finally, water availability plays a central role in enzymatic mobility and microbial metabolism during fermentation-based processes, with controlled moisture levels being critical for efficient deglycosylation in solid matrices. Protein–isoflavone interactions, glucose accumulation, and structural barriers may limit conversion unless process conditions are optimized. The efficiency of isoflavone bioconversion can be constrained by multiple factors, including binding-related protein–isoflavone interactions, inhibitory effects arising from glucose accumulation, and diffusional resistance imposed by structural barriers, underscoring the importance of optimizing processing conditions to enhance conversion yields [13,22,25]. Importantly, prior biotransformation enhances aglycone bioaccessibility during gastrointestinal digestion, reinforcing the relevance of processing-driven structural modulation [9,31,33].

3. Soy Food Processing Technologies and Their Effects on Isoflavone Profile Alteration

Food processing technologies influence the chemical profile of soybean isoflavones through distinct physicochemical and biochemical mechanisms. Depending on the processing conditions, these technologies may promote enzymatic deglycosylation, chemical interconversion among conjugated forms, or increased accessibility of substrates within the food matrix. Among the available approaches, fermentation processes generally promote the most extensive conversion of glycosylated isoflavones into aglycones, whereas thermal and emerging non-thermal technologies tend to induce partial conversion or structural rearrangements. A schematic overview of the main processing technologies and their general effects on isoflavone deglycosylation is presented in Figure 3.

To facilitate comparison among the different processing approaches, each technology discussed in the following sections will be evaluated according to four key aspects: the underlying mechanism responsible for isoflavone bioconversion, the main conversion outcomes achieved, particularly regarding aglycone formation, the critical process variables influencing conversion efficiency, and the major limitations associated with industrial application and process scalability.

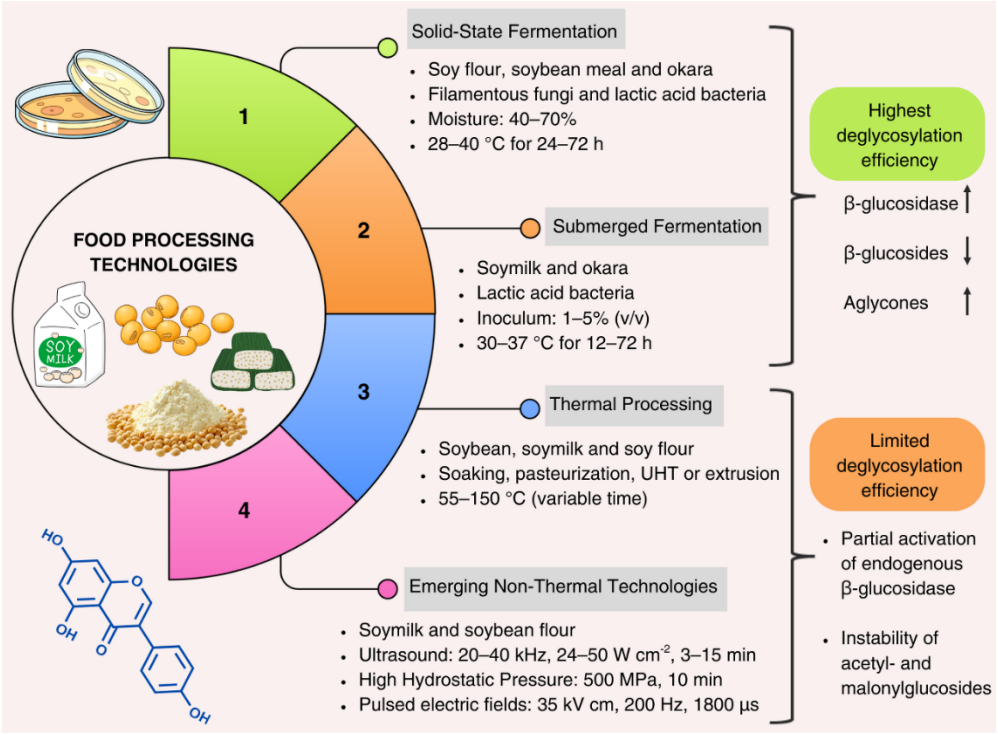


Figure 3. Comparison of solid-state fermentation, submerged fermentation, thermal processing and emerging non-thermal technologies regarding their operating characteristics and relative efficiency for isoflavone deglycosylation.

The following sections discuss the main characteristics of each processing approach and their reported effects on isoflavone profile alteration.

3.1. Solid-State Fermentation

Solid-state fermentation (SSF) involves microbial growth on solid substrates under low water activity, in which the matrix serves simultaneously as physical support and nutrient source. This configuration resembles traditional grain-based fermentations and has been widely applied to soybean matrices to modulate bioactive compound profiles, particularly isoflavones [34–36]. SSF operates under heterogeneous microenvironments characterized by gradients of moisture, oxygen, and temperature, which directly influence microbial metabolism and enzymatic expression, potentially enabling biotransformation pathways not typically observed in liquid fermentations. Fungal and bacterial SSF differ not only in microbial physiology but also in their bioconversion performance. Fungal fermentations are often associated with the secretion of a broad range of extracellular enzymes, including cellulases, hemicellulases, proteases, and β-glucosidases, which promote extensive modification of the soybean matrix and facilitate the release of bound bioactive compounds [37]. In contrast, bacterial fermentations, particularly those involving lactic acid bacteria, contribute to isoflavone bioconversion through specific enzymatic activities, such as β-glucosidase production, as well as acidification effects that enhance the conversion of glucosides into aglycones. Consequently, fungal SSF is generally associated with greater substrate deconstruction, whereas bacterial SSF is more closely related to targeted biochemical transformations that improve the bioavailability of phytochemicals [38,39].

Across diverse matrices and microbial systems, SSF consistently promotes the conversion of glycosylated isoflavones into aglycones, leading to marked increases in the fraction of biologically more available forms. As summarized in Table 1, substantial depletion of β-glucosides and concomitant accumulation of aglycones have been reported in whole soybean flour, defatted flour, okara, and intact grains under various fermentative conditions [34–36,40,41]. These convergent findings position SSF as one of the most effective technological strategies for driving the transition from conjugated forms to aglycones in solid soy matrices.

Filamentous fungi and lactic acid bacteria are the predominant microbial groups employed in SSF, with conversion efficiency largely determined by strain-specific β-glucosidase production and invasive growth capacity. Fungal systems, particularly those based on *Monascus purpureus* and *Aspergillus oryzae*, are characterized by extensive extracellular enzyme secretion and hyphal penetration into the substrate, enhancing enzyme–substrate contact and aglycone accumulation. Bacterial SSF, including systems based on *Bifidobacterium longum*, also achieves substantial conversion, frequently facilitated by localized acidification that favors enzymatic hydrolysis [34,41,42]. Nevertheless, the magnitude of aglycone enrichment remains strongly matrix- and condition-dependent, reinforcing the need for rational strain selection and precise processing conditions control.

Process parameters critically modulate SSF performance. Substrate moisture governs enzymatic mobility and oxygen diffusion within the fermentative bed, with intermediate moisture levels generally maximizing aglycone yield [41]. Matrix structure further influences substrate accessibility, explaining the variability observed among whole soybean flour, defatted flour, okara, and intact grains [34–36,40]. Fermentation time defines optimal conversion windows; prolonged incubation may lead to stabilization, secondary modification, or partial degradation of free aglycones [40]. Notably, fungal SSF systems frequently generate not only aglycones but also structurally modified derivatives, suggesting that SSF may expand the diversity of free isoflavones beyond simple deglycosylation [35].

From a technological perspective, SSF offers advantages, including reduced water consumption and low effluent generation. However, industrial-scale implementation remains challenging due to difficulties in controlling bed homogeneity, heat and oxygen transfer, fine moisture regulation, and real-time monitoring of enzymatic activity in dense substrates [40,41]. Furthermore, incomplete quantification of malonyl- and acetylglucoside intermediates in several SSF studies limits a comprehensive understanding of intermediate conversion pathways and reaction kinetics.

Despite the advances in SSF research, direct comparison among studies remains challenging due to differences in substrate heterogeneity, moisture basis, inoculum preparation, and reporting practices. Variations in the expression of process parameters and response variables may significantly affect the interpretation of fermentation performance and reaction kinetics, highlighting the need for greater methodological standardization across studies [38,39].

Table 1. Effect of solid-state fermentation on the isoflavone profile of soy-based foods.

Soy Product	Microorganisms	Processing Conditions	Isoflavone Concentration before Processing	Isoflavone Concentration after Processing	References
Whole soybean flour	*Lactobacillus casei*	Fermentation: 42 °C for 24 h–72 h	β-glucosides: 80.5 mg/100 g Aglycones: 10.1 mg/100 g Malonylglucosides: 19.2 mg/100 g Acetylglucosides: 24.3 mg/100 g	β-glucosides: 10.2 mg/100 g Aglycones: 72.9 mg/100 g Malonylglucosides: 13.1 mg/100 g Acetylglucosides: 13.9 mg/100 g	[36]
Soybean Meal	*Bifidobacterium longum*	Inoculum: 4% (*v*/*w*) Moisture: 50, 55, 65, 75, 80% Temperature: 31, 33, 37, 41, 43 °C	β-glucosides: not investigated Aglycones: 20 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: not investigated Aglycones: 56 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[41]
Okara	*Monascus purpureus*	30 °C for 25 days	β-glucosides: 205.7 mg/100 g Aglycones: not detected Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: not detected Aglycones: 228.9 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[42]
Soybean	*Eurotium cristatum*	Inoculum: 5% (*v*/*w*) 28 °C for 15 days	β-glucosides: 168.5 mg/100 g Aglycones: 15.4 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: 51.1 mg/100 g Aglycones: 144.6 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[40]
Defatted soybean flour	*Monascus purpureus*	30 °C for 48 h, pH 6.0	β-glucosides: 109.3 mg/100 g Aglycones: 15.5 mg/100 g Malonylglucosides: 47.7 mg/100 g Acetylglucosides: 24.9 mg/100 g	β-glucosides: 23.9 mg/100 g Aglycones: 78.2 mg/100 g Malonylglucosides: 53.7 mg/100 g Acetylglucosides: 5.4 mg/100 g	[34]
Defatted soybean flour	*Aspergillus oryzae*	30 °C for 48 h, pH 6.0	β-glucosides: 107.8 mg/100 g Aglycones: 20 mg/100 g Malonylglucosides: 68.4 mg/100 g Acetylglucosides: 22.1 mg/100 g	β-glucosides: 71.8 mg/100 g Aglycones: 36.2 mg/100 g Malonylglucosides: 67 mg/100 g Acetylglucosides: 18.1 mg/100 g	[34]
Okara	*Saccharomyces cerevisiae*	28 °C for 72 h	β-glucosides: not investigated Aglycones: not investigated Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: not investigated Aglycones: 311 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[43]

3.2. Submerged Fermentation

Submerged fermentation (SmF) is a liquid-based process conducted in nutrient-rich media under controlled conditions of temperature, pH, aeration, and agitation, enabling homogeneous systems and scalable production of microbial metabolites [44–46]. Owing to its high water activity, SmF enhances microbial growth and enzymatic mobility, making it the predominant fermentation mode for liquid soy matrices, particularly soymilk. Furthermore, the homogeneous mass transfer and more uniform enzyme–substrate contact provided by liquid media enhance substrate accessibility, favoring efficient β-glucosidase-catalyzed hydrolysis of isoflavone glucosides into aglycones [44,45].

Across different studies, SmF consistently drives the conversion of glycosylated isoflavones into aglycones (Table 2), primarily through microbial β-glucosidase activity. Fermentations conducted with lactic acid bacteria and bifidobacteria in soymilk and soy residues report substantial reductions in β-glucosides accompanied by significant increases in daidzein, genistein, and glycitein levels [32,47–50]. These findings confirm SmF as a robust technological strategy for reshaping isoflavone profiles in liquid soybean-based substrates.

Conversion efficiency, however, is strongly strain-dependent. Lactic acid bacteria such as *Limosilactobacillus fermentum*, *Levilactobacillus brevis*, *Lacticaseibacillus rhamnosus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* exhibit markedly different β-glucosidase activities, resulting in variable aglycone yields [47,48,51]. Effective strain screening is therefore critical, as not all fermentative cultures possess sufficient enzymatic capacity for efficient deglycosylation. Moreover, interactions between microbial metabolism and matrix composition modulate enzyme expression and catalytic performance, ultimately determining the final isoflavone profile.

Soymilk represents the most extensively investigated substrate due to its low viscosity and high substrate accessibility. Under optimized conditions, fermentation with selected lactic acid bacteria can achieve near-complete conversion of β-glucosides into aglycones, with concurrent increases in antioxidant activity and bioaccessibility [48,49,51]. Therefore, aglycone enrichment is not only relevant from a nutritional standpoint but also represents an important technological objective in the development of soy-based foods and ingredients. Processing strategies capable of modulating isoflavone profiles provide opportunities to enhance the functional value of soybean-derived products through controlled bioconversion of conjugated forms [3,7]. Mixed microbial consortia, such as kefir and kombucha cultures, frequently outperform single strains, likely due to complementary enzymatic systems and synergistic metabolic interactions [49,52,53].

Recent strategies have focused on intensifying SmF-driven bioconversion. Adaptive laboratory evolution of *Lacticaseibacillus gasseri* enhanced β-glucosidase activity and aglycone production from soybean germ extract, illustrating the potential of strain engineering to optimize processing outcomes [54]. Similarly, fungal SmF with *Ganoderma lucidum* promoted complete depletion of β-glucosides and accumulation of aglycones, accompanied by the formation of hydroxyisoflavones, indicating that submerged systems may induce structural diversification beyond simple deglycosylation [35].

Process integration strategies further enhance SmF performance. The combination of microbial fermentation with plant-derived β-glucosidases or auxiliary enzymes such as tannase significantly increases aglycone yields compared with fermentation alone [50,55]. Pre-germination of soybeans prior to fermentation activates endogenous enzymes and improves substrate accessibility, accelerating glucoside hydrolysis and aglycone accumulation during SmF [47].

From a technological perspective, SmF offers superior operational control and reproducibility, facilitating optimization under well-defined pH, temperature, and aeration conditions. However, large-scale application presents challenges related to contamination risk, effluent management, and downstream processing, particularly when aglycones are targeted as purified ingredients rather than components of fermented foods. Additionally, as observed in SSF systems, incomplete quantification of malonyl- and acetylglucosides in several studies limits full elucidation of intermediate conversion pathways, and the formation and biological relevance of hydroxyisoflavones remain insufficiently characterized.

Table 2. Effect of submerged fermentation on the isoflavone profile of soy-based foods.

Soy Product	Microorganisms	Processing Conditions	Isoflavone Concentration before Processing	Isoflavone Concentration after Processing	References
Okara in liquid medium	*L. fermentum* 44197	Fermentation: 37 °C for 24 h	β-glucosides: 63 mg/100 g Aglycones: 70.7 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: 13.7 mg/100 g Aglycones: 82.5 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[32]
Soymilk due to sprouting of soybean	*L. brevis* KCTC 3320	Fermentation: 30 °C for 72 h	β-glucosides: 106.3 mg/100 g Aglycones: 8.1 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: 5.4 mg/100 g Aglycones: 49.1 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[47]
Soybean embryo homogenate	*Ganoderma lucidum*	Inoculum: 5% (*v*/*v*). Fermentation at 26 °C for 7 days, and 120 rpm	β-glucosides: 260 mg/100 g Aglycones: 140 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: not detected Aglycones: 220 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[35]
Soymilk	Mixed culture of lactic acid bacteria (LAB) and kombucha bacteria.	Inoculum: 4.2% (*v*/*v*). Fermentation: 32 °C until 48 h, with indirect shock ventilation every 2 h for 5 min	β-glucosides: 33.7 mg/100 g Aglycones: 5.4 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: not detected Aglycones: 35.9 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[49]
Soymilk	*L. plantarum* CQPC01	Inoculum: 105 CFU/mL. Fermentation: 37 °C for 12 h	β-glucosides: 1.4 mg/100 g Aglycones: 0.4 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: 1 mg/100 g Aglycones: 1.1 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[48]
Soymilk	*L. bulgaricus* MB153	Inoculum: 1% (*v*/*v*). Fermentation: 37 °C, 50 rpm for 24 h under anaerobic conditions	β-glucosides: 23 mg/100 g Aglycones: 1.9 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: 3.6 mg/100 g Aglycones: 14.2 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[50]

3.3. Thermal Processing of Soybean Matrices and Isoflavone Modulation

Thermal processing is a cornerstone of food technology, widely applied to ensure microbial safety, enzyme inactivation, and sensory stabilization, while simultaneously inducing chemical transformations in food matrices. In soybean-based products, thermal treatments consistently reshape isoflavone profiles, with the magnitude and direction of these changes strongly dependent on temperature severity, time, and moisture conditions [13,56]. Among the different isoflavone forms, malonylglucosides are the most thermolabile and are preferentially converted into acetylglucosides and β-glucosides, whereas aglycones generally represent a smaller fraction and are less responsive to heat alone [13,57]. Representative processing conditions and their compositional effects are summarized in Table 3. However, thermal processing does not necessarily result in aglycone enrichment, as its primary effect is often the redistribution of isoflavones among malonyl-, acetyl-, and β-glucoside conjugates through temperature-induced interconversion reactions [13,58].

Hydrothermal processing, which involves soaking and heating in aqueous systems, is commonly employed during the manufacture of soymilk, tofu, and fermented soy products. Under controlled hydration and moderate temperatures, endogenous β-glucosidases may remain transiently active, promoting hydrolysis of β-glucosides into aglycones, while malonylglucosides undergo thermal decarboxylation to acetyl derivatives [1,23,59]. Water availability is a critical determinant of isoflavone fate: excessive hydration may favor leaching losses, whereas controlled water ratios combined with mild pre-incubation enhance aglycone formation while preserving total isoflavone content [23,59]. Hydrothermal treatments that integrate limited water, moderate incubation, and short high-temperature steps therefore represent a cost-effective strategy to exploit endogenous enzymatic activity before inactivation, yielding profiles enriched in aglycones and β-glucosides with reduced malonylglucosides [1,52]. Thus, under mild hydrothermal conditions, heat functions as a bioconversion-enabling factor by transiently preserving endogenous β-glucosidase activity, whereas in conventional thermal processing it is primarily used for preservation, enzyme inactivation, and structural modification of soy matrices [1,23,56].

Traditional cooking methods, including boiling, steaming, roasting, and frying, effectively reduce antinutritional factors but induce substantial rearrangements in isoflavone conjugation patterns. In both wet- and dry-heating systems, malonylglucosides exhibit the highest thermal sensitivity, being converted predominantly into acetylglucosides and, to a lesser extent, β-glucosides, while aglycones often show limited net accumulation [60,61]. Moist heat tends to intensify malonylglucoside degradation and leaching losses, whereas dry heat favors acetyl derivative formation through decarboxylation [61]. Comparative studies indicate that temperature-induced shifts up to 120 °C largely reflect interconversion among chemical forms rather than extensive degradation [58]. However, changes in aglycone levels alone may mask substantial redistribution among conjugated intermediates; therefore, quantification of the malonylglucoside and β-glucoside fractions is essential for accurately interpreting processing-induced changes [62].

Thermoplastic extrusion combines high temperature, pressure, and shear to restructure proteins and produce textured soy ingredients. Under typical conditions (100–150 °C, low moisture), extrusion destabilizes malonylglucosides and promotes acetyl conjugate formation while rapidly inactivating β-glucosidases, thereby limiting enzymatic aglycone formation [2]. As a result, extrusion-induced modulation of isoflavone profiles is predominantly driven by chemical rearrangement and partial degradation rather than controlled bioconversion. Similar patterns are observed in industrial expansion processes, where reductions in glycosylated forms are not necessarily accompanied by aglycone accumulation, reinforcing that thermomechanical severity favors rearrangement and loss over enzymatic transformation [63].

Pasteurization and ultra-high-temperature (UHT) treatments are essential for stabilizing liquid soy beverages. These processes generally reduce malonylglucosides while increasing β-glucosides and acetyl derivatives, with aglycone formation depending on time–temperature severity and residual enzymatic activity [64,65]. Moderate pasteurization often preserves total isoflavone content and induces mainly interconversion among conjugated forms [66,67]. In contrast, UHT processing rapidly inactivates endogenous enzymes, favoring purely chemical decarboxylation and rearrangement reactions [68]. Post-processing storage may further modulate profiles, with gradual increases in aglycones under refrigerated conditions attributed to slow deesterification and residual enzymatic activity [66,67].

Collectively, thermal processing reshapes soybean isoflavone profiles primarily through the thermal instability of malonylglucosides and their sequential conversion into acetylglucosides and β-glucosides. Meaningful aglycone enrichment depends on the transient preservation of enzymatic activity under controlled hydrothermal conditions before thermal inactivation. In contrast, severe thermomechanical treatments such as extrusion and UHT suppress enzymatic pathways and favor chemical rearrangements and partial degradation.

Integrating controlled hydration, mild incubation, and short heat treatments, therefore, emerges as a rational strategy to balance microbial safety, structural conversion, and functional value in soy-based foods.

3.4. Emerging Non-Thermal Technologies

Emerging non-thermal technologies have gained prominence as alternatives to conventional heat-based preservation, which, although effective for microbial inactivation, may compromise nutritional quality and sensory attributes, particularly in matrices rich in heat-sensitive bioactives [69]. Driven by consumer demand for minimally processed foods, the industry has increasingly adopted non-thermal approaches capable of ensuring safety while preserving nutritional functionality [4,10]. In soybean systems, these technologies extend beyond preservation and function as process-intensification tools, enhancing extractability and facilitating the conversion of glycosylated isoflavones into more bioavailable aglycones, an outcome that remains challenging under conventional processing [70].

Representative applications and compositional shifts are summarized in Table 4. Rather than promoting complete deglycosylation as standalone treatments, these technologies primarily function as process-intensification tools that improve matrix permeability and enzyme accessibility, thereby facilitating subsequent bioconversion [4,13,70]. Among available technologies, ultrasound (US), pulsed electric fields (PEF), and high hydrostatic pressure (HHP) stand out due to their combined capacity to disrupt cellular structures, modulate enzyme activity, and promote controlled structural interconversion. Although all three technologies enhance substrate accessibility and facilitate isoflavone bioconversion, they operate through distinct mechanisms: acoustic cavitation in ultrasound (US), membrane electroporation in pulsed electric fields (PEF), and uniform matrix compression accompanied by disruption of non-covalent interactions in high hydrostatic pressure (HHP) treatments [4,14,71,72].

Ultrasound induces acoustic cavitation, generating localized shear forces, microjets, and transient high-pressure zones that disrupt cell walls and enhance matrix permeability [4,73]. In soy matrices, ultrasound treatments consistently increase total phenolics, antioxidant activity, and aglycone isoflavone levels, particularly when applied as a pre-treatment before soaking or extraction [74–76]. These effects are attributed to improved release of insoluble-bound phenolics and enhanced enzyme–substrate interactions following matrix disintegration. However, processing intensity is critical: mild ultrasound conditions may stimulate β-glucosidase activity, whereas excessive exposure can partially inactivate the enzyme [77,78], underscoring the need for precise parameter optimization.

Pulsed electric field processing promotes electroporation of cell membranes, increasing permeability and facilitating interaction between endogenous β-glucosidases and isoflavone glucosides [13]. In soymilk systems, PEF pre-treatment has been shown to modulate glucoside levels while increasing genistein and daidzein contents, indicating enhanced enzymatic hydrolysis [71]. Similarly, [79] reported that high-intensity pulsed electric fields and high-pressure processing improved the bioaccessibility of isoflavones in a soymilk-based beverage, suggesting that non-thermal treatments can facilitate the release and accessibility of these compounds without the detrimental effects commonly associated with conventional thermal processing. At moderate intensities, PEF may induce conformational modifications in β-glucosidase, enhancing its catalytic activity and promoting the hydrolysis of isoflavone glucosides into their corresponding aglycones, with conversion efficiencies approaching 95% under optimized conditions [80]. This effect is particularly relevant for soy-based products, as aglycones exhibit greater bioavailability and biological activity than their conjugated counterparts. Consequently, PEF-assisted bioconversion represents a promising technological strategy for improving the functional quality and added value of soybean-derived foods and ingredients. Furthermore, because PEF operates under low thermal loads and short treatment times, it aligns with sustainable processing approaches while minimizing undesirable thermal degradation of bioactive compounds [4,10].

High hydrostatic pressure further complements these strategies by inducing uniform compression and selectively disrupting non-covalent interactions within proteins and polysaccharides, thereby altering matrix architecture and enhancing phytochemical release [14,72]. In soybean matrices, pressures between 100 and 500 MPa increase extractable isoflavone content and improve bioaccessibility, particularly at intermediate pressure levels [72]. At higher intensities, partial decreases in specific glucosides and aglycones may reflect pressure-induced interconversion among malonyl-, β-glucoside, and aglycone forms rather than extensive degradation [13]. From a sustainability standpoint, HHP is associated with reduced water use, minimal emissions, and lower additive dependency, aligning with clean-label and green-processing principles [4,81].

Table 3. Effect of thermal processing on the isoflavone profile of soy-based foods.

Soy Product	Thermal Processing	Processing Conditions	Isoflavone Concentration before Processing	Isoflavone Concentration after Processing	References
Soybean	Hydrothermal	Soybean:buffer (1:1.5, g:g), pH 6.0 55 °C, 6 h	β-glucosides: 42.8 mg/100 g Aglycones: 2.8 mg/100 g Malonylglucosides: 448.4 mg/100 g Acetylglucosides: not investigated	β-glucosides: 7.8 mg/100 g Aglycones: 31.0 mg/100 g Malonylglucosides: 236.6 mg/100 g Acetylglucosides: not investigated	[23]
Soymilk	Pasteurized	95 °C for 30 s	β-glucosides: 59.9 mg/L Aglycones: 8.5 mg/L Malonylglucosides: 63.43 mg/L Acetylglucosides: 2.5 mg/L	β-glucosides: 56.8 mg/L Aglycones: 7.81 mg/L Malonylglucosides: 60.0 mg/L Acetylglucosides: 2.46 mg/L	[67]
Soymilk	Thermal treatment	97 °C for 25 min	β-glucosides: 87.3 mg/100 g Aglycones: 4.9 mg/100 g Malonylglucosides: 314.4 mg/100 g Acetylglucosides: not investigated	β-glucosides: 54 mg/100 g Aglycones: 99.1 mg/100 g Malonylglucosides: 158.5 mg/100 g Acetylglucosides: not investigated	[52]
Raw soy flour	Steamed	95 °C for 1 h and dried at 30 °C for 48 h	β-glucosides: not investigated Aglycones: 43.7 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: not investigated Aglycones: 43.88 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[62]
Mature soybean	Dry heating	100 °C for 120 min	β-glucosides: 23.7 mg/100 g Aglycones: 2.1 mg/100 g Malonylglucosides: 284.6 mg/100 g Acetylglucosides: 3.1 mg/100 g	β-glucosides: 60.5 mg/100 g Aglycones: 1.3 mg/100 g Malonylglucosides: 98.5 mg/100 g Acetylglucosides: 41.5 mg/100 g	[61]
Mature soybean	Wet heating (steaming)	100 °C for 120 min	β-glucosides: 23.7 mg/100 g Aglycones: 2.1 mg/100 g Malonylglucosides: 284.6 mg/100 g Acetylglucosides: 3.1 mg/100 g	β-glucosides: 91.6 mg/100 g Aglycones: 1 mg/100 g Malonylglucosides: 48.6 mg/100 g Acetylglucosides: 22.2 mg/100 g	[61]
Soybean	Boiled	100 °C for 5 min	β-glucosides: 22.3 mg/100 g Aglycones: 20.5 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: 25.8 mg/100 g Aglycones: 11.1 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[58]
Soybean	Fried	120 °C for 5 min	β-glucosides: 22.3 mg/100 g Aglycones: 20.5 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: 56.6 mg/100 g Aglycones: 22.2 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[58]
Soymilk prosoy	UHT	140 °C for 5 s	β-glucosides: 55.8 mg/100 g Aglycones: 5 mg/100 g Malonylglucosides: 226.6 mg/100 g Acetylglucosides: not investigated	β-glucosides: 59.4 mg/100 g Aglycones: 4.5 mg/100 g Malonylglucosides: 189.3 mg/100 g Acetylglucosides: not investigated	[68]
Soymilk prosoy	UHT	120 °C for 80 s + 140 °C for 4 s	β-glucosides: 55.8 mg/100 g Aglycones: 5 mg/100 g Malonylglucosides: 226.6 mg/100 g Acetylglucosides: not investigated	β-glucosides: 74.1 mg/100 g Aglycones: 5.8 mg/100 g Malonylglucosides: 171.9 mg/100 g Acetylglucosides: not investigated	[68]

Table 3. *Cont.*

Soy Product	Thermal Processing	Processing Conditions	Isoflavone Concentration before Processing	Isoflavone Concentration after Processing	References
Soymilk black soybean	Stove cooking	90 °C water bath, 20 min boiling, ice-bath cooling	β-glucosides: 47.4 mg/100 g Aglycones: 12.8 mg/100 g Malonylglucosides: 137.2 mg/100 g Acetylglucosides: not investigated	β-glucosides: 84.1 mg/100 g Aglycones: 13.1 mg/100 g Malonylglucosides: 102.6 mg/100 g Acetylglucosides: not investigated	[68]
Soymilk black soybean	UHT	140 °C for 5 s	β-glucosides: 47.4 mg/100 g Aglycones: 12.8 mg/100 g Malonylglucosides: 137.2 mg/100 g Acetylglucosides: not investigated	β-glucosides: 53.7 mg/100 g Aglycones: 12.6 mg/100 g Malonylglucosides: 126.4 mg/100 g Acetylglucosides: not investigated	[68]
Soymilk black soybean	UHT	120 °C for 80 s + 140 °C for 4 s	β-glucosides: 47.4 mg/100 g Aglycones: 12.8 mg/100 g Malonylglucosides: 137.24 mg/100 g Acetylglucosides: not investigated	β-glucosides: 59.8 mg/100 g Aglycones: 11.1 mg/100 g Malonylglucosides: 112.2 mg/100 g Acetylglucosides: not investigated	[68]

Table 4. Effects of non-thermal technologies on the isoflavone profile in soy matrices.

Soy Product	Non-Thermal Processing	Processing Conditions	Isoflavone Concentration before Processing	Isoflavone Concentration after Processing	References
Soymilk	Ultrasound	20 kHz and 50 W cm^{-2} for 3 min	β-glucosides: 20.3 mg/100 g Aglycones: 6.6 mg/100 g Malonylglucosides: 88.8 mg/100 g Acetylglucosides: not investigated	β-glucosides: 12.1 mg/100 g Aglycones: 16 mg/100 g Malonylglucosides: 80 mg/100 g Acetylglucosides: not investigated	[78]
Soybean	Ultrasound	24 W cm^{-2}, 55 °C for 15 min	β-glucosides: 43.2 mg/100 g Aglycones: 16.2 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: 32.1 mg/100 g Aglycones: 8.1 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[74]
Soybean powder	HHP	500 MPa, 25 °C for 10 min	β-glucosides: 92.7 mg/100 g Aglycones: 5.6 mg/100 g Malonylglucosides: 115.1 mg/100 g Acetylglucosides: not investigated	β-glucosides: 57.4 mg/100 g Aglycones: 67.4 mg/100 g Malonylglucosides: 115.1 mg/100 g Acetylglucosides: not investigated	[72]
Soymilk	HIPEF (PEF)	35 kV/cm, 4 µs, 200 Hz, 1800 µs (bipolar)	β-glucosides: 11.9 mg/100 g Aglycones: 5.3 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	β-glucosides: 14.5 mg/100 g Aglycones: 5.2 mg/100 g Malonylglucosides: not investigated Acetylglucosides: not investigated	[79]

Collectively, non-thermal technologies provide versatile tools for reshaping soy isoflavone profiles by enhancing matrix permeability, improving enzyme accessibility, and promoting controlled interconversion among conjugated forms. While typically less effective than fermentation for complete deglycosylation, these approaches offer complementary pathways for aglycone enrichment under milder processing conditions. Strategic integration with controlled hydrothermal steps or fermentation may therefore maximize conversion efficiency while preserving nutritional and sensory quality, advancing the development of functional soy-based foods within sustainability-oriented processing paradigms.

Taken together, fermentation promotes true enzymatic deglycosylation, thermal treatments primarily drive chemical interconversion, and non-thermal technologies enhance accessibility and partial conversion. Therefore, isoflavone modulation should be understood as a process-design outcome rather than an isolated technological effect.

4. Conclusions

Food processing decisively shapes the structural fate of soy isoflavones, governing not only total retention but, more critically, the transition from conjugated derivatives to bioavailable aglycones. Among the technologies examined, microbial fermentation emerges as the most efficient and reliable strategy for driving deglycosylation, with both solid-state and submerged systems consistently promoting substantial aglycone enrichment when appropriate strains and moisture conditions are selected. In contrast, thermal processing primarily induces chemical interconversions among malonyl-, acetyl-, and β-glucoside forms, with meaningful aglycone formation occurring only when endogenous β-glucosidase activity is transiently preserved under carefully controlled hydrothermal conditions. Emerging non-thermal technologies, including ultrasound, pulsed electric fields, and high hydrostatic pressure, complement these strategies by enhancing matrix permeability and enzyme accessibility, enabling controlled structural modulation while minimizing nutritional and sensory losses.

From a technological standpoint, no single process fully optimizes isoflavone conversion. Instead, rational integration of controlled hydration, mild pre-incubation, targeted fermentation, and selected non-thermal intensification strategies offers the most promising pathway to maximize aglycone enrichment while maintaining product quality and sustainability. This integrated perspective shifts the focus from isolated treatments to process design guided by mechanistic understanding.

Nevertheless, progress remains constrained by incomplete profiling of intermediate conjugated forms and limited characterization of secondary transformations, including hydroxyisoflavone formation during prolonged fermentations. Future research should prioritize standardized analytical methodologies, deeper investigation of process–structure–bioaccessibility relationships, and scalable process integration frameworks. Advancing these fronts will enable the translation of laboratory-scale insights into industrially viable technologies and support the development of soy-based foods and ingredients with optimized isoflavone profiles and enhanced functional performance.

Author Contributions

M.F.G.G.: conceptualization, data curation, writing—original draft preparation, visualization, writing—reviewing and editing. C.L.H., T.R.B., C.d.S.P. and H.G.F.: writing—original draft preparation. F.S.d.L.: visualization, writing—reviewing and editing. T.C.P.: conceptualization, supervision, project administration, writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant numbers 303017/2026-0 and 303893/2022-2, and by the Fundação Araucária, grant number ERC2026581000001.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments

The authors would like to thank the "Coordination for the Improvement of Higher Education Personnel" (CAPES; Financial code 001), "National Council for Scientific and Technological Development" (CNPq; 303017/2026-0, and 303893/2022-2), and the Fundação Araucária, grant number (ERC2026581000001).

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

During the preparation of this work, the authors used ChatGPT (OpenAI) to assist with language editing, grammar correction, text refinement, and manuscript organization, and SciSpace to support the identification and selection of relevant scientific literature. After using these tools, the authors critically reviewed and edited the content as needed and take full responsibility for the content of the published article.

References

1. Kuligowski, M.; Sobkowiak, D.; Polanowska, K.; Jasińska-Kuligowska, I. Effect of different processing methods on isoflavone content in soybeans and soy products. *J. Food Compos. Anal*. **2022**, 110, 104535.
2. Villares, A.; Rostagno, M.A.; García-Lafuente, A.; et al. Content and profile of isoflavones in soy-based foods as a function of the production process. *Food Bioprocess Technol.* **2011**, *4*, 27–38
3. Zaheer, K.; Akhtar, M.H. An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 1280–1293
4. Agagunduz, D.; Ayakdas, G.; Katicioglu, B.; et al. Advances in non-thermal food processing: A comprehensive approach to nutrient retention, food quality, and safety. *Sustain. Food Technol.* **2025**, *3*, 1284.
5. Messina, M.; Badger, T.M. Health effects of isoflavones misrepresented. *Food Chem.* **2017**, *225*, 289–292.
6. Vitale, D.C.; Piazza, C.; Melilli, B.; et al. Isoflavones: Estrogenic activity, biological effect and bioavailability. *Eur. J. Drug Metab. Pharmacokinet.* **2013**, *38*, 15–25.
7. Izumi, T.; Osawa, S.; Obata, A.; et al. Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J. Nutr.* **2000***, 130*, 1695–1699.
8. Kano, M.; Takayanagi, T.; Harada, K.; et al. Bioavailability of isoflavones after ingestion of soy beverages in healthy adults. *J. Nutr.* **2006**, *136*, 2291–2296.
9. Macedo, G.A.; Caria, C.R.E.P.; Barbosa, P.D.P.M.; et al. Bioaccessibility evaluation of soymilk isoflavones with biotransformation processing. *Foods* **2023**, *12*, 3401.
10. Al-Sharify, Z.T.; Al-Najjar, S.Z.; Anumudu, C.K.; et al. Non-thermal technologies in food processing: Implications for food quality and rheology. *Appl. Sci.* **2025**, *15*, 3049.
11. Harahap, I.A.; Suliburska, J.; Karaca, A.C.; et al. Fermented soy products: A review of bioactives for health from fermentation to functionality. *Compr. Rev. Food Sci. Food Saf.* **2025**, *24*, e70080.
12. Nurmilah, S.; Frediansyah, A.; Cahyana, Y.; et al. Biotransformation and health potential of isoflavones by microorganisms in Indonesian traditional fermented soy products: A review. *J. Agric. Food Res.* **2024**, *18*, 101365.
13. Ramasinghe, C.; Xu, B. Critical review on effects of thermal and non-thermal food processing on soy isoflavones. *Food Rev. Int.* **2025**, *41*, 3267–3285
14. Wang, Y.; Ma, C.; Yang, Y.; et al. Effect of high hydrostatic pressure treatment on food composition and applications in food industry: A review. *Food Res. Int.* **2024**, *195*, 114991
15. Liu, K. *Soybeans: Chemistry, Technology and Utilization*; Chapman & Hall: New York, NY, USA, 1997.
16. Nikolić, I.L.; Savić-Gajić, I.M.; Tačić, A.D.; et al. Classification and biological activity of phytoestrogens: A review. *Adv. Technol.* **2017**, *6*, 96–106.
17. An, J.H.; Ko, M.J.; Chung, M.S. Thermal conversion kinetics and solubility of soy isoflavones in subcritical water extraction. *Food Chem.* **2023**, *424*, 136430.
18. Colletti, A; Attrovio, A.; Boffa, L; et al. Valorisation of by-products from soybean *(Glycine max* (L.) Merr.) processing. *Molecules* **2020**, *25*, 2129.
19. Fujita, A.; Alencar, S.M.; Park, Y.K. Conversion of isoflavone glucosides to aglycones by partially purified β-glucosidases from microbial and vegetable sources. *Appl. Biochem. Biotechnol.* **2015**, *176*, 1659–1672.
20. Kumari, S.; Chang, S.K. Changes in isoflavones, phenolic acids and antioxidant activities of soybean products as affected by fermentation and non-fermentation processing techniques. *J. Food Nutr. Sci.* **2020**, *2*, 1.

21. Aguiar, C.L.; Haddad, R.; Eberlin, M.N.; et al. Thermal behavior of malonylglucoside isoflavones in soybean flour analyzed by RPHPLC/DAD and eletrospray ionization mass spectrometry. *LWT-Food Sci. Technol.* **2012**, 48, 114–119.
22. Cao, H.; Zhang, Y.; Shi, P.; et al. A highly glucose-tolerant GH1 β-glucosidase with greater conversion rate of soybean isoflavones in monogastric animals. *J. Ind. Microbiol. Biotechnol.* **2018**, *45*, 369–378.
23. de Lima, F.S.; Ida, E.I. Optimisation of soybean hydrothermal treatment for the conversion of β-glucoside isoflavones to aglycones. *LWT-Food Sci. Technol.* **2014**, *56*, 232–239.
24. Hsiao, Y.H.; Hsieh, J.F. The conversion and deglycosylation of isoflavones and anthocyanins in black soymilk process. *Food Chem.* **2018**, *261*, 8–14.
25. de Lima, F.S; Handa, C.L.; Fernandes, M.S.; et al. Kinetic modeling of the conversion and losses of isoflavones during soybean soaking. *J. Food Eng*. **2019**, 261, 171–177.
26. Delgado, L.; Heckmann, C.M.; Di Pisa, F.; et al. Release of soybean isoflavones by using a β-glucosidase from *Alicyclobacillus herbarius*. *ChemBioChem.* **2021**, *22*, 1223–1231.
27. Yang, H.; Li, T.; Cao, W. Optimizing pre-treatment of alkaline hydrolysis RP-HPLC for enhancing accuracy of soy isoflavone determination. *J. Chromatogr. B* **2022**, *1207*, 123382.
28. Paventi, G.; Di Martino, C.; Coppola, F.; et al. β-Glucosidase activity of *Lactiplantibacillus plantarum*: A key player in food fermentation and human health. *Foods* **2025**, *14*, 1451.
29. Yan, F.Y.; Xia, W.; Zhang, X.X.; et al. Characterization of β-glucosidase from *Aspergillus terreus* and its application in the hydrolysis of soybean isoflavones. *J. Zhejiang Univ.-Sci. B* **2016**, *17*, 455–464.
30. Handa, C.L.; Lima, F.S.; Guelfi, M.F.G.; et al. Parameters of the fermentation of soybean flour by *Monascus purpureus* or *Aspergillus oryzae* on the production of bioactive compounds and antioxidant activity. *Food Chem.* **2019**, *271*, 274–283.
31. Zhang, J.; Xia, N.; Teng, J.; et al. Effect of lactic acid bacteria fermentation on extractable and non-extractable polyphenols of soybean milk: Influence of β-glucosidase and okara. *Food Biosci.* **2023**, *56*, 103326.
32. Phomkaivon, N.; Pan-Utai, W.; Surojanametakul, V.; et al. Isoflavone aglycone-rich powder from soybean residue submerged fermentation using *Lactobacillus fermentum* 44197. *NFS J.* **2023**, *33*, 100157.
33. Fernandes, M.S.; Lima, F.S.; Rodrigues, D.; et al. Evaluation of the isoflavone and total phenolic contents of kefir-fermented soymilk storage and after the in vitro digestive system simulation. *Food Chem.* **2017**, *229*, 373–380.
34. Handa, C.L.; Couto, U.R.; Vicensoti, A.H.; et al. Optimisation of soy flour fermentation parameters to produce β-glucosidase for bioconversion into aglycones. *Food Chem.* **2014**, *152*, 56–65.
35. Kim, G.Y.; Kwon, Y.S.; Gebru, Y.A.; et al. Dynamic changes of nutrients, isoflavone composition and antioxidant activities during liquid-state fermentation of soybean embryo homogenate by *Ganoderma lucidum*. *Food Chem*. *X* **2025**, *26*, 102283.
36. Li, S.; Jin, Z.; Hu, D.; et al. Effect of solid-state fermentation with *Lactobacillus casei* on the nutritional value, isoflavones, phenolic acids and antioxidant activity of whole soybean flour. *LWT* **2020**, *125*, 109264.
37. Barrios-González, J. Solid-state fermentation: Physiology of solid medium, its molecular basis and applications. *Process Biochem.* **2012**, *47*, 175–185.
38. Feng, X.; Ng, K.; Ajlouni, S.; et al. Effect of Solid-State Fermentation on Plant-Sourced Proteins: A Review. *Food Rev. Int.* **2024**, *40*, 2580–2617.
39. Perwez, M.; Asheh, S.A. Valorization of agro-industrial waste through solid-state fermentation: Mini review. *Biotechnol. Rep.* **2025**, *45*, e00873.
40. Chen, Y.; Wang, Y.; Chen, J.; et al. Bioprocessing of soybeans (*Glycine max* L.) by solid-state fermentation with *Eurotium cristatum* YL-1 improves total phenolic content, isoflavone aglycones, and antioxidant activity. *RSC Adv.* **2020**, *10*, 16928–16941.
41. Olmos, A.R.; Garro, O.A.; Garro, M.S. Behavior study of *Bifidobacterium longum* using solid state fermentation from commercial soybean meal. *LWT* **2022**, *157*, 113101.
42. Kim, H.; Lee, H.; Park, S.; et al. Value-adding okara via *Monascus purpureus* solid-state fermentation: Microstructural remodeling, metabolite enrichment, and bioactivity enhancement. *Food Chem.* **2025**, *495*, 146443.
43. Santos, V.A.Q.; Nascimento, C.G.; Schmidt, C.A.P.; et al. Solid-state fermentation of soybean okara: Isoflavones biotransformation, antioxidant activity and enhancement of nutritional quality. *LWT* **2018**, *92*, 509–515.
44. Mascarin, G.M.; Golo, P.S.; Ribeiro-Silva, C.S.; Muniz, E.R.; Franco, A.O.; Kobori, N.N.; Fernandes, É.K.K. Advances in submerged liquid fermentation and formulation of entomopathogenic fungi. *Appl. Microbiol. Biotechnol*. **2024**, 108, 451.
45. Sun, W.; Shahrajabian, M.H.; Lin, M. Research progress on fermented functional foods and protein factory-microbial fermentation technology. *Fermentation* **2022**, *8*, 688.
46. Yatmaz, E.; Turhan, I. Liquid state bioreactor. In *Essentials in Fermentation Technology*; Berenjian, A., Ed.; Springer: Cham, Switzerland, 2019; pp. 1–18.
47. Hwang, C.E.; Haque, M.A.; Lee, J.H.; et al. Bioconversion of γ-aminobutyric acid and isoflavone contents during the fermentation of high-protein soy powder yogurt with *Lactobacillus brevis*. *Appl. Biol. Chem.* **2018**, *61*, 409–421.

48. Liu, B.; Xiao, X.; Zhou, X.; et al. Effects of *Lactobacillus plantarum* CQPC01-fermented soybean milk on activated carbon-induced constipation through its antioxidant activity in mice. *Food Sci. Nutr.* **2019**, *7*, 2068–2082.
49. Peng, X.; Liao, Y.; Ren, K.; et al. Fermentation performance, nutrient composition, and flavor volatiles in soy milk after mixed culture fermentation. *Process Biochem.* **2022**, *121*, 286–297.
50. Queirós, L.D.; Ávila, A.R.A.; Botaro, A.V.; et al. Combined isoflavones biotransformation increases the bioactive and antioxidant capacity of soymilk. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 10019–10031.
51. Lodha, D.; Das, S.; Hati, S. Antioxidant activity, total phenolic content and biotransformation of isoflavones during soy lactic fermentations. *J. Food Process. Preserv.* **2021**, *45*, e15583.
52. Baú, T.R.; Garcia, S.; Ida, E.I. Changes in soymilk during fermentation with kefir culture: Oligosaccharides hydrolysis and isoflavone aglycone production. *Int. J. Food Sci. Nutr.* **2015**, *66*, 845–850.
53. Yang, M.; Yang, X.; Chen, X.; et al. Effect of kefir on soybean isoflavone aglycone content in soymilk kefir. *Front. Nutr.* **2020**, *7*, 587665.
54. Lee, S.H.; Lee, N.R.; Ji, J.H.; et al. Evolution-aided enhancement of β-glucosidase activity for improved conversion of isoflavone glucosides to aglycones by *Lactobacillus gasseri*. *Food Chem.* **2025**, *493*, 146055.
55. Hati, S.; Ningtyas, D.W.; Khanuja, J.K.; et al. β-Glucosidase from almonds and yoghurt cultures in the biotransformation of isoflavones in soy milk. *Food Biosci.* **2020**, *34*, 100542.
56. Lewis, M.J.; Jun, S. Thermal processing. In *Food Processing Handbook*; CRC Press: Boca Raton, FL, USA, 2011; pp. 31–75.
57. Coward, L.; Smith, M.; Kirk, M.; et al. Chemical modification of isoflavones in soyfoods during cooking and processing. *Am. J. Clin. Nutr.* **1998**, *68*, 1486S–1491S.
58. Chen, L.; Teng, H.; Xiao, J. A value-added cooking process to improve the quality of soybean: Protecting its isoflavones and antioxidant activity. *Food Sci. Hum. Wellness* **2019**, *8*, 195–201.
59. Salces, F.R.; Rostagno, M.A.; Amaya-Farfan, J. Novel process of hydration, followed by incubation and thermal processing, for high isoflavone bioconversion in soybeans. *Food Res. Int.* **2019**, *121*, 691–696.
60. Andrade, J.C; Mandarino, J.M.G.; Kurozawa, L.E.; et al. 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.
61. Qu, S.; Kwon, S.J.; Duan, S.; et al. Isoflavone changes in immature and mature soybeans by thermal processing. *Molecules* **2021**, *26*, 7471.
62. Shin, D.J.; Kim, W.; Kim, Y. Physicochemical and sensory properties of soy bread made with germinated, steamed, and roasted soy flour. *Food Chem.* **2013**, *141*, 517–523.
63. Privatti, R.T.; Capellini, M.C.; Thomazini, M.; et al. Profile and content of isoflavones on flaked and extruded soybeans and okara submitted to different drying methods. *Food Chem.* **2022**, *380*, 132168.
64. Deak, T. Thermal treatment. In *Food Safety Management*; Elsevier: Amsterdam, The Netherlands, 2014; Chapter 17, pp. 423–442.
65. Kelly, A.L.; Waldron, D.S.; Datta, N.; et al. Heat exchangers, pasteurizers and UHT equipment—Design and operation. In *Encyclopedia of Dairy Sciences*, 3rd ed.; Elsevier: Amsterdam, The Netherlands, 2022; pp. 321–335.
66. Ruiz de la Bastida, A.; Peirotén, Á.; Langa, S.; et al. Effect of storage and heat treatment on the levels of bioactive flavonoids produced in fermented soy beverages. *LWT* **2022**, *154*, 112872.
67. Toro-Funes, N.; Bosch-Fusté, J.; Veciana-Nogués, M.T.; et al. Changes of isoflavones and protein quality in soymilk pasteurised by ultra-high-pressure homogenisation throughout storage. *Food Chem.* **2014**, *162*, 47–53.
68. Zhang, Y.; Chang, S.K.; Liu, Z. Isoflavone profile in soymilk as affected by soybean variety, grinding, and heat-processing methods. *J. Food Sci.* **2015**, *80*, C983–C988.
69. Jadhav, H.B.; Annapure, U.S.; Deshmukh, R.R. Non-thermal technologies for food processing. *Front. Nutr.* **2021**, *8*, 657090.
70. Zhao, Y.; Fu, Y.; Du, P.; et al. Efficient preparation and bioactivity evaluation of aglycone soy isoflavones via a multi-enzyme synergistic catalysis strategy. *Processes* **2025**, *13*, 1831.
71. Peña, M.M.; Welti-Chanes, J.; Martín-Belloso, O. Impact of pulsed electric field pre-treatment on the isoflavone profile of soymilk. *Beverages* **2022**, *8*, 19.
72. Yan, X.; Yi, L.; Wang, X.; et al. Effects of high hydrostatic pressure on folate and isoflavone contents, bioaccessibility, and physicochemical properties of soybean powder. *LWT* **2025**, *215*, 117056.
73. Lee, M.; Lee, K.-G. Effect of ultrasound and microwave treatment on the level of volatile compounds, total polyphenols, total flavonoids, and isoflavones in soymilk processed with black soybean (*Glycine max* (L.) Merr.). *Ultrason. Sonochem.* **2023**, *99*, 106579.
74. Falcão, H.G.; Handa, C.L.; Silva, M.B.R.; et al. Soybean ultrasound pre-treatment prior to soaking affects β-glucosidase activity, isoflavone profile and soaking time. *Food Chem.* **2018**, *269*, 404–412.
75. Falcão, H.G.; Silva, M.B.R.; de Camargo, A.C.; et al. Optimizing the potential bioactivity of isoflavones from soybeans via ultrasound pretreatment: Antioxidant potential and NF-κB activation. *J. Food Biochem.* **2019**, *43*, e13018.

76. Zhao, C.-C.; Kim, P.-H.; Eun, J.-B. Influence of high-intensity ultrasound application on the physicochemical properties, isoflavone composition, and antioxidant activity of tofu whey. *LWT* **2020**, *117*, 108618.
77. Shahid, F.; Yeo, J. Insoluble-bound phenolics in food. *Molecules* **2016**, *21*, 1216.
78. Silva, M.B.R.; Falcão, H.G.; Kurozawa, L.E.; et al. Ultrasound- and hemicellulase-assisted extraction increase β-glucosidase activity, the content of isoflavone aglycones and antioxidant potential of soymilk. *J. Food Bioact.* **2019**, *6*, 140–147.
79. Rodríguez-Roque, M.J.; De Ancos, B.; Sánchez-Vega, R.; et al. 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.
80. Lu, C.; Li, F.; Yan, X.; et al. Effect of pulsed electric field on soybean isoflavone glucosides hydrolysis by β-glucosidase: Investigation on enzyme characteristics and assisted reaction. *Food Chem.* **2022**, *378*, 132032.
81. Rostamabadi, H.; Karaca, A.C.; Nowacka, M.; et al. How high hydrostatic pressure treatment modifies the physicochemical and nutritional attributes of polysaccharides? *Food Hydrocoll.* **2023**, *137*, 108375.