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Comparison of coagulant-induced changes in isoflavone content, and texture profile of Himalayan black soybean-based tofu

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Introduction: This research examined the isoflavone levels and texture characteristics of tofu produced from Himalayan black soybean, utilizing two distinct soymilk processing techniques and both synthetic and natural coagulants.
Method: Soymilk was prepared following two different methos and then it was coagulated using 10 different coagulants (natural and synthetic).
Results: The tofu made with *Rhododendron arboreum* floral juice exhibited the highest isoflavone levels, irrespective of the method used to prepare the soymilk. The texture quality was consistently good for tofu made with acetic acid, regardless of the soymilk preparation technique employed.
Discussion: The findings indicate that the isoflavone composition of the final product is affected by both the coagulant and the preparation techniques, with β-glucosides being more common than aglycones. The observation that the okara fraction contains a higher concentration of aglycones supports the notion that these compounds are less soluble in water and may be lost during the tofu production process. The interaction between coagulants and soymilk proteins affects tofu's texture. Upon heating, proteins unfold, exposing negatively charged disulfides that counteract the coagulants, resulting in protein aggregation. These results were further validated through factor and cluster analysis. Research has explored the effects of different coagulants and processing methods on tofu texture, including the use of natural coagulants to improve texture and flavor.

KEYWORDS

Himalayan black soybean, tofu, isoflavones, textural profile analysis, natural and synthetic coagulants

1 Introduction

Soybean-based foods, both fermented and non-fermented, are consumed worldwide because of their outstanding nutritional value that supports a balanced human diet. Black soybean [*Glycine max* (L) Merrill], a member of the Fabaceae family, is traditionally consumed in various forms in Asian countries, including the northwestern Indian Himalayan region, Japan, Korea, Indonesia, and China. Some popular dishes include Chunjang (black soybean paste), Jajang (black soybean sauce), soybean touhua (soft soybean curd), tofu, natto, cinema, and cookies (Bhartiya et al., 2020). This soybean variety is distinguished by its black seed coat, which is rich in isoflavones, anthocyanins (mainly cyanidin-3-glucoside), flavonoids, and c-tocopherol (Bhartiya et al., 2020). Black soybeans are associated with numerous health benefits, such as lowering the risk of cardiovascular diseases, preventing low-density lipoprotein (LDL) oxidation, offering anti-obesity and hypolipidemic effects, reducing the likelihood of breast and ovarian cancers, preventing lipid peroxidation, managing diabetes, fighting osteoporosis, easing menopausal symptoms, and providing hypocholesterolemic, hemolytic, immune-stimulatory, antiviral, and hepatoprotective effects (Li et al., 2024).

Tofu is a well-known, cost-effective, non-fermented, and adaptable soybean product that serves as a substitute for meat and cheese. It is popular globally due to its mild taste, porous texture, cholesterol-free nature, and rich content of proteins, minerals, vitamins, and omega-3 fatty acids (Zheng et al., 2020). The protein in tofu is considered the most balanced form suitable for human consumption. Regular tofu consumption may help reduce the risk of developing chronic diseases, including various types of cancer (Fan et al., 2022), cardiovascular ailments (Naghshi et al., 2024), menopausal symptoms (Levis and Griebeler, 2010), and osteoporosis (Zheng et al., 2016). The final quality attributes of tofu, such as texture and nutritional properties, can be influenced by factors including the variety of soybean seeds used as raw materials (Wang and Murphy, 1994), processing conditions, coagulant concentration, heating and stirring/mixing methods employed during soymilk coagulation, and pressing time.

The initial step in producing tofu from soymilk involves coagulation under optimal conditions. At this stage, the protein molecules in soymilk create crosslinks when a coagulant is present (Geng et al., 2024). A variety of coagulants are used in tofu production, such as glucone delta lactone, calcium and magnesium salts, lemon juice, and natural plant extracts, which behave differently depending on their acidic, basic, or neutral natures (Ezeama and Dobson, 2019). Foods derived from soybeans are beneficial to health because they contain phytochemicals, such as isoflavones (Lee et al., 2004). These compounds have been found to help with various health conditions, including lowering the risk of cardiovascular diseases (Naghshi et al., 2024), reducing total cholesterol and LDL cholesterol levels (Rimbach et al., 2008), easing postmenopausal symptoms and osteoporosis risks (Messina et al., 2022), fighting oxidative stress through their antioxidant properties (Zhang et al., 2013), and decreasing cancer incidence and mortality (Applegate et al., 2018; He and Chen, 2013). Therefore, adding isoflavones to the diet can offer multiple health advantages. These phytochemicals are referred to as phytoestrogens because of their estrogen-like activities (Vitale et al., 2013), and are categorized into two types: aglycones and glycosidic conjugates. Daidzin, genistin, and glycitin are glycosidic isoflavones, while daidzein, genistein, and glycitein are aglycone isoflavones. The coagulants used in making tofu affect its texture, including attributes such as springiness, hardness, adhesiveness, cohesiveness, gumminess, chewiness, and resilience (Shi et al., 2020). This study aimed to explore how different preparation methods, such as heating soymilk (with and without okara) and using various coagulants (acidic, basic, and natural), affect the isoflavone content and texture of black soybean-based tofu.

2 Materials and methods

2.1 Chemicals

High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile, along with analytical reagent (AR)-grade orthophosphoric acid and isoflavone standards such as genistin, daidzein, daidzin, glycitin, glycitein, and genistein, were sourced from Sigma-Aldrich (Germany). Food-grade coagulants, including acetic acid (TAA), citric acid (TCA), calcium sulfate (TCS), lemon juice (TL), magnesium sulfate (TMS), magnesium chloride (TMC), calcium lactate (TCL), and calcium acetate (TCAT), were obtained from Anmol Chemicals (Mumbai, India). Additionally, apple cider vinegar (TACV) and *Rhododendron arboreum* floral juice (TRD) were acquired from Himka, Almora, Uttarakhand, India.

2.2 Sample processing

Black soybean seeds were sourced from villages in the Almora District of Uttarakhand. Before soymilk production, the seeds were soaked in water at a 1:4 seed-to-water ratio for 12 h at 25°C. After soaking, the seeds were drained and blended with four parts of distilled water. The resulting mixture underwent two different processing methods before tofu was prepared. In the first method, the entire mixture, including okara, was heated for 15 min (initially at 90°C for 5 min, then at 70–80°C for the remaining 10 min) and strained to separate the soymilk from the okara. The second method involved filtering the mixture to separate soymilk and okara, then heating the soymilk (without okara) for 15 min (90°C for 5 min, followed by 70–80°C for 10 min) before proceeding to tofu preparation. These methods were labeled as Tofu processing method I (TPMI) and Tofu processing method II (TPMII), respectively (Figure 1). The soymilk obtained from these methods was used to produce tofu with various food-grade coagulants. These coagulants included acetic acid (TAA), citric acid (TCA), calcium sulfate (TCS), lemon juice (TL), magnesium sulfate (TMS), magnesium chloride (TMC), calcium lactate (TCL), calcium acetate (TCAT), apple cider vinegar (TACV), and *Rhododendron arboreum* floral juice (TRD). Chemical coagulants were used at a concentration of 0.5%, while natural coagulants such as lemon juice, apple cider vinegar, and *R. arboreum* floral juice (supplemented with 0.25% citric acid at the production level) were used at a concentration of 1%. The addition of these coagulants triggered the separation of protein-rich tofu from soymilk. The mixture was strained through a muslin cloth and pressed for 30 min to remove excess liquid, resulting in the final tofu product.

2.3 Sample drying

Prior to performing nutritional analysis, the tofu samples were subjected to a slow drying process using a water bath at 30°C. After drying, the samples were finely milled into powder and stored in sealed containers. These containers were labeled and kept at −20°C for later analysis.

2.4 Isoflavones extraction procedure

Isoflavones were extracted following Klump et al. (2001) with some modifications in sample-to-solvent ratio, decreasing the NaOH volume and increasing the duration of the saponification step (Prabhakaran et al., 2006). The process began by combining

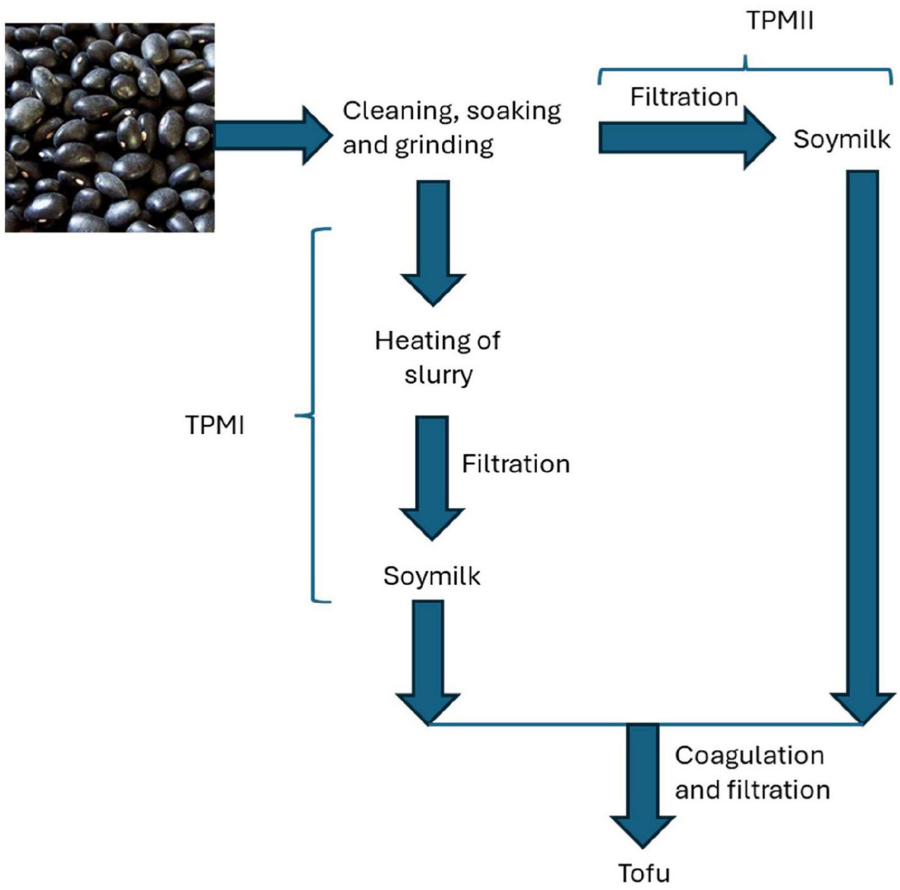

FIGURE 1
Tofu processing.

1 g of the sample with 20 mL 80% MeOH, followed by maceration at 65°C for 2 h in an incubator shaker (Jeiotech, Korea). After cooling, 1.5 mL of 2 M NaOH was added for saponification, and the mixture was agitated for 20 min at room temperature using an orbital shaker (Remi, India). The solution was neutralized with glacial acetic acid (GAA) and diluted to a total volume of 25 mL with distilled water. The resulting mixture was filtered through Axiva No. 1 filter paper, and the filtrate (2.5 mL) was further diluted to 5 mL using distilled water and methanol. Finally, 1 mL of this solution was centrifuged at 7,000 g for 5 min to obtain the supernatant for RP-HPLC analysis.

2.5 RP-HPLC analysis of isoflavones

Isoflavone content was analyzed using a Waters 2695 separation module equipped with a quaternary pump delivery system, inline degasser, and Waters 2996 photodiode array (PDA) detector. Isoflavones were separated on a C-18 column (4.6 × 250 mm Analytical Column Waters ® SPHERISORB ® 5 μm OD 52). The mobile phase consisted of 0.02% orthophosphoric acid and acetonitrile in a 35:65 volume ratio (mobile phase A) and 0.2% orthophosphoric acid (mobile phase B). The elution gradient was programmed as follows: 75% B from 0 to 10 min, 60% B from 10 to 15 min, and maintained at 10% B for 30 min. The solvent flow rate was set at 1 mL/min, and the entire run lasted 40 min (Montero et al., 2018). A sample volume of 10 μL was injected and post-chromatographic analysis was performed using the "Empower Software System" (Waters Corp., Milford, USA). The analysis was conducted at room temperature using two different solvent systems. Daidzin, daidzein, glycitin, glycitein, genistin, and genistein were detected at 254 nm. Standard calibration curves were established at concentrations ranging from 0 to 50 mg/L.

2.6 Texture profile analysis

A texture profile analyzer (TAXT2i) from the Stable Micro System UK was employed to assess the texture profile of the tofu samples. Textural analysis of the tofu was conducted on a Heavy-Duty Platform (HDP/90) using a stainless-steel probe (75 mm diameter Compression Platen—P/75). The analysis involved two compression cycles, using a probe. The peak force at the conclusion of the first compression cycle represented the hardness or firmness of the sample, whereas the other parameters were calculated and provided by the equipment software. Tofu samples measuring 1 cm^3 were taken from each batch for textural parameter analysis. The TPA settings were as follows: load cell capacity, 50 kg; pre-test speed, 10 mm/s; test speed: 2.0 mm/s; post-test speed, 10 mm/s;

trigger force, 5 g; and trigger type, auto. Each measurement was performed in triplicate. Texture profile analysis curves provided data on various textural parameters, including hardness, springiness, adhesiveness, cohesiveness, gumminess, chewiness, and resilience. Textural properties were evaluated according to the standard method described by (Bourne, 1982).

2.7 Statistical analysis

Experiments were conducted on three separate occasions. Basic statistical analysis was performed using MS Excel and SPSS 16.0, while Statistica 8.0 was used for factor and cluster analyses to identify the primary factors influencing variability and to assess the similarities among various tofu samples. Before performing multivariate analysis, the data were normalized using log transformation, followed by z-score calculation. This approach was adopted to minimize the influence of different variables in the study and to address discrepancies arising from diverse measurement units. The standardized variable data generated the factor scores. Varimax rotation was applied to minimize the variance in the squared loadings. Kaiser's criterion determines the number of factors that need to be retained. To investigate the similarities between tofu samples prepared with different coagulants, cluster analysis was performed on the normalized data, employing Ward's method for linkage analysis and Euclidean distances as a measure of similarity. The findings are illustrated in a dendrogram that displays clusters and their interconnections (Agnihotri et al., 2020).

3 Results and discussion

3.1 Effect of processing condition on isoflavones concentration

Isoflavones are a group of phytoestrogenic compounds found in adequate quantities in soybeans and are responsible for various biological activities in soybean products (Benedetti et al., 2015; Kim et al., 2005). The isoflavones daidzein, genistein, glycitein, daidzin, genistin, and glycitin detected in tofu samples are shown in Figure 2 and Table 1. From the results, it was observed that isoflavones from the β-glucoside family were present in higher concentrations than aglycones. The concentrations of daidzein, genistin, and glycitin were high in the raw soybean sample, while the other analyzed isoflavones were high in okara. Based on the statistical analysis among all the isoflavones, daidzin was the most abundant, followed by genistein and glycitin; however, these values were not statistically different from those of other samples (Table 1). Between both tofu preparation methods, isoflavone content was found to be higher in the tofu samples prepared following TPMI than in those prepared following TPMII. Among the tofu samples prepared using the first processing method (TPMI), apple cider vinegar-based tofu contained the highest amount of daidzein (0.0505 ± 0.00014 mg 100 mg^{-1} DW of sample) and genistein (0.0776 ± 0.0008 mg 100 mg^{-1} DW of sample), the highest concentration of daidzin (0.237 ± 0.0043 mg 100 mg^{-1} DW of sample), and glycitein (0.0077 ± 0.00 mg 100 mg^{-1} DW of sample) was found in citric acid-based samples, while rhododendron juice and calcium lactate-based tofu samples retained the maximum amount of genistin and glycitin, respectively, however, statistical analysis indicates that these values are comparable to other samples (Table 1).

Among the samples prepared using the second processing method (TPMII), glycitin (0.0666 ± 0.003 mg 100 mg^{-1} DW), glycitein (0.0057 ± 0.0005 mg 100 mg^{-1} DW), daidzin (0.388 ± 0.0053 mg 100 mg^{-1} DW), and daidzein (0.0526 ± 0.00095 mg 100 mg^{-1} DW) were found to be highest ($p > 0.05$) in Rhododendron arboreum floral juice-based tofu; genistein (0.0643 ± 0.0005 mg 100 mg g^{-1} DW) was highest ($p > 0.05$) in calcium lactate-based tofu, and genistein (0.448 ± 0.0026 mg 100 mg g^{-1} DW) was highest ($p > 0.05$) in aple cidar vinegar-based tofu. Overall, isoflavone content was higher in Rhododendron arboreum floral juice-based tofu prepared by both methods.

Liu et al. (2005) reported the isoflavones concentration in freeze-dried tofu where aglycones such as daidzein, genistein, and glycitein were present at the concentration of 336–416, 600–683, and 90–135 μg/g, respectively, whereas glycosides such as daidzin, genistein, and glycitin were present at the concentration of 23–36, 106–151, and 6.2–14.3 μg/g respectively. Prabhakaran et al. (2006) examined the use of calcium lactate and calcium acetate as coagulants and found that calcium lactate was more effective in retaining isoflavones. This finding challenges the assertion by Thompson et al. (2006) that most soy-based products have higher concentrations of genistein compared to daidzein and glycitein. The results suggest that isoflavones belonging to the β-glucoside family are generally found at higher concentrations in tofu samples than in aglycones. This phenomenon may be explained by the limited water solubility of aglycones, as some studies have suggested that isoflavones can form complexes with soy protein and potentially be discharged with okara (Jackson et al., 2002). Obatolu (2007) observed the impact of calcium and magnesium ions in coagulants on isoflavone binding capacity and stability.

In this study, okara seeds had more aglycones than soybean seeds, which could be attributed to their poorer water solubility than that of their glucoside counterparts. The tofu-making process involves different steps, including soaking, grinding, heating, and coagulation, which significantly influence the isoflavone content in the final product (Jackson et al., 2002; Anjum et al., 2023). Soybean processing increases the hydrolysis of isoflavone glucosides, resulting in higher concentrations of aglycones (Qu et al., 2021). Wang and Murphy (1996) also reported that more aglycones are present in okara than in soymilk. Aglycones are more readily absorbed and bioavailable than glucosides because their structure allows for easier passage through the intestinal wall and does not require enzymatic hydrolysis prior to absorption (Yu-Hsuan et al., 2020). Minor discrepancies in isoflavone concentrations could be attributed to the type of coagulant used or the tofu preparation method. According to Jackson et al. (2002), some coagulants may be better and more efficient for isoflavone retention in tofu than others. Kao et al. (2003) stated that most of the isoflavones do not remain in soymilk and are discarded with whey (Góes-Favoni et al., 2010), so it might be possible that, due to the boiling of soymilk with okara, exclusion of isoflavones into soymilk occurs due to which concentration might have increased in TPMI.

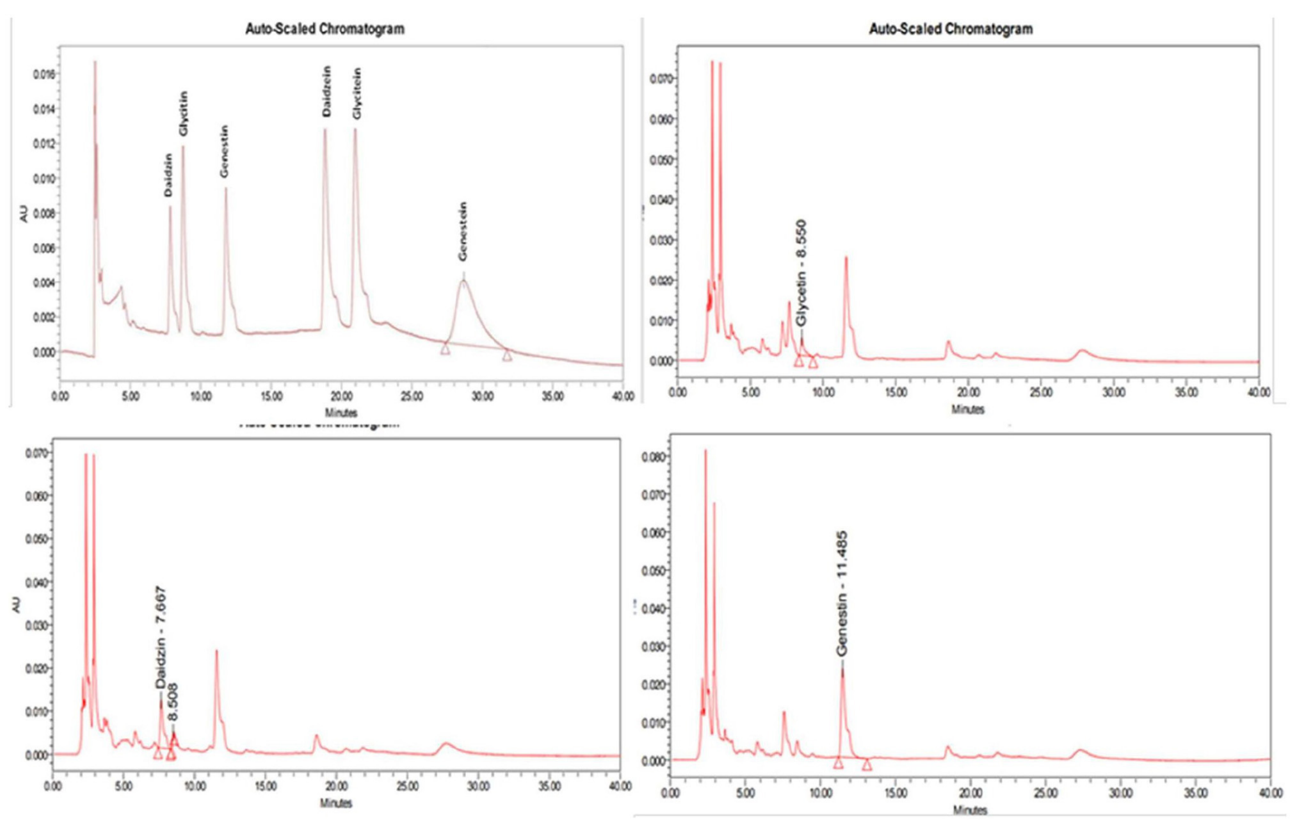

FIGURE 2
HPLC chromatogram of isoflavone standards and tofu samples.

3.2 Effect of processing condition on texture profile

Table 2 presents the values for hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience of tofu samples made using various processing techniques and coagulants. The findings indicated significant differences ($p < 0.05$) among the tofu samples. Tofu made with calcium sulfate was the hardest ($p < 0.05$) among those prepared using TPMI, while tofu with calcium acetate had the lowest hardness ($p < 0.05$). For TPMII, apple cider vinegar tofu exhibited higher ($p > 0.05$) textural hardness, whereas tofu coagulated with calcium sulfate showed lower ($p > 0.05$) hardness. The hardness in tofu is defined as the peak force during the initial compression cycle or the force required to deform the tofu structure (Shi et al., 2020). In TPMI, tofu coagulated with citric acid had the highest cohesiveness, whereas magnesium sulfate-based tofu had the lowest cohesiveness; however, statistical analysis indicates that these values are comparable to other samples (Table 2). Conversely, in TPMII, tofu made with calcium lactate showed the highest ($p < 0.05$) cohesiveness, and calcium sulfate-based tofu had the lowest ($p > 0.05$) cohesiveness. Cohesiveness refers to a material's ability to endure a second deformation compared to its resistance to the first, often termed "structural recoverability" (Nishinari and Fang, 2018). The low cohesiveness of tofu made with calcium sulfate and magnesium sulfate suggests inferior textural quality. Although calcium sulfate and magnesium chloride are common salt coagulants, undissolved calcium sulfate or rapid magnesium chloride release often leads to a coarse texture, indicating a poor quality (Meng et al., 2016; Ting et al., 2009). In the TPMI process, lemon juice-based tofu exhibited the highest ($p > 0.05$) springiness, whereas magnesium sulfate-based tofu had the lowest ($p > 0.05$) springiness. In contrast, lemon juice-based tofu showed the lowest ($p > 0.05$) springiness and calcium lactate-based tofu had the highest ($p > 0.05$) springiness in TPMII. Springiness relates to the elasticity of the sample, measured as the height recovered between the end of the first bite and the start of the second bite. It was calculated by dividing the distance during the second compression by the original compression distance (Zheng et al., 2021). A typical graph of the textural parameters of tofu samples is shown in Figure 3.

Adhesiveness is estimated in terms of the negative force required for the first bite, and represents the work required to overcome the attractive forces between the surface of food and materials in contact with food. Higher values represent softer textures. Among the tofu samples prepared using both methods (TPMI and TPMII), the highest ($p > 0.05$) adhesiveness was observed in calcium lactate-based tofu, and the lowest ($p < 0.05$) was observed for the tofu prepared using calcium sulfate. Gumminess, which is the product of hardness and cohesiveness, was found to be the highest for calcium sulfate-based tofu and lowest for calcium acetate-based tofu samples prepared following

TABLE 1 Isoflavone content (mg/100 mg dry weight) in raw soybean, okara, and tofu samples prepared with different coagulants and processing methods.

Samples	Glycitin (mg 100 mg^{-1})	Glycitein (mg 100 mg^{-1})	Genistin (mg 100 mg^{-1})	Genistein (mg 100 mg^{-1})	Daidzin (mg 100 mg^{-1})	Daidzein (mg 100 mg^{-1})
BS	0.063 ± 0.0019^{a}	0.0015 ± 0.0002^{b}	0.536 ± 0.0074^{a}	0.020 ± 0.0002^{b}	0.140 ± 0.0010^{a}	0.0534 ± 0.0003^{b}
OK	0.045 ± 0.0002^{b}	0.0058 ± 0.0006^{a}	0.328 ± 0.0020^{a}	0.038 ± 0.0021^{a,b}	0.153 ± 0.0013^{a}	0.0310 ± 0.0013^{c}
TPMI						
TAAI	0.0507 ± 0.0004^{d,e}	0.0076 ± 0.0005^{c}	0.389 ± 0.0043^{e,f}	0.0570 ± 0.0006^{a}	0.217 ± 0.0036^{c,d}	0.0460 ± 0.0014^{b,c}
TCAI	0.0501 ± 0.0019^{c,d,e}	0.0077 ± 0.0005^{c}	0.411 ± 0.0037^{f}	0.0551 ± 0.0081^{a}	0.237 ± 0.0043^{e}	0.0447 ± 0.00054^{b,c}
TCSI	0.0349 ± 0.0011^{a}	0.0069 ± 0.0005^{c}	0.291 ± 0.0016^{a}	0.0574 ± 0.0046^{a}	0.165 ± 0.0032^{a}	0.0406 ± 0.00067^{a,b}
TLI	0.0494 ± 0.00^{c,d,e}	0.00 ± 0.00^{a}	0.413 ± 0.0019^{f}	0.0539 ± 0.0016^{a}	0.198 ± 0.0054^{b}	0.0384 ± 0.0026^{a}
TMSI	0.0425 ± 0.0003^{b}	0.0024 ± 0.0006^{b}	0.381 ± 0.023^{d,e,f}	0.0512 ± 0.0012^{a}	0.192 ± 0.0064^{b}	0.0488 ± 0.00081^{c}
TMSCI	0.0442 ± 0.0008^{b}	0.0056 ± 0.0003^{c}	0.325 ± 0.0031^{a,b,c}	0.0675 ± 0.0009^{b}	0.204 ± 0.0022^{b,c}	0.0414 ± 0.00031^{a,b}
TCLI	0.0512 ± 0.0007^{e}	0.0064 ± 0.0004^{c}	0.386 ± 0.0063^{e,f}	0.0752 ± 0.0022^{c}	0.230 ± 0.0038^{d,e}	0.0461 ± 0.0015^{b,c}
TCATI	0.0457 ± 0.0009^{b,c}	0.0069 ± 0.0002^{c}	0.342 ± 0.0016^{b,c}	0.0739 ± 0.0002^{b,c}	0.199 ± 0.0027^{b}	0.0478 ± 0.00056^{c}
TACVI	0.0465 ± 0.0003^{b,c,d}	0.0070 ± 0.00^{c}	0.369 ± 0.0025^{c,d,e}	0.0776 ± 0.0008^{c}	0.191 ± 0.0013^{b}	0.0505 ± 0.00014^{c}
TRDI	0.0439 ± 0.0004^{b}	0.0071 ± 0.0006^{c}	0.664 ± 0.005^{g}	0.0752 ± 0.0006^{c}	0.192 ± 0.0014^{b}	0.0498 ± 0.0011^{c}
TPMII						
TAAII	0.0378 ± 0.0002^{a,b}	0.0043 ± 0.0001^{c,d}	0.306 ± 0.0011^{b}	0.0562 ± 0.0018^{b,c,d}	0.180 ± 0.0004^{d}	0.0373 ± 0.0004^{b,c}
TCAII	0.0416 ± 0.0003^{b}	0.0020 ± 0.0008^{b}	0.351 ± 0.0036^{e,f}	0.0484 ± 0.0007^{a,b}	0.215 ± 0.0008^{f}	0.0342 ± 0.0004^{b}
TCSII	0.0326 ± 0.0001^{a}	0.0025 ± 0.0003^{b,c}	0.273 ± 0.0003^{a}	0.0512 ± 0.0016^{a,b,c}	0.159 ± 0.0007^{c}	0.0293 ± 0.0003^{a}
TLII	0.0329 ± 0.0008^{a}	0.00 ± 0.00^{a}	0.336 ± 0.0047^{d,e}	0.0429 ± 0.0020^{a}	0.104 ± 0.0042^{a}	0.0354 ± 0.0003^{b}
TMSII	0.0416 ± 0.0002^{b}	0.0009 ± 0.0004^{a,b}	0.256 ± 0.0036^{a}	0.0499 ± 0.0014^{a,b}	0.138 ± 0.0031^{b}	0.0333 ± 0.0019^{a,b}
TMCII	0.0418 ± 0.00^{b}	0.0055 ± 0.0002^{d}	0.308 ± 0.0018^{b,c}	0.0636 ± 0.0005^{d}	0.195 ± 0.0029^{d,e}	0.0416 ± 0.00^{c,d}
TCLII	0.0429 ± 0.0004^{b}	0.0048 ± 0.0001^{d}	0.331 ± 0.0018^{d}	0.0643 ± 0.0005^{d}	0.205 ± 0.0003^{e,f}	0.0414 ± 0.0003^{c,d}
TCATII	0.0421 ± 0.0002^{b}	0.0048 ± 0.0001^{d}	0.360 ± 0.0081^{f}	0.0642 ± 0.0021^{d}	0.217 ± 0.0065^{f}	0.0426 ± 0.0016^{d}
TACVII	0.0522 ± 0.0018^{c}	0.0042 ± 0.0001^{c,d}	0.448 ± 0.0026^{g}	0.0626 ± 0.0037^{d}	0.207 ± 0.0021^{g}	0.0448 ± 0.00017^{d}
TRDII	0.0666 ± 0.003^{d}	0.0057 ± 0.0005^{d}	0.325 ± 0.0039^{c,d}	0.0612 ± 0.0031^{c,d}	0.388 ± 0.0053^{h}	0.0526 ± 0.00095^{e}

TAAI, Acetic acid tofu; TCAI, Citric acid tofu; TCSI, Calcium sulfate tofu; TLI, Lemon tofu; TMSI, Magnesium sulfate tofu; TMCI, Magnesium chloride tofu; TCLI, Calcium lactate tofu; TCATI, Calcium acetate tofu; TACVI, Apple cider vinegar tofu; TRDI, Rhododendron arboreum tofu. TAAII, Acetic acid tofu; TCAII, Citric acid tofu; TCSII, Calcium sulfate tofu; TLII, Lemon tofu; TMSII, Magnesium sulfate tofu; TMCII, Magnesium chloride tofu; TCLII, Calcium lactate tofu; TCATII, Calcium acetate tofu; TACVII, Apple cider vinegar tofu; TRDII, Rhododendron arboreum tofu; Values are presented as mean ± standard deviation.
Within each column, values with different superscript letters (a, b, c, etc.) are significantly different ($p < 0.05$) according to *post-hoc* multiple comparison tests. Values sharing the same letter are not significantly different from each other.

the TPMI process, while in the samples prepared following the TPMII process, gumminess was the highest for calcium lactate-based tofu and lowest for calcium sulfate-based tofu. However, statistical analysis indicates that these values are comparable to other samples marked with the same superscript letter in Table 2. Higher gumminess is caused by a higher hardness (Rahman and Al-Mahrouqi, 2009). Chewiness, which is the product's gumminess and springiness, was significantly highest ($p < 0.05$) for calcium sulfate-based tofu and lowest ($p > 0.05$) for magnesium sulfate-based tofu samples prepared following the TPMI process. Among the samples prepared following the TPMII process, chewiness was significantly highest ($p < 0.05$) for calcium lactate-based tofu and the lowest ($p > 0.05$) for tofu prepared using calcium sulfate. It is a measure of the energy required to masticate food. Resilience was highest ($p > 0.05$) for citric acid-based tofu and lowest ($p > 0.05$) for magnesium sulfate-based tofu samples prepared following the TPMI process, whereas it was highest ($p > 0.05$) in tofu samples prepared using calcium sulfate and lowest ($p > 0.05$) in samples prepared using citric acid following the TPMII process. Sample recovery from deformation, in terms of force and speed, determines resilience (Chandra and Shamasundar, 2015).

Tofu is preferred by people based on its textural differences, as people of Taiwan prefer soft, hard, and dry tofu (tou-kan). Chinese tofu (Tou-chi) also has a firm texture and is suitable for boiling soy sauce and seasoning mixtures. This Chinese tofu has a chewy, meaty feel, similar to textured soy protein. In several western countries, smooth and firm tofu is favored over hard and rubbery tofu (Tsai et al., 1981). The choice of coagulant in tofu preparation significantly affects its textural properties, which have been extensively studied across various types

TABLE 2 Texture profile of tofu samples prepared with different coagulants and processing methods.

Tofu samples	Hardness (g Force)	Adhesiveness (g.s)	Springiness	Cohesiveness (g.mm)	Gumminess (g Force)	Chewiness (g.mm)	Resilience
TPMI							
TAAI	6120.07 ± 267.8[f]	−32.63 ± 6.3[d]	0.663 ± 0.016[b,c]	0.346 ± 0.003[b]	2121.86 ± 113.3[c]	1409.6 ± 85.2[c]	0.098 ± 0.003[s]
TCAI	3773.50 ± 257.6[d,e]	−78.98 ± 3.2[b]	0.6 ± 0.026[b]	0.413 ± 0.033[b]	1562.40 ± 166.8[b]	948.31 ± 132.9[b]	0.101 ± 0.012[s]
TCSI	7059.69 ± 141.1[g]	−106.94 ± [a]	0.696 ± 0.052[b,c]	0.356 ± 0.014[b]	2508.86 ± 124[c]	1733.55 ± 97.09[d]	0.096 ± 0.004[s]
TLI	3654.48 ± 77.4[d]	−56.67 ± 5.1[c]	0.793 ± 0.017[c]	0.363 ± 0.008[b]	1329.38 ± 61.5[b]	1056.56 ± 69.9[b]	0.042 ± 0.001[a]
TMSI	4407.5 ± 33.4[e]	−85.78 ± 3.3[b]	0.334 ± 0.013[a]	0.214 ± 0.007[a]	229.33 ± 5.56[a]	76.56 ± 2.84[a]	0.037 ± 0.0006[a]
TMCI	1161.66 ± 33.5[a,b]	−58.84 ± 13.2[c]	0.391 ± 0.036[a]	0.222 ± 0.004[a]	258.37 ± 10.14[a]	100.26 ± 5.98[a]	0.039 ± 0.0003[a]
TCLI	1204.20 ± 48.5[a,b]	−19.44 ± 14.4[d]	0.564 ± 0.045[b]	0.277 ± 0.006[a]	333.14 ± 7.41[a]	188.61 ± 18.7[a]	0.070 ± 0.003[c]
TCATI	785.88 ± 47.03[a]	−27.04 ± 23.3[d]	0.559 ± 0.025[b]	0.248 ± 0.01[a]	194.54 ± 11.47[a]	108.93 ± 8.07[a]	0.062 ± 0.001[b,c]
TACVI	1939.12 ± 41.4[c]	−76.74 ± 4.1[b]	0.34 ± 0.03[a]	0.253 ± 0.009[a]	491.41 ± 26.87[a]	168.57 ± 24.5[a]	0.048 ± 0.001[a,b]
TRDI	1623.02 ± 96.6[b,c]	−73.16 ± 12.3[b]	0.369 ± 0.045[a]	0.229 ± 0.01[a]	370.31 ± 6.6[a]	136.91 ± 17.2[a]	0.046 ± 0.002[a]
TPMII							
TAAII	4179.83 ± 34.5[e]	−2.83 ± 0.3[d,e]	0.548 ± 0.010[c]	0.302 ± 0.014[b,c]	1262.04 ± 56.9[e]	691.52 ± 20.9[c]	0.087 ± 0.003[a]
TCAII	1552.63 ± 70.4[b,c]	−41.77 ± 19.6[b]	0.389 ± 0.036[b]	0.251 ± 0.007[a,b]	390.55 ± 24.8[b,c]	152.66 ± 19.7[a]	0.064 ± 0.002[a]
TCSII	493.18 ± 39.9[a]	−90.23 ± 10.6[a]	0.222 ± 0.021[a]	0.233 ± 0.005[a]	115.22 ± 10.8[a]	25.67 ± 3.63[a]	0.128 ± 0.1[a]
TLII	675.494 ± 20.6[a]	−32.53 ± 3[b]	0.219 ± 0.021[a]	0.236 ± 0.002[a]	159.46 ± 5.07[a,b]	34.83 ± 3.06[a]	0.075 ± 0.0003[a]
TMSII	1068.54 ± 117.9[a,b]	−5.37 ± 4.1[d,e]	0.714 ± 0.019[d,e]	0.355 ± 0.009[c]	1561.91 ± 20.01[f]	1115.66 ± 34.2[d]	0.065 ± 0.027[a]
TMCII	2553.07 ± 25.03[d]	−16.65 ± 3.3[c]	0.614 ± 0.043[c,d]	0.342 ± 0.01[c]	912.76 ± 30.79[d]	562.94 ± 55.5[b,c]	0.075 ± 0.006[a]
TCLII	4923.09 ± 439.1[e,f]	−1.53 ± [e]	0.782 ± 0.002[e]	0.510 ± 0.027[d]	2490.08 ± 121.6[g]	1947.32 ± 88.9[f]	0.194 ± 0.017[a]
TCATII	1960.83 ± 116.4[c,d]	−12.48 ± 0.7[c,d,e]	0.609 ± 0.031[c,d]	0.333 ± 0.003[c]	654.07 ± 34.7[c,d]	398.49 ± 30.7[b]	0.085 ± 0.0006[a]
TACVII	5112.45 ± 158.2[f]	−11.02 ± 23.5[c,d,e]	0.758 ± 0.007[e]	0.354 ± 0.004[c]	1813.18 ± 64.1[f]	1372.81 ± 35.1[e]	0.091 ± 0.001[a]
TRDII	2055.45 ± 131.9[c,d]	−12.81 ± 5.7[c,d]	0.636 ± 0.006[c,d]	0.300 ± 0.015[b,c]	618.16 ± 57.1[c]	393.73 ± 38.7[b]	0.097 ± 0.009[a]

TAAI, Acetic acid tofu; TCAI, Citric acid tofu; TCSI, Calcium sulfate tofu; TLI, Lemon tofu; TMSI, Magnesium sulfate tofu; TMCI, Magnesium chloride tofu; TCLI, Calcium lactate tofu; TCATI, Calcium acetate tofu; TACVI, Apple cider vinegar tofu; TRDI, Rhododendron arboreum tofu; TAAII, Acetic acid tofu; TCAII, Citric acid tofu; TCSII, Calcium sulfate tofu; TLII, Lemon tofu; TMSII, Magnesium sulfate tofu; TMCII, Magnesium chloride tofu; TCLII, Calcium lactate tofu; TCATII, Calcium acetate tofu; TACVII, Apple cider vinegar tofu; TRDII, Rhododendron arboreum tofu.

Values are presented as mean ± standard deviation. Within each column, values with different superscript letters (a, b, c, etc.) are significantly different ($p < 0.05$) according to *post-hoc* multiple comparison tests. Values sharing the same letter are not significantly different from each other.

of coagulants. The primary coagulants used in tofu production include magnesium chloride ($MgCl_2$), calcium sulfate ($CaSO_4$), glucono-δ-lactone (GDL), and various indigenous and alternative coagulants. Wang et al. (2018) reported firmer texture and high rigidity with less elasticity in the tofu prepared using $CaSO_4$ while tofu prepared using $MgCl_2$ was found smoother in texture with high moisture content.

The interaction of coagulants with different types of proteins present in soymilk may affect the textural properties of tofu samples. Upon heating, the proteins present in soymilk are unfolded, and disulfide bonds are exposed. These di-sulfides are negatively charged, which neutralizes the addition of coagulants and causes the aggregation of soy proteins (Prabhakaran et al., 2006). In the tofu-making process, many people rely on coagulants such as glucono-δ-lactone (GDL) and salts (magnesium sulfate or calcium sulfate) because the final products often have a gritty and loose texture as the coagulant type affects the tofu texture (Li et al., 2013). This type of tofu is generally used in traditional Sichuan dishes such as "mapo tofu" (Shi et al., 2020). Previously, researchers also studied the effect of coagulants and various processing conditions on the texture profile of tofu and found significant differences among samples. Joo and Cavender (2020) reported lower chewiness, cohesiveness, and hardness on a similar line with few exceptions in tofu samples coagulated with tri magnesium citrate, nigari, and gypsum. Li et al. (2014) studied the bittern-solidified tofu for quality improvement and hardness and springiness values are in accordance with the present work for different tofu samples. Natural coagulants are also being used for tofu gel preparation with improved texture and flavor (Jun et al., 2019; Shen and Kuo, 2017).

3.3 Multivariate analysis

To understand the primary factors affecting the differences among tofu samples, a correlation matrix was developed using

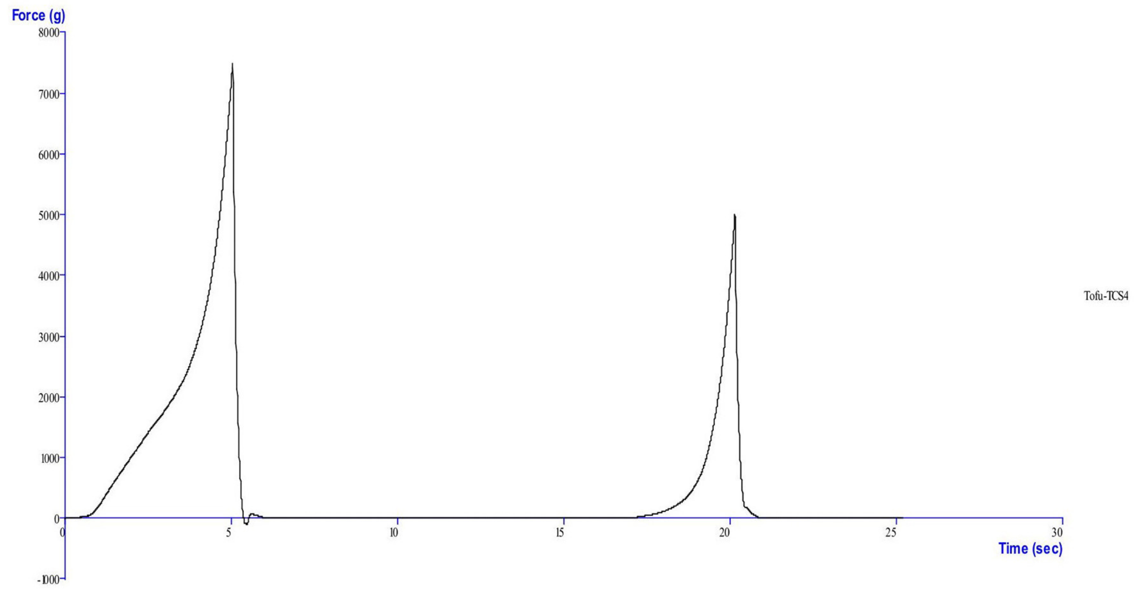

FIGURE 3
Typical graph of textural analysis for tofu coagulated with Calcium sulfate.

TABLE 3 Factors affecting the differences among the tofu samples following two different types of processing methods and coagulants used.

Parameters	TPMI			TPMII			
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 4
Daidzein	−0.46	0.44	−0.38	0.28	**0.81**	0.42	0.15
Daidzin	0.12	**0.89**	−0.17	0.31	**0.91**	−0.09	0.10
Genistein	−0.38	−0.02	**−0.90**	0.29	0.16	**0.94**	0.04
Genistin	0.07	**0.92**	0.34	−0.06	**0.96**	0.17	0.01
Glycitein	0.56	0.01	**−0.80**	0.29	0.16	**0.94**	0.04
Glycitin	0.03	**0.97**	−0.15	0.36	**0.87**	0.15	−0.10
Hardness	0.58	−0.17	0.69	**0.80**	0.38	0.12	0.17
Springiness	**0.84**	0.12	0.08	**0.90**	0.32	0.23	−0.05
Cohesiveness	**0.95**	0.19	0.18	**0.86**	0.02	0.30	0.36
Gumminess	**0.92**	−0.07	0.25	**0.97**	0.11	0.20	0.02
Chewiness	**0.96**	−0.02	0.22	**0.96**	0.18	0.21	0.00
Resilience	**0.85**	0.05	−0.23	0.12	0.06	0.04	**0.98**
Expl.Var	5.12	2.87	2.43	4.53	3.52	2.25	1.17
Prp.Totl	0.43	0.24	0.20	0.38	0.29	0.19	0.10
Eigenvalue	5.43	2.96	2.03	6.77	2.26	1.39	1.07
% Total variance	45.23	24.67	16.96	56.38	18.80	11.61	8.93
Cumulative Eigen value	5.43	8.39	10.42	6.77	9.02	10.41	11.49
Cumulative %	45.23	69.89	86.85	56.38	75.18	86.79	95.71

Factor loadings greater than 0.7 (highlighted in bold letter) are considered significant contributors to the corresponding principal component.
Expl.Var = Explained variance; Prp.Totl = Proportion of total variance explained.

factor analysis. The number of factors was identified through a Scree plot, focusing on eigenvalues >1 (Liu et al., 2003). This analysis revealed three factors in the TPMI and four in the TPMII (Table 3), explaining 86.85 and 95.71% of the total variance, respectively. In the TPMI, the first factor accounted for 45.23% of the total variance, with strong positive correlations

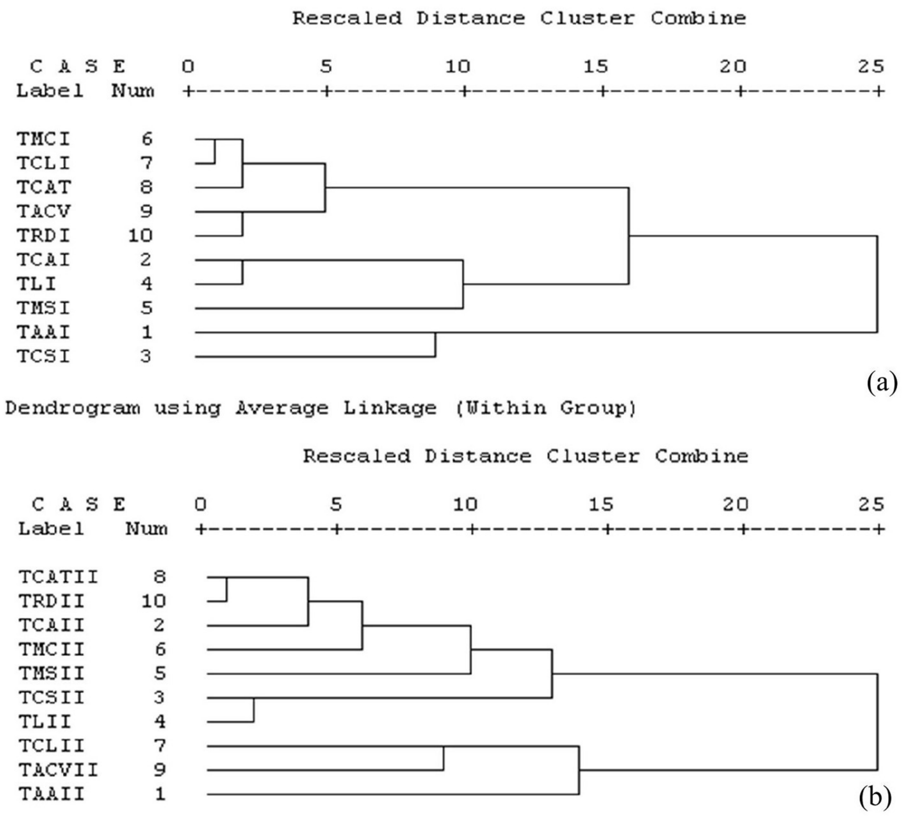

FIGURE 4
Clustering of different tofu samples prepared using **(a)** TPMI **(b)** TPMII based on their analyzed properties.

(>0.75) with springiness, cohesiveness, gumminess, chewiness, and resilience. The second factor, which explained 24.67% of the total variance, showed positive correlations (>0.75) with daidzin, genistin, and glycitin. The third factor, responsible for 16.96% of the variance, had negative correlations (>0.75) with genistein and glycetein. In the TPMII, the first factor explained 56.38% of the total variance, with high correlations (>0.75) with hardness, springiness, cohesiveness, gumminess, and chewiness. The second factor, which accounted for 18.80% of the total variance, exhibited strong correlations with daidzein, daidzin, genistin, and glycitin. The third and fourth factors, explaining 11.61 and 8.93% of the total variation, respectively, showed positive correlations (>0.75) with resilience, genistein, and glycitein levels.

Cluster analysis indicated that all tofu samples prepared using each processing method could be divided into two main clusters with sub-clusters, showing less difference in the Dlink/Dmax value in TPMI and a greater difference in TPMII (Figures 4a, b). In TPMI, tofu made with acetic acid and calcium sulfate formed separate clusters, whereas the other eight samples were grouped into another cluster. In TPMII, tofu prepared with acetic acid, apple cider vinegar, and calcium lactate were in the same cluster, whereas the remaining tofu samples were grouped into another cluster. Factor and cluster analyses suggested that processing conditions affect isoflavone concentrations and textural properties of tofu samples.

4 Conclusion

This study examined the influence of different coagulants on isoflavone content, and textural properties of Himalayan black soybean-based tofu prepared using two distinct methods. Our findings indicate that tofu made with soymilk using the TPMII process contains higher total isoflavone levels than tofu produced from soymilk with okara removed after boiling (TPMI). Textural attributes varied between the TMPI and TMPII tofu samples, except when acetic acid was used as a coagulant. Texture was identified as the primary factor contributing to the differences among tofu prepared using various coagulants and methods. This study explored *R. arboreum* floral juice as a coagulating agent, which showed promising results in terms of isoflavone content. Given its medicinal properties, this natural coagulant needs further investigation and its potential adoption by tofu manufacturers for flavored tofu production.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SA: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. VA: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. SR: Data curation, Formal analysis, Methodology, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

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