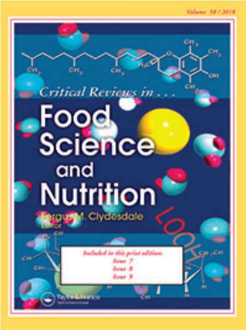

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Research progress in tofu processing: From raw materials to processing conditions

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ABSTRACT

As a traditional soybean product with good quality and a healthy food with many functional components, tofu is increasingly consumed in people's daily life. Traditional tofu processing consists of numerous steps, including the soaking and grinding of soybean seeds, heating of the soybean slurry, filtering, and addition of coagulants, and others. The properties of soybean seeds, processing scale, soaking and heating conditions, type and concentration of coagulant, and other factors collectively impact the processing steps and the final tofu quality. The generation of whole soybean tofu with more nutritive value comparing with traditional tofu has been successfully reported by several studies. As one of the most important functional component, isoflavones and their presence in tofu are also influenced by the above-mentioned factors, which influence the nutritive value of tofu. Research investigating the influence of tofu processing conditions on the quality and isoflavone profiles of tofu are the subject of this review. Issues that should be further studied to investigate the influence of processing conditions on the quality and nutritive value of tofu are also introduced.

Glossary of acronyms: CA, calcium acetate; CC, calcium chloride; CL, calcium lactate; CS, calcium sulfate; GDL, glucono-δ-lactone; LOX, lipoxygenase; MC, magnesium chloride; MS, magnesium sulfate; MTGase, microbial transglutaminase; MW, molecular weight; OCC, optimal coagulant concentration; RH, relative humidity; SC, sodium chloride; SEM, scanning electron microscopy; SPI, soybean protein isolate; UHPH, ultra-high pressure homogenization; WHC, water-holding capacity; WST, whole soybean tofu

KEYWORDS
Tofu; processing technology; processing conditions; coagulant; whole soybean tofu; isoflavones

Introduction

Tofu, also known as bean curd, was first and unintentionally invented during the Western Han Dynasty from 202 BC to 8 AD in China. However, another more reasonable perspective indicates that tofu first appeared during the middle to late Tang Period from 836 AD to 907 AD is more reasonable (Shurtleff and Aoyagi, 2013). After long-term practice and innovations, the technique used to produce tofu has been handed down in China and has spread worldwide.

As one of the best source of plant protein, tofu is also rich in beneficial lipid, vitamins, and minerals, as well as other bioactive compounds, such as isoflavones, soyasaponin, and others. Therefore, reducing the risk of many diseases, such as hypertension, hyperlipidemia, hypercholesterolemia, arteriosclerosis, coronary heart disease, and breast cancer, and others, has been widely reported with tofu consumption (White et al., 2000; Zhang et al., 2003; Takahashi and Konishi, 2011). Therefore, tofu is currently increasingly consumed on a daily basis by people who value it for its characteristic taste and substantial nutritive value.

It is well known that tofu is a highly hydrated gel-type food, such that the formation of tofu is attributed to the gelation properties of soybean protein (Singh et al., 2008). Together with confirmation of the theory for tofu formation and the development of advanced food processing techniques, the diversity of tofu products has satisfied the different consumption demands. At present, firm tofu and soft tofu manufactured by different processes, or pressed tofu and packed tofu derived from different coagulants, are representative types of commercial tofu, as shown in Table 1. It is worth noting that the classification of tofu differs in different countries, such as northern tofu and southern tofu for Chinese-style soft and firm tofu, and momen tofu and silken tofu for Japanese-style soft and firm tofu (Shurtleff and Aoyagi, 2013). Therefore, discrepancies among the different types of tofu usually result from the different processing methods and conditions. However, different manufacturing processes for different types of tofu using similar coagulants utilize the same processing mechanism.

In general, the pretreatment of raw soybean seeds, soaking, grinding, slurry heating, filtering, solidifying with coagulants, and pressing, and others are the main operations involved in traditional tofu processing. All of the parameters related to these operations can influence the final quality attributes of

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Table 1. Representative types of commercial tofu and their processing steps.

Type of tofu		General characterization	Sensory evaluation	Key process	Shelf life	Syneresis	Industrialization
Pressed tofu	Firm	Processed by pressing broken bean curd; ~80% water content	Compact internal structure; tough taste and strong beany flavor	Heat denaturation of soymilk protein; suitable stirring speed and time after coagulant addition; gelation at high temperature, such as 75–85°C; selection of appropriate pressing pressure.	Several days in bulk	High	Industrialized production; workshop-style
	Soft	Processed by pressing unbroken bean curd; ~90% water content	Soft cheese-like texture but firm enough to retain shape after slicing; light beany flavor	Heat denaturation of soymilk protein; suitable stirring speed and time after coagulant addition; gelation at high temperature, such as 75–85°C; selection of appropriate pressing pressure; selection of appropriate sterilization conditions.	Several months in a pouch pack	Low	Industrialized production
Packed tofu	Soft	Gelation in a pouch pack and molding without pressing; ~90% water content; relatively high yield due to little loss of water soluble protein	Bland taste and fine texture; soft cheese-like texture but firm enough to retain shape after slicing; light beany flavor	Heat denaturation of soymilk protein; suitable stirring speed and time after coagulant addition; gelation at low temperature, such as 40°C or ambient temperature; selection of appropriate sterilization conditions.	Several months in a pouch pack	High	Industrialized production

Note: Adapted from Shurtleff and Aoyagi (2013).

tofu from the perspective of intact food processing. Based on previous investigations, the variety and storage of soybean seeds, composition of soybean protein, soaking and heating conditions, coagulant types and operating conditions, and processing scale, and others, are the most-studied factors that significantly influence the manufacturing of tofu. In addition, the processing of whole soybean products has become common in the soybean industry. Therefore, progress in research examining the relationships between processing conditions and quality attributes and isoflavone profiles of tofu and the development of whole soybean tofu (WST) are introduced in this review.

Soybean seeds

The nature or quality of food material is always closely related to the final quality of the corresponding products, which is also suitable for tofu production. Soybean seeds with good quality can produce tofu with high quality and economic benefit. In general, the composition of soybean seeds, including protein, lipid, sugar, phytic acid, and other chemical components, directly affects the yield and quality of tofu. The variety, growing environment, and storage condition indirectly affect the yield and quality of tofu by influencing the composition of soybean seeds. For example, different varieties of soybean seeds mainly refer to the different chemical compositions, storage protein profiles, and other characteristics of soybean seeds. Among these factors, protein composition is regarded as the most direct and important factor for tofu production and the final tofu quality.

Protein composition of soybean seeds

Protein is contained in most soybean varieties, making up approximately 35%–50% of their content. This high amount of protein determines the suitability of producing tofu from soybean seeds. Soybean protein is not only complete protein with the perfect composition of amino acids but it is also an important material with favorable processability for the food industry. In general, the higher the protein content, the greater the yield and quality of tofu (Bhardwaj et al., 1999; James and Yang, 2016). However, not all the proteins contained in soybean seeds are related to the final quality of tofu. Soybean proteins that are closely related to tofu quality consist mainly of glycinin with a centrifugal sedimentation coefficient of 11S and β-conglycinin with a centrifugal sedimentation coefficient of 7S. These two proteins are also collectively termed as storage proteins, making up approximately 70% of the total soybean protein.

In general, the content of glycinin in soybean protein influences the hardness of tofu; however, its springiness is related to the content of β-conglycinin. When protein content and pH value were set as the fixed values, the heat-induced gel of β-conglycinin was more prone to fracture than the heat-induced gel of glycinin (Utsumi and Kinsella, 1985; Renkema et al., 2001). This phenomenon is related to the different molecular structures of the heat-induced gels. Briefly, the glycinin gel can be produced in the form of a stable three-dimensional network structure with the help of electrostatic interactions and disulfide bonds under conditions of heating and coagulation. However, under the same conditions, formation of the β-conglycinin gel is completed only by hydrogen bonding (Utsumi et al., 1997). Thus, the textural properties of the heat-induced glycinin gel are superior to that of the β-conglycinin gel. The rheological properties of glycinin and β-conglycinin gels coagulated by glucono-δ-lactone (GDL) were investigated to elucidate the gelation of tofu (Kohyama and Nishinari, 1993). Under the same coagulant concentration and temperature, the gelation rate of β-conglycinin was much slower than that of glycinin, and correspondingly, the gelation time for β-conglycinin was longer than that for glycinin. In addition, the gelation time increased when the concentration of β-conglycinin was increased in the mixture of β-conglycinin and GDL with a fixed mass ratio of 10:1. Moreover, the minimum concentration of β-conglycinin needed to form a gel (0.479%) was lower than that of glycinin (1.03%) under the same coagulant concentration and operation parameter conditions.

Physicochemical properties of GDL-induced gels of β-conglycinin and glycinin mixtures, such as pH value, water-holding capacity (WHC), texture, and color, were also studied to evaluate the gel properties derived from different soybean proteins (Tay and Perera, 2006). Consequently, the gel obtained from β-conglycinin and glycinin mixture with a high mass ratio of 11S/7S exhibited the highest level of hardness, cohesiveness, gumminess, and L^* values. The gelation rate of glycinin was significantly higher than that of β-conglycinin. In addition, the hardness and WHC of calcium sulfate (CS)-induced tofu were positively related to the denaturation degree of glycinin and the particle size of soybean protein isolate (SPI) aggregates which were closely influenced by the heating temperature of SPI dispersions (65, 75, 85, and 90°C) (Zhao et al., 2016). Moreover, as the degree of denaturation and the particle size of SPI aggregates increased, the formed gel displayed a more uniform and denser network structure. Thus, glycinin is clearly more suitable for gelation, and the resultant gel has better quality than that gelled by β-conglycinin. The selection of SPI for preparation of tofu-type gels can reduce the impact of nonprotein components on the gelation, which is convenient to investigate the different gelation properties between β-conglycinin and glycinin using the same coagulant.

To identify the specific reason for different gelation properties of glycinin and β-conglycinin, discrepancies in their molecule structures have been extensively investigated. Basically, the different gelation properties of these storage proteins can be reflected by differences among their subunits (Mujoo et al., 2003). In general, glycinin consists of six subunits and each subunit has an acidic polypeptide chain (A, with a molecular weight [MW] of ~35,000) and a specific basic polypeptide chain (B, with a WM of ~20,000) linked by a disulfide bond. Five genetic variants which are divided into group-I ($A_{1a}B_2$, $A_{1b}B_{1b}$, and A_2B_{1a}) and group-II (IIa or A_3B_4 and IIb or $A_5A_4B_3$) based on the homology of their subunit sequences, are observed in glycinin. However, β-conglycinin is a trimeric glycoprotein that consists of three subunits, namely, α (with a WM of ~72,000), α' (with a WM of ~68,000), and β (with a WM of ~52,000) (Tezuka et al., 2000; Poysa et al., 2006). From the perspective of molecular microstructure, glycinin contains two sulfydryl groups and 20 sulfur-sulfur single bonds; however, only two sulfur-sulfur single bonds and no sulfhydryl are contained in β-conglycinin (Pazdernik et al., 1996). This phenomenon is related to differences in the content of sulfur-containing amino acids (methionine and cysteine) between these two storage proteins. As a result, different gelation properties are observed when tofu is produced from soybean varieties with different mass ratios of 11S/7S. Therefore, 11S/7S was regarded as a reliable index for the selection of soybean varieties for suitably producing tofu products (Meng et al., 2016).

According to previous studies, the hardness values of gels generated using varying glycinin subunits displayed an increase as follows: group-IIb, group-IIa, and group-I (Yagasaki et al., 2000); those of gels formed from β-conglycinin subunits exhibited an increase order of α, α', and β (Mohamad Ramlan et al., 2004). In addition, soybean cultivars with 11SIIa null, 7Sα' null, and higher contents of 11SI and 11SIIb were more prone to produce tofu with increased hardness (Poysa et al., 2006). Moreover, significant correlations were observed between the profiles of $11SA_3B_4$ and $11SA_5A_4B_3$ and the gelation speed, gel strength, and transparent density of the soybean protein gel (Utsumi, 1989). Differences in the hardness, WHC, and color of silken tofu produced by varying soybean seeds with different protein contents and 11SA4 or 11SA4 null were investigated by James and Yang (2014). A positive correlation was observed between the hardness and WHC of the formed tofu and the high content of protein and 11SA4 null in soybean. Good texture properties of silken tofu formed by an 11SA4 null soybean variety were also observed. Furthermore, although the protein content of the 11SA4 null soybean variety was relatively low, the formed silken tofu had a similar hardness to the tofu formed by soybean variety with higher protein content and the 11SA4 subunit. Based on these findings, the selection of a dedicated soybean variety for tofu production could be meaningfully achieved by analyzing the protein content, storage protein composition, and protein subunit composition.

However, the specific composition of protein subunits is influenced by the protein profile of raw soybean seeds, which is determined by the variable genotypes and planting environments. Significant variability in the mass ratio of β-conglycinin and glycinin fractions was observed among 90 Brazilian soybean cultivars sown in several different regions of Brazil. Moreover, sowing location was also significantly affected by the protein fraction contents (Carrão Panizzi et al., 2008). Thus, the production of tofu using different varieties of soybean seeds could originate from the genetic variation and environmental effects.

Other chemical components of soybean seeds

Although the formation of tofu depends on the storage protein composition of soybean seeds, tofu processing is also indirectly influenced by other constituents, such as lipid, carbohydrate, and phytic acid or its salts. The lipid contents (dry basis) of 20 types of soybean collected from Beijing were reported to positively correlate with the yield and volume of wet tofu and the yield of dry tofu (Chen et al., 2004). In addition, the hardness, springiness, cohesiveness, and resilience were influenced by both the lipid, starch and the content of soluble protein contents of soybean seeds. These different results revealed the negative correlations between these textural properties of tofu and the contents of lipid and starch (Song et al., 2013). Systematic research investigating the relationship between tofu yield and soybean composition were conducted (Poysa and Woodrow, 2002). According to the results, the yield, hardness, and firmness of tofus coagulated with GDL and CS were positively correlated to both protein and stachyose content. However, soybean seed oil, free sugar, sucrose, and the remaining contents were negatively correlated to the aforementioned tofu quality. Tofu-type gel prepared from SPI showed a hard-packed texture and enhanced quality when nonpolar lipids (soybean oil and palm oil) were added (Zhou et al., 2009). However, the fracture stress of this tofu-type gel decreased with the nonpolar lipid contents increased. In contrast, the WHC of tofu-type gel increased to a certain degree when polar lipids (monoacylglycerol and phospholipid) were added to the SPI material.

Antinutritional factors, such as phytic acid or its salts and lipoxygenase (LOX), have been also reported to be closely related to the yield and quality of tofu. Similar to the protein profile in soybean seeds, the profile of phytic acid or phytate is another important index for evaluating the processability of soybean seeds because phytic acid can influence both the quality and processing properties of tofu. Two explanations have been proposed to interpret this influence. First, phytic acid can modify the molecular structure to change the physicochemical properties of storage proteins. Second, coagulants (such as CS and magnesium chloride [MC]) can be reacted with phytic acid to form complex compounds and lose their gelation properties. During the 1990s, the relationship between phytic acid profile and tofu quality were preliminary investigated (Schaefer and Love, 1992). Excluding the significant association of tofu hardness and springiness with protein content, the content of phytic acid in soybean seeds was significantly correlated with the calcium content in tofu ($r = 0.90$), which directly influenced its hardness ($r = 0.73$) and springiness ($r = 0.83$). According to the results reported by Song et al. (2013), phytic acid influenced the gelation of proteins and coagulants by reacting with these gelatinous substances to increase the tofu yield and decrease the tofu hardness.

The mechanism underlying the influence of phytate on tofu quality was clearly explained by studying the distribution of phytate during tofu processing (Ishiguro et al., 2008). The results showed that 38% of the total phytate was bound to soluble protein, 3% was linked to particulate protein, and the other 59% was in free form in soymilk. Along with the formation of tofu, the content of phytate bound to particulate protein increased, resulting in the increase in total phytate content in tofu. In addition, an increasing content of phytate in soymilk resulted in an increased concentration requirement of MC for gelation. Thus, the presence of phytate changed the optimal coagulant concentration (OCC) of coagulant to decrease the hardness and to increase the fracturability and viscosity of tofu during tofu processing.

However, the influence of phytate profile in soybean seeds on tofu quality could be varied using different coagulant concentrations (Ishiguro et al., 2006). The breaking stress of tofu made using 0.25% MC was significantly negatively correlated to the phytic acid content in soymilk obtained from various types of soybean seeds ($p < .001$). Nevertheless, when the MC concentration increased (>0.25%), the correlation decreased between the breaking stress of tofu and phytic acid content. Moreover, no significant correlation between them was observed when the breaking stress reached a maximum at a particular MC concentration (>0.35%). This phenomenon might be attributed to the buffer role of phytic acid for coagulation retardation of soybean proteins and coagulants (Toda et al., 2006). Therefore, the effects of phytic acid on tofu quality could be influenced by the adopted processing conditions.

In addition, undesirable flavors, such as beany, greasy, and bitter, are generally present in tofu due to the appearance of LOX in soybean seeds. Three major LOXs, LOX-1, LOX-2, and LOX-3, in mature soybean seeds react with polyunsaturated fatty acid to influence the odor quality of soybean seeds or soybean products. Sensory evaluations of tofu performed using LOX-1 null, LOX-1,2 null, LOX-1,2,3 null, and LOX-containing soybean seeds were comparatively assessed by a trained descriptive taste panel (Yang et al., 2015). As a result, soybean seeds lacking LOX-1,2,3 produced tofu with the lowest rancid or grassy odor; however, sweeter and less bitter tastes were identified in tofu made from LOX-1,2 null and LOX-1,2,3 null soybean. Thus, the LOX null soybean could produce tofu with more acceptable aromas.

Cultivars and growing environment

As the major genetic encoding substance, storage protein composition has been considered to be closely related to the soybean variety and the planting environment. Twelve soybean genotypes (protein content ranging from 31.42% to 48.07%) harvested from four locations in southern America were used as raw materials to produce GDL-induced tofu, and the quality attributes were comparatively evaluated (Bhardwaj et al., 1999). First, the solid content of soymilk and hardness value of the formed tofu were significantly influenced by the varying sowing locations ($p < .05$). Second, the yield of GDL-induced tofu was extremely significantly influenced by the varying genotypes of raw soybean ($p < .01$). Third, soybean seeds with a high content of protein imparted a higher yield and better springiness and hardness to the GDL-induced tofu. However, the influence of sowing location and planting season on tofu quality was weaker than that of genotype because soybean breeding using genetic technique could directly change the protein composition of soybean seeds (Min et al., 2005). This observation confirmed that the protein profile is the most important factor for determination of the processing properties and quality of tofu.

Research examining the roles of genotype, environment, and genotype $\times$ environment interactions in the glycinin and β-conglycinin profiles of soybean seeds revealed that the contents of these two storage proteins and their mass ratio remained unchanged with varying planting years and locations (Fehr et al., 2003). However, the varying environments involved in the different sowing locations displayed a significant phenotypic correlation with the contents of these two storage proteins. Therefore, environmental factors remarkably influence the biochemical composition of soybean (Kumar et al., 2006). For example, the total protein content was negatively related to the latitude ($p < .05$) and rainfall ($p < .001$) of the growing location, and positively related to the daily mean temperatures ($p < .05$) of the growing location. In the contrast, the contents of oil and linoleic acid were positively and negatively related to the latitude and daily mean temperature, respectively. A significant correlation was observed between the contents of phytic acid or heat-stable antinutritional factors of soybean seeds and the genotype and genotypic $\times$ locational interaction. Recently, by investigating the influence of genotype and sowing location on the subunits of storage proteins, Yang and James (2014) found that soybean seeds with varying globulin subunit profiles could impart tofu with different yields and quality properties. Moreover, the specific globulin subunit composition of the varying genotypic soybean seeds could be influenced by the different growing locations to finally affect the yield, hardness, and WHC of tofu. Based on the aforementioned description, genotypic features and planting environmental factors could indirectly affect the processing properties of tofu by influencing the globulin subunit composition of soybean seeds.

It is worth noting that different conclusions regarding the influence of soybean cultivars on the processing properties of tofu can be found in previous studies due to the different processing methods and parameters utilized. The yield and quality of tofu derived from three types of soybean seeds (Proto, Vinton, and Sturdy) and the fractionated 11S and 7S proteins at varying ratios of 1.6:3.2 were comparatively studied. The results showed that soybean cultivar had a more substantial effect on the yield or textural quality of tofu than the 11S/7S ratio (Ji et al., 1999). However, the 11S protein content or the 11S/7S ratio was positively related to the hardness of sodium chloride (SC)-induced tofu when the fractionated storage proteins were purified (KangII et al., 1991). In addition, the influence of storage protein composition on the yield and quality of tofu could also be related to processing scales, such as bench, pilot, and production methods (Cai and Chang, 1999). Therefore, many complex physicochemical factors, such as protein composition of soybean seeds, processing methods, heating slurry, and coagulant types, and others, influence the yield and quality of tofu during tofu processing.

Storage of soybean seeds

A relatively long storage of soybean seeds is usually required after harvest due to the further postripening and necessary transportation. In general, storage treatment is an essential step for the usage of soybean seeds because the physicochemical properties of soybean can change in a manner that favors or hinders the production of soybean food. Thus, the selection of suitable storage conditions, including initial water content of soybean seeds, storage humidity, temperature, and time, is vital for the control of soybean quality. For example, after the storage of soybean seeds under high temperature and humidity conditions, an increase in acidity and color darkening of soybean seeds, and a decrease in the water imbibition rate and increase in solid matter leakage during soaking were observed (Lambrecht et al., 1996; Hou and Chang, 2004a).

Soybean seeds stored at a series of relative humidity (RH) from 70% to 75% and 4°C displayed no changes during the storage (<15 months) in color and solid matter of soybean seeds, pH value of soymilk, or textural properties of CS-induced tofu (Kong et al., 2008). However, when the storage temperature was >30°C, soybean seeds deterioration decreased the yield and quality of tofu. The optimum mixing time of soymilk and coagulant was reduced to produce a low yield of tofu when soybean seeds were stored under adverse conditions. The OCC during tofu processing was also affected by the storage time (Kamizake et al., 2016). Under accelerated (84% of RH and 30°C, up to nine months) and natural (ambient RH and temperature, up to 18 months) aging conditions, the OCCs for all soybean seeds were reduced with an increasing storage time. This phenomenon might be attributed to the decreased content of soymilk soluble solids. Correspondingly, tofu made from these materials showed a characteristic of low yield and quality.

In essence, the composition of storage proteins (β-conglycinin and glycinin) is altered during storage to influence tofu processing. Under adverse conditions of 84% RH and 30°C, the yield of crude glycinin extraction gradually declined as the storage time increased during the first three months (Hou and Chang, 2004b). The crude yield was then significantly decreased and barely be extracted after storage for nine months. Moreover, the total sugar content increased from 0.19% in the crude glycinin extract for fresh soybean seeds to 1.04% in the crude glycinin extract for the soybean seeds stored for nine months. However, no significant changes in these two indexes were observed when soybean seeds were stored in 57% RH at 20°C, in the cold (4°C), or an uncontrolled ambient garage for 18 months. From the microstructural analysis, as the storage time increased under adverse conditions, the content of total free hydrosulfuryl decreased and the content of total disulfide bond increased in glycinin. In addition, there was a significant decrease in surface hydrophobicity and a significant increase in contents of total free hydrosulfuryl and total hydrosulfuryl including disulfide bonds in β-conglycinin when soybean seeds were stored under adverse conditions after six months (Hou and Chang, 2004c). By analyzing the protein composition of soymilk obtained from soybean seeds stored under adverse condition, a clear change in protein composition was observed as the storage time increased (Kong and Chang, 2013). This finding might be attributed to the aggregation of proteins with the formation of aggregated proteins having higher MWs and insoluble property. Thus, the reduced tofu yield of soybean seeds stored under adverse condition was not due to the decreased total protein content in soymilk but to the protein denaturation resulting from the aggregation of β-conglycinin and glycinin. Therefore, the conditions adopted for the necessary storage of soybean seeds before processing are closely related to the final tofu quality.

Processing conditions

When the characteristics of soybean seeds, such as protein content and composition of protein subunits, and others, are suitable for the desired tofu quality, the processing methods and corresponding conditions involved in tofu processing are another important consideration for the yield, textural properties, and taste of tofu. These conditions mainly include the parameters used in the processing steps of soaking soybean seeds, heating soymilk, and coagulation, and others. Thus, although soybean seeds that are specialized for high-quality tofu processing, the final quality of the tofu is also determined by the use of appropriate processing methods and conditions.

The principle or objective of tofu processing is the thermal denaturation of soybean proteins and the formation of tofu with a compact network structure with the help of coagulant. As a result, as long as a method can promote the gelation of soybean proteins, it can be used to produce tofu. Therefore, tofu processing methods are characteristically diversity.

Processing scale

Many factors are involved in tofu processing and complex interactions can occur to affect the final tofu quality. In contrast, the influence of these factors on tofu processing can be adjusted by processing scales which are determined by the practical production statement. Two processing scales, designated the bench scale (139 g soybean by the manual method) and the production scale (6,500 g soybean by the automated machine method) were comparatively performed to study differences among manufacturing qualities influenced by processing scales (Cai et al., 1997). As a result, the yield, color, texture, and contents of water, protein, lipid, and ash of tofu were significantly related to the selected two processing scales. Moreover, greater varietal effects on tofu yield, moisture content, hardness, and elasticity were observed in bench scale-prepared tofu compared with those made using the production scale. According to the different results, the bench scale could be suitable for the selection of a dedicated soybean variety for tofu processing and the production scale could be used to evaluate potential of soybean varieties for the production of commercial tofu. The same result was obtained in a study reported by Evans et al. (1997). In addition, based on these two processing scales, the influence of a pilot scale (900 g soybean by the manual method) on the manufacturing quality of tofu was also examined (Cai and Chang, 1999). Between the pilot scale and production scale, no significant changes in glycinin contents and 11S/7S ratios of the 13 soybean varieties were found from soymilk to tofu. Therefore, different processing scales influenced the tofu yield, hardness, and sensory quality by changing the glycinin and β-conglycinin contents of tofu.

Soaking of soybean seeds

As an important operating step in tofu processing, soaking directly modifies the structural characteristics and grinding properties of soybean seeds. In addition, sprouting and freezing of soaked soybean seeds can also modify the chemical components and texture of soybean seeds to influence the tofu quality.

Specifically, soaking can accelerate protein extraction from raw soybean seeds and, consequently, lead to higher tofu protein contents (Yang and James, 2016). First, to obtain the same soaking effect, the higher the soaking temperature, the less time is used. This phenomenon might be related to the influence of soaking temperature on the time required for obtaining the maximum water absorption. Second, grinding properties were not related to the soaking parameters but only to the final water content of soaked soybean seeds. Finally, the solid content of soaked soybean seeds was significantly reduced due to the leaking of proteins and carbohydrates as the soaking temperature increased (Pan and Tangratanavalee, 2003). However, this solid loss could decrease the solid content of soymilk, directly varying the final textural properties of tofu (Shih et al., 1997). Thus, soaking not only modifies the processability of soybean seeds to facilitate tofu processing but also decreases the solid content of soybean seeds to alter the yield and protein content of tofu products. Therefore, the selection of appropriate soaking conditions, such as the soybean seeds-to-water ratio, temperature, and time, and others, is very crucial for tofu processing.

The yield, basic composition, and textural properties of tofu made from varying soybean seeds-to-water ratios (1:9, 1:10, 1:11, and 1:12) for 8 h using a mixture of CS and natural nigari containing 29% moisture were comparatively investigated (Cai and Chang, 1997). When the soybean seeds-to-water ratio decreased within the selected range, the solid content of soymilk declined as follows: 9.2, 8.4, 7.8, and 7.1 °Brix. However, tofu gelled for the longest time (among 5, 10, and 15 min) had the highest water content to supply the highest tofu yield at the increased soybean seeds-to-water ratio. However, tofu derived from the soybean-to-water ratio of 1:9 showed the highest textural property values, such as fracturability, hardness, cohesiveness, springiness, and chewiness. The influence of soaking temperature set as 10, 20, 30, 40, 50, 60, and 70°C, on tofu processing was also investigated (Li and Cao, 1998). The solid and protein contents in soymilk were the highest to produce tofu with the best gel strength and WHC when soybean seeds were soaked at 20°C for approximately 14 h. However, although the soaking time was shortened and a satisfying tofu quality was obtained when soybean seeds were soaked at 60°C, the solid loss was higher than that at 20°C. Similarly, tofu coagulated by GDL with the best quality was produced when soybean seeds were soaked at 20°C for 17 h (Zhang et al., 2006). Therefore, the solid content in soymilk could be adjusted by controlling the soaking time and temperature to improve the tofu quality.

Sprouting of soybean seeds not only improves the quality of soybean products but also reduces the adverse effects resulting from the subsequent high-temperature treatment of thermally sensitive nutritional ingredients. Specifically, removal of trypsin inhibitor, flatulence factor, phytic acid, and off-flavor by thermal processing could be replaced by the appropriate sprouting of soybean seeds (Mostafa et al., 1987; Agrahar-Murugkar and Jha, 2009; Agrahar-Murugkar, 2015). Soybean seeds sprouted under conditions of 25°C and 90% RH for 72 h were used to produce CS-coagulated tofu (Agrahar-Murugkar, 2014). According to the results, no significant differences were observed between the yields of tofu from sprouted soybean seeds and unsprouted soybean seeds. However, significant increases in protein content, cohesiveness, springiness, and sensory quality, and decreases in fat content, hardness, and chewiness in these two types of tofu were observed. These phenomena might be ascribed to the slight increase in protein content and the significant decrease in contents of trypsin inhibitor and phytic acid after sprouting.

Another effective treatment of soybean seeds for shortening the boiling time of soymilk and improving the tofu quality is appropriate freezing after soaking (Gu et al., 2016). In general, freezing (such as −20°C for 5 h) could vary the water solubility of soybean proteins (Noh et al., 2006). In essence, the formation of disulfide bonds among the protein molecules was promoted under the low-temperature condition to increase the hydrophobicity of the original proteins. Moreover, the time required to cook soybean seeds that had been frozen after soaking was significantly reduced and the quality of the cooked soybean seeds was also modified. The processing qualities of CS-induced tofu made from soybean seeds that had been frozen at −20°C for 5 min and unfrozen soybean seeds were comparatively studied (Noh et al., 2005). For conventional quality attributes, although yield, water content, and fat content of tofu from frozen soybean seeds were lower compared to those of tofu derived from unfrozen soybean seeds, there was an increase in protein content and syneresis of tofu generated from frozen soybean seeds. In addition, textural properties, including hardness, springiness, gumminess, and chewiness, were remarkably increased for tofu obtained from frozen soybean seeds. These changes were also reflected by the more orderly and denser network structure of tofu derived from frozen soybean seeds than that from unfrozen soybean seeds, as shown in Figure 1. According to these results, the increase in protein content and textural parameters, and the modification of network structure of tofu derived from frozen soybean seeds was attributed to the increased protein concentration and the denser combination of protein molecules in soymilk, which was correlated to the elevated protein hydrophobicity.

Preparation methods and properties of soymilk

Preparation methods

For traditional tofu processing, soybean slurry generated from soaked soybean seeds must be filtrated to obtain soymilk. According to the sequence of filtration and heating treatment of soybean slurry, three types of soymilk preparation methods can be characterized as uncooked slurry, cooked slurry, and hot water-mixed slurry. The uncooked slurry is the method for producing tofu using soymilk filtrated from uncooked slurry, which is commonly seen in soybean product enterprises in China. The cooked slurry method indicates that soybean slurry is first heat-treated for a certain time and then filtrated to obtain soymilk to produce tofu. This method is well known in the soybean processing enterprise in Japan. The hot water-mixed slurry method refers to the preparation of soymilk with a temperature above 65°C by adding hot water to soybean slurry, sufficiently stirring, and filtrating. Yields and edible qualities of the tofus made using these three soymilk preparation methods are variable. It has been reported that yield, WHC, and water content of tofu made using the cooked slurry method were significantly higher than those made using the other two methods (Chen et al., 2011). The hardness and adhesiveness values of tofu made using the uncooked slurry method were higher than those made using the other two methods. In essence, during filtration of cooked slurry, the temperature was high enough to facilitate the leaking of protein from okara into soymilk, which increased the protein content in the obtained soymilk and tofu yield when the cooked slurry method was used (Toda et al., 2007). In addition, polysaccharides were more easily diluted in soymilk to strengthen the WHC of tofu under a relatively high temperature during heating and filtration of soybean slurry. Because of the negative correlation between textural properties and WHC or water content, the hardness of tofu made using the uncooked slurry method was clearly very high. Different soymilk preparation methods can used to produce tofu with different quality attributes.

Heating techniques

Heating of soymilk is indispensable for the prompt formation of tofu during processing. In theory, there is an increase in hydrophobicity and sulfydryl content of proteins, promoting protein aggregation due to the presence of hydrophobic groups in response to the thermal treatment. Tofu is then formed after the generation of a stable protein network structure using an appropriate coagulant. Therefore, selection of parameters for the heating of soymilk is vital for the formation of tofu. Soymilk boiled for varying times (0, 12, 30, and 60 min) was performed to study the influence of heating time on tofu quality (Elias et al., 1986). The results showed that the contents of protein and fat and the sensory evaluation of tofu were not obviously changed as the boiling time increased; however, a significant decrease in values of hardness, cohesiveness, and chewiness

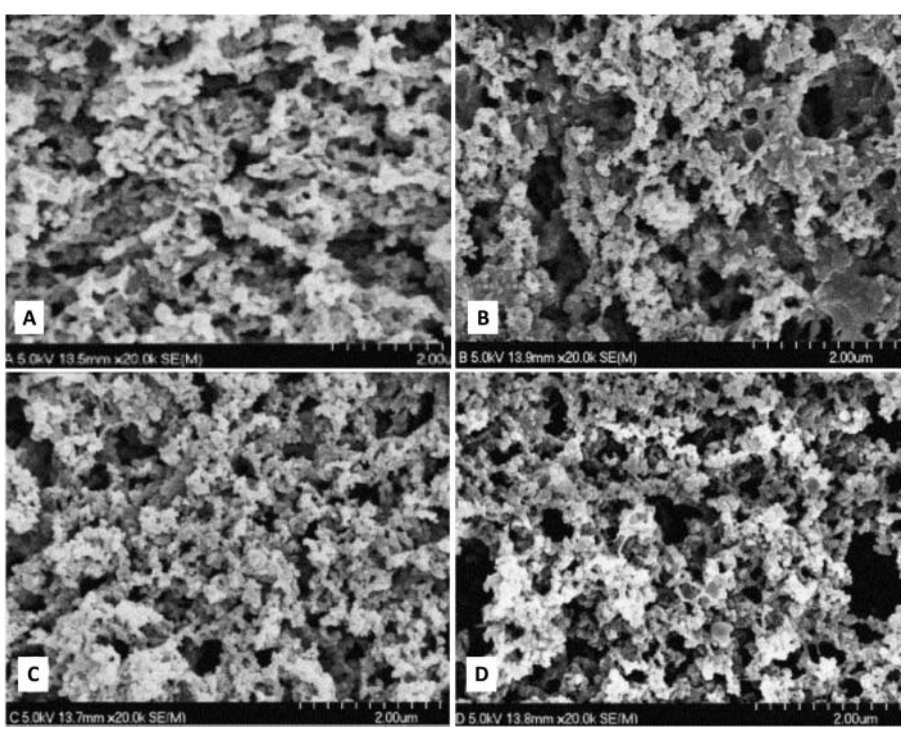

Figure 1. Scanning electron microscopy (SEM) images (20.0×) of tofus prepared using (A) unfrozen soybean seeds and soymilk heated for 2.5 min; (B) unfrozen soybean seeds and soymilk heated for 5 min; (C) frozen soybean seeds and soymilk heated for 2.5 min; (D) frozen soybean seeds and soymilk heated for 5 min. Adapted from Noh and others (2005).

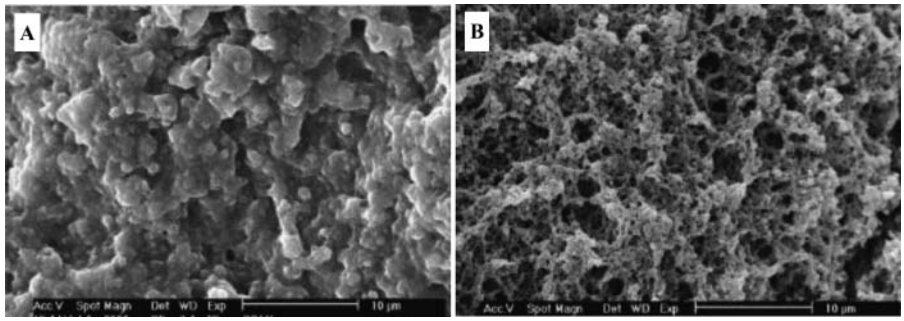

Figure 2. SEM images (×3,200) of tofu prepared from soymilk preheated under various conditions. The tofu was induced by 150 units per 100 mL soymilk by MTGase at 37°C for more than 16 h. (A) heated at 75°C for 30 min; (B) heated at 95°C for 5min. SEM, scanning electron microscopy; MTGase, microbial transglutaminase. Adapted from Tang (2007).

was observed when boiling time was extended beyond 30 min. In addition, as reported by Tang (2007), the temperature and time of thermal treatment of soymilk were more important than the concentration of microbial transglutaminase (MTGase) for the hardness value of tofu. Specifically, at any concentrations (50, 100, 150, and 200 units per 100 mL soymilk), the hardness values of tofu were significantly higher when soymilk was heated at 75°C for 10 or 30 min compared with other tested groups ($p < .05$). Moreover, as shown in Figure 2, a unique coral-like structure was shown in tofu when soymilk was heated at 75°C for 30 min, which was much more continuous and homogenous than that in tofu produced from soymilk heated at 95°C for 5 min.

As described earlier, traditional thermal treatment of soymilk is performed by heating to the targeted temperature followed by temperature maintenance for a certain time. However, this one-step heating of soymilk overlooks the different thermal denaturation temperatures of glycinin and β-conglycinin to obtain tofu with a nonoptimum manufacturing quality. In general, the heat denaturation temperature of glycinin (85–95°C) is approximately 20°C higher than that of glycinin (65–75°C) (German et al., 1997). Thus, two-step heating of soymilk is more suitable for efficient denaturation and gelation of soybean proteins (Shin et al., 2015). Soymilk was first heated at 75°C for 5 min and then heated at 95°C for 5 min using an ohmic technique to obtain soymilk with a higher viscosity and GDL-induced tofu with a higher apparent Young's modulus and lower syneresis than those obtained from soymilk treated by one-step heating (95°C, 5 min) (Liu et al., 2004). According to the SEM images of the obtained tofus, tofu prepared using two-step ohmic heating showed a denser, finer, and more homogeneous network structure, as shown in Figure 3. Based on the high viscosity of soymilk obtained by two-step heating operation, the β-conglycinin subunits dissociated after the first step of heating and would unfold and self-aggregate to form network structure before the second heating. When the glycinin subunits dissociated, further association occurred between these glycinin subunits and the former formed β-conglycinin aggregate to generate a larger, finer, and more orderly macrocomplex. Conversely, unordered aggregation and incomplete networking could occur between the dissociated subunits of β-conglycinin and glycinin when one-step heating of soymilk was used. Optimum two-step heating conditions using ohmic heating operation, namely, the combination of 70°C for 10 min and 100°C for 5 min at a temperature rate of 40°C/min, were studied (Wang et al., 2007). Under these conditions, in comparison to one-step heating, apparent breaking strength, apparent Young's modulus, and yield of packed GDL-induced tofu increased by 12.2%, 16.2%, and 4.5%, respectively. Moreover, syneresis rate was reduced by 21.8%. Therefore, two-step heating of soymilk is more suitable for tofu processing from the perspective of increasing the product quality.

Traditional heating of soymilk is completed at atmospheric pressure, which normally leads to the nonuniform heating of soymilk to reduce uniform quality of tofu. Recently, therefore, soymilk heated at relatively high pressure and temperature (0.17 MPa at 115°C) was performed to study the influence of high-pressure heating on the yield and textural properties of tofu (Zuo et al., 2016). The results showed that, in comparison to tofu made using traditional heating of soymilk under the

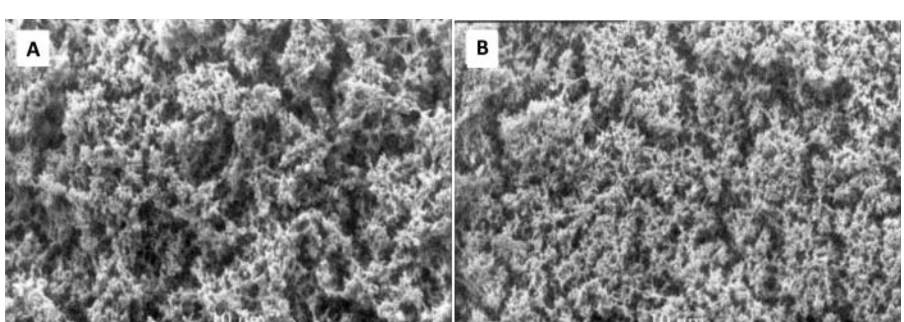

Figure 3. Effect of selective thermal denaturation on the microstructure of filled tofu: (A) one-step heating and (B) two-step heating. Adapted from Liu and others (2004).

conditions of atmospheric pressure at 97°C, tofu produced by heating at a relatively high pressure and temperature showed higher yields and better textural properties. This finding might be attributed to the increased protein particle contents in soymilk exposed to high pressure and temperature and thus forming a dense network of tofu, which could retain more water and solid contents and thus improve the textural properties. Thus, there are a variety of ways to obtain heat-denatured soybean proteins for tofu processing, and each has its own advantages, providing various selection protocols for the production of tofu with different eating qualities.

Soymilk properties

Apart from the effective protein denaturation in heating of soymilk, other inherent properties of soymilk, such as protein content and composition, pH, and particle size distribution, are also important for tofu quality during tofu processing. For example, tofu yield was positively related to the pH value and total solid content of soymilk (Lim et al., 1990). In addition, under the same processing conditions, soymilk obtained by dissolving SPI in distilled water at a high concentration (5%, 6%, 7%, 8%, and 9%) resulted in a GDL-induced protein gel with increased break stress and hardness (Cheng et al., 2005). Guo and Ono (2005) reported that the content and composition of protein particles in soymilk significantly influenced the formation of tofu. Soymilk samples were prepared by blending the glycinin-rich and β-conglycinin-rich soybean seeds together at ratios of 0:4, 1:3, 2:2, 3:1, and 4:0. As a result, the highest content of protein particles was observed in soymilk prepared using glycinin-rich soybean seeds, which formed tofu with the largest breaking stress and the hardest texture. When the protein particle content increased, the possibility and frequency of protein particle aggregation increased, resulting in the formation of a firmer gel network structure.

In general, the particle size of soymilk, which mainly depends on the composition of storage proteins, closely affects the textural properties of tofu (Nik et al., 2009). The particle size of soymilk can be modified by certain processing operations, such as heating and grinding. Heating of soymilk is an essential step during tofu processing. However, grinding of soymilk is not common. Improved textural properties of tofus were observed when soymilk was separately treated using a colloid mill, an ultrasound, and a homogenizer compared with the untreated soymilk (Yu and Wang, 2014). The optimum conditions of these three methods for obtaining GDL-induced tofu with enhanced textural quality were a plate gap of 10 μm for 2 min, an ultrasound power of 2 W/g for 3 min, and a speed of 1,000 r/min for 8 min, respectively. The average particle sizes were reduced 4.2, 1.6, and 1.4 μm from the original value of 8.6 μm, respectively, consequently modifying the hardness, elasticity, and cohesion of tofu.

According to these research results, various preparation methods of soymilk are capable of providing the same objective of soybean protein denaturation and generating soymilk with different physicochemical properties. For practical tofu production, soymilk properties can be used as an index to predict the tofu quality and the efficiency of tofu processing. In addition, soymilk properties, such as solid content and particle size, and others, can be adjusted to produce tofu that satisfies different consumption demands.

Addition of coagulant

As introduced earlier, aggregation occurs among soybean storage proteins due to the appearance of hydrophobic groups when soymilk is thermally treated. This aggregation can be significantly promoted and enhanced by low pH and metal ionic strength to form tofu (Kohyama et al., 1995; Renkema et al., 2002; Peng et al., 2016). Therefore, the selection of an appropriate coagulant for supplying the effect of salt ions and pH is a very important issue during tofu processing.

To date, as shown in Table 2, four types of coagulant, namely, salt, acid, enzymatic coagulants, and other coagulants, are used in tofu processing according to their different chemical properties. As a traditional coagulant, salt, such as CS, MC, and calciumchloride (CC), and others, is commonly used. Acid includes several organic acids, such as gluconic acid, citric acid, and lactic acid. Among these acids, gluconic acid is the most commonly used coagulant and is used in the form of GDL. Enzymatic coagulants, such as MTGase, trypsin, and bromelain, were recently studied and developed as coagulant for previously denatured soybean proteins. The remaining investigated coagulants or coagulant aids mainly include some polysaccharides, chitosan, inulin, and carrageenan. In addition, two or more coagulants, namely, mixed coagulant, are usually simultaneously used to modify the gelation of soybean proteins to increase tofu yield and quality. These substances promote formation of protein network structure and can be used as the coagulants for tofu processing. Therefore, various coagulants can be selected to prepare different types of tofu with different eating qualities.

Salt

Salts, such as gypsum (mainly CS) and nigari (consisted of MC hexahydrate, magnesium sulfate [MS], CC and others), have long been applied to tofu processing. The taste and aroma of tofu products made using this type of coagulant are most familiar and accepted by consumers. Although the application of salts dates back to the invention of tofu, the precise mechanism of salts on the formation of tofu has not been uniformly defined. Thus far, four theories have prevailed in academic circles of tofu research (Liu et al., 2000; Qi et al., 2006). The Cation Bridge theory considers the cross-linking between heat-induced soybean proteins and metal ions to accelerate the speed of protein gelation and increase the stability of the formed three-dimensional network structure (Kao et al., 2003), ultimately increasing the strength and hardness of tofu. The salting-out effect is based on the salting-out phenomenon resulted from the dehydration of the thermally denatured soybean proteins following the addition of salts. The third theory concerns the reduction of pH to the isoelectric point of soybean proteins after the addition of salts as the main reason for the aggregation of heat-denatured proteins by electrostatic force to promote gelation. Based on the Cation Bridge theory, the formation of protein particles in the presence of specific metal ions is considered the fourth theory. Briefly, calcium ions first react with phytic acid to form nonionizing products that can weaken the

Table 2. Basic properties and application of typical coagulants used to produce tofu.

Type	Typical coagulant	Type of tofu	Possible mechanism	Use form	Use concentration	Reference
Salt	CC	Soft or pressed tofu	Cation Bridge theory; more rapid coagulation than sulfate salts	Freshly prepared by dissolving in cold water; added to heated soymilk	0.4% or 0.5% based on the amount of soymilk	Prabhakaran et al., 2006
	CS	Soft or pressed tofu "Southern tofu"	Cation Bridge theory	Preparation of saturated solution that was used in time; added to heated soymilk	0.4% based on the amount of soymilk	Kao et al., 2003; Prabhakaran et al., 2006
	MC	Firm or pressed tofu "Northern tofu"	Cation Bridge theory; more rapid coagulation than sulfate salts	Freshly prepared by dissolving in cold water; added to heated soymilk	0.4% based on the amount of soymilk	Prabhakaran et al., 2006; Toda et al., 2003
	MS	Rarely used alone as a coagulant but used as modified nigari; firm or pressed tofu	Cation Bridge theory	Freshly prepared by dissolving in cold water; added to heated soymilk	0.3% soymilk weight	Hou et al., 1997; Prabhakaran et al., 2006
Acid	GDL	Soft or packed tofu	Reduction of pH to the isoelectric point of soybean protein, and isoelectric precipitation via hydrophobic and electrostatic interaction	Freshly prepared by dissolving in cold water; added to cooled heated soymilk	0.6% based on the amount of soymilk	Grygorczyk and Corredig, 2013; Murekatete et al., 2014
Enzymatic coagulants	MTGase	Soft or packed tofu	Formation of cross-links or ε-(γ-glutamyl)lysine isopeptide bonds in protein polymers with high molecular weights	Before soymilk boiling: directly added to soymilk at 50°C; after soymilk boiling: added when soymilk had cooled to 50°C	150 units per 100 mL soymilk	Tang et al., 2007; Yasir et al., 2007
Others	Chitosan	Soft or pressed tofu	Cross-linking among the heat-denatured soybean protein due to polycationic properties; extended the shelf life due to antimicrobial properties	Freshly prepared by dissolving in acetic acid; added to heated soymilk at 80°C; coagulation at 80°C for 15 min	Chitosan solution (1% of chitosan dissolved in 1% acetic acid) to soymilk ratio: 1:8	No and Meyers, 2004
	Guar gum	Firm or pressed tofu	Stabilization, thickening, and emulsibility characteristics; used as an additive to modify coagulation in the presence of magnesium chloride	Added together with magnesium chloride; added to heated soymilk at 80°C; coagulation at 80°C for 20 min	0.6 g guar gum combined with 2.93 g magnesium chloride for 100 g soybean	Li et al., 2015

Note: CC, calcium chloride; CS, calcium sulfate; MC, magnesium chloride; MS, magnesium sulfate; GDL, glucono-δ-lactone; MTGase, microbial transglutaminase.

electrostatic screening effects of ions on protein molecules and lead to interactions between calcium ions and nonparticle soybean proteins to form the new protein particles. The formed protein particles then associate with one another to form the final gel network structure (Wang et al., 2015). However, each theory has its own limitation for practical gelation of soybean proteins. Therefore, the formation mechanism of tofu in the presence of metal ions requires further investigation.

Due to the various types of salts, different tofu yields and eating qualities can be obtained. Six salts, CC, CS, MC, MS, calcium acetate (CA), and calcium lactate (CL), were compared to evaluate the differences in yield, color, and textural properties of tofu (Prabhakaran et al., 2006). There were no significant differences among the color values of tofus generated by all the coagulants. The lowest hardness and chewiness and the highest water content of tofu were observed when soymilk was coagulated with MS. This finding might be attributed to the formation of an incompact protein network structure with large air voids due to the occurrence of incomplete soybean proteins precipitation following the addition of MS to the hot soymilk. The CA-induced tofu showed the greatest hardness, gumminess, and chewiness among all the tofus, likely because of the formation of compact hydrogen bond and calcium ion-protein bridges for obtaining tofu with increased hardness when CA was used as a coagulant (Zhang et al., 2013). However, among these salts, CS was the most suitable coagulant in terms of high yield and the production of a firm, but not hard, tofu texture during the processing of Harovinton soybean seeds.

Apart from the type of salt, the concentration, stirring speed, duration, and temperature were the main parameters influencing the gelation effectiveness. Furthermore, interactions among these parameters also influence the yield, texture, and taste of tofu. An analysis of the microstructure of tofu using a series of concentration of dihydrate CS, 0.20%, 0.25%, 0.30%, 0.35%, 0.40%, 0.45%, and 0.50% (w/v), demonstrated that tofu made using 0.40% dihydrate CS had the most uniform and continuous network structure (Kao et al., 2003). As the concentration was increased from 0.20% to 0.40%, textural properties steadily increased, such as hardness, chewiness, cohesiveness, springiness, and gumminess. Six soybean varieties with different protein contents were compared for the preparation of tofu using a series of concentration of MC, 0.20%, 0.25%, 0.30%, 0.35%, 0.40%, 0.45%, and 0.50% (w/v) (Toda et al., 2003). The breaking stress of tofu increased when the concentration of MC increased and peaked at 0.40%. At this concentration, the breaking stress of tofu was significantly positively related to protein contents of the selected soybean beans ($p < .001$, $r = 0.87$). However, tofu made from soybean seeds with higher protein content using MC in a recommended concentration of 0.25% displayed reduced breaking stress. Thus, similar to protein content, to obtain the maximum breaking stress, the coagulant concentration is also an important index for quality evaluation of soybean seeds in the field of tofu processing.

The yields and textural quality of tofu prepared using CS as the coagulant at a series of temperatures of 80, 85, 90, and 95°C were assessed (Mathare et al., 2009). The yield was positively related to the coagulation temperature. The hardness, cohesiveness, chewiness, and adhesiveness all increased to a maximum at 90°C; however, the springiness increased to a maximum at 85°C, remained unchanged from 85 to 90°C and finally decreased when the temperature increased to 95°C. A high coagulation temperature might prompt further denaturation of soybean proteins and the formation of tofu with a uniform network structure. Significant differences was observed among the yield and quality of tofu made using CS and modified nigari as the coagulants at different stirring speeds (137, 207, and 285 rpm) and for different coagulation times (5, 10, 15, 20, 25, and 30 s) (Hou et al., 1997). Under these conditions, a higher stirring speed resulted in a lower yield and an increased hardness, brittleness, and elasticity observed. Additionally, the tofu yield was reduced with as increased stirring time.

As indicated previously, the fundamental effect of salt-induced gelation is mainly related to the type and concentration of metal ions. Therefore, the controlling of metal ion concentration can adjust the tofu quality to meet the various consumption preferences in the practical tofu industry. To date, dissolving the metal ions in water/oil or oil/water emulsions has been the most successfully studied method. One of the outstanding advantages of this method is the decreased release rate of metal ions (such as magnesium ions) from water/oil emulsions to decelerate the gelation rate and thereby increase or modify the yield and textural properties of tofu (Li et al., 2014; Zhu et al., 2015). According to results reported by Zhu et al. (2016), as the MC concentration in water/oil emulsions increased (0.4, 1.2, 2.0, and 2.6 mol/L), the yield and WHC of tofu decreased; however, the protein content, crude fat content, and hardness value of tofu increased. This finding was related to the rapid reaction between magnesium ions and soybean proteins at the higher MC concentration in water/oil emulsions. The best WHC and microstructural properties were observed in tofu by using 2 mol/L MC in a water/oil emulsion. Therefore, the effective concentration of MC for gelation can be regulated by containing in water/oil emulsifier to process tofu with different eating qualities.

It is worth noting that these optimum types of coagulant and operation parameters were obtained using specific soybean seeds and processing conditions. Therefore, the practical utilization of coagulant types and their operation levels are closely influenced by material property, other operation steps and conditions, and others. By analyzing the contents of β-conglycinin and glycinin and their subunit profiles in tofu using acetic acid, lactic acid, GDL, and CS dihydrate, the CS dehydrate-induced tofu contained the highest content of glycinin (Syah et al., 2015). Moreover, the glycinin subunits mainly consisted of acidic peptides (A_1, A_2, A_3, and A_4) which were relatively rich in cysteine. These results also indicated that preparation of tofu with a hard and viscous texture using salt was closely related to the retention of higher glycinin content in tofu. As previously introduced, the solid content of soymilk is positively related to the yield and textural properties of tofu. Thus the suitable coagulant type and its optimum operation concentration can be modified by adding water to soymilk (Rekha and Vijayalakshmi, 2013). To obtain similar yields, protein contents, and textural properties of tofu, both the concentration of CS and mixing time were reduced by stirring with a large three-blade (4 W × 7 L cm) propeller compared with stirring with a small three-blade propeller (3 W × 5 L cm) (Cai and Chang, 1998). Furthermore, coagulant concentration had a reduced effect on the tofu quality when the small propeller was used.

Therefore, the yield and quality of tofu made using different soybean varieties could be modified by using the suitable coagulant and the corresponding operation concentrations for practical tofu processing.

Acid

Application of acid in tofu processing is mainly based on the provision of hydrions to reduce pH to the isoelectric point of soybean proteins and then to prompt isoelectric precipitation of the heat-denatured soybean proteins. Among the studied acids, GDL has demonstrated the best application effect on the manufacturing of packed tofu. GDL can slowly decompose to gluconic acid and release protons into water at certain temperatures, satisfying the characteristic gradual process for the formation of a continuous soybean protein network structure via hydrophobic and electrostatic interactions (Liu and Kuo, 2011). Therefore, a higher yield, stronger elasticity, higher WHC, and smoother, and softer texture of tofu made using GDL are usually observed in comparison to the use of traditional salts (Shen et al., 1991). In addition, based on the formation of uniform protein network structure, water can be homogeneously trapped inside this structure to omit the pressing step in traditional tofu processing.

As reported by Nik et al. (2011), the pH was approximately 5.8 at the beginning of gelation coagulated by GDL, which was mainly influenced by soybean variety with different storage protein subunits, but not GDL concentration. However, an acid environment caused by GDL was requisite for the formation of an acid-induced tofu. In addition, different acids had different pH values for the gelation of heat-denatured soybean proteins. Specifically, a higher gelation pH value of 6.29 ± 0.05 was observed following lactic acid bacterial acidification, compared with 5.90 ± 0.04 for gelation induced by GDL (Grygorczyk and Corredig, 2013). Thus, the lactic acid-induced gel formed earlier than the GDL-induced gel. This phenomenon might be attributed to the longer time needed for lactic acid to rearrange the solid particles in soymilk in comparison to the fast acidification by GDL.

In comparison of the coagulation efficiency of CS, GDL-induced tofu displayed a greater hardness and firmness, and 20% more yield (Lim et al., 1990; Shen et al., 1991). However, a higher syneresis rate was observed for the GDL-induced gel compared with the salt-induced tofu (Murekatete et al., 2014). Similar to the salts, the concentration and operation parameters of GDL are also important for tofu processing. As reported by Chang et al. (2009), gelation rate of black soybean touhua, which has a structure similar to that of tofu but with a softer texture, increased with an increasing GDL concentration and operation temperature. Correspondingly, gelation time was reduced when these two parameters were increased.

Recently, lactic acid bacteria-fermented acidic whey generated during traditional tofu squeezing procedures has been successfully used to produce acid-induced tofu. Moreover, the development of this type of tofu also takes advantage of the by-products of salt-induced tofu processing. In general, acid whey tofu has the characteristics of good WHC, a fine and smooth texture, a light but sweet taste, and a low syneresis rate. The critical step for producing acid whey tofu is the preparation of acid slurry which can be obtained via both natural fermentation and controlled lactic acid bacteria fermentation. Using single factor experiment, the optimum temperature and time of natural fermentation for the preparation of acid whey with a pH of 3.3–3.5 were 42°C and 30–35 h, respectively (Zhang et al., 2014). Tofu produced using this acid whey exhibited better sensory quality and textural properties in comparison to the sold MC-induced tofu in market. However, natural fermentation is an experiential operation and is of uncontrollability. Therefore, fresh soymilk was fermented by addition of *Lactobacillus plantarum* to replace the naturally fermented acid whey to produce acid-induced tofu (Guan et al., 2009). The results showed that the directly fermented tofu had similar WHC, textural properties, and aroma compared with traditional acid whey-induced tofu; however, it has shorter processing time. In addition, lactic acid bacteria screened from fermented acid whey were used to ferment soymilk to prepare acid-induced tofu (Qiao et al., 2014). Consequently, the color, texture, aroma, taste, and total acceptability of sensory evaluation of soymilk-fermented tofu were not significantly different from those of traditional MC-induced tofu. Based on these results, there are diverse methodologies to decrease the pH of the protein gelation system.

Enzymatic coagulants

Application of enzymatic coagulants can increase the controllability of tofu processing and produce tofu with a better aroma than that of acid-induced tofu. Furthermore, enzymatic coagulants can continuously degrade the soybean proteins in tofu to reduce hardness texture and modify taste. Enzymatic coagulants are extensively distributed in plants, animals, and microorganisms. Recently, transglutaminase has been the most commonly applied enzymatic coagulant with the best coagulation effect.

Transglutaminase, also known as protein-glutamine: amine γ-glutamyltransferase (EC 2.3.2.13), can catalyze intra- or intermolecular cross-linking reactions within or among proteins. In essence, an acyl-transfer reaction between γ-carbonxyamide groups of protein-bound glutaminyl residues (acyl donor) and primary amines of amine compounds (acyl acceptor) is catalyzed by this enzyme to form the cross-links or ε-(γ-glutamyl)lysine isopeptide bonds in protein polymers with a high MW (Kang et al., 1994; Motoki and Seguro, 1998). Finally, the elasticity, WHC, and stability of proteins are altered, and the textural properties of protein-containing food materials are modified. By analyzing the gelation quality and formation mechanism of MTGase-induced protein gel prepared from glycinin-rich and β-conglycinin-rich SPI, a discrepancy was observed between the mechanisms for the MTGase-induced gelation of different storage proteins (Tang et al., 2006). With the aid of MTGase catalysis, covalent cross-links, hydrophobic and hydrogen bonding, and disulfide bonds were the main chemical forces involved in the formation and maintenance of the glycinin-rich SPI gel. However, the principal forces responsible for the maintenance of the β-conglycinin-rich SPI gel structure were hydrophobic and hydrogen bonding.

Packed tofu induced in a sealed cylinder at a lower incubation temperature of 25 or 37°C was prepared using cooled heat-treated soymilk (20°C) which has been previously heated at 95°C for 5 or 15 min from 20°C at a rate of 6.3°C/min (Tang et al., 2007). Similarly, the hardness, gumminess, springiness, and cohesiveness of this packed tofu were also affected by the amount of MTGase, operation temperature, pH of soymilk, and addition of SC. As reported by Yasir et al. (2007), the MTGase-induced tofu had an increased firmness and fracture force compared with traditional CS-induced tofu. From a microstructural perspective, finer-stranded and uniform networks were observed in the MTGase-induced tofu, and little, if any, cross-linking occurred in the MTGase-induced tofu matrix at the protein molecule level. Instead, MTGase side reaction, such as the hydrolysis of glutamine residues, were responsible for the functional properties of this type of tofu. In addition, different timing of MTGase addition can be used to prepare packed tofu with different manufacturing qualities to satisfy the varying consumption demands.

Apart from the direct application of MTGase for tofu gelation, MTGase can also be used as an additive to facilitate protein gelation. Among the four factors, concentration of CS, coagulation temperature, coagulation time, and additive amount of MTGase, the additive amount of MTGase had the greatest impact on the tofu yield (Jiang et al., 2007). Moreover, an appropriate MTGase amount (3 U/100 g soymilk) prepared the tofu with a certain mechanical strength and moderate cross-linking of soybean proteins, resulting in the best WHC and yield. As the additive amount of MTGase increased, the values of storage modulus (*G*'), loss modulus (*G*''), complex viscosity (η^*), hardness, and gumminess of packed tofu coagulated with agar increased (Chang et al., 2011). Thus, the viscoelasticity and some textural properties were positively related to the MTGase concentration.

The short shelf life (2–4 days) has hindered the industrialization of traditional tofu. Fortunately, this problem has been acceptably solved during the manufacturing of packed tofu. To obtain tofu with a longer shelf life, sterilization after the packaging step is an essential operation. Nevertheless, most sterilization methods are completed by heating at a high temperature which has an adverse effect on the texture of tofu, producing so-called retorted tofu with a lower water content and tough texture. The addition of MTGase during the processing of GDL-induced packed tofu successfully suppressed the retort-induced water-release, induced the occurrence of tiny holes, and hardened the tofu packed with water in a retort-pouch (Nonaka et al., 1996). These satisfactory results mainly depended on the occurrence of polymerized soybean proteins in the enzyme-treated tofu. Furthermore, the addition of MTGase was more suitable for suppressing retort-induced water release to produce retort-resistant tofu coagulated with GDL compared with addition of glucose at ≤5 mg/mL (Kwan and Easa, 2003). Therefore, the application of MTGase during the manufacturing of packed tofu was very important for implementation in the tofu industrialization.

Other coagulants or additives

To date, the other most well-studied coagulants or additives during the coagulation of tofu are types of polysaccharides (Kim et al., 2007; Buescher and Margaritis, 2008; Shin et al., 2010; Hsiao et al., 2016). In general, polysaccharides can interact with protein polymers present in foods via chemical bonding to increase the consistency, stability, and gelation of foods (van de Velde et al., 2015). The addition of appropriate amounts of carbohydrates, including carrageenan, guar gum, chitosan, and inulin, during tofu gelation can modify the gelation process and the gelling effect.

As a type of polysaccharide with cations derived from the deacetylation of chitin, chitosan has been broadly used for food preservation and processing due to its characteristics of thickening and antibacterial effect (Rinaudo, 2006). Chitosan dissolved in acetic acid can be directly used as a coagulant to produce tofu. For example, soymilk coagulated with 0.08% chitosan dissolved in 1.2% acetic acid at 80°C provides tofu with an optimal yield and better textural properties than that of the sold CS-induced tofu (Li et al., 2014). Chitosan with varying MWs dissolved in acetic acid were used to produce tofu (No and Meyers, 2004). First, chitosan with a MW of 28 kDa was dissolved in 1% acetic acid aqueous solution to obtain 1% chitosan. Second, this chitosan-acetic acid solution was mixed with heat-treated soymilk at a ratio of 1:8. Third, tofu was formed at 80°C for 15 min. Under these conditions, the prepared tofu showed the best yield, textural properties, and shelf life. When the 1% acetic acid aqueous solution was replaced with 1% acetic acid and 1% lactic acid aqueous solution, the prepared tofu had a better sensory evaluation, for example, the sourness was reduced. Furthermore, the chitosan-induced tofu had a lower ash content, higher protein content and a significantly increased shelf life compared with the sold CC-induced tofu.

Chitosan was also used as an additive to modify the gelation of soybean proteins and tofu quality. It was added in a series of amounts to produce tofus induced by GDL, CS, and acetic acid (Chang et al., 2003). The results showed that the tofu quality was influenced not only by the type of coagulants but also by the amount and deacetylation degree of chitosan. Specifically, the addition of 2% chitosan with varying degrees of deacetylation resulted in an increased gel strength and shelf life of tofus induced by these three coagulants by 5%–305% and 2–10 days, respectively.

As a group of linear sulfated polysaccharides of D-galactose and 3,6 anhydro-D-galactose extracted from red edible seaweeds, carrageenan has also been used during food processing because of its thickening, stabilizing, and gelling properties (Trius and Sebranek, 1996). The addition of carrageenan had no significant impact on the yield and hardness of GDL-induced tofu, but it increased the WHC (Karim et al., 1999). However, the yields of CS- and CA-induced tofus were increased by 33% and 46.7%, respectively, accompanied by a reduction of hardness of these two types of tofu. These two phenomena were related to the increase in water content of these two tofus. From a microstructural perspective, the formation of network structure with more stable and tiny holes among carrageenan, protein, and calcium cations was observed when carrageenan was present. Varying types of carbohydrates, namely glucose, maltodextrin, soluble starch, and carrageenan, were added to the preparation of GDL-induced tofu at concentrations of 0%, 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% (Yu and Wang, 2012). Unexpectedly, the hardness, chewiness, elasticity, and viscosity of GDL-induced tofu, especially the hardness, were all reduced as the concentration of carbohydrates increased. Moreover, among the studied carbohydrates, carrageenan had the strongest effect on the texture of GDL-induced tofu based on principal component analysis. Therefore, the addition of carrageenan or other carbohydrates during tofu processing is very effective for the modification of tofu quality.

Guar gum, also named guaran, mainly consists of galactomannan and possesses the functions of stabilization, thickening, and emulsibility (Mudgil et al., 2014). Therefore, the addition of guar gum has been reported to increase the yield and quality of MC-induced tofu (Li et al., 2014). In general, MC induces a rapid rate of soybean protein gelation to produce the tofu with a lower WHC and relatively rough texture. By contrast, the addition of guar gum (0.6 g/100 g soybean) reduced the rate of MC-induced gelation to produce tofu with reduced hardness, similar textural properties and better aroma compared with CS-induced tofu (Li et al., 2015).

As another type of energy storage form in natural plants, inulin polymerized between β-D-fructofuranose and a handful of pyran inulin residues also has the ability to modify texture and flavor in food industry (Niness, 2010). The addition of both inulin and oligofructose significantly increased the cross-linking reaction among heat-treated SPI to create a tofu-type gel with a denser protein cross-linking structure and reduced pore sizes (Tseng et al., 2008). Moreover, inulin has been shown to be more capable of improving SPI gelation than oligofructose, which might be attributed to the improved thermal stability and rheological properties of SPI gel induced by inulin with a high polymerization degree of fructose. In addition, the changes in quality resulting from the addition of inulin during the preparation of GDL-induced silken tofu were evaluated by the same research group (Tseng and Xiong, 2009). According to the experimental result, 2% inulin reduced the onset gelation temperature from 46.7°C to 43.9°C and significantly increased the storage modulus, loss modulus, hardness, and rupture force of the formed silken tofu.

Based on these research findings, as long as a food grade substance can promote the formation, modify the manufacturing quality, or improve the shelf life of tofu, it can be used as a coagulant or additive to produce tofu in an appropriate amount. Therefore, the selection of coagulant or additive resulted in considerable yields, good sensory evaluations, and extension of shelf life is very crucial for tofu processing.

Mixed coagulants

In general, CS-induced tofu has a high yield, soft texture, and a bitter, and astringent taste. Merits of a natural beany flavor and excellent reprocessability and shortcomings of a low yield and hard texture are usually observed in MC-induced tofu. GDL-induced tofu has the merits of a high yield and tender texture and the shortcomings of a plain taste and lack of reprocessability. Enzyme-induced tofu exhibits the features of delicate taste, moderate operation conditions, and low gel strength. Therefore, the utilization of a mixed coagulant could overcome the aforementioned shortcomings when a single coagulant was used. By analyzing the yield, textural properties, and sensory characteristics of tofus induced by CS and MC in varying proportions, tofu with the best quality was prepared using a CS to MC ratio of 4:6 (Shi et al., 2007). As reported by Song et al. (2011), tofus induced by 2.5% CS together with 0.4% citric acid and 1.5% CL combined with 2.0% GDL showed excellent yields, textural properties, and sensory characteristics. However, it is noteworthy that different manufacturing qualities can be obtained using different mixed coagulants, satisfying the various consumption demands in terms of aroma and taste.

Whole soybean tofu

Along with the increased health consciousness of consumers, requirement for a comprehensive utilization of food materials in food industry, and development of modern food processing techniques, research examining the processing of WST is currently a major focus. From the perspective of nutrition, WST retains the most nutritional ingredients in soybean seeds, especially dietary fiber and isoflavones. Consequently, the production of WST can effectively increase the nutritive value, enhance the utilization of soybean seeds, reduce the generation of okara waste, and decrease the material cost. From the quality perspective, however, the gel formation and palatability of WST are difficult or poor due to the high content of soybean fiber. This phenomenon was validated by a comparison of the results obtained for WST and traditional tofu (Lee et al., 2009). Consequently, the processing conditions and the corresponding equipment required to produce WST are more rigorous than those needed to produce traditional tofu. Thus, problems, such as loose texture and rough taste of WST, caused by the high content of fiber are an important topic that must be solved during the practical manufacturing.

High content of fiber reduces the cross-linking degree among heat-treated proteins and leads to reduced gel strength. Therefore, the conditions used for traditional tofu processing are not suitable for producing WST. High-pressure homogenization treatment was performed separately before or after the soymilk heating operation to produce WST with high gel strength, excellent WHC, fine texture, and proper elasticity (Sun et al., 2003). However, the specific parameters of high-pressure homogenization were not investigated. Whole soybean flour suspension treated by ultra-high pressure homogenization (UHPH) for two or three cycles was used to produce GDL-induced tofu with a texture that was similar to the traditional GDL-induced tofu (Liu et al., 2013). Tofu with the best textural properties was made using a 20% flour suspension homogenized at 150 MPa for three cycles. The UHPH not only denatured soybean proteins to a certain degree but also reduced the particle size of soymilk. According to the SEM images, WST obtained using UHPH showed a regular, continuous honeycomb-like structure, as shown in Figure 4. Moreover, the homogenization treated WST had excellent WHC after storage for 24 h at 4°C. Thus, homogenization of soymilk for reducing the particle size could be an effective choice for producing WST with satisfactory quality.

Because the gel strength was weakened by soybean fiber during the gelation of WST, some substances were selected to enhance the gel strength during the practical production (Wang et al., 2015). Based on the effective cross-links formed during tofu gelation by MTGase and the increased hydrophilic properties of soybean proteins by SC, certain amounts of MTGase and SC were used together to produce WST (Liu and

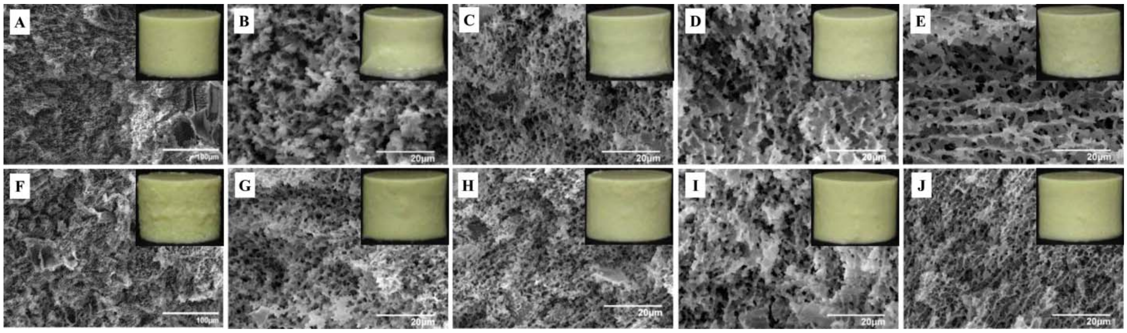

Figure 4. Appearances of GDL-induced tofu samples produced using various concentrations (15% and 20%) of soybean flour suspension with UHPH, and the corresponding scanning electron micrographs. (A) 15%, thermal treatment; ([B] and [C]) 15%, 100MPa UHPH for two and three cycles; ([D] and [E]) 15%, 150 MPa UHPH for two and three cycles; (F) 20%, thermal treatment; ([G] and [H]) 20%, 100 MPa UHPH for two and three cycles; ([I] and [J]) 20%, 150 MPa UHPH for two and three cycles. GDL, glucono-δ-lactone; UHPH, ultra-high pressure homogenization. Adapted from Liu and others (2013).

Qian, 2013). Using a single factor and an orthogonal experiment, WST with a compact texture, moderate elasticity, and rich beany flavor was coagulated with 1.2 U/mL soymilk MTGase and 0.125% SC at 50°C for 2 h. Furthermore, due to the more complex constituents contained in whole soybean soymilk, the heating treatment conditions were performed at 90°C for 10 min. MTGase was also used to prepare WST using micronized full-fat soybean powder (25.4 μm average particle size) as the material (Joo et al., 2011). The micronized full-fat soybean powder suspension (15%) heated at 95°C for 5 min and then coagulated with 10% MTGase for 1 h could produce WST with optimal textural properties and apparent structure. To obtain WST with more satisfactory textural properties, 0.5% gelatin was added to the suspension and coagulant mixture. According to these studies, the utilization of MTGase combined with an appropriate amount of SC provides a new route for the production of WST with good processing quality.

Based on the research progress in WST processing, the production of WST can be come realized. The factors determining the final-product quality include quantity of soybean seeds, micronization degree of particle size of soymilk, and type of coagulant and its operation conditions, and others. However, to achieve the commercial process of WST, more research investigating modifications of taste, quality stability, suitable fabrication machinery and processing maneuverability, and others, should be performed in future analyses.

Changes in isoflavones during tofu processing

It is well known that tofu processing is a complex physicochemical process. On the one hand, many beneficial components are lost, accompanied by the filtration of okara during the manufacturing of traditional tofu. As a result, research has been conducted to produce WST. On the other hand, the constituents of soymilk are either extracted or contained in tofu at the time of aggregation among the heat-treated soybean proteins or dissolved in whey during coagulation process. From the perspective of constituent polarity, the possibility of water-soluble components in whey is higher than those extracted in tofu. However, hydrogen bond might form between water-soluble components and proteins; thus, some of the beneficial components are retained in tofu. Therefore, the processing steps and operation conditions used during tofu processing might influence the distribution of nutritional components to impact the nutritive value of tofu. Changes in the composition of isoflavones during tofu processing are introduced in the following section.

As a type of phytoestrogen with a molecular structure similar to that of estrogen, isoflavones are extensively distributed and studied in beans, grains, and fruits. To date, twelve isoflavones have been isolated and identified in soybean seeds, including three aglycones (free isoflavones) of genistein, daidzein, and glycitein, and their respective three glucosidic conjugates. These nine glucosides include three β-glucosides of genistin, daidzin, and glycitin, three malonylglucosides of 6″-*O*-malonylgenistin, 6″-*O*-malnoyldaidzin, and 6″-*O*-malonylglycitin, and three acetylglucosides of 6″-*O*-acetylgenistin, 6″-*O*-acetyldaidzin, and 6″-*O*-acetylglycitin (Kudou et al., 1991; Wang and Murphy, 1994). The resources and compositions of isoflavones have been extensively studied because this type of phytoestrogen can lower the risk of breast cancer, prostate cancer, cardiovascular disease, osteoporosis, and climacteric syndrome and its related diseases (Vitale et al., 2013; Zaheer and Akhtar, 2015). In general, the ratio among these three types of aglycones and their derivatives is approximately 6:3:1 in soybean seeds. However, the specific composition of isoflavones in soybean seeds or soybean products varies due to the different varieties, planting environments, product forms, storage conditions for the materials and products, processing methods, and established operation conditions (Franke et al., 1999; Villares et al., 2011).

As indicated earlier, together with the occurrence of complex physicochemical reactions, the composition of isoflavones in product or intermediate product obtained during every processing step is clearly different. By analyzing the changes in isoflavone composition during the preparation of momen tofu induced by GDL, the processing steps responsible for the loss of total mass of isoflavones were soaking raw soybean seeds, filtrating soybean slurry, and coagulation step, with loss ratios of 4%, 31%, and 18%, respectively (Jackson et al., 2002). For specific isoflavone, the content of aglycones increased to a maximum value after heating soybean slurry and was then reduced together with tofu processing. 6″-*O*-acetylgenistin displayed a similar trend. However, the contents of β-glucosides and malonylglucosides were continuously reduced throughout tofu processing. These results might be due to the thermal instability of malonylglucosides, resulting in decomposition into their corresponding acetylglucosides and aglycones and higher water solubility in whey and okara (Kudou et al., 1991). Moreover, the loss of isoflavones during gel formation was related to the combination with protein to dissolve in whey. However, the content of aglycones is not significantly changed during the grinding, heating, coagulation, and other operation steps due to their relative water insolubility. Therefore, the operation conditions, such as soybean seeds-to-water ratio used for soaking, heating temperature and time, coagulant type and operation parameters, and others, used during tofu processing have a large connection with the changes in composition of isoflavones.

Kao et al. (2004a) studied the influence of varying soybean seeds-to-water ratios (1:11, 1:10, 1:9, 1:7, and 1:5) in the soaking step on changes in composition of isoflavones during the production of CS-induced tofu. The contents of the extracted daidzin and genistin in soymilk increased to the maximum values as the soybean seeds-to-water ratio increased from 1:9 to 1:5 and were then remained nearly unchanged. However, the contents of daidzein and genistein were not affected by the adopted soybean seeds-to-water ratios and were twofold to fourfold greater than those in raw soybean seeds. The increase in contents of daidzein and genistein might be attributed to the enzymatic hydrolysis of their corresponding β-glucosides and malonylglucosides during the soymilk production. The contents of daidzein and genistein were highest in tofus produced using soybean seeds-to-water ratios of 1:9 and 1:10, probably resulting from the formation of a fine homogeneous network microstructure of tofu in which these two hydrophilic isoflavones could be easily retained via hydrophilic interaction. Correspondingly, due to the difficulty associated with retaining free hydrophobic isoflavones in a protein network structure, the contents of daidzein and genistein in tofus were significantly reduced as the soybean seeds-to-water ratio increased from 1:11 to 1:7. The influence of soaking temperature (25, 40, 55, and 70°C) and time (1, 2, 3, 4, 5, 6, and 7 h) on the changes in contents of isoflavones were also investigated (de Lima et al., 2014). As a result, the aglycone content in soybean seeds soaked at 55°C for 5 h was sixfold higher than that in raw soybean seeds, and the soluble protein content and hardness of the soaked soybean seeds attained acceptable levels. The same results were observed in a study conducted by Kao et al. (2004b). Briefly, the contents of the extracted aglycones increased but the contents of the other nine glucosides declined as the soaking temperature or time increased. Therefore, the soaking conditions should be meticulously selected for consideration of the bioavailability of soybean products. In addition, the temperature and time used for denaturing the soybean proteins are also closely related to the composition of isoflavones. After the heating at 100°C for different durations (10, 20, and 30 min), no significant changes were observed in the content of aglycones, but the content of β-glucosides increased as the heating time increased. Moreover, as the heating time increased, the content of malonylglucosides decreased, mainly due to the hydrolysis of malonylglucosides under the hygrothermal conditions.

On the one hand, the efficiency of coagulation has a strong influence on the retention rate of isoflavones in tofu. For example, according to the data shown in Table 3, loss rates of the total content of isoflavones relative to the original content of raw soybean seeds in the soaked water, okara, and whey were 0.46%, 11.92%, and 43.92%, respectively (Wang and Murphy, 1996). This finding might be attributed to the formation of soybean protein-isoflavone complex, which dissolved easily in whey and resulted in a loss of water insoluble isoflavones. This type of loss was probably related to the type of coagulant applied for gelation (Jackson et al., 2002). On the other hand, the utilization of different coagulants with varying concentrations leads to different formation rates and mechanisms of tofu gelation, resulting in the different amounts of isoflavone loss via dissolving in whey in the form of soybean protein-isoflavone complex. For example, daidzein and genistein were probably bound to glycinin or β-conglycinin and were then coprecipitated into the soymilk pellet fraction obtained by centrifugation when CC was used as a coagulant (Hsiao et al., 2015). As the concentrations of CS and CC (0.3%, 0.5%, and 0.7%) increased, the total contents of isoflavones in tofu products were reduced. Moreover, at the same concentration, the total content of isoflavones in CS-induced tofu was higher than that in CC-induced tofu (Kao et al., 2004b). The same result was reported by Prabhakaran et al. (2006), who observed the highest total content of isoflavones in CS-induced tofu using a concentration of 4% among the studied coagulants, which included CC, CS, MC, MS, CA, and CL.

In general, aglycones are the main molecular forms for generating the aforementioned function in the human body and are also observed in increasing trend of total content during tofu processing. Therefore, the transformation of glucosides to aglycones using β-glycosidase during the soaking of soybean

Table 3. Changes in the contents of isoflavones during the production of CS-induced tofu.

(Intermediate) products	Yield (g)	Total daidzein (mg)	Total genistein (mg)	Total glycitein (mg)	Total isoflavones (mg)
Raw soybeans	600	59.3 ± 20.0^{a}	124.0 ± 35.0^{a}	$33.9 \pm 7.7^{a,b}$	217.2 ± 63^{a}
Soaked soybeans	1297	$54.2 \pm 31.0^{a,b}$	114.7 ± 47.0^{a}	27.8 ± 4.1^{b}	196.8 ± 82^{a}
Soaking water	1063	0.5 ± 0.3^{d}	0.3 ± 0.0^{c}	0.2 ± 0.0^{d}	1.0 ± 0.3^{c}
Heated slurry	5976	67.9 ± 17.0^{a}	121.6 ± 22.0^{a}	37.7 ± 2.7^{a}	227.2 ± 41^{a}
Soymilk	5581	63.6 ± 12.0^{a}	103.7 ± 16.0^{a}	27.6 ± 1.9^{b}	194.8 ± 30^{a}
Okara	717	4.1 ± 0.8^{d}	$14.0 \pm 0.6b^{c}$	7.8 ± 21^{c}	$25.9 \pm 0.7^{b,c}$
Whey	5140	$35.9 \pm 1.3^{b,c}$	50.4 ± 1.8^{b}	9.1 ± 0.1^{c}	95.7 ± 3.2^{b}
Tofu	1390	$16.0 \pm 1.5^{c,d}$	40.2 ± 3.4^{b}	14.6 ± 2.9^{c}	70.8 ± 4.7^{b}

[a]CS, calcium sulfate. Individual isoflavone glucosides and aglycone forms were normalized in terms of their molecular weight differences and summed to estimate the total isoflavone amounts. Values represent the mean ± standard deviation; $n = 3$. Values in a column with different superscripts are significantly different ($p < .05$).
[b]Calculated on a dry basis.
[c]Adapted from Wang and Murphy (1996).

seeds can effectively increase the functionality of tofu. This conclusion has been clearly confirmed by research focused on the influence of addition of β-glycosidase during various steps of tofu processing on the composition of isoflavones (Ryu et al., 2010). Specifically, the addition of 0.02% β-glycosidase before coagulation operation at 55°C for 30 min provided a maximum 84.5% conversion of glycosides to aglycones. Thus, the addition of β-glycosidase at an appropriate step during tofu processing could be a promising method for controlling the loss of isoflavones during the production of soybean foods.

Conclusions and outlook

As an important type of soybean product, numerous studies have been conducted to assess the possibility of modifying the quality and manufacturing of tofu. From the raw material to the final product, tofu processing consists of a complex physicochemical course in which the composition of many nutrients and product shapes are altered. The processing of tofu with satisfactory quality is influenced by many factors, such as the soybean variety, composition of soybean proteins and subunits, pretreatment of raw soybean seeds, processing technology and the corresponding operation conditions, and others. Among these possible factors, the protein profile, soymilk heating, and coagulation are the most important steps in the processing of tofu due to the nature of tofu product. During tofu processing, the total content of isoflavones is reduced, because of a decrease in the content of β-glucosides and an increase in the content of aglycones. The factors involved in tofu processing are interdependent and their interactions determine the yield, textural properties, sensory quality, and nutritive value of tofu. In addition, in comparison to traditional tofu, WST with promising prospects has a greater advantage from the perspective of nutrition.

Based on the changes in consumption demand for tofu and research progress on tofu processing, outstanding areas of study are as follows.

(1) The further elucidation of the coagulation mechanism underlying tofu gelation when a new type of coagulant or additive, such as an enzymatic coagulant or saccharide, is used.
(2) The study of a novel method for lengthening the shelf life and also maintaining the quality of tofu.
(3) The enhancement of the reprocessing performance of tofu through the use of suitable processing methods, such as the optimization of processing conditions for critical steps and the addition of additives during the coagulation process, and others.
(4) The illumination of the relationships between the operation conditions for critical steps and the yield and quality of tofu that can be readily adjusted in practice to satisfy the increasing variation in consumption demands.
(5) The appropriate utilization of by-products, such as okara and whey, generated during tofu processing to promote the modification of tofu quality.
(6) The investigation of an effective processing technology to ensure both the manufacturing of tofu with stable quality attributes and the retention of valuable isoflavones with reduced loss throughout processing.
(7) The development of WST with increased nutritive value and modified quality using a different or alternative processing technology and corresponding equipment compared with traditional tofu processing.

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