

The Impact of Processing Methods and Conditions on Nutritional Properties of Soybean-Based Tofu: A Review

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Abstract: The soybean seed is used for the preparation of protein rich tofu. Along with protein, it is also rich in many other nutrients including carbohydrate, crude fibre, carbohydrate, fat, minerals, and isoflavones. Antinutrients are also present in tofu, although the concentration is less than the raw grains. The nutrient content is affected by the tofu preparation method used starting from selection of suitable soybean seed, seed soaking, sprouting, soymilk production and coagulation using different types of natural and artificial coagulants. These procedures also affect the textural properties of the tofu and their shelf life.

Keywords: Soybean; Coagulation; Shelf life; Nutraceutical properties; Texture.

1. Introduction

Tofu, which is also known as bean curd, is a traditional soy-based food product. In the tofu preparation mechanism, coagulant forms soy protein gel matrix that helps in entrapping lipids, water, and constituents to form a curd, thereafter pressurized to form solids structure [1,2]. It is first recorded in China nearly 2000 years ago during the Chinese Han dynasty, however, it became popular in China during the Song Dynasty [3–5]. It became popular in Japan during the Edo period and was later introduced to southeast Asia and the rest of the world [6]. Being originated in China, it is called doufu later Japanese coined the term tofu in 1182. The global tofu market was estimated to be worth USD 2.31 billion in 2018 and is expected to grow at a compound annual growth rate (CAGR) of 5.2 percent from 2019 to 2025 [7].

There are many health benefits associated with the consumption of tofu. Reduced incidents of breast cancer were reported due to consumption of tofu [8]. Saponins present in tofu, are compounds that are thought to have heart-protective effects and are capable of improving blood cholesterol and increasing bile acid disposal, both of which can help to lower the risk of heart disease [9,10]. Furthermore, a study found that supplementing with 30 g of isolated soy protein lowered blood glucose levels, improved insulin levels by 8.1 percent, insulin resistance by 6.5 percent, cholesterol by 7.1 percent, and total cholesterol by 4.1 percent in diabetic postmenopausal women [11]. Tofu, consumption is associated with 32% to 51% lower risk of developing prostate cancer [12,13]. Consuming more tofu was also associated with a 61% lower risk of stomach cancer [14].

2. Different types of tofu preparations

2.1 Regular tofu

Regular tofu is prepared by heating of soymilk at 100°C for 10 min and then cooling till 75°C followed by addition of coagulant (3%), stirring for 3s, coagulation for 10 min, moulding and pressing for 45 min and cooling till 10°C in 30 min approximately.

2.2 Firm tofu

Firm tofu contains 76-81% moisture content and it is prepared mainly by the addition of coagulant, removal of excess water, and pressed through cheesecloth. Due to the removal of excess water by putting heavyweights, it is firm in texture [15]. Its preparation method includes soaking of soybeans for 12 h at ambient temperature. The

slurry is obtained by the addition of soybean and water in a 1:8 ratio and heated for 45 min at 85°C with continuous stirring. The hot slurry is filtered through a cheesecloth and soymilk is obtained. Later soymilk is heated up to 95°C for 5 min followed by the addition of coagulant, stirred for 5 min and allowed to coagulate for 15 min. Further pressed to form tofu with double cheesecloth for 20 min at 1 kg pressure initially and 0.5 kg for next 20 min [16].

2.3 Silken/soft tofu

As the name suggests, silken tofu is soft and smooth in texture containing a moisture content of about 87-90% [17,18]. Silken tofu is prepared by soaking 55g of soybean seeds in 200 ml of deionized water for 16-17 h at 15°C. After that soybean are drained and 400 ml of water is added to make a slurry. The slurry is heated at 98°C for 15 min. Extraction of soymilk is done with the help of a centrifugal liquid extractor. 1.5-5g/kg coagulants are used. 7 ml of coagulant is mixed in 220 ml of soymilk in a container with a lid containing holes to avoid gas formation and, stirred vigorously and thoroughly. The sealed container is heated indirectly at 85°C for 35 min for curd formation [19].

2.4 Dried tofu

Dried tofu is the type of tofu that contains the least moisture content below 76% prepared by breaking soybean curd vigorously in order to exclude extra water before pressing. It is prepared by soaking of soybeans in a 1:6 ratio of water at 21-22°C for 8h. Now excess water is removed and soaked grains are ground in a high-speed grinder followed by centrifugation to obtain soymilk. Soymilk is boiled with 1 g of antifoaming agent for 20 min and allowed to stand for 3 min at 96°C. The coagulant is added and allowed to stand for 20 min and the curd is formed. The curd is again stirred for few seconds and coagulated again. Further, it is transferred to a wooden mould lined with cloth and pressurized for up to 30 min [1].

2.5 Fermented tofu (sufu)

Fermented tofu, also called sufu, can be made using various strains namely *Actinomyces elegans*, *Mucor racemosus*, *M. sufu*, *M. dispersus*, *M. racemosus*, *M. wutuongkiao*, *Aspergillus* sp. Chou and Hwan prepared sufu using *Aspergillus taiwanensis* (CCRC 31159) and *A. selegans* (CCRC 31342). To prevent contamination by putrefactive organisms, tofu pieces were soaked at 95°C for 6 min followed by drying in a laminar air-flow hood for 2 h. Test microorganisms were inoculated by the spraying of 2 ml of the culture on the surface of each tofu piece and then incubated at 30°C for 48 h. Thereafter, the ageing of sufu was done at 25°C for 75 days, mixed with brine solution [20].

3. Tofu gelation mechanism

There are various gel formation theories given by different scientists. Kohyama et al. proposed the gelation mechanism of tofu that includes two steps- denaturation of protein and hydrophobic coagulation. In the first step, the denaturation of protein produces negatively charged ions. In the second step, geneses of protons are triggered by coagulants and because of that, the net charge of protein becomes neutralized. Due to this better hydrophobic interaction occurs between the neutral protein molecules and induces aggregation of molecules of protein, leading to the formation of curd [21]. Another model for soy gel formation integrates lipids, soy proteins, and small charged molecules (phytates and polyacid ions). In this mechanism, 11S protein coagulates in the presence of coagulants and, small molecules prevent the protein-lipid interface from coagulation. The lipid globules are wrapped with 11S proteins micelles and later with soluble 7S rich particles. During this, lipids get coated with oleosins (structural proteins). These micelles appear like a sandwich, many such protein-lipid interfaces form soymilk gel matrix [22]. Another theory of the tofu gel mechanism is illustrated by Clark et al. In their hypothesis, formation of gel largely depends on the intermolecular electrostatic repulsion. The repulsion might be due to surface charges and that is influenced by various factors including the nature of the particle, pH, and concentration of the coagulant [23].

4. Nutritional composition of tofu

4.1 Proximate composition

Numerous studies on the quality characteristics of tofu on a fresh and dry basis have been reported (Table.1). All these studies have suggested that tofu contains a fair amount of protein, carbohydrates, fat, ash, and fiber. These quality characteristics make it automatically attractive to the consumer as nowadays people are quite aware and attracted towards healthy and nutritious diets.

Table 1. Proximate nutrition composition of tofu prepared by different groups

No. Ref.	Tofu condition (wet or dry basis)	Protein	Carbohydrate	Fat/Oil/Lipid	Ash	Crude/Total fiber	Moisture
1 [24]	Fresh weight basis	6.58-9.56	2.95-4.14	5.17-5.43	1.46-1.68	–	81.4-82.4
2 [25]	Dry weight basis	5.21-5.56	–	2.46-2.78	0.43-0.53	–	–
3 [1]	Dry weight basis	54.5-57.7	–	20.4-22.5	–	–	–
4 [26]	Dry weight basis	51.93- 56.66	–	18.13- 20.55	5.64- 5.76	–	–
5 [27]	Dry weight basis	54.76-64.57	–	–	2.4-4.4	–	76.5-84.2
6 [28]	Fresh weight basis	13.3-17.6	3.8-7.2	4.9-6.2	0.5-0.7	–	71.9-73.9
7 [16]	Dry weight basis	56.3- 72.5	–	22.0- 27.8	2.0-2.8	–	71.2- 80.4
8 [29]	Fresh weight basis	12.1- 16.5	–	4.9- 6.9	–	–	–
9 [30]	Dry weight basis	32.10-39	22.60-28.26	8.70-35.20	3.30-3.60	–	3.70-5.50
10 [31]	Dry weight basis	47.29-50.92	–	–	7.78-10.50	–	–
11 [32]	Dry weight basis	8.53- 10.5	5.33- 7.34	5.82- 7.58	2.87- 4.54	–	72.01- 75.18
12 [33]	Fresh weight basis	14.6- 15.9	–	7.1- 8.7	–	–	71.1- 73.9
13 [34]	Dry weight basis	39.42- 44.73	23.56- 26.91	15.81- 21.15	3.93- 5.75	6.78- 11.98	67.88- 68.33
14 [35]	Fresh weight basis	11.97-13.72	–	2.92-4.87	1.12-2.08	–	76.10-82.07
15 [36]	Fresh weight basis	6.93- 12.07	–	4.27- 6.77	–	–	73.03- 85.9
16 [37]	Fresh weight basis	10.18-12.90	2.50-3.28	1.13-6.03	1.17-1.27	–	79.34-82.25
17 [38]	Fresh weight basis	10.88 - 12.9	–	0.12-5.03	0.90-1.13	–	77.6- 85.83
18 [39]	Dry weight basis	53.04-56.73	–	27.92-33.88	1.81-2.22	0.36-1.13	–
19 [40]	Fresh weight basis	11.80-12.40	17-26	1.80-2.20	1-1.80	0.11-0.12	58-65.8

4.2 Antinutrient content

Despite being highly nutritious and having so many health benefits, soy products are still less acceptable among consumer because of antinutrients present in both the soybean and soy-foods. Tofu is no exception and contains various antinutrients (Fig 1). Tofu has been reported for having a high content of oxalate which also raises a concern regarding the risk of kidney stones in humans [41]. American Dietetic Association recommends 10 mg oxalate content consumption to humans having history of calcium oxalate kidney stones (American Dietetic Association: Chicago 2000). Oxalate content in tofu was earlier reported and varies among different studies. Massey et al. found 0.5-2.8 mg/ [41], and Al-Wahsh et al. reported 0.02-0.13 mg/g [42].

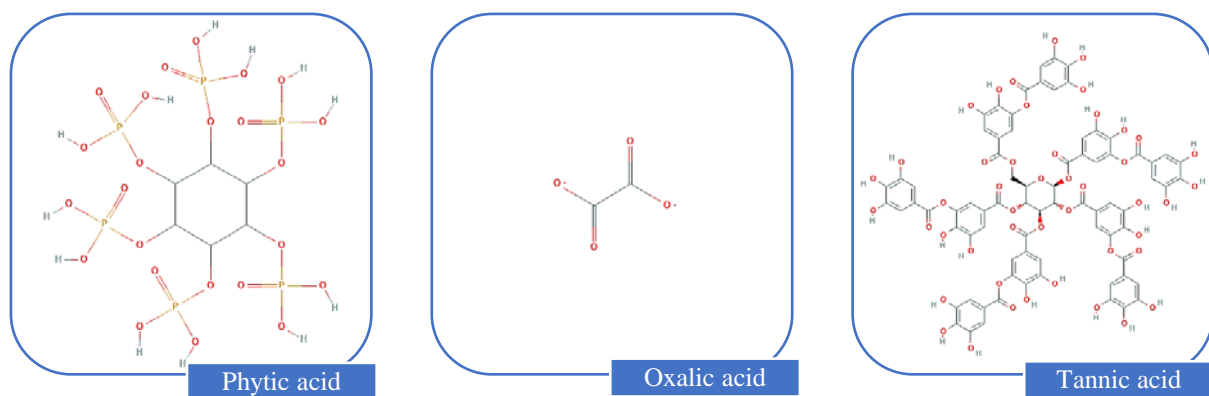


Fig. 1. Structure of Phytic acid, Oxalic acid and Tannic acid

Phytic acid (hexaphosphate salt of myoinositol) is another important antinutrient present in soybean as it reduces the bioavailability of minerals present in the grains. However, it was also suggested earlier that phytate content also exhibits anticarcinogenic activity against different cancers [43]. Phytate accounts for about 70-80% of phosphorus in soybean seeds [44]. Hou and Chang evaluated the effect of storage conditions on the phytate content of soybean, soymilk, and tofu (on a wet and dry basis). They reported that as the storage time is increasing, phytate content is also increasing in tofu samples and phytate content ranged from 8.72-13.32 mg/g (soybean seeds), 1.84-8.83 mg/g (tofu on a wet basis), 15.65-27.05 mg/g (tofu on dry basis) [45]. Al-Wahsh et al. also analyzed phytic acid content of the same tofu samples for which they have analyzed oxalate content and reported phytate concentrations ranging from 0.89 to 6.21 mg/ g for 11 tofu samples out of 19 while other samples did not exhibit

phytate [42]. Phytic acid in tofu samples from three different cultivars of soybean ranged between 4.6-25.7 mg/g [46]. Saio et al. examined the effect of phytic acid on the physical characteristics of tofu and stated that due to its presence in soybean or upon addition in soymilk it slowed the coagulative reaction [47]. Phytic acid also plays a role in pH decrease in soymilk after calcium is added [48,49].

4.3 Isoflavone content

Processed soy foods contain a lesser amount of isoflavones than unprocessed soybeans. Tofu possesses 6-20% of the isoflavone amount in unprocessed soybeans [50] (Fig. 2). Wang and Murphy reported the total value of isoflavones (0.532 mg/g) in tofu [51]. Isoflavones in tofu from two different brands were analyzed by Coward et al. and they reported 0.031 and 0.015 mg/g of genistein, 0.249 and 0.269 mg/g of genistin, 0.016 and 0.249 mg/g of daidzein, and 0.015 and 0.269 mg/g of daidzin [52]. In another work, raw and cooked tofu contained a total of 0.297 and 0.258 mg/g isoflavone content [53]. Grun et al. showed a large variation in the genistein and daidzein series in tofu caused by specific heat treatments, however, could not be explained specific reasons for this [54]. Kao et al. worked on the Influence of concentration (0.3, 0.5, 0.7%) of magnesium sulphate and magnesium chloride as coagulants on isoflavones in tofu samples and values of daidzein were ranged from 387-416 µg/g and 336-387 µg/g, daidzin 31.3-36 µg/g and 23.2-33 µg/g, genistein 630-683 µg/g and 600.5-679 µg/g, genistin 139.4-151.9 µg/g and 106.8-138.5 µg/g, glycitein 132-135.3 µg/g and 90.8-133.7 µg/g, glycitin 13.1-14.3 µg/g and 6.2-11.8 µg/g for calcium sulphate and calcium chloride respectively [55]. Isoflavone content in soybean seeds, tofu, and fermented tofu (also called *sufu*) was reported 1.15, 0.57, 0.23 mg/g, respectively. It was also stated that aglycones gradually increased, whereas the contents of glycosides decreased from raw soybeans to fermented tofu during processing [56]. Values for daidzin, glycitin, genistin, 6-O-malonyldaidzin, 6-O-malonylglycitin, 6-O-malonylgenistin, 6-O-acetyldaidzin, 6-O-acetylglycitin, 6-O-acetylgenistin, daidzein, glycitein, genistein are reported 25.7, 4.1, 35.1, 4.2, 0.5, 1.3, 0.8, 0.5, 6.4, 1.7, 0.8, 2.5 mg/100g, respectively, in freeze-dried tofu [57]. The effect of coagulant type and concentration on the isoflavone content was also evaluated by Prakaran et al. reported total isoflavones ranges from 1279.54-1424 µg [58]. Total isoflavone content was reported 937, 1481, and 1571 µg in tofu coagulated with calcium sulphate, calcium chloride, and magnesium chloride, respectively [59]. The amount of isoflavones (aglycones and glucosides) in tofu coagulated with synthetic and natural coagulants (Lemon, Tamarind, Garcinia, Gooseberry, Passion fruit) is reported earlier. Tofu coagulated with synthetic and plant origin coagulants had the isoflavone aglyconic content ranging from 0.308 to 0.430 mg/g and glucosidic content ranged from 0.310 to 0.471 mg/g [16]. Isoflavones content in two varieties of soybean and tofu prepared from it was reported by Mo et al. and the value of daidzein for the first and second variety of soybean is 51 and 56 mg/kg, glycitein 3 and 4 mg/kg, genistein 69 and 47 mg/kg, respectively. While for tofu, values of daidzein are 608 and 1331 mg/kg, glycitein 28 and 180 mg/kg, genistein 927 and 1407 mg/kg [31].

5. Texture properties

Textural properties of tofu are equally important attributes as nutritional quality for its acceptance by consumers and producers, respectively. Hardness, brittleness, cohesiveness, springiness, gumminess, and chewiness are some textural parameters that ultimately affect the quality of tofu. de Man et al. reported that the microstructure and textural property of tofu is largely influenced by the type of coagulating agent and stated that tofu prepared by gluconic delta lactone and calcium sulphate was finer and more uniform than the tofu prepared with calcium chloride, magnesium chloride, and magnesium sulphate [15]. Kao et al. analysed that an increased concentration of calcium sulphate from 0.2% to 0.4% causes a steady increase in hardness, cohesiveness, springiness, chewiness, and gumminess [60]. Obatolu showed significant variation in texture parameters of tofu coagulated with various coagulants namely calcium sulphate, epsom salt, lemon juice, alum, topwater of fermented maize coagulant. Tofu coagulated with Epsom salt has the highest value for hardness (1008.5 ± 24.1 (g)c), chewiness (4.5 ± 0.03 kg), and the brittleness (3678 ± 38.6 (g)c) [61].

Tofu's texture properties are dependent on its water content and tofu coagulated with water-in-oil-in-water (W/O/W) emulsion coagulant are much less hard than regular tofu thus having superior textural quality [62]. John Dzikunoo et al. done the textural analysis of tofu prepared with different coagulants and concluded that tofu prepared with acid coagulants are harder, less springy, and more fructurable than tofu made from that of salt precipitation [63]. Effect of trimagnesium citrate, nigari, and gypsum on the physical properties of tofu was also studied. It was reported that tofu coagulated with trimagnesium citrate had a lower value of hardness and chewiness while trimagnesium citrate and nigari-based tofu is less cohesive than gypsum-based tofu that suggests that it has strong internal bonding [64].

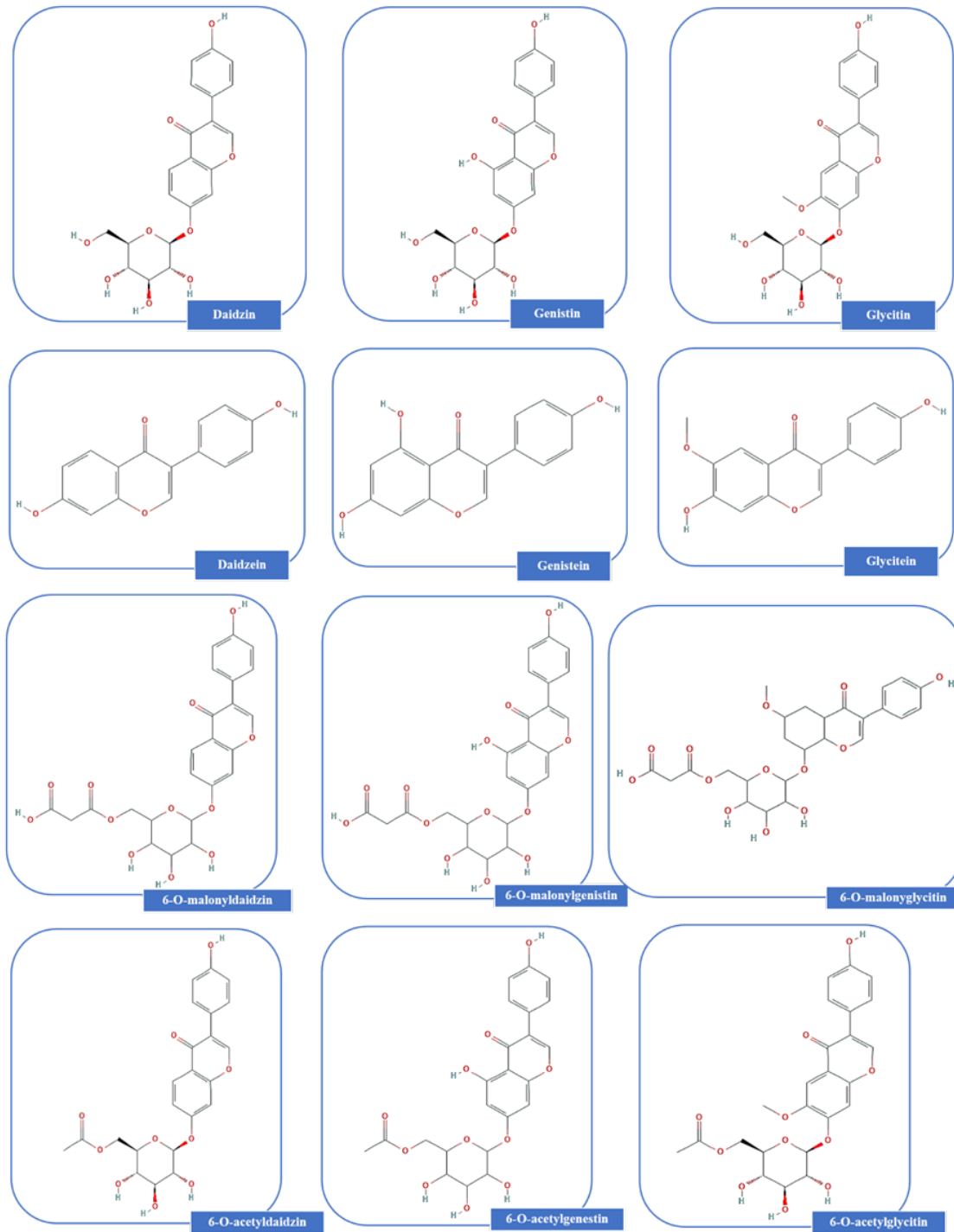


Fig. 2. Structure of 12 Isoflavones present in soybean

6. Shelf life

Due to the presence of high moisture and protein, tofu is a favourable medium for microorganism growth [65]. Tofu gets spoiled within 1-3 days without the use of any preservation method [66]. Many researchers have previously attempted to study the spoiling microorganisms responsible for tofu spoilage in order to know the methods to increase its shelf life [67,68]. Tofu spoilage by lactic acid bacteria and *Escherichia coli* was earlier reported [69,70]. Due to spoilage, tofu protein decomposes rapidly, and a change in pH occurs [70]. *Streptococcus* sp., *Pediococcus* sp, *Lactobacillus* sp. *Pseudomonas putida* and *Pseudomonas aeruginosa*. *Enterobacter agglomerans* and *Enterobacter cloacae* were reported for tofu spoilage by Tuitemwong and Fung in different brands

of Tofu [71]. Microorganisms like *Acinetobacter calcoaceticus* var. *anitratus* and *Klebsiella pneumoniae* subgroup *pneumoniae* have been reported being the major tofu spoiler of tofu [72]. Fouad and Hegeman analyzed that tofu is spoiled mainly by lactic acid bacteria, *Serratia liquefaciens*, and *Pseudomonas* species [73]. Joo and others found *Acinetobacter calcoaceticus*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *Xenorhabdus luminescens* to be major bacteria present in tofu samples [74]. Various pathogenic bacteria, namely *Pseudomonas* spp., *Escherichia coli*, *Enterococcus* spp., lactic acid bacteria, *Bacillus cereus*, *Staphylococcus* spp., *Salmonella* spp., *Yersinia* spp., and *Cronobacter sakazakii* were reported in tofu samples sold in Thailand [75]. *Bacillus cereus* reported to contaminate tofu samples purchased from the supermarket of Seoul, South Korea [76].

7. Storage and preservation method

Preservation of tofu is affected by various factors such as storage temperature, air composition, bacterial load, and processing techniques [77,78]. Previously various preservation methods have been explored to increase the shelf-life of tofu. Zhao and others used electrolyzed water for tofu manufacturing [79]. Different packaging methods also have been reported for the increment of shelf life of tofu such as after preparation packaging in PE films coated with nisin incorporated methylcellulose and hydroxypropyl methylcellulose solution [80]. Indian holy basil *Ocimum sanctum* has been used as a natural preservative to increase the shelf life of tofu [65]. In another work done by researchers, chitosan is used as a coagulant in order to explore it as a natural preservative. Chitosan is a natural biopolymer that is derived by the deacetylation of chitin a major component of the shells of crustacea such as shrimp, crab, and crawfish [27,81]. Citrus fruits' essential oils have been reported to possess antimicrobial activity against various pathogens. Tofu coated with this essential oil has been reported to be effective in delaying the microbial degradation of tofu [82]. Freezing is also explored for shelf-life elongation. Although in freezing there is ice formation occurs due to which textural quality, taste, appearance, and colour is affected [83–86]. Therefore, freezing was done by some researchers with the addition of some other techniques like static electric field-assisted freezing, freezing with ultrasound, freezing with a pressure shift, freezing assisted with the magnetic field [83,85,87–91]. Cold Atmospheric Pressure Plasma (CAPP) is used for shelf-life elongation of tofu. CAPP is a treatment method for food preservation in which food is exposed to ionized gases, generated by applying electric discharges [92].

8. Factors affecting the quality attributes of tofu

8.1 Soybeans seeds

Soybean seeds variety significantly affects the tofu quality. Good quality seeds can produce tofu with more nutrient content. Soybean seeds' quality entirely depends on the cultivar, environmental conditions, soil quality, etc. [93]. It was reported earlier that the quality attributes of tofu were significantly affected by soybean genotypes [94]. Two proteins namely glycinin with a centrifugal sedimentation coefficient of 11S and β -conglycinin with a centrifugal sedimentation coefficient of 7S can overall influence the tofu yield and quality. The higher the protein in seeds, the higher will be the tofu quality and yield [94,95]. Soybean seeds storage is another important factor that can indirectly affect tofu quality by affecting the seed composition. Seeds stored at a temperature higher than 30°C get deteriorated and that finally results in lower quality of tofu [96]. The amount of two important proteins (glycinin and β -conglycinin) can also be declined by the storage of soybean seeds in adverse conditions. It was supported by the fact that these proteins gradually decreased when stored in 84 % relative humidity and 30°C for 3 months [97]. Apart from soybean proteins, other chemical components also influence the tofu quality such as carbohydrates and lipids. Chen et al. reported that the lipid content of various soybean samples is positively correlated with the yield and quality of dry and wet tofu [98]. Along with nutrients, antinutrients present in soybean seeds significantly affect the tofu. Phytic acid content affects the tofu hardness by influencing the protein gelation by reacting with gelatinous substances [99].

8.2 Soaking and sprouting of soybeans

Soaking of soybean seeds is the first and important step in tofu production that also affects the tofu quality. Soaking of soybean can help in the acceleration of protein extraction from raw seeds that apparently leads to the higher protein content of tofu [100]. Consequently, soybean seeds and water ration, soaking time, soaking temperature affects the texture, yield, and tofu nutrient content [1,101–104]. Along with the soaking effect, many researchers have also investigated the effect of soybean seeds sprouting on soybean products. It was reported that sprouting of seeds not only improves the quality of products but also reduces the antinutrients components that are the reason for adverse effects of these products like removal of trypsin inhibitor, flatulence factor, phytic acid, and off-flavour [33,105,106]. Agrahar-Murugkar (2014) compared the tofu quality prepared from sprouted and non-sprouted soybean and observed an increase in the amount of protein, springiness, cohesiveness, and improved sensory quality, and decreased fat content, chewiness, and hardness in sprouted soybean-based tofu [107].

8.3 Soymilk preparation methods

The soymilk is reportedly prepared mainly following three methods- uncooked slurry, cooked slurry, and hot water mixed slurry. In uncooked slurry soymilk is filtered without boiling the slurry. In cooked slurry soymilk is heated to a certain temperature and then filtered. While in hot water mixed slurry soymilk is prepared with a temperature of 65°C and hot water is added to it with continuous stirring. It was observed earlier that tofu prepared with cooked slurry method has better yield, water holding capacity, and water content than the other two methods [108]. Tofu prepared from the uncooked slurry method has higher adhesiveness and hardness. In the cooked slurry method during the filtration, the temperature is high which eventually facilitates the protein from okara to soymilk and because of that protein content was found to be high in tofu made from the cooked slurry method [109].

8.4 Heating techniques

Theoretically, due to thermal treatment, protein aggregation occurs due to the presence of hydrophobic groups. Elias et al. performed boiling of soymilk for various timings to study its effect on tofu quality and reported that boiling time did not affect the fat content, protein content, and sensory quality but significantly affects the hardness, cohesiveness, and chewiness when boiling was beyond 30 min [110]. The denaturation temperature of two important proteins of soymilk namely glycinin and β -conglycinin are 65-75°C and 85-95°C, respectively [111]. Thus, due to this two-step heating process is apparently more suitable for effective denaturation and gelation of soybean proteins [112]. Liu et al. compared heating methods i.e., two step heating (soymilk boiled at 75°C for 5 min and then at 95°C for 5 min using an ohmic technique) and one-step heating (soymilk boiling at 95°C for 5 min) and suggested that soymilk boiled with a two-step heating technique has a higher viscosity, a denser, finer, and more homogeneous network structure than those obtained from soymilk treated by the traditional one-step heating technique [113]. Traditional heating of soymilk is happened at atmospheric pressure where non-uniform heating of soymilk occurs that leads to a decrease in tofu structure uniformity.

Table. 2 List of coagulants used for tofu preparation

Coagulants	Reference
Salt	
<i>Calcium sulphate</i>	[60,61,64,101,118–120]
<i>Calcium chloride</i>	[58,118]
<i>Calcium lactate</i>	[58,118]
<i>Calcium acetate</i>	[58]
<i>Magnesium chloride</i>	[58,62,64,120,121]
<i>Magnesium sulphate</i>	[58,101]
<i>Trimagnesium citrate</i>	[64]
<i>Alum</i>	[61]
Acid	
<i>Acetic acid</i>	[58]
<i>Citric acid</i>	[122]
<i>l-[-]-malic acid</i>	[122]
<i>Tartaric acid</i>	[122]
<i>Glucone delta lactone</i>	[113,122–125]
Natural	
<i>Citrus limonum</i>	[16,61,126]
<i>Citrus aurantifolia</i>	[127]
<i>Averrhoa bilimbi</i>	[127]
<i>Tamarindus indica</i>	[16,126]
<i>Garcinia indica</i>	[16,126]
<i>Phyllanthus acidus</i>	[16,126]
<i>Passiflora edulis</i>	[16,126]
<i>Averrhoa carambola</i>	[126]
<i>Averrhoa bilimbi</i>	[126]
<i>Phyllanthus distichus</i>	[126]
<i>Hibiscus cannabinus</i>	[126]
Enzymatic	
<i>MTgase</i>	[128,129]
Other	
<i>Chitosan</i>	[27]
<i>Guar gum</i>	[35]
<i>Top water of fermented maize</i>	[61]
<i>Crab shell powder and ash</i>	[130]

8.5 Effect of coagulants

Coagulation of the soymilk results due to the cross-linking of protein molecules with divalent cations [114]. Coagulant type and concentration are critical in order to determine the tofu texture, yield, and quality [115]. Therefore, the selection of coagulating agents and optimization of its concentration is one of the most critical steps in tofu production. Lately, many salts, acids, natural, enzymatic, and some other coagulants are used in tofu manufacturing (Table 2).

Apart from the type and concentration of coagulant, stirring speed, temperature, duration are the parameters that directly influence the tofu quality [60]. Various coagulant stirring speed (137, 207, and 285 rpm) for different coagulation times (5, 10, 15, 20, 25, and 30s) significantly affects the tofu yield and quality of tofu [116]. It was earlier reported that tofu yield and texture are positively correlated to coagulation temperature [117].

9. Conclusion

The nutritionally rich tofu is now becoming a favourable food choice worldwide. The proper preparation, preservation and storage methods affects the nutritional properties of tofu and can increase the life of this protein rich food material. The selection of suitable coagulants are also found to affect the nutritional and textural properties of tofu along with many other processing method.

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