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Effect of different coagulants on the isoflavone levels and physical properties of prepared firm tofu

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Abstract

The objective of this study was to understand the effect of different coagulants in their ability to retain isoflavones in prepared firm tofu. Harovinton beans were processed to obtain soymilk and a specific amount of this soymilk was coagulated using different types of coagulants for the preparation of tofu. A reversed phase high performance liquid chromatographic method was used for determining the concentration of isoflavones in tofu and whey. Quality parameters such as the texture, color and moisture of tofu, prepared from different coagulants were also determined in this study. Tofus coagulated with different coagulants were found to contain different amounts of isoflavones. Calcium sulfate was found to be the most suitable coagulant for tofu making in terms of its high yield, retention of maximum amount of isoflavones and in obtaining a firm, but not hard texture of tofu.

Keywords: Tofu; Whey; Isoflavone; Coagulant; Texture

1. Introduction

Soybeans have been transformed into various forms, among which tofu being the most widely accepted soy food. Tofu is a nutritional, gel-like soy food. Its preparation generally includes soaking and grinding of soybeans in water, filtering, boiling and coagulation of soymilk, molding and pressing. It is consumed in significant amounts in Asian countries because of their inexpensive, high quality protein (Koury & Hodges, 1968). Moreover, isoflavones in tofu have been credited with performing several health-promoting functions, like lowering the incidence of several types of cancers (Messina, Persky, Setchell, & Barnes, 1994) including the breast cancer (Adlercreutz et al., 1991; Lee et al., 1991), prostate cancer (Severson, Nomura, Grove, & Stemmermann, 1989), colon cancer (Kurzer & Xu, 1997); and reducing the risk of various diseases like cardiovascular problem (Anderson, Johnstone, & Cook-Newell, 1995), osteoporosis (Anderson, 1999), menopausal symptoms (Adlercreutz, Hamalainen, Gorbach, & Goldin, 1992), etc. Daidzein, glycitein and genistein are the three main soy isoflavones existing in its aglycon form, while daidzin, glycitin and genistin are the respective glucosides. Acetyl daidzin, acetyl glycitin, acetyl genistin, malonyl daidzin, malonyl glycitin and malonyl genistin are conjugates of glucosides having either an acetyl or a malonyl β-glucoside. Thus there are a total of 12 soy isoflavone conjugates (Wang & Murphy, 1994).

Coagulation of soymilk is the most important step in the tofu-making process. Calcium sulfate, calcium chloride, magnesium sulfate and magnesium chloride are many of the different types of coagulants used on an industrial scale for the preparation of tofu. Coagulation occurs due to the cross-linking of protein molecules in soymilk with the divalent cations (Saio, Koyama, & Watanabe, 1967). After the coagulation process, whey is removed for the preparation of a firm tofu. The thermal denaturation, aggregation and gelation properties of soy proteins during tofu making

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have been intensively investigated by many researchers (Lakemond, de Jongh, Gruppen, & Voragen, 2002; Lakemond et al., 2003; Mujoo, Trinh, & Ng, 2003; Renkema, Knabben, & Vliet, 2001). However, the loss of isoflavones might be significant when the soy beverage is coagulated to form tofu and the type of coagulant may have a different influence towards the retention of isoflavones in tofu and whey. But, little information is available on the effect of various coagulants on the level of isoflavones in tofu. Moreover the released whey has proteins associated with it and a certain amount of isoflavones is lost into it. But to our knowledge, there was no literature available which evaluated the retention of isoflavones in tofu or whey with regard to the coagulant used for the tofu preparation. The objective of this study was to understand the effect of different coagulants towards the retention of isoflavones in the respective tofu and whey. To the best of our knowledge, this is the first set of studies, which analyzed wheys in a very sequential manner to identify the loss of isoflavones. Wheys are currently not utilized as a protein or isoflavone source except that in certain parts of Indonesia, it is re-used as a coagulating agent for further preparations of tofu (Shurtleff & Aoyagi, 1979b).

The physical properties such as the hardness, texture, moisture and color of tofu determine the quality of tofu, especially because of its bland nature. Hence, evaluation of these quality parameters along with the isoflavone levels in tofu prepared from different coagulants, were carried out in this study. Our study also highlights the need for utilization of an ideal coagulant along with other processing conditions to optimize the retention of maximum isoflavones in the prepared tofu.

Unless otherwise stated, tofu refers to a '*firm tofu*' throughout, in this particular study.

2. Materials and methods

2.1. Chemicals

The coagulants used were calcium sulfate $CaSO_4 \cdot 2H_2O$, calcium chloride $CaCl_2 \cdot 2H_2O$, calcium acetate $Ca(CH_3COO)_2$, Calcium lactate $(CH_3CHOHCOO)_2Ca \cdot 5H_2O$, magnesium chloride $MgCl_2 \cdot 6H_2O$, magnesium sulfate $MgSO_4 \cdot 7H_2O$ and acetic acid CH_3COOH. The above chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA), while a 5% acetic acid solution in water was prepared in the lab by diluting glacial acetic acid and were used for the preparations of tofu.

Isoflavone standards, such as daidzin, glycitin, genistin, daidzein, glycitein and genistein were purchased from LC Laboratories (Woburn, MA, USA). All solvents used were of HPLC grade and were purchased from Sigma Chemical Co. (Jefferson City, MO, USA). Linear responses were obtained for the six standards and calibration curves were plotted by using peak area versus corresponding isoflavone concentrations.

2.2. Preparation of soymilk

Soymilk was prepared in the pilot plant of a local soymilk processing industry by the traditional method. Identity preserved soybean seeds ''Harovinton'' were obtained from a local supplier and was used as the raw material for this study. Soybean seeds were soaked in water at ambient temperature for a period of 5 h, rinsed and ground with water at a bean:water ratio of 1:4. The resultant slurry was cooked at 100 °C for more than 15 min and passed through a centrifugal separator to remove the soy residue (okara), to obtain a soymilk of 12° brix. The brix of soymilk was measured using a refractometer (Abbe, Model 3T, Atago, Japan). A single batch of soymilk was prepared from the bean and was used for the preparation of tofu using each coagulant. This was done in order to avoid any changes in the milk composition and or concentration factors.

2.3. Preparation of tofu

A 500 g portion of soymilk was heated to a temperature of 80 °C under stirring. The time period of heating, and stirring speed were kept constant for every tofu preparations. Tofu was prepared by coagulating the soymilk using any one of the coagulants, calcium chloride, calcium sulfate, magnesium chloride, magnesium sulfate, calcium acetate or calcium lactate. Coagulants were used at two different concentration levels to better understand the level of coagulant required and possible effects if any, on isoflavone concentrations. The coagulant concentrations used were 0.4% and 0.5% based on the amount of soymilk used. These coagulant concentrations (0.4% and 0.5%) were chosen based on a preliminary set of experiments on coagulant concentrations carried out prior to this study. Each coagulant was dissolved completely in 20 ml of cold water and was used immediately. Calcium sulfate was not completely soluble in water, and hence saturated solutions of calcium sulfate obtained with the above concentrations were used. The hot soymilk and coagulant solution were poured simultaneously into a glass container ensuring good mixing without stirring. The soymilk-coagulant suspensions were allowed to stand undisturbed for a period of 20 min to ensure that coagulation occurred. The curds thus formed were broken thoroughly and transferred into a specially designed mould ($9 \times 9 \times 8$ cm) lined with cheesecloth. The mould had perforations on sides and bottom. The whey was drained off naturally for 10 min and the curd was pressed for 1 h using a pressure of 28.0 g/cm^2. After pressing, tofu and whey were weighed separately. The tofu was transferred into a plastic bag and stored in a refrigerator till further analysis. Tofu was also prepared using 20 ml of 5% acetic acid solution as a coagulant following the above procedure.

2.4. Extraction of isoflavones

Tofu samples were freeze-dried and dried samples equivalent to 2 g of fresh tofu were used for isoflavone analyses.

Whey was used as such for isoflavone extractions. The extraction procedure followed the methodology of Klump, Allred, MacDonald, and Ballam (2001), where the samples were incubated at 65 °C with 80% methanol, saponified, neutralized and made up to a volume of 50 ml. A portion of the sample was further used for HPLC analysis.

2.5. HPLC instrumentation and chromatographic conditions

Isoflavone analysis were carried out on a Waters 2695 separations module equipped with an inline degasser, a binary pump delivery system and a Waters 2996 photodiode array detector. A sample of 20 μl was loaded onto a YMC-pack ODS-AM 303 column (5 μm, 25 cm × 4.6 mm i.d., Waters Corp., Milford, USA) through an autosampler. The diode array detector was set from 200 to 350 nm, and the eluting components were monitored at 260 nm, while processing was carried out using the Waters "Empower Software System" (Waters Corp., Milford, USA). The mobile phase consisted of (A) 0.1% glacial acetic acid in filtered Milli-Q water and (B) 0.1% glacial acetic acid in acetonitrile. The components were eluted using the following solvent gradient: from 0 to 5 min 10%B; from 5 to 50 min 10–35%B; then held at 35%B for another 10 min and re-equilibrated back to 10%B. The solvent flow rate was 0.8 ml/min with a total run time of 80 min. The identity and purity of isoflavones in the samples were confirmed by matching the mass spectrum analysis of the standards, which was carried out on a Finnigan Quadrupole Mass Spectrometer (Finnigan MAT LCQ, San Jose, CA, USA). Isoflavones were separated on the YMC pack column itself using the same gradient run.

2.6. Moisture determination

Moisture content of soybean seed powders and tofu (homogenized) were obtained by drying a weighed amount of samples to a constant weight at 105 °C in an oven for 24 h (AOAC, 2000). Total solids in the whey were determined by drying 5 ml of the whey at 105 °C in an air oven for 24 h.

2.7. pH measurement

pH of the wheys were measured using a ThermoOrion model 410A pH meter equipped with a standard line (model no. 91-56) electrode (ThermoOrion, Beverly, MA, USA). Commercially prepared buffer solutions of pH 4.00 and 7.00 were used to standardize the pH meter.

2.8. Texture measurement

Texture profile analysis of tofu was carried out using a TA.XT2i texture analyzer (Stable Micro Systems, Godalming, UK). A sample from the central part of tofu was always used for texture evaluation. A test speed of 2.0 mm/s and 10 mm dia probe was used for this purpose. Cylindrical samples (1.5 cm diameter × 1 cm height) were prepared from the central portion of tofu with a stainless steel boring tube and a wire cutter. The samples were compressed to 50% deformation. Six replicate tests were carried out for every coagulant tofu. Hardness was defined as the height of the peak force on first bite, which was the force necessary to attain a given deformation. Hardness, cohesiveness and gumminess of individual tofu samples were determined from the TPA curve as described by Bourne (2002).

2.9. Yield and color analysis

The yield of tofu was calculated as the weight of fresh tofu obtained from a specified amount of the soymilk used for its preparation. The color of tofu, expressed in L, a^* and b^* values, according to the CIE definition were measured using Minolta spectrophotometer model CM-3500d (Osaka, Japan). The measurements were replicated six times and the mean value was obtained.

2.10. Statistical analysis of data

All extraction experiments and analysis were performed in triplicate and the isoflavone values are reported as means ± SD as obtained. Analysis of variance was conducted for every data collected, using SPSS version 12.0 (SPSS Inc., Chicago, USA). The significance between the mean values was determined at $p \leqslant 0.05$ levels.

3. Results and discussion

Results of LC MS analysis showed no significant difference in the chromatographic resolution obtained between HPLC-UV detection and HPLC-MS. Selected peaks were extracted using the positive and negative acquisition mode over an m/z range of 160–800. Details of ions observed in the positive and negative ion spectra of different isoflavones generated by electrospray ionization (ESI) mass spectrometry are shown in Table 1. Results of analysis showed that

Table 1
Ions observed in positive and negative ion spectra of isoflavone glucosides and aglycones generated by ESI–MS

Isoflavone[a]	+ve ions	−ve ions
Daidzin	255 $[M - Glc]^+$	253.1 $[M - H - Glc]^-$
	417.2 $[M + H]^+$	415 $[M - H]^-$
		475 $[M - H + CH_3COOH]^-$
Glycitin	285 $[M + H - Glc]^+$	283.1 $[M - H - Glc]^-$
	447.2 $[M + H]^+$	445.2 $[M - H]^-$
		505 $[M - H + CH_3COOH]^-$
Genistin	271.1 $[M + H - Glc]^+$	269.1 $[M - H - Glc]^-$
	433.1 $[M + H]^+$	431.3 $[M - H]^-$
		491 $[M - H + CH_3COOH]^-$
Daidzein	255.4 $[M + H]^+$	253.5 $[M - H]^-$
Glycitein	285.3 $[M + H]^+$	283.5 $[M - H]^-$
Genistein	271.4 $[M + H]^+$	269.5 $[M - H]^-$

[a] Isoflavones were separated by 0.1% acetic acid in water and acetonitrile gradient profile using YMC pack ODS column.

an HPLC-MS run using ESI was sufficient to obtain the most sensitive and structurally useful information about the different isoflavones.

The amounts of daidzin, glycitin, genistin, daidzein and genistein in soybeans used for this study were determined, and the contents were found to be 1022.19 ± 13.2, 111.52 ± 7.8, 1049.32 ± 15.4, 37.14 ± 0.77 and 16.90 ± 0.53 μg/g, respectively, on a dry weight basis. Extraction followed by a mild saponification, utilized in this study converted all the isoflavone ester forms into their corresponding glucosides. The total isoflavone contents in soy samples were expressed in aglycone equivalents after adjusting for their molecular weight differences (Klump et al., 2001). This was obtained by summing the concentrations of daidzein, glycitein and genistein and adding this total to the sum of aglycon equivalent concentrations of daidzin, glycitin and genistin. Thus the soybeans contained 1405.50 ± 17.3 μg/g of isoflavones on a dry weight basis. Soymilk was prepared in bulk from a single batch of soybeans and the prepared soymilk was also analyzed for its isoflavone concentrations. The prepared soymilk had a total of 182.19 ± 0.36 μg/g of isoflavones on a wet weight basis, which was equivalent to 1570.64 μg/g on a dry matter basis. However, since the aim of this study was in evaluating the concentration of isoflavones in tofu and whey prepared from this soymilk, a mass balance study on isoflavone concentrations during the soymilk preparation was not carried out and hence the soy residue (okara) was neglected.

Isoflavone levels in tofu are considered important in terms of its functional food value, but the texture of tofu is an equally important attribute that affects the product acceptability. Texture, hardness, cohesiveness and chewiness of tofu are the several parameters that ultimately determine the quality of tofu. For e.g., a firm tofu with a greater hardness means harder and firmer, with greater cohesiveness. It requires more work to break down the internal bonding. Tofu with high springiness possesses higher elasticity and greater chewiness, making it more difficult to eat. Texture profile analysis was therefore carried out to study the effects of different coagulants on the firmness of tofu matrix. The typical TPA curve obtained for tofu sample is shown in Fig. 1.

With regard to the color, either white or creamy white color is the desirable tofu characteristic. Every tofu produced in our laboratory had a creamy white color. The L values for the tofus ranged from 85.93 to 86.41 while a^* and b^* values ranged from 0.003 to 0.657 and 22.36 to 24.95, respectively. The different coagulating agents were found to have no significant difference ($p \leqslant 0.05$) on the color of tofu, especially when using a single lot bulk soymilk, as in this case (Table 2).

The moisture content of tofu samples varied with the use of different coagulants. The variation in the moisture content of tofu prepared with different coagulants is probably due to the differences in gel network influenced by different anions and its ionic strengths towards the water holding

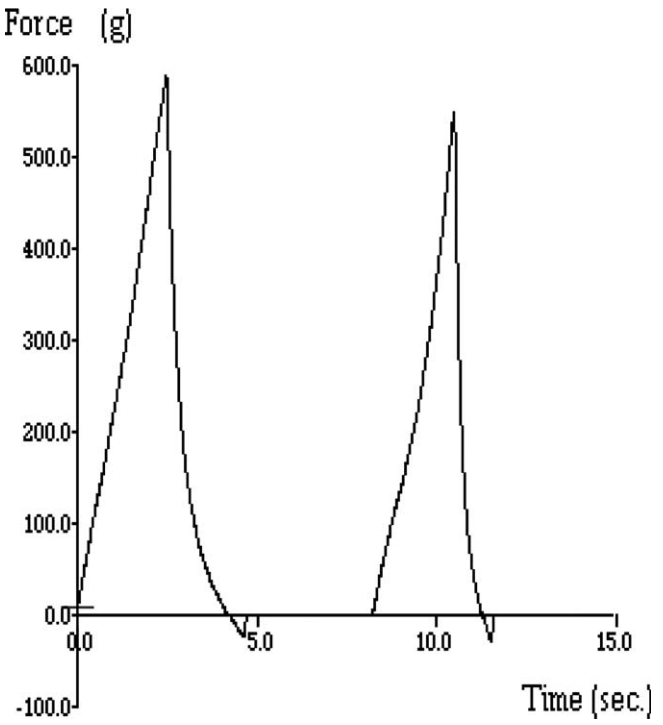

Fig. 1. Typical texture profile analysis curve obtained for a firm tofu.

capacity of soy protein gels. It has also been reported that the coagulant concentration and type of anion might affect the hardness of tofu (Sun & Breene, 1991; Tay, Tan, & Perera, 2005; Wang & Hesseltine, 1982). The high moisture content accounted for a higher tofu yield since tofu yield and moisture contents are highly correlated (Cai, Chang, Shih, Hou, & Ji, 1997). Tofu with high moisture content appeared smooth while tofu with low moisture content had a coarse texture by visual examination.

Obtaining a tofu with the desired texture, color and moisture together with its maximized functional value might be critical, but achievable. Recent studies by Jackson et al. (2002), also illustrated the probability of leaching of isoflavones into the whey, and that the type of coagulant may have differences in their ability to retain isoflavones in the tofu. Moreover, whey being a waste material, its nutritive value is always neglected. Hence, an analysis of its isoflavone contents together with the level of isoflavones in the prepared tofu will help to understand the influence of different coagulants towards the ability to retain isoflavones in the product (tofu) and byproduct (whey) of the process. This can further help in process optimization that becomes essential in retaining maximum nutritive value in the tofu especially when the choices of coagulants are plenty.

3.1. Choice of coagulant concentrations for the study

All the calcium salts, magnesium salts and the acidic coagulant (acetic acid) used in this study precipitated the soymilk proteins. However, only two concentrations of

Table 2
Comparison in the yield, color and isoflavone concentrations in soybean curds prepared using different coagulants

Coagulant type	Usage level (% w/w)	Tofu M%	Tofu obtained (g)	Isoflavones in tofu (μg/g) dry matter basis	Color determination		
					L	*a**	*b**
Calcium sulfate	0.5	79.2	225.36[a]	1317.88 ± 6.77[d]	85.65	0.535	24.39
Calcium sulfate	0.4	79.5	232.49[a]	1424.00 ± 0.67[e]	85.82	0.281	24.95
Calcium chloride	0.5	76.6	210.18[b]	1301.06 ± 2.43[dh]	86.66	0.421	23.01
Calcium chloride	0.4	76.1	209.99[b]	1295.85 ± 2.41[dg]	86.57	0.388	22.79
Magnesium sulfate	0.5	79.6	246.26[a]	1333.77 ± 0.19[d]	86.41	0.003	23.00
Magnesium sulfate	0.4	78.7	234.49[a]	1282.16 ± 1.21[f]	85.78	0.359	23.74
Magnesium chloride	0.5	78.3	203.46[b]	1279.54 ± 4.69[f]	86.56	0.435	23.12
Magnesium chloride	0.4	78.7	219.27[a]	1279.54 ± 1.11[f]	86.98	0.280	23.30
Calcium acetate	0.5	77.3	195.14[c]	1280.18 ± 4.24[f]	85.96	0.549	23.56
Calcium acetate	0.4	77.4	192.75[c]	1285.30 ± 3.98[f]	85.91	0.614	24.19
Calcium lactate	0.5	79.0	220.55[a]	1301.81 ± 6.01[dh]	86.53	0.320	23.30
Calcium lactate	0.4	79.7	232.20[a]	1292.36 ± 4.20[dg]	85.97	0.287	23.72
Acetic acid 5%	20 ml	76.8	212.50[b]	1280.18 ± 7.03[f]	86.73	0.657	22.36

Tofu M% = percentage moisture in tofu. Isoflavones in tofu given as the means ± SD.
Like superscripts in the same column do not differ significantly at $p \leqslant 0.05$.

the coagulants were applied for this study. The coagulant concentrations used for tofu preparation were 0.4% and 0.5% based on the amount of soymilk used. The lowest concentration of coagulant applied by Lu, Carter, and Chung (1980) to obtain coagulation was 0.1% for calcium chloride, 0.2% for calcium lactate and 0.3% for calcium sulfate. Tofu is a perishable food product and its preparation over a wider range of coagulants (0.1–0.6%) for many different coagulants was not possible in this study, since the physico-chemical properties along with its isoflavone evaluation need to be carried out immediately after its preparation. Hence, during our experiments involving many different types of coagulants, the concentration of coagulants utilized for the preparation of tofu was 0.4–0.5%. Moreover, the concentration level of the coagulants so chosen was also the typical amounts used for tofu formations.

3.2. Properties of tofu coagulated by calcium and magnesium sulfates and chlorides

Table 2 shows the effect of different soy protein coagulants on the level of isoflavones in tofu. While using calcium sulfate as a coagulating agent, it was observed that the tofu prepared from a lower concentration of calcium sulfate (0.4%) gave a higher product yield than when 0.5% concentration was used. The same tofu coagulated with 0.4% calcium sulfate was also found to contain a higher concentration of isoflavones than when the higher concentration of calcium sulfate was used. The decrease in yield of tofu with increasing calcium sulfate concentration could be due to increasing syneresis and loss of whey from the curd as more bonding occurred thus making the protein matrix denser and compact (Sun & Breene, 1991). A firm, but not hard tofu was obtained after using calcium sulfate as a coagulant. Moreover, calcium sulfate was also found to be a better coagulant than calcium chloride in terms of the yield of tofu and in the retention of isoflavones in the tofu. Calcium chloride was quick in coagulating the soy protein than calcium sulfate. However, the quick coagulating power of calcium chloride must have resulted in more exclusion of isoflavones into the whey during the coagulation process. This caused the calcium chloride coagulated tofu to have lesser concentration of isoflavones in it than the calcium sulfate coagulated tofu. Moreover, using calcium chloride at 0.4% or 0.5% level did not cause a significant difference in the yield of tofu ($p \leqslant 0.05$), though there was a slight difference in the concentration of isoflavones in them (Table 2).

The textural properties of tofu prepared using different coagulants are shown in Table 3. When magnesium sulfate was used as a coagulant the corresponding tofu had textural properties much different from others. The hardness and chewiness of magnesium sulfate coagulated tofu was less than the hardness and chewiness of every other tofu prepared in this series. While the moisture content of this tofu was higher, that of its whey was lower. Probably an incomplete precipitation of soy proteins occurred and instead of having a compact protein network, it had a loose network encompassing many air gaps within it. Comparatively higher levels of isoflavones were found in this tofu since the amount of released whey was less. The pH of tofu whey was 5.91 when the concentration of coagulant used was either at 0.4% or at 0.5% level. A decrease in pH was always described as essential for the coagulation of soy proteins by many researchers (Kamel & de Man, 1982; Lu et al., 1980). However, when compared to calcium sulfate and calcium chloride, the decrease in pH caused by magnesium sulfate was far less. According to Wang and Hesseltine (1982), cross-linking between protein molecules along with the presence of calcium ions are required for soy protein coagulation. But magnesium ions can also be used instead of calcium ions, since this divalent cation can form cross-linking between protein molecules. However, the sites of cross-linking in the protein molecules may be different for both calcium and magnesium causing the latter to form a loose network. This might be another

Table 3
Textural properties of tofu prepared using different coagulants

Coagulant type	Usage level (% w/w)	Hardness (g)	Springiness (mm)	Cohesiveness	Gumminess (g)	Chewiness (kg mm)
Calcium sulfate	0.5	416.82	9.66	0.649	247.51	2.39
Calcium sulfate	0.4	458.53	9.69	0.635	262.47	2.54
Calcium chloride	0.5	515.29	9.88	0.645	301.46	2.97
Calcium chloride	0.4	484.16	9.68	0.648	286.75	2.77
Magnesium sulfate	0.5	292.49	9.86	0.644	171.89	1.69
Magnesium sulfate	0.4	293.36	9.89	0.639	168.34	1.66
Magnesium chloride	0.5	414.56	10.00	0.663	255.32	2.55
Magnesium chloride	0.4	395.49	9.88	0.661	238.94	2.36
Calcium acetate	0.5	607.77	10.00	0.657	368.84	3.68
Calcium acetate	0.4	617.68	9.81	0.652	368.69	3.61
Calcium lactate	0.5	370.54	9.82	0.660	226.41	2.22
Calcium lactate	0.4	339.42	9.84	0.657	205.39	2.02
Acetic acid 5%	20 ml	436.01	9.37	0.656	263.83	2.47

reason why magnesium sulfate is rarely used alone as a coagulant for firm tofu preparation. It is commonly used after mixing with other coagulants such as magnesium chloride and calcium chloride. "Modified nigari" is a popular name used for such type of coagulants (Hou, Chang, & Shih, 1997).

The chloride salts, calcium chloride and magnesium chloride were found to be rapid in its action of coagulating the soy proteins. A similar observation was also noticed by other researchers (de Man, de Man, & Gupta, 1986). After the addition of these coagulants into the soymilk, a quick formation of curd was observed with separation of whey. Visualized by naked eye as the separation of whey occurred, the coagulating speeds of the respective sulfate salts were found to be very slow. A time period of more than 8 min was required for the curd formation to occur when using the calcium and magnesium sulfate salts. The slow acting coagulants also gave a better yield of tofu than rapid acting ones. Used at the same concentration levels, sulfates gave a better yield of tofu than their respective chlorides. They also retained a higher concentration of isoflavones than those prepared from chlorides. However, among the sulfate and chloride salts, calcium sulfate at 0.4% level gave a higher yield of firm, but smooth tofu with a higher concentration of isoflavones in it.

3.3. Properties of tofu coagulated by other calcium salts

Calcium lactate and calcium acetate are rarely used as coagulants for tofu manufacture. However in this study, we checked the possibility of using these as suitable coagulants and their impact on the level of isoflavones in the prepared tofu. Calcium lactate was found to be a better coagulant than calcium acetate, in terms of the yield and retention of isoflavones in tofu. A tofu with lower yield contained less water and would be harder, which was well observed in the case of calcium acetate coagulated tofu. Details of the amount of whey, its pH and isoflavone contents are given in Table 4. The amount of whey released was also higher for calcium acetate tofu than for calcium lactate tofu. pH of these tofu wheys were found in a very narrow range of 5.90–5.95. A decrease in pH to less than 6 might be essential for soy protein coagulation (Lu et al., 1980), but even at the same pH, different coagulants behaved differently. For example, the hardness, gumminess and chewiness of the calcium acetate tofu were the highest while these values were the least for magnesium sulfate coagulated tofu (Table 3), though both the tofu wheys had similar pH.

3.4. Properties of tofu coagulated by acetic acid solution

A mild acetic acid solution is commonly used as a tofu coagulant, especially in Indonesia (Shurtleff & Aoyagi, 1979b). It is mainly aimed at increasing the shelf life of tofu and for imparting a mild acidic taste. In this study, a 5% acetic acid solution was used for the preparation of tofu. The yield of acetic acid coagulated tofu was found to be nearly the same ($p \leqslant 0.05$) as when using calcium chloride or magnesium chloride as coagulants (Table 2). The mechanism of action of an acidic coagulant in causing the soy protein to coagulate is probably different from that of calcium or magnesium salts, but the retention of isoflavones in acetic acid coagulated tofu is similar to some calcium or magnesium salt coagulated tofu. There are not any reports which analyzed the acetic acid coagulated tofu or its respective whey for their isoflavone contents. Neither could we find any reports that studied the isoflavone levels in tofus prepared by calcium and magnesium salts and an organic acid. The concentration of isoflavones in acetic acid coagulated tofu was found to be very similar ($p \leqslant 0.05$) to that of the isoflavone concentrations in magnesium chloride and calcium acetate coagulated tofu (Table 2). The pH of the whey separated from acetic acid coagulated tofu was found to be the least (5.29) among all the others. The pH decrease caused by acidic coagulants like acetic acid might aggregate proteins by weakening the electric repulsion and liberates the hydrated water of protein, while the binding of calcium to carboxyl groups of proteins might have brought about the association of proteins (Ono, Kaminogawa, Odagiri, & Yamauchi, 1976). Results of these studies showed the option of using a mild acetic acid solution as

Table 4
Amount of whey, pH and isoflavone concentrations in the released wheys obtained during the preparation of tofus

Coagulant type	Usage level (% w/w)	Whey M%	Whey obtained (g)	Whey pH	Isoflavones in whey (μg/g) wet wgt basis
Calcium sulfate	0.5	96.2	257.08	5.76	101.30 ± 0.47
Calcium sulfate	0.4	96.2	249.27	5.77	102.12 ± 0.18
Calcium chloride	0.5	95.9	275.02	5.62	99.23 ± 0.45
Calcium chloride	0.4	96.1	271.10	5.70	103.35 ± 0.72
Magnesium sulfate	0.5	95.8	238.36	5.91	106.47 ± 0.13
Magnesium sulfate	0.4	95.8	246.89	5.91	111.11 ± 1.34
Magnesium chloride	0.5	95.8	279.11	5.74	103.88 ± 0.58
Magnesium chloride	0.4	96.2	265.60	5.84	103.73 ± 0.38
Calcium acetate	0.5	95.8	292.79	5.90	98.98 ± 0.58
Calcium acetate	0.4	96.0	293.15	5.91	104.72 ± 0.83
Calcium lactate	0.5	96.2	262.02	5.92	105.19 ± 0.15
Calcium lactate	0.4	96.2	247.45	5.95	109.51 ± 0.54
Acetic acid 5%	20 ml	96.7	263.43	5.29	102.79 ± 1.04

Whey M% = percentage moisture in whey. Isoflavones in whey given as the means ± SD.

an alternative for calcium or magnesium salts for tofu preparations. However, the color measurement of this tofu showed the highest a^* value (0.657), indicating an increase in the redness and a low b^* value (22.36), indicating a decrease in the yellowness for this particular tofu.

3.5. Isoflavones in whey

Tofu whey is the residual liquid separated during the production of firm tofu. Removal of whey by pressing is requisite, in order to obtain a compact and close network of firm tofu. The amount of whey released also varied with the type of coagulant used for tofu preparation. This variation in the amount of whey was due to the different water holding capacities of tofu. Tofu whey consists of complex sugars and are flatulence causing factors. It is a waste material and accounts for a major disposal material of concern for tofu manufacturers (Uzzan & Labuza, 2004). There are not any reports about the utilization of tofu whey either as a nutritional liquid or as a modified product suitable for human consumption. Recently, it has been used as a medium for the production of lactic starters (Thi, Champagne, Lee, & Goulet, 2003). However, wheys are also a source of minor proteins (Kao, Su, & Lee, 2003). Isoflavones might form complexes with protein in soybean, which can be released together with whey during the coagulation process. Wheys were analyzed for their isoflavone concentrations in this study and a significant amount of isoflavones were found to be lost into the whey. The isoflavones in tofu whey obtained by different coagulants are shown in Table 4. The amount of isoflavones lost into the whey varied with the type of coagulants used for tofu preparation. On an average 104.02 μg of isoflavones per gram of whey were lost into the tofu wheys, expressed on a wet weight basis. Release of wheys might be essential during a firm tofu preparation, however, steps to minimize the drain of proteins and associated isoflavones into the whey is crucial in order to enhance the functional value of the tofu. The need for proper utilization of this low cost by-product as a source of isoflavones or as a functional liquid product becomes very essential.

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