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Effect of carrageenan on yield and properties of tofu

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Abstract

The effects of the polysaccharide carrageenan and three types of coagulants namely, glucono-δ-lactone (GDL), calcium sulphate (CS) and calcium acetate (CA) on yield and physical properties of tofu (soybean curd), were investigated. Moisture content and yield of GDL-coagulated tofu were higher than that coagulated with CS and CA. Addition of carrageenan did not increase yield of GDL-tofu significantly but increased yield of CS-tofu and CA-tofu by 33 and 46.7%, respectively. Texture of CS-tofu was harder than CA- and GDL-tofu. Carrageenan brought about a significant decrease in hardness of CS- and CA-tofu which was more pronounced at the higher gum concentration. Hardness of GDL-tofu was not significantly altered. The tofu gels exhibited syneresis on storage at 4°C for 24 h in the order: CS-tofu < CA-tofu < GDL-tofu. Addition of carrageenan increased the syneresis, which was higher in CA-tofu than CS-tofu. On the other hand, GDL-tofu showed a decrease in syneresis in the presence of carrageenan.
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1. Introduction

Tofu (soybean curd) is made by coagulation of heated soya milk with a coagulant, followed by moulding and pressing the curd to draw the whey. The resulting curd or tofu is valued for its flavour and texture. Yield, quality and texture of tofu are influenced by several factors (Sun & Breene, 1991) such as variety of soybeans and storage conditions, time and temperature of soaking the soybeans, extent of heat-treatment of soymilk, type and concentration of coagulant and rate of stirring and coagulation temperature.

Various coagulants have been used in the preparation of tofu, each coagulant resulting in a product with textural characteristics varying from soft to firm and with moisture content ranging from 70 to 90% (de Man, J.M., de Man, L., & Gupta, 1986). Although calcium sulphate and glucono-δ-lactone are the coagulants of choice, other soluble salts of calcium, such as calcium acetate and calcium chloride have also been recommended for the coagulation of soymilk (Lim, de Man, J.M., de Man, L., & Buzzel, 1990; Tsai, Lan, Kao, & Chen, 1981; Shen, de Man, L., Buzzel, & de Man, J.M., 1991; Wang & Hesseltine, 1982; Sun & Breene, 1991).

The rheological and other physicochemical properties of many processed and convenience foods are determined to a large extent by the behaviour of proteins and polysaccharide components (Samant, Singhal, Kulkarni, & Rege, 1993). The protein–polysaccharide interaction has generated considerable research interest (Bernal, Smadja, Smith, & Stanley, 1987; Lin & Hansen, 1970; Stainsby, 1980; Tolstoguzov, 1986) and the industrial significance of these interactions for food texture modification has been documented (Antonov, Grinberg, Zhuravskaya, & Tolstoguzov, 1980). Understanding the mechanisms involved in the interactions between these components is important to exploit their potential to meet new technological requirements.

Polysaccharides play a key role in modifying the textural properties of protein food systems. Carrageenans, a group of sulphated linear polysaccharides of D-galactose, and 3,6 anhydro-D-galactose are used extensively in the food industry for their thickening, stabilizing and gelling properties (Trius & Sebranek, 1996). Being negatively charged polysaccharides over a wide range of pH, carrageenans are capable of forming complexes with proteins in the presence and absence of calcium ions (Bernal, Smadja, Smith, & Stanley, 1987; Glicksman, 1983). There have been several studies on carrageenan-milk protein interaction (Hansen, 1968; Hood & Allen, 1977; Ozawa, Niki, & Arima, 1984; Schmidt & Smith, 1992; Xu, Stanley, Goff, Davidson, & Le Maguer, 1992). The unique

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interaction of carrageenan with the casein micelle has led to its widespread usage in the dairy industry. Addition of κ- or ι-carrageenan to milk has been shown to increase yield of cottage cheese by 10–20% (Kailasapathy, Hourigan, & Nguyen, 1992). Studies on carrageenan-meat protein interaction, and the effect on functional properties, have also been carried out (Foegeding & Ramsey, 1987; DeFreitas, Sebranek, Olson, & Carr, 1997; Shand, Sofos, & Schmidt, 1994; DeFreitas, Sebranek, Olson, & Carr, 1997).

Tofu manufacturers are concerned, firstly with the yield which is of economic importance, and secondly, texture which determines its acceptability. In the course of tofu preparation, proteins in the soymilk are coagulated with a concomitant release of curds and whey; the curds are filtered off and moulded into shape under pressure. A large amount (approximately one third) of the original soybean mass is unavoidably lost in the whey during the first filtration step (van der Riet, Wight, Cilliers, & Datel, 1989). The whey is a byproduct of relatively low nutritive value and has the disadvantage of containing appreciable amounts of flatulence-causing carbohydrates (Liener, 1981). The objective of our study at the outset was to study the feasibility of increasing the yield of tofu by exploiting the carrageenan–protein interaction, and to determine how the yield and physical properties of tofu are influenced by carrageenan addition to soymilk prior to coagulation by calcium sulphate, calcium acetate and glucono-δ-lactone.

2. Materials and methods

2.1. Materials

Green maple leaf brand of Canadian soybean variety was purchased from a local supplier. The seed density was 18.8 g/100 beans.

Food grade calcium sulphate and glucono-δ-lactone were procured from a local chemical supplier. Calcium acetate was purchased from BDH Chemicals Ltd., Poole, England. Food grade carrageenan (Grindsted SL 320), with 26% galactose and 21% sulphate, was obtained through the courtesy of Ms. Danisco Ingredients, Penang.

2.2. Preparation of tofu

Tofu was prepared by a modification of the methods proposed by Escueta, Bourne, and Hood (1986), Lim, de Man, de Man, L., Buzzel (1990) and Sun and Breene (1991). Washed soybeans (approximately 72 g) were soaked in 1 litre beakers at room temperature (28°C) for 16 h in 500 ml tap water. After the stipulated soaking time, the beans were drained and ground with 725 ml tap water in a Waring blender for 2 min at high speed. The mash was strained through a muslin cloth and pressed to obtain soymilk. The soymilk (710 ml) was heated to 95–98°C for 15 min in a steam-jacketed kettle and then cooled rapidly to 30°C in an ice bath and volume made up to 710 ml by adding cooled, boiled water. The required quantity of carrageenan (1 or 2 g l^{-1}) was added to the soymilk and heated to 85°C in a water bath. The hot soymilk (700 ml) was poured into 100 ml water at 70°C containing coagulant at the respective concentration in a 1 litre beaker. Calcium sulphate (CS) and glucono-δ-lactone (GDL) were added at a level of 0.02 mol l^{-1} while calcium acetate (CA) was added at 0.01 mol l^{-1}. The 800 ml soymilk-coagulant suspension was held at 70°C for 15 min in a water bath for 15 min to ensure that coagulation occurred. The curd thus formed was transferred to a specially designed mould (11 cm diameter and 10.4 cm height) lined with cheese cloth. The whey was drained off naturally for 10 min and the curd was pressed for 30 min by placing a 1.3 kg weight on the 86.6 cm^2 plate covering the curd.

2.3. Analyses

2.3.1. Yield, solid recovery and proximate analyses

Yield of tofu was expressed as fresh weight of tofu obtained from 700 ml of soymilk. Moisture content was determined by drying 5 g of fresh tofu at 105°C in an air oven to constant weight (Tsai, Lan, Kao & Chen, 1981). Total solids in whey was determined by drying 10 ml of the whey at 105°C in an air oven for 24 h. Total protein was determined by the micro Kjeldahl method (AOAC, 1975) on air-dried tofu samples and using the factor N × 6.25 to convert nitrogen to protein. pH of the tofu samples was measured with a digital pH meter with a glass electrode (Metrohm AG, Switzerland).

2.3.2. Syneresis

A modified method of Amstrong, Hill, Schrooyen, and Mitchell (1994) was employed. Three pieces of tofu samples of 1.5 cm diameter were weighed and filled into Visking tubing (2.5 cm diameter). The tube was wrapped with plastic wraps and tied to a wire frame placed over a 2 litre beaker in a hanging position for 24 h at 4°C. Percentage syneresis was calculated as the weight of water released from the tofu in 24 h divided by the weight of sample and multiplied by 100.

2.3.3. Texture profile of tofu

Textural properties of tofu were evaluated with TA.XT2 Texture Analyser (Stable Micro Systems, Goldaming, Surrey, UK) fitted with a 5 kg load cell. Cylindrical samples of tofu (20 mm diameter, 16 mm height) were compressed by a flat plate (4×4 cm) to 80% deformation. The parameter setting and operation of the instrument were accomplished through a PC with Texture Expert software version 1.0. The force and probe calibration procedure for the system were followed before actual tests. The test mode was set to 'Texture Profile in Compression'. The pre-test, test and post-test speeds were set to 2, 2 and 4 mm s^{-1}, respectively. Each test was repeated 10 times at room temperature. Coefficients of variation for all determinations were less than 8%.

2.4. Colour

Colour evaluation was performed on fresh tofu samples using a Hunterlab Model D25 Tristimulus Colorimeter, equipped with a D25 circumferential optical sensor. A standard white tile with reflectance values of $X = 83.24$, $Y = 85.23$ and $Z = 100.92$ was used as a reference. A representative sample was placed into a 6 cm Petri dish and covered to avoid stray light. Hunter L (lightness), +a (red) to −a (green), and +b (yellow) to −b (blue) were then determined for each sample. Each value represented a mean value of five replicate determinations. Coefficient of variations for all measurements were less than 3%.

2.5. Sensory evaluation

Nineteen untrained panellists, composed of adult males and females who were familiar with tofu, were used in each tasting session. Tests on overall acceptability, colour, flavour and mouthfeel were conducted using a 9-point hedonic scale. Samples were steamed before being served in the form of a cube (1.5 cm) in 90 ml vegetable soup. All samples were coded and always presented in a randomized arrangement.

2.5.1. Statistical design and analysis

Experiments were based on a randomised complete block design. Data were analysed using an ANOVA procedure of the MINITAB software version 10 for Windows (MINITAB Inc.). A two-way analysis of variance was conducted, with carrageenan concentrations and coagulant types as factors. When significance was indicated, means were separated using Fisher's Least Square Difference Test. Statistical tests were conducted at the 5% probability level.

3. Results and discussion

3.1. Yield and composition of tofu

One of the critical steps in the preparation of tofu is the addition of salt to precipitate soy protein. Calcium sulphate (CS) and glucono-δ-lactone (GDL) are traditionally used as coagulants in tofu manufacture, yielding tofu of uniform texture with high moisture content. Being practically insoluble (Lu, Carter, & Chung, 1980), use of CS demands skill to avoid variation in quality. Lu et al. (1980) proposed use of soluble salts such as calcium acetate (CA), which yielded tofu of acceptable quality at half the levels of CS and GDL. Hence, in the present study, we decided to use CS and GDL at 0.02 mol l^{-1} and CA at 0.01 mol l^{-1}.

Yield of tofu in this experiment was of the order: GDL tofu > CS-tofu > CA-tofu, which is in agreement with the findings of Tsai et al. (1981) and Shen et al. (1991) (Fig. 1). Moisture content of GDL-tofu was higher than CS- and CA-tofu, which is reflected in the lower yield of whey (Table 1). Moisture retention, on a protein basis, was higher for GDL-tofu, followed by CA-tofu and CS-tofu. The lower pH of GDL-tofu reflects the isoelectric precipitation of soyproteins by the release of protons from GDL (Smith & Circle, 1972). Both CS- and CA-tofu gave a clear whey, indicating that the level of coagulant added was sufficient for complete coagulation of the soyproteins. The solids in whey are most probably soluble sugars and low molecular weight proteins. The variation in the whey volume was most likely due to a change in the water-holding capacity of tofu, which is affected by carrageenan.

Addition of carrageenan to soymilk prior to coagulation resulted in significant increases in yields of tofu ($p < 0.05$). At a concentration of 1 g l^{-1} and 2 g l^{-1}, carrageenan increased the yield of CS-tofu by approximately 9.5 and 33%, respectively. Corresponding increases in yield for CA-tofu were 17.1 and 46.7%, respectively. The increase in yield was reflected by the higher moisture content of CS- and CA-tofu and a corresponding decrease in yield of whey (Table 1). With GDL-tofu, carrageenan at 1 g l^{-1} resulted in a significant increase in yield ($p < 0.05$) and decrease in whey volume, without affecting other parameters. Higher concentration of carrageenan, however, did not sustain the observed changes.

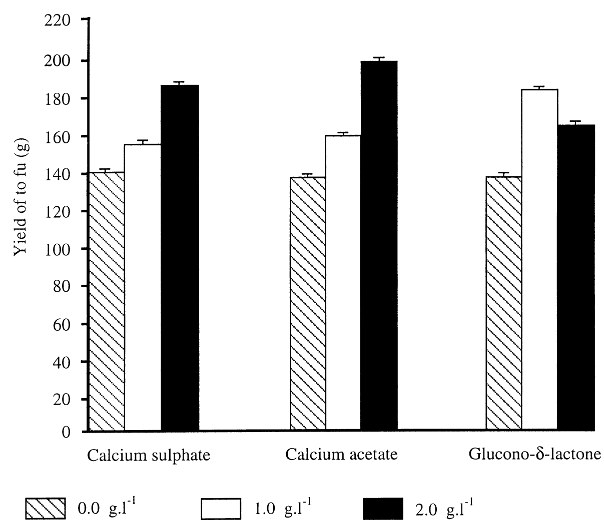

Fig. 1. Effect of coagulants and carrageenan on yield of tofu.

Table 1
Effect of carrageenan and coagulants on the composition of tofu[a]

Coagulant[b]	Carrageenan (g l^{-1})	Yield[c] (g)	Moisture (%)	Protein[d] (%)	Moisture retention[e] (g g^{-1})	pH	Whey (ml)
	0	140.5[f]	78.1[f]	11.5[f] (52.30)	6.82[f]	5.57	623[f]
Calcium sulphate	1.0	153.8[g]	81.3[g]	9.49[f] (50.83)	8.57[g]	5.62	590[g]
	2.0	186.9[h]	82.8[h]	8.77[f] (50.88)	9.44[h]	5.60	558[h]
	0	136.3[f]	79.0[f]	11.0[f] (52.29)	7.20[f]	5.62	622[f]
Calcium acetate	1.0	159.6[g]	82.4[g]	8.93[f] (50.86)	9.23[g]	5.82	596[g]
	2.0	200.0[h]	84.6[g]	7.45[g] (48.46)	11.36[g]	5.76	532[h]
	0	161.1[f]	81.3[f]	9.41[f] (50.29)	8.64[f]	4.52	586[f]
Glucono-δ-lactone	1.0	183.5[g]	83.1[f]	8.29[f] (48.94)	10.02[f]	4.55	561[f]
	2.0	164.2[f]	79.7[f]	10.41[f] (51.4)	7.66[f]	4.52	580[f]

[a] Means of quadruplicates. Coefficient of variation <5%. For each tofu, means with the same superscripts in the same column are not significantly different ($p > 0.05$).
[b] Calcium sulphate at 0.02 mol l^{-1}; Glucono-δ-lactone at 0.02 mol l^{-1}.
[c] Fresh weight basis from 700 ml soymilk.
[d] Figures in parentheses represent protein content on dry weight basis.
[e] Gram per gram protein basis (dry).

A two-step mechanism for the gelation of tofu proteins has been proposed by Kohyama, Sano, and Doi (1995). The first step is the heat-induced denaturation of the soyprotein which exposes the hydrophobic regions of the protein molecules in the native state to the outside. The denatured soyprotein is negatively charged (Kohyama & Nishinari, 1993) and the release of protons induced by GDL or calcium ions from the coagulant neutralises the net charge of the protein. As a result, the hydrophobic interaction of the neutralised protein molecules becomes more predominant and induces the random aggregation of proteins, leading to gel formation (de Man, de Man, L., & Gupta, 1986).

Being a sulphated polysaccharide, carrageenan can exist as a negatively charged polymer over a wide range of pH. Above the isoelectric point, polyvalent metal ions such as Ca^{2+} can form bridges between the negatively charged carboxyl groups of the protein and the ester sulphate groups of the polysaccharide. In such protein–polysaccharide–calcium systems, both the protein and the polysaccharide can interact independently with the calcium ions (Hughes, Ledward, Mitchell, & Summerlin, 1980). The extensive network structure formed by carrageenan could trap more water in the interstitial spaces of the gel, and reduce syneresis, resulting in the observed yield increase. The higher moisture retention in CA-tofu, compared to CS-tofu, is probably due to the differences in the gel network effected by the ionic strengths of the coagulants and/or the effect of the different anions on the water-holding capacity of the soyprotein gels (Wang & Hesseltine, 1982).

The observed effects of carrageenan in the present study can also be explained on the basis of the two component type II mixed gel networks proposed by Morris (1986). In the first instance, normal aggregated soyprotein gels are formed on the addition of calcium salts or GDL. When calcium salts are employed, the pH being above the isoelectric point, soyprotein molecules will be negatively charged. The protein and the polysaccharide can interact on their own with the metal ions or with each other, with or without the involvement of metal ions. Similar ionic interactions between κ-casein and κ-carrageenan and the resulting mixed coupled gel networks are held responsible for the stabilization of milk products (Lin & Hansen, 1970; Ozawa, Niki & Anna, 1984; Schmidt & Smith, 1992). The formation of a coupled gel network of carrageenan and soyprotein around the aggregated tofu gel could bind more water, increasing yield and reducing hardness. On the other hand, addition of GDL as a coagulant results in a tofu gel at isoelectric point which precludes formation of coupled mixed gel networks of carrageenan and soyprotein. However, weak gels of carrageenan with the calcium or potassium constituents of soymilk would still be possible. This may explain the lack of effect of carrageenan on the yield and hardness of GDL-tofu as compared to CA- and CS-tofu, although syneresis was reduced perhaps due to better moisture retention.

3.2. Textural properties

Textural parameters of the tofu were evaluated according to definitions given by Bourne (1982). Fig. 2 shows a typical force-time curve obtained after the application of 80% uniaxial compression. Similar curves were obtained from tofu made with the addition of carrageenan. As shown in Table 2, hardness, cohesiveness, elasticity, gumminess and chewiness decreased with increasing concentration of carrageenan for CA- and CS-tofu. However, effect of carrageenan on the other two parameters, i.e., adhesiveness and fracturability was not consistent. In most cases, CS-tofu

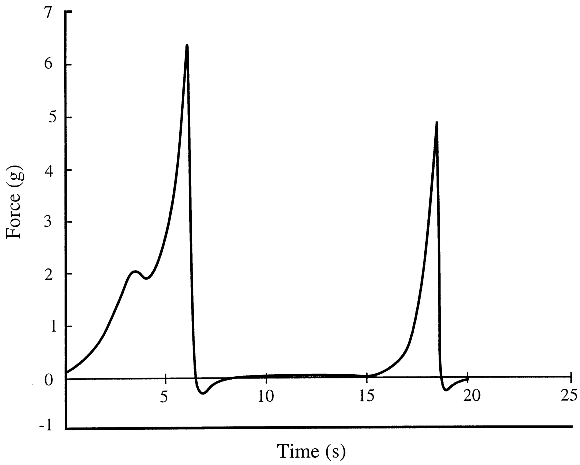

Fig. 2. Typical texture profile curve for tofu subjected to 80% compression.

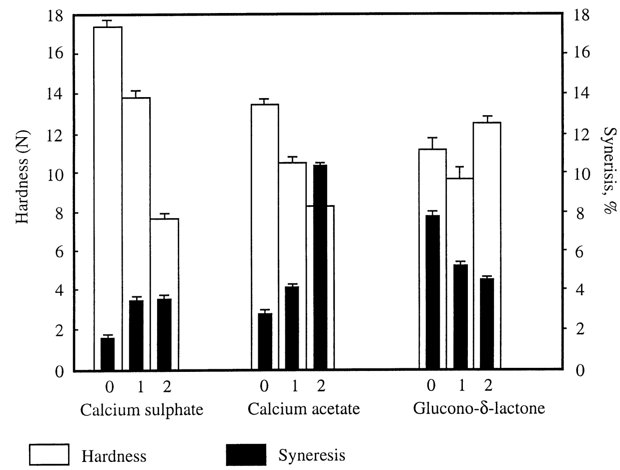

Fig. 3. Effect of coagulants and carrageenan on hardness and syneresis of tofu. The numbers on x-axis represent carrageenan concentrations (g l^{-1}).

(without carrageenan) showed highest values for hardness, gumminess, elasticity and chewiness but, with addition of carrageenan, especially at 2 g l^{-1}, GDL-tofu showed highest values for hardness and gumminess.

Fig. 3 shows the effect of coagulants and carrageenan on hardness and syneresis of tofu. Texture analysis revealed that CS-tofu was harder than CA- and GDL-tofu, which is in contrast to the findings of Shen et al. (1991). It has been reported that coagulant concentration and type of anion affect hardness of tofu (Sun & Breene, 1991; Wang & Hesseltine, 1982). Probably the way protein interacts with calcium and other constituents, e.g. phytic acid, in soy milk and anions to form the microstructure could determine the hardness of tofu (Lim, de Man, de Man, L., & Buzzel, 1990; Wang & Hesseltine, 1982). Addition of carrageenan, at 1 g l^{-1} and 2 g l^{-1} resulted in a significant decrease ($p<0.05$) in hardness of CS-tofu (Fig. 2) by 21.2 and 55.9% and that of CA-tofu by 21.6 and 38.3%, respectively. However, the effect of carrageenan on hardness of GDL-tofu was insignificant ($p>0.05$).

Gelation of food protein involves heat denaturation followed by aggregation. If aggregation is relatively slower than denaturation an ordered structure will be promoted, by allowing the denatured molecules to orient themselves in a systematic fashion prior to aggregation (Hermansson, 1978). Conditions that retard intermolecular interaction will result in a more homogeneous and regular network and consequently a stronger gel (Bernal, Smadja, Smith & Stanley, 1987). Recently, soybean protein composition has been related to texture of tofu (Murphy, Chen, Hauck, & Wilson, 1997). Contrary to expectations, interaction between soyprotein and carrageenan resulted in reduction of the hardness of tofu, which is a desirable sensory attribute.

Table 2
Texture profile analysis of tofu[a]

Types of tofu	Carrageenan (g l^{-1})	Texture parameters				
		Hardness (N)	Springiness (mm)	Chewiness (N mm)	Cohesiveness	Gumminess (N)
	0.0	17.59[b]	0.09[b]	0.40[b]	0.18[b]	3.87[b]
CS-tofu	1.0	13.86[c]	0.08[c]	0.20[c]	0.07[c]	2.45[c]
	2.0	7.75[d]	0.02[d]	0.02[d]	0.00[d]	0.54[d]
	0.0	13.49[b]	0.07[b]	0.18[b]	0.18[b]	2.46[b]
CA-tofu	1.0	10.58[c]	0.05[c]	0.07[c]	0.13[c]	1.42[b]
	2.0	8.33[c]	0.03[d]	0.04[c]	0.08[c]	0.66[c]
	0.0	11.15[b]	0.06[b]	0.12[b]	0.13	1.50[b]
GDL-tofu	1.0	9.72[b]	0.04[b]	0.06[b]	0.11	1.07[b]
	2.0	12.56[b]	0.07[b]	0.16[b]	0.15	1.89[b]

[a] Means of ten replicates. Coefficient of variation <8%. For each type of tofu, the same superscripts in the same column are not significantly different ($p>0.05$).

3.3. Syneresis

On storage at 4°C for 24 h, syneresis was low in CS- and CA-tofu, but higher in GDL-tofu (Fig. 2). Carrageenan enhanced syneresis in CA-tofu compared to CS-tofu. Interestingly, syneresis was reduced by carrageenan in GDL-tofu. Increase of syneresis from the curd could be due to increased bonding occurring during storage, making the protein matrix more dense or compacted (Sun & Breene, 1991). In GDL-tofu, the decrease in syneresis could result from enhanced water retention in the gel microstructure.

3.4. Colour

Tofu of good quality is generally white or light-yellow in colour. All the tofu samples prepared in this study had a light yellow colour. CA-tofu with 2 g l^{-1} carrageenan showed a slightly higher L, 'a' and 'b' values (Table 3). The greenness ('a' value) increased with increasing concentration of carrageenan in CS-tofu. In contrast, 'a' values decreased with increase in carrageenan concentration for GDL-tofu. No significant effect ($p > 0.05$) on 'a' value was noted for CA-tofu. Two-way analysis of variance indicated that both coagulant and carrageenan affected the colour of tofu significantly. The effect of coagulants on colour of tofu was greater than the effect of carrageenan. In addition, an interaction between coagulant and carrageenan was evident.

3.5. Sensory characteristics

Tofu samples were evaluated by a panel for colour, flavour, mouthfeel and overall acceptability on a 9-point scale. Tofu prepared with CA/2 g l^{-1} carrageenan and CA and CS alone (without carrageenan) were evaluated (Table 4). These three representative samples were chosen for sensory evaluation because of the pronounced effect on the hardness of CA-tofu brought about by addition of carrageenan. The acceptability scores ranged from 5.05 to 7.53 which are moderate even though tofu is very much a customary part of the Malaysian diet. Highest scores were given to CA-tofu with 2 g l^{-1} carrageenan, which had a smooth, soft but firm texture.

Table 3
Effect of coagulants and carrageenan on the colour of tofu[a]

Types of tofu	Carrageenan (g l^{-1})	Hunter values		
		L	a	b
CS-tofu	0.0	84.13[b]	−0.27[b]	14.17[b]
	1.0	83.83[b]	−0.73[c]	14.20[b]
	2.0	83.77[b]	−0.87[c]	14.82[b]
CA-tofu	0.0	84.05[b]	0.08[b]	14.87[b]
	1.0	83.73[c]	0.20[b]	14.87[b]
	2.0	84.47[d]	0.12[b]	14.90[b]
GDL-tofu	0.0	82.75[b]	0.33[b]	13.82[b]
	1.0	82.25[b]	0.25[b]	13.82[b]
	2.0	82.53[b]	−0.07[c]	13.60[b]

[a] Means of six replicates. Coefficient of variation <3%. For each type of tofu, the same superscripts in the same column are not significantly different ($p > 0.05$).

Table 4
Effect of coagulants and carrageenan on sensory characteristics of tofu[a]

Sensory parameters	CA + carrageenan tofu[b]	CA-tofu[c]	CS-tofu[d]
Colour	7.53	7.05	7.00
Flavour	6.74	6.26	5.84
Mouthfeel	6.68	5.95	5.05
Overall acceptability	6.63	6.21	6.05

[a] On the 9-point hedonic scale.
[b] Calcium acetate 0.1 mol l^{-1} and carrageenan at 2 g l^{-1}.
[c] Calcium acetate only at 0.1 mol l^{-1}.
[d] Calcium sulphate only at 0.2 mol l^{-1}.

4. Conclusions

The present study has confirmed the feasibility of replacing calcium sulphate by calcium acetate for the coagulation of soybean milk in the manufacture of tofu. Furthermore, the study has demonstrated the possibility of increasing the yield and moisture content of tofu through use of a plant hydrocolloid, i.e. carrageenan. Carrageenan at relatively low concentration (2 g l^{-1}) has been shown to enhance the water holding capacity of the soybean protein gel without affecting the hardness of the GDL-tofu significantly but brought about a significant decrease in hardness of CS- and CA-tofu. Carrageenan has also been shown to have an interaction with the coagulant. Optimization of conditions for gelation of soyprotein and the carrageenan-induced modifications of the gel network may be useful in modifying the tofu processing technology with tangible commercial advantages.

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