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Production of hard tofu from calcium fortified soybean milk and its chemical and sensory properties

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Abstract

Hard tofu, a product from soybean milk, is one of the common food products in Asia. Soybean milk with soybean to water ratio varying from 1:6 to 1:8 was prepared in triplicate. The weight of soybean used in all experiments was 200 g. Calcium salt used to fortify soybean milk was 120 mg Ca/100 ml together with sequestering and stabilizing agents. Calcium-fortified hard tofu was produced by coagulation of the calcium-fortified soybean milk with saturated $MgSO_4$. Nutritive values and bioaccessibility of calcium in calcium-fortified tofu were determined. The yields of total solid and protein in calcium-fortified tofu prepared from soybean milk with a soybean to water ratio of 1:6 were the lowest (78.9±5.4 and 33.0±1.6 g, respectively). In contrast, the highest yields of both parameters (total solid, 90.5 ±3.8 g and protein, 45.4±4.2 g) were obtained in tofu prepared from soybean milk with the ratio of 1:8. The tofu also contained the highest amount of total calcium (1947±54 mg). As a result, the soybean to water ratio of 1:8 was selected to be used for calcium-fortified tofu production with and without adding of sequestering and stabilizing agents. The yield of total solid in the calcium-fortified tofu of both types was in the same range. However, the total protein yield of tofu with additives was significantly higher than that without the additives, 45.4±4.2 g compared to 37.0±1.8 g, respectively. Carrageenan, used as stabilizing agent in preparation of the fortified tofu, may play an important role in holding water and protein, resulted in higher tofu yield with higher protein content. The addition of the sequestering and stabilizing agents did not show any significant effects on the total amount of calcium in the calcium-fortified tofu. The calcium fortified tofu was well accepted, however, its calcium bioaccessibility was lower than that of milk powder which is likely due to the presence of phytate.

Keywords: Calcium-fortification, Hard tofu, Soybean milk

1. Introduction

Calcium plays important roles in human body. Adequate calcium intake prevents osteoporosis and reduces the risk of hypertension (1). The Thai Recommended Daily Intake (Thai RDI), based on 2,000 kcal intake for adults and children at 6 or more years of age, is 800 mg/day (2). The survey of food and nutritional status conducted by the Ministry of Public Health in 1995 revealed that Thai people did not have adequate calcium intake, the average calcium intake was 344 mg/person/day.

Soybean, whole dry seed, contains 343 mg Ca/100g (3). Based on the amount of calcium per serving (30 g) which is about 13% Thai RDI, it can be considered as a good source of calcium. Soybean can be processed into many food products such as soybean milk, tofu, fermented soybean, tempeh, soy sauce, soybean oil (4, 5). However, soybean milk has been recognised to contain much lower calcium than cow's milk (20-30 mg/100 ml compared to 120 mg/100 ml, respectively). In 2000, Chaiwanon *et. al.* (6) developed calcium-fortified soybean milk with good calcium bioaccessibility and sensory acceptability. Soybean curd or tofu is a high protein product widely consumed in Asian countries and among vegetarians. It is generally made by protein coagulation of heated soybean milk with a coagulant, followed by moulding and pressing the curd to drain the whey (5, 7). Calcium-fortified tofu could provide another choice of calcium source to the consumers. Therefore, this research is aimed to produce hard tofu from calcium-fortified soybean milk and to evaluate its chemical and sensory properties.

2. Materials and method

Soybean seeds, Chiangmai-60 variety, were purchased from Chiangmai province in the northern part of Thailand. They were kept in a cold room at 7-8^{0}C until used and were allowed to reach room temperature before tofu processing.

Food grade coagulant ($MgSO_4.7H_2O$) was provided by Vidhyasom Co., Ltd. Bangkok, Thailand. Food grade calcium salt (tri-calcium phosphate) and sequestering agent (potassium citrate) were obtained from Tinnakorn Chemical and Supply Co., Ltd., Thailand. Stabilizing agent (carrageenan) was obtained from Wang Chemicals Co., Ltd., Thailand.

The Central-Healthy Electric Food Grinder (Central Industrial Supply, Co., Ltd., Bangkok, Thailand) was used as a grinder to extract soybean milk and the residue was automatically separated.

2.1 Preparation of calcium-fortified soybean milk

Calcium-fortified soybean milk containing 120 mg Ca/100 ml was prepared according to Chaiwanon *et al.* (6), with the proportion of soybean:water varying from 1:6 to 1:8 (w/v). Three individual sets of each formula were prepared using an initial amount of 200 g soybean seeds. The volume of the prepared soybean milk, their total solid, protein and calcium content were determined, and their yields (amount of components per total volume of heat-treated calcium-fortified soybean milk) were calculated.

2.2 Preparation of hard tofu from calcium-fortified soybean milk

High calcium hard tofu was prepared by coagulation of the heat-treated calcium-fortified soybean milk with saturated magnesium sulphate. The proportion of saturated $MgSO_4$ to soybean seeds was 10 ml to 100 g. While mixing, saturated $MgSO_4$ was added little by little into calcium-fortified soybean milk until clear separation of soy protein curd from whey appeared. The coagulated soy protein was immediately transferred into the wooden mould, size 8.5 cm x 8.5 cm x 4 cm, lined with muslin-cloth. Then the cloth was folded over the top of bean curd. The whey was drained off naturally for 3-5 min and the curd was evenly pressed by placing a 4.5 kg weight on the 595 cm^2 plate (7.5 g/ cm^2) on top of the curd for an hour or until dripping ceased. Three individual sets of tofu from each formula of calcium fortified soybean milk were prepared. Tofu from non-fortified soybean milk (1:8 soybean to water ratio) prepared by the same method was used as a control. All the tofu were weighed, and analysed for total solid, protein and calcium content. Their yields and per cent recoveries were calculated. The optimum soybean to water ratio for soybean milk preparation was identified based on these parameters.

2.3 Effect of sequestering and stabilizing agents on tofu quality

The optimum proportion of soybean to water ratio obtained from the above experiment was used to prepare calcium-fortified tofu. Three individual sets of tofu were prepared with and without adding of sequestering and stabilizing agents in the soybean milk, Yields and per cent recoveries of total solid, protein and calcium in the prepared calcium-fortified tofu were compared.

2.4 Chemical and sensory evaluation

The Official Methods of Analysis of AOAC International (8) were used for the determination of total solid, crude protein, calcium, phosphorus and phytate in calcium-fortified soybean milk and tofu. Two types of calcium-fortified tofu, prepared from calcium-fortified soybean milk (with and without adding sequestering and stabilizing agents), were investigated for calcium bioaccessibility following the simulated gastrointestinal digestion procedure of Miller *et. al.* (9). Dry milk powder and non-fortified tofu were used as control samples.

Sensory evaluation (general appearance, odor, taste, texture and overall liking) was conducted with 50 panelists to determine the liking of calcium-fortified tofu. The panelists were faculty staffs and graduate students of the Institute of Nutrition, Mahidol University (INMU) who have been familiar with tofu. Tests on odor, taste, texture and overall liking were conducted using a nine-point hedonic scale (1 - dislike extremely; 5 - neither like nor dislike; 9 - like extremely). Five categories just-about-right scales, ranging from 1 - much too light/ soft, 3 - just about right, 5 - much too dark/hard, were used to evaluate sensory characteristics of colour and hardness. All tofu samples were stored overnight in water at 5-7°C and steamed before being served in the form of a cube (1.5 cm x 1.5 cm x 0.5 cm) with soy sauce. They were coded in three-digit numbers and presented in a randomised arrangement.

2.5 Statistical analysis

The data on tofu quality and sensory properties are given as Mean $\pm$ S.D. Any significant difference in the tofu quality due to the difference in the soybean to water ratio of the soybean milk was evaluated using SPSS v.9. Kruskal-Wallis H test type. Mann-Whitney U test was used to do pair wise comparison to indicate any soybean to water ratios which showed significant difference in the tofu quality. It was also used to evaluate the sensory properties of the prepared tofu (non-fortified and calcium-fortified tofu). The significant difference was established at *p-value* ≤ 0.05.

3. Results and discussion

3.1 Effects of soybean to water ratio on the quality of prepared calcium-fortified tofu

There are many factors involved in the quality of soybean milk which affect the quality of tofu, i.e., soybean variety, type of soybean seeds (whole or split), coagulant (types and concentration), soybean to water ratio (7, 10-15). Soybean seeds, Chiangmai-60, have been widely used in Thailand for making soybean products. The whole seeds were used in order to get the better soybean milk quality in terms of freshness, flavour, and extractability of protein (10). Soybean milk is the initial product affecting the tofu quality. The effects of using different soybean to water ratios to prepare calcium fortified soybean milk on the extracted total solid and protein were then evaluated.

As shown in Table 1, the calcium-fortified tofu produced from 1:8 soybean milk gave the highest total solid and protein yields (90.5±3.8 and 45.4±4.2 g, respectively), whereas that produced from 1:6 soybean milk gave the lowest yields (78.9±5.4 and 33.0±1.6 g, respectively). Total calcium yield in the calcium-fortified tofu prepared from various formulas of soybean milk were significantly different, ranged from 1559±40 to 1947±54 mg. Per cent recovery of total solid (82±9% to 85±6%), protein (84±3 to 100±9%) and calcium (103±7 to 107±8%) in the prepared calcium-fortified tofu were not significantly affected by varying of the soybean to water ratios except that of protein from 1:6 soybean to water ratio. This implied that the amount of the coagulant (saturated $MgSO_4$) was sufficient to completely precipitate protein from various calcium-fortified soybean milk and all calcium in the calcium fortified soybean milk was incorporated into the tofu curd along with the protein.

According to the high yields and recoveries of total solid, protein and calcium of the tofu prepared from soybean to water ratio of 1:8 (Table 1), it was selected to be used for studying the effect of sequestering and stabilizing agents on tofu quality.

Table 1. Yield of total solid, protein and calcium and their per cent recoveries (Mean ± SD.) in calcium-fortified tofu prepared from different soybean to water ratios

Soybean : Water	Total solid		Protein		Calcium	
	Yield (g)	% Recovery	Yield (g)	% Recovery	Yield (mg)	% Recovery
1 : 6	78.9 ± 5.4^{b}	83 ± 3	33.0 ± 1.6	84 ± 3^{b}	1559 ± 40^{c}	107 ± 8
1 : 7	85.0 ± 8.0^{ab}	82 ± 9	43.3 ± 0.6	100 ± 6^{a}	1705 ± 61^{b}	103 ± 7
1 : 8	90.5 ± 3.8^{a}	85 ± 6	45.4 ± 4.2	100 ± 9^{a}	1947 ± 54^{a}	104 ± 3
Significant	*	ns	ns	*	*	ns

^{a,b} Mean values showing different superscript letter differ significantly ($p<0.05$)

ns = not statistically significant

3.2 Effect of sequestering and stabilizing agents in calcium-fortified soybean milk on tofu quality

The quality of calcium-fortified tofu prepared from soybean milk with (Tofu-A) and without (Tofu-NA) sequestering and stabilizing agents are shown in Table 2. The appearance of Tofu-NA was closer to the hard tofu purchased from the market than Tofu-A. Although total solid yields (about 90 g) and their recoveries (about 85%) in two types of tofu were not different, the Tofu-NA had lower protein yield and its recovery than Tofu-A (37.4±1.7 g compared to 45.4±4.2 g and 87±6% compared to 100±9%, respectively). In addition, the derived Tofu-NA had slightly lower in total weight than Tofu-A (298±10 and 364±14 g/ 200 g initial soybean seeds, respectively). It was likely that carrageenan which was used as a stabilizing agent in calcium-fortified soybean milk plays an important role in holding water and protein in the derived tofu. Being a sulphated polysaccharide, carrageenan can exist as a negatively charged polymer over a wide range of pH and can form complexes with protein in the presence and absence of calcium ions (16). Above the isoelectric point, which was the pH of soybean milk mixture (pH about 6.2) during tofu production, polyvalent metal such as Ca^{2+} can form bridges between the negative charged carboxyl groups of the protein and the sulphate groups of the polysaccharide (carrageenan). The extensive network structure formed by carrageenan could trap more water in the interstitial spaces of the gel (16), resulted in the higher protein yield and slightly higher weight in the calcium-fortified tofu with adding the agents than that without the agents.

Potassium citrate was normally used as a sequestering agent to protect protein coagulation during heating of soybean milk fortified with some forms of calcium salt, i.e. calcium lactogluconate (17). It was found to extend the stability of calcium salt in calcium-fortified soybean milk (6). However, in this study, there was no effect of using sequestering and stabilizing agents on calcium recovery in calcium-fortified tofu. The amount of total calcium and its per cent recovery in calcium-fortified tofu with and without adding the agents were not significantly different.

Although the use of additives provided a slightly higher yield and per cent recovery of protein in the calcium-fortified tofu, it did not significantly affect those parameters of total solid and calcium. Furthermore, the tofu without additives was more similar in appearance to commercial tofu found in the market. Therefore, it was possible to omit the use of additives in order to reduce the cost and avoid the complexity of this processing step. Tofu without additives was then selected for sensory evaluation.

Table 2. Yield of total solid, protein and calcium and their per cent recoveries (Mean ± SD.) in calcium-fortified tofu with and without adding sequestering and stabilizing agents

Soybean : Water[1]	Total solid		Protein		Calcium	
	Yield (g)	% Recovery	Yield (g)	% Recovery	Yield (mg)	% Recovery
1 : 8 A	90.5 ± 3.8	85 ± 6	45.4 ± 4.2 [a]	100 ± 9	1947 ± 54	104 ± 3
1 : 8 NA	88.9 ± 3.2	86 ± 6	37.4 ± 1.7[b]	87 ± 6	1901 ± 180	100 ± 7
Significant	ns	ns	*	ns	ns	ns

[1] tofu was prepared from calcium-fortified soybean milk with (A) or without (NA) adding agents

[a,b] Mean values showing different superscript letter differ significantly ($p<0.05$)

ns not statistically significant

3.3 Chemical and sensory properties of calcium-fortified tofu

Total solid, crude protein, calcium, phosphorus, phytate content in non-fortified and calcium-fortified tofu, with and without adding sequestering and stabilizing agents, and their *in vitro* calcium bioaccessibility are presented in Table 3. Calcium content in 100 g of non-fortified and calcium-fortified tofu with and without the additives were 62, 518 and 693 mg, respectively. Calcium fortification provided higher content of phosphorus in the derived tofu because the tri-calcium phosphate fortificant was used, resulting in the better calcium phosphorus ratio of 1:1.2, compared to 1:9.4 of non-fortified tofu. Similar level of phytate content was found in non-fortified and calcium-fortified tofu, 530 and 516 mg/100 g, respectively.

Table 3. Nutritive values of tofu and calcium-fortified tofu[1]

Nutritive values per 100 g	Unfortified tofu	Calcium-fortified tofu	
		With agents	Without agents
Total solid, g	28.3	24.8	32.2
Protein (total N x 5.71), g	15.4	12.4	14.4
Lipid, g	9	NA	9
Calcium, mg	62	518	693
Phosphorus, mg	580	NA	815
Ca:P ratio	1:9.4	NA	1:1.2
Phytate, mg	530	NA	516
Ca dialysability[2], %	14	13	11
Calcium dialysability of dry milk powder = 26%, based on 25 mg Ca = 24%, based on 250 mg Ca			

[1] tofu prepared from soybean to water ratio of 1:8

[2] based on tofu with 7 g protein

NA not analyzed

The calcium bioaccessibility (based on the amount of one serving tofu which contained 7 g protein and 290-330 mg Ca) of calcium-fortified tofu with additives was 13% compared to 11% of that without additives and 14% of non-fortified tofu. These values were much lower than those of dry milk powder, 26% at 25 mg Ca and 24% at 250 mg Ca. The finding was in accordance with those reported for soy-based formula (11.4%) by Miller *et al.* (cited by 18) and for cooked soybean seeds (11.1%) by Kamchan *et al.* (19). High phytate, naturally present in the protein complex of soybean, is believed to be the main factor involving in the low bioaccessibility of calcium in both types of prepared tofu (contained more than 500 mg phytate/100 g tofu). This is because phytate is the hexaphosphate ester of inositol, the stereologic conformation of its highly polar compound reveals its high affinity toward di- and tri-valent cations, such as copper, iron, zinc and calcium (20).

For the effect of additives, potassium citrate has been used as a sequestering agent. This is because of its chelating property - citric ions in the molecule can chelate with calcium, thus forming a chain structure. The adjacent chains of cation-citrate complex are linked by extensive hydrogen bonding through water molecule (21). However, the effect of potassium citrate on calcium bioaccessibility has not been reported. Carrageenan was used as the stabilizing agent in calcium fortified soybean milk for tofu production. At the pH of the soybean milk during tofu production (about 6.2), Ca^{2+} can form bridges between the negatively charged carboxyl groups of the protein and the sulphate groups of the polysaccharide of carrageenan. The presence of carrageenan in calcium-fortified soybean products was reported to reduce calcium bioaccessibility (22, 23). The effect of each sequestering and stabilizing agent on calcium bioavailability should be investigated.

The sensory liking scores of non-fortified and calcium fortified tofu without adding sequestering and stabilizing agents are presented in Table 4. Commercial tofu was used as a control sample. Control and non-fortified tofu showed similar characteristics, except hardness, to those of calcium-fortified tofu. General appearance scores of the prepared tofu before tasting were in the range of "like slightly", with the average score of 6.5. The overall liking scores which represent the liking degree of all sensory characteristics of both prepared tofu were about 6, which means "like slightly". The liking scores of taste and texture were also about 6. Scores of odor were in the range of "like slightly to like moderately" (6.3, 6.4 and 6.9 for control tofu, non-fortified tofu and calcium fortified tofu, respectively). The prepared tofu showed significant difference in liking scores of hardness. The hardness score of control and non-fortified tofu (3.0 and 3.2, respectively) was just-about-right. Although the appearance of calcium-fortified Tofu-NA was closer to the hard tofu in the market, its hardness score from sensory evaluation was significantly higher than the non-fortified tofu. The higher total solid content found in the prepared Tofu-NA (32.2 g compared to 28.3 g of non-fortified tofu) is likely to be reflected in the harder texture. Other sensory characteristics were not different from the control. However, their overall liking scores were the same, about 6 (like slightly).

Table 4. Sensory liking scores[1] (Mean ± SD., n=50) of non-fortified tofu and calcium fortified tofu

Sensory Characteristics		Tofu[3]			Significant
		Control	Non-fortified	Ca-fortified	
Before taste	Appearance	6.3±1.3	6.5±1.2	6.6±1.1	ns
	Colour[2]	3.3±0.7	3.0±0.4	2.9±0.4	ns
After taste	Overall liking	6.6±1.3	6.4±1.4	6.3±1.7	ns
	Odor	6.3±1.7	6.4±1.4	6.9±1.4	ns
	Taste	6.7±1.3	6.4±1.5	6.2±1.6	ns
	Texture	6.6±1.3	6.3±1.4	5.9±1.8	ns
	Hardness[2]	3.0±0.5[a]	3.2±0.7[a]	3.6±0.9[b]	*

[a,b] Mean values showing different superscript letter differ significantly different ($p<0.05$)

ns not statistically significant

[1] nine-point hedonic scale ranging from dislike extremely, 1; neither like nor dislike, 5; like extremely, 9.

[2] five categories Just About Right scale ranging from much too light / soft, 1; just about right, 3; much too dark or hard, 5.

[3] non-fortified tofu purchased from a local market (control) and prepared from soybean to water ratio of 1:8 (non-fortified); calcium fortified tofu was prepared without adding agents

4. Conclusion

Various soybean to water ratios used to prepare calcium fortified soybean milk did not considerably affect the derived tofu quality. The soybean to water ratio of 1:8 was the optimum proportion for calcium-fortified soybean milk to produce tofu. Not including thesequestering and stabilizing agents in the calcium-fortified soybean milk did not affect the yield and recovery of total solid and calcium in the prepared tofu. However, it produced tofu with slightly lower total weight and protein yield. The derived calcium-fortified tofu contained calcium about 10 times higher than that of non-fortified tofu. Its calcium bioaccessibility was lower than that of dry milk powder (24-26%) due to the presence of high phytate. The sensory liking score of calcium-fortified tofu without additives was not different from that of non-fortified tofu and tofu purchased from the market. Their liking scores were "like slightly". The calcium-fortified tofu obtained from this research could be a new choice of calcium source to consumers and could decrease a calcium deficiency problem in Thailand.

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6. References

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