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Effect of partial replacement of soybean with chickpea to the nutritional and textural properties of tofu

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Abstract— Tofu is an oriental food that originated from China and later became a traditional cuisine in many South East Asia Countries, e.g. Indonesia, Singapore, and Thailand. The tofu was made by coagulating soy milk with salt to form curds, which further compress to form a solid block. Tofu usually uses soybeans as the main ingredients; however, in this experiment, the soybeans were partially replaced with chickpeas to increase the amount of dietary fibre and reduce fat levels in the final products. The tofu was made of soybean and chickpea at a ratio of 100:0 (control), 90:10, 80:20 and, 70:30. The products were evaluated based on their nutritional contents (crude protein, crude fat, and crude fibre), and textural properties using Texture Profile Analysis. Some analytical parameters, such as pH, moisture content, and colour were also studied. Partial replacement of soybean with chickpea decreased overall crude protein and fat content, whereas increased crude fibre. The presence of chickpea in the blends, up to 30% (w/w), had affected the tofu curd's protein stability, lowered their pH, and moisture content. A denser tofu curd was obtained by adding more chickpea to the product. The 70:30 blend has the highest hardness value compared to the other formulations. This low soy content mixture, however, can not produce a cohesive and springy tofu gel. The CIELAB colour space of the soybean/chickpea tofu cubes records a trend of increase in redness and yellowness values for tofu with chickpea.

Keywords— **tofu, soybean; chickpea; texture; nutritional properties**

I. INTRODUCTION

Tofu is a soybean curd produced by the protein coagulation technique. This protein denaturation develops a variety of textures ranging from soft to firm characteristics. Modification of tofu texture depends on various physical and chemical factors. Salt and acid are two coagulants generally commercially used for tofu processing [11]. Epson salt (or magnesium sulfate), lime juice, and tamarind juice were studied to determine their coagulation time. The results revealed that the Epson salt had the least coagulation time resulted from a higher soy protein content in the coagulate [5]. Calcium chloride was also suggested as an alternative coagulant to increase salt particle distribution in protein matrix, resulting in a firmer texture of finished tofu [9]. The textural properties of tofu are affected by the type of coagulating agents, amount of coagulants, temperature, time, soybean/water ratio (or total soluble solid), and the varieties of soybean [7,13].

The coagulation stage is an essential step to make tofu. The addition of salts such as chloride salt, sulfate, citrate at their optimum concentration triggers aggregation of a high-density soy glycinin protein (11S globulin) and beta-conglycinin (7S globulin) in the presences of cations (Cl^{2+}, Mg^{2+}). The ions could promote the formation of a bridge between the negatively charged protein and cationic molecules. This "salting-out" mechanism is part of the tofu process, specifically during the soybean curd formation [4]. The protein coagulation determined the nutritional value of tofu in protein content, fat content, crude fibre, and essential minerals (Ca, Mg, K, P, Na, and Fe) [5,9].

Chickpea is one of the plant-based protein sources. The pulse seeds are largely grown in India. There are two major types of chickpeas; the light seeded Kabuli type and the dark seeds Desi type (Fig.1). The chickpeas are known for their nutritional value, namely dietary fibre, polyunsaturated fatty acids, vitamin A, vitamin E, vitamin C, folate, magnesium, potassium, and iron [14]. Chickpea protein has similar properties as soybean protein. Both legume proteins are soluble in an alkali solution (pH>10). The isoelectric point of chickpea protein is 4.5 [6], while the soybean protein has a broad range of 4.7-5.2 [13]. Moreover, chickpea protein has the ability to precipitate in the system containing 1.5% $CaSO_4$ and 2.3-3.0% protein concentration [3]. However, chickpeas' protein content is about 20% lower than soybeans (approximately 36%).

Fig. 1 Soybeans (left), Chickpeas (Kabuli type, middle), and Chickpeas (Dasi type, right)

This research aimed to study the nutritional and physical properties (texture and colour characteristics) of fresh tofu due to the partial replacement of soybeans with chickpea.

II. Materials and Methods

A. *Materials*

Chickpea (Kabuli type) and soybeans were purchased from the Warorot market (Chiang Mai, Thailand). The legumes used for a protein source were up to moisture standard (<13%). The magnesium sulfate with high purity (99%) was a food-grade protein precipitation agent. Drinking water used for tofu processing was qualified by The Thai Food and Drug Administration, Ministry of Public Health. The water quality regards to Notification of the Ministry of Public Health No. 61 1981 drinking water in sealed containers.

There are six steps involved in tofu making, namely flour grinding, soaking, boiling, coagulating, pressing, and cutting into cubes. The Soybeans and chickpeas were ground into flour using a hammer mill (Hammertec™, Foss, Switzerland) before sieving with an electromagnetic sieve shaker (Octagon 200, Italy) to obtain 60 mesh size powder. The soybeans and chickpeas were mixed in different ratios, as seen in Table 1. The 500 grams flour mixture was prepared and soaked in 5 liters of sterilized water for six hours. Next, the unsuspended parts were sieved with a muslin cloth, and the aqueous part (milk) was collected. The 1.2 liters of milk was allowed to boil for 30 minutes and set aside at ambient temperature (27°C) for two minutes. An amount of magnesium sulfate was added to make up the concentration of 0.5% (w/v, magnesium sulfate to milk), which served to initiate the legumes' milk coagulation. After the coagulation step, the coagulated milk was poured into basket molds, and the whey was pressed out using a presser for an hour to drain the water residue. The fresh tofu was immediately used for textural analysis, and the rest was kept in a plastic box at 4 °C for further analysis.

Table 1 Flour formulation for tofu making

Soybean/chickpea (%)	Soybean/chickpea (g)
100:0	500:0
90:10	450:50
80:20	400:100
70:30	350:150

B. *Nutritional analysis*

Crude protein The protein content was carried out by the Kjeldahl methods modified by Onwuka [10]. One gram of the fresh tofu was digested by heating the sample with 15 ml of concentrate H_2SO_4 (98% w/w) in the presence of one selenium catalyst tablet. Protein digestion was processed under a fume cupboard for two hours. The aliquot was mixed with 80 ml distilled water and 50 ml 40% NaOH solution in a Kjeldahl apparatus and distilled. The distillate was collected in 25 ml 4% boric acid solution contained mixed indicator (methyl red/bromocresol green). A total of 50 ml green distillate was collected and titrated against 0.1N HCl to a deep red endpoint. A reagent blank was also digested, distilled, and done titration as described above. The total N_2 was calculated, and the protein content was obtained using the soy conversion factor of 5.71 based on glycinin. The average percentage of crude protein was based on triplicate.

Crude fat Fat content was determined by the continuous solvent extraction in Soxhlet reflux apparatus (Soxtec2055, FOSS Sweden). The tofu was dried prior to the fat analysis to avoid interference of water components during the fat analysis. However, the quantification was later calculated on a wet basis. One gram of dried tofu sample was wrapped in a filter paper and put in a Soxhlet reflux filter. An extraction flask was pre-weighed before adding 120 ml petroleum ether. The reflux flask was mounted on the extraction flask and connected to a condenser. The solvent was boiled on an electrothermal heater. The vaporized petroleum ether was condensed into the reflux flask to completely submerge the wrapped tofu and extract its fat. When the reflux flask was filled, it refluxed, carrying the extracted oil back into the extraction flask while the boiling continued. The cycle of boiling, vaporisation, condensation, extraction, and reflux was carried out for an hour prior to the solvent was recovered, leaving the extracted oil in the flask. The flask with oil residue was further dried in the oven at 105°C for 30 minutes to remove the remaining solvent. It was then cooled in the desiccators and weighed. The crude fat was calculated by the ratio of the weight of oil/weight of fresh tofu times 100. The experiment was triplicate, and the average percentage of crude fat was calculated.

Crude fibre The crude fibre was analysed by Fibretec 8000, FOSS, Sweden. A gram of the dried tofu was weighted in a glass crucible. The sample was digested by 150 ml of 0.1M H_2SO_4 solution for 30 minutes. It was then washed with several portions of distilled water and the washed sample was digested by 150 ml of 0.1M NaOH solution for 30 minutes, washed as before with water and allowed to drain dry. It was then very carefully transferred quantitatively to be weighed in a dried crucible. The sample in the crucible was finally burnt to ashes in a muffle furnace at 525^{o}C for three hours, cooled and weighed. The percentage of crude fibre content was determined by the weight difference between residue after acid/base digestion and ash divided by dried tofu weight times 100. The experiment was triplicate, and the average percentage of crude fibre was calculated.

C. Chemical analysis

The determination of the pH value of tofu was carried out following this method. Twenty-five grams of the tofu were in 25 ml distilled water. The sample was blended with a high-speed blender and then the mixture was wrapped with sheet cloth and manually squeezed to obtain a filtrate. The filtrate was analysed pH value using a pH meter (Mettler Toledo, Switzerland). The moisture content was determined by the hot air method [1]. The dried aluminium tin be weighted, and then 1 gram of fresh tofu was put in the can. The sample was heated at 105°C for six hours. The sample was weighed after every two hours until a constant weight was obtained. The moisture content was a percentage of wet basis and calculated based on the weight loss of tofu after drying divided by the fresh sample weight times 100. The experiment was triplicate, and their average percentage was calculated.

D. Textural measurements

Textural characteristics of fresh tofu were investigated using a texture analyser (TA.XT plus, Stable Micro System, UK). The texture profile analysis or TPA was obtained by two consecutive compression modes at 27°C. The experiments were carried out using 100 mm (diameter) cylindrical probe)TA-100 (and a heavy-duty platform (HDP/90) using a 25 kg load cell. Each tofu cube was cut with a sharp knife to obtain symmetrical cubes of 15x15x15 mm^3. The cube was placed on the platform and the cylindrical probe was pressed against the sample surface up to 1.5 mm in depth. The test speed was set at 2 mm/s. The typical TPA analysis results showed a relationship between the three parameters: force (N), time (second), or distance (millimeter). The hardness is calculated as the peak force of the first compression. The cohesiveness is the ratio of the area of the second peak compression (A2) and the area of the first peak compression (A1). The springiness is the distance at which deformed tofu went back to its non-deformed stage after the compression force was removed during the second compression. The adhesiveness is the negative area after the deforming force was removed during the first compression (A3). The results were expressed as an average of five measurements.

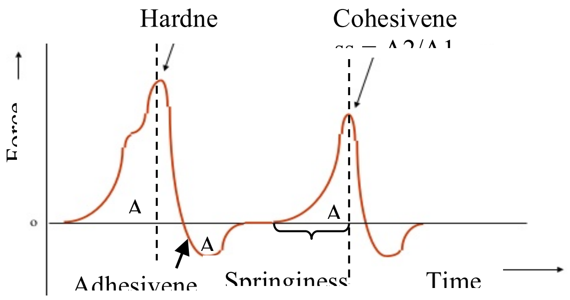

Fig. 2 The hardness, cohesiveness, springiness, and adhesiveness of tofu at all varians [15].

E. Colour measurements

The surface colour of the tofu was carried out using a chromameter (Minolta CR-400) at ambient temperature (27°C). Colour system was expressed in CIE L* (darkness/brightness), +a* (redness), and +b*(yellowness). For each treatment, all sides of the tofu cube or six measurements were performed, and data were reported as the average number.

F. Statistical analysis

The collected data from the experiments were expressed as the mean value ± standard deviation (SD). The Completely Randomised Design (CRD) was applied to nutritional, textural, chemical, and colour measurements. The effects of chickpea addition on the soybean tofu characteristics were evaluated using analysis of variance (ANOVA). Duncan's multiple range test ($p<0.05$) was used to describe the difference among treatments. The statistical analysis was performed using IBM SPSS version 23 (IBM, USA).

III. Results and Discussion

A. Nutritional characteristics

Proximate analysis was applied to all tofu made from soybean/chickpea blends in order to estimate the nutritional content and the effect of these macromolecules on the textural properties of the tofu curd as presented in Table 2.

Table 2 Nutrients of soybean/chickpea tofu at different blends

Soybean/ chickpea	Crude protein* (% wet basis)	Crude fat* (% wet basis)	Crude fibre* (% wet basis)
100:0	12.26±0.33 ^{d}	4.18±0.69 ^{d}	0.01±0.00 ^{d}
90:10	11.49±0.23 ^{c}	3.47±0.75 ^{c}	1.90±0.12 ^{c}
80:20	10.99±0.44 ^{b}	3.10±0.43 ^{b}	2.40±0.15 ^{b}
70:30	10.22±0.11 ^{a}	2.36±0.56 ^{a}	2.80±0.11 ^{a}

*$^{a-d}$ following the mean value (n=3) suggested a significant difference between treatments at $p<0.05$

The total protein of all tofu was in between 10.22% and 12.26% with 100:0 ratio tofu was ranked the highest among all ratios. This was similar to the previous study reporting the protein content of fresh tofu between 11.80 and 12.40% [5]. Partial replacement soybeans with chickpea reduced the total crude protein. This may be due to the protein content in chickpea seeds is about half of that in soybean seeds resulted in lower total protein in this formulated tofu. Similarly, fat content in all samples reflected the same trend as protein content for all treatments. The crude fibre in soybean/chickpea tofu in all preparation increased as more chickpea was added to the mixture [13]. The soybean tofu typically lacks crude fibre because it is removed during the pressing process [2].

B. Chemical analysis

Moisture content for all preparations was ranging from 69.65-82.56% as showed in Table 1. Moisture in the tofu was affected by its protein content which played an important role in holding water within the tofu matrix. The gel formation in the tofu was initiated by Mg^{2+} cations which formed crosslink with protein. Protein coagulation was affected by the pH. The pH value of all tofu blended with chickpea protein was significantly different (P<0.05) from soybean tofu. The pH of chickpea flour is approximately 6.4, slightly lower than the pH of soy flour at 6.7. However, the pH values of all systems were far from the isoelectric point of chickpea proteins at 4.5, where the solubility protein was strongly influenced by the zero net charge of the amino acids [6].

Table 3 Moisture and pH values of soybean/chickpea tofu at different blends

Soybean/ chickpea	Moisture* (% wet basis)	pH value*
100:0	82.56±0.47 [a]	6.70±0.04 [a]
90:10	71.04±0.49 [b]	6.37±0.02 [b]
80:20	70.91±0.38 [b]	6.22±0.08 [b]
70:30	69.65±0.44 [b]	6.20±0.48 [b]

*a-d following the mean value (n=3) suggested a significant difference between treatments at p<0.05

C. Textural characteristics

Fig. 3 presents the textural characteristics of soybean tofu and its blending with chickpea. The TPA reveals the four parameters, namely hardness, adhesiveness, cohesiveness, and springiness.

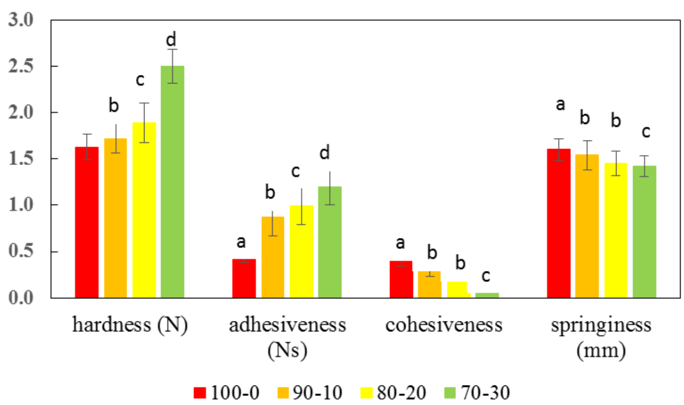

Fig.3 Textural characteristics of soybean/chickpea tofu at different blends.
*a-d following the mean value, n=3, suggested a significant difference between treatments at p<0.0

In Fig.3, the soybean tofu (ratio 100:0) showed the least hardness compared to the other three preparations with chickpea. The fibres in chickpea fill up the space between soy protein gels and therefore strengthen the gel network. The presence of chickpeas gradually increases the adhesiveness of tofu mixtures. The adhesiveness properties might be related to the water-binding properties of the tofu's protein fraction, which creates a more sticky texture for the tofu [9]. In contrast, the cohesiveness and springiness reduce in line with the increase in the degree of soybean substitution. As more chickpeas present in the systems, the tofu mixtures are unable to retain their original form. In other words they are brittle and less elastic or chewy.

D. Colour characteristics

Colour properties of tofu analysed on Minolta CR-400 are presented in Table 4. The brightness of soybean tofu is significantly different from those tofu preparations with chickpeas. However, there is no difference between all treatments (10-30% soybean replacement) in the mixture. The intensity of brown-yellowish colour was increase as more chickpeas presence in the mixture (Table 4). The tofu with more chickpea added appears brown in colour (Fig. 4). The degrees of light redness and yellowish of the tofu was a result of the natural pigments e.g. xanthophyll, anthocyanin in chickpea [8].

Table 4 Colour of soybean/chickpea tofu at different blends

Soybean/ chickpea	Brightness (L*)	Redness (+a*)	Yellowness (+b*)
100:0	84.17±0.95 a	0.73±0.23 a	18.86±0.44 a
90:10	81.33±0.49 b	0.93±0.11 b	21.99±0.23 b
80:20	81.11±0.38 b	1.12±0.17 b	22.22±0.59 b
70:30	81.05±0.48 b	1.46±0.16 c	23.57±0.98 c

*a-d following the mean value (n=6) suggested a significant difference between treatments at p<0.05

100:0 90:10 80:20 70:30

Fig. 4 Colour of soybean/chickpea tofu at different blends of the two legumes.

IV. CONCLUSIONS

Partial replacement of soybean with chickpea in tofu formulation has shown that the nutritional composition of the mixture (protein, fat, and mineral) affects the overall textural profile of tofu. As more chickpeas present in the mixture, the total protein, fat, moisture, and pH of the systems reduce significantly. The total fibre, on the contrary, increases significantly, especially for 70:30 preparation. An increase in fibre content promotes a harder structure but reduces the cohesiveness and springiness of the tofu. Tofu appears to be more brittle and unable to retain its texture as more chickpeas are added to the system. Darker colour was monitored visually and analytically with chromameter for all formulations with chickpeas.

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