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EFFECTS OF pH ON *MƠ* TOFU QUALITIES

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Abstract. Tofu is a common food in Vietnamese and global cuisine. It has high nutritional value, good flavor, and texture, that it is easy to produce other food from it. In Ha Noi, *Mơ* tofu is a traditional product produced from tofu- whey, which is commonly produced by small-scale producers. This study focused on the effects of coagulating pH on *Mơ* tofu qualities. The results showed that *Mơ* tofu quality, which was produced by coagulating soy protein at pH 5.8, had the highest moisture of 83.71 %, highest water holding capacity of 8.96 g/g protein, low hardness, rather high elasticity, S-S bonding number of 41.12 nmol/mg protein, and the Ho index (phobicity) of 285.1.

Keywords: Mơ tofu, hardness, water holding capacity, phobicity, pH.

Classification numbers: 1.4.4, 1.3.4, 1.1.3.

1. INTRODUCTION

Tofu is a food made from soybean by coagulation of bean milk, a very familiar food product in Asian countries, including Viet Nam. The tofu has high nutritional value, good taste, easy to coordinate with various dishes depending on local food culture, easy to digest. Especially, for people suffering from diabetes, high blood pressure, etc., tofu is a nutrition source that guarantees and enhances health.

In the world, there were many studies of coagulated protein process in soymilk to form tofu, primarily by the chemical coagulation method by calcium salts, magnesium, glucono-delta-lactones, and acids. Several studies were on the influence of the specifications during coagulation such as temperature, pH to the quality of tofu but only studied with the above agents. In Viet Nam, tofu is a familiar product daily and is made with sour water, which is a common agent. However, there is little research on the effects of specification on tofu from sour water. In fact, tofu is coagulated by sour water, which is the popular product in Viet Nam; especially in the north, the most famous one is the *Mơ* tofu.

Coagulation is the most important in the production of tofu. The purpose is to precipitate proteins and lipids in soymilk to form a curd. Curd formation can be explained via a 2 step process: (1) conjugation of protein particles on the surface of lipid globules following the addition of the coagulant, and (2) formation of a network off non-repulsive lipid globules covered with protein particles [1]. Tofu curd is stabilized based on such of molecular forces: hydrogen bonding, hydrophobic interaction, ionic and disulfide bonds. Moreover, salts, reducing agents, and water-soluble solvents also affected the curd gel properties [2, 3]. The various coagulants are used to produce various tofu types. There are three types of coagulated agents commonly used: salts, acids, and enzymes, which can be used separately or in combination with each other. Usually, coagulants add to soybean milk solution while stirring at 70 – 90 °C [4]. Heating increased free sulphydryl groups in soybean protein and resulted in harder tofu [2]. The disulfide bond is one of 4 comment bonds of protein which has an energy of 120 - 140 kJ/mol [5].

The calcium sulfate (gypsum) is the coagulating agent used before and until now, it is still the most common method used much in China, and western countries. Tofu is quite hard, smooth, slightly brittle state, non-effect to product, and the highest tofu yields if tofu is coagulated by calcium [4]. Beside calcium, the most commonly used acid is Glucono delta lactone (GDL, also known as grape sugar). GDL is hydrolyzed to gluconic acid, to decrease the pH of soymilk, and so proteins are coagulated. GDL is used primarily to the production of tofu in Japan, or called as young tofu. The product has a soft structure, smooth, high moisture, a little bit sour. It can be used in combination with calcium sulfate to produce soft and smooth tofu. Other acids, such as acetic acid and citric can also be used, but they create unwanted flavors, less commonly used [4]. In 2000, Renkama *et al*. found that the essence of the freezing nodes was different in pH, because at high pH only 51 - 69 % of total proteins were combined in the gel (mainly polypeptide), whereas at low pH, most proteins are coagulated and contained in coagulated mass. Higher water retention was observed at higher pH [6]. Catriona *et al*. studied the influence of pH on gel-generating properties of fat-separated soybeans, and pure glycinin transformed [7]. At high pH, the smoother gel is formed that characterized by low G value, and the surface is smooth, and quite opaque; whereas at low pH, the raw gel is obtained with high stiffness and structure is harder. The low G value, as found in high pH related to the high solubility of glycinin and soybeans isolated proteins (about 50 percent) after heating at low protein concentration. Further, gel-making mechanisms seem to be affected by pH. Thus, this research focused on the effect of pH on the properties of *Mơ* tofu, which was produced by naturally fermented whey.

2. MATERIALS AND METHODS

2.1. Tofu processing method

Soybean was obtained from Vietnam Academy of Agricultural Science and stored in sealed plastic bags in dry place. Soybean was soaked on tap water at 30 ^{o}C for 4 hours, then wet milled with dry soybean/ water ratio of 1/10. The slurry was run through the N^{o}70 sieve to remove the coarse part of the bean and the soymilk slurry was boiled for 2.5 min, cooled down to 85 ^{o}C. The soymilk was coagulated by whey collected from the 1st price- house-hold *Mo* tofu producer in Mai Dong traditional village where Mơ tofu was produced. Coagulation of soymilk was carried out by coagulant of naturally fermented whey (so-called "sour water" in traditional tofu processing). The curd was transferred into a laboratory-designed tofu box (5 × 5 × 100 cm) lined with cloth for molding. The cloth was folded over the top of the curd and pressing was achieved with a weight for 2 hours. The tofu was then unwrapped from cloth and properties of tofu were determined.

2.2. Determination of tofu moisture. The moisture of tofu was determined according to TCVN 4326: 2001.

2.3. Determination of water holding capacity of Mơ tofu

The water holding capacity of *Mo* tofu was determined based on the method of Khatib *et al*. [8]. 5 - 10 g of tofu was weighed, put into falcon tube and centrifuged at 9000 rpm for 12 min. After that, the amount of drained water was recorded. The water holding capacity of tofu was calculated by the percentage of the water weight difference before and after draining and protein content of the sample.

2.4. Determination of disulfide (S-S) bond of Mơ tofu [9]

Disulfide bond was calculated by the diffference between free -SH group and total -SH group in tofu.

Determination of the free SH group

0.02 g of sample was dissolved in 2.5 ml of buffer solution of Tris 0.086 M, glycine 0.09 M, EDTA 4 mM, urea 8 M, pH = 8 by homogenizing for 3 minutes. The sample solution was then centrifuged at 10000rpm for 15 min. 1 ml solution after centrifugation was added 0.01 ml of Ellman reagent (10 mM DTNB), then incubated at room temperature for 5 min. The absorbance of the sample was measured at a wavelength of 412 nm. The result was calculated using the following formula:

$$C_o = A/(\varepsilon.l)\ D,$$

where: C_o: concentration of -SH group, A: adsorption at 412 nm, ε: molecular molar absorption = 13600 $M^{-1}.cm^{-1}$, l: thickness of cuvette, D: dilution factor

Determination of the total –SH group in soy milk and tofu

0.02 g of sample was dissolved in 2.5 ml of buffer solution of Tris 0.086 M, glycine 0.09 M, EDTA 4 mM, urea 8 M, 2-ME 0.1 M, pH8 by homogenizing for 3 minutes. The solution was then centrifuged at 10000 rpm for 15 min. 1 ml of sample solution was added with 0.01 ml of Ellman reagent (10 nM DTNB) and incubated at room temperature for 5 min. The absorbance of the sample was measured at a wavelength of 412 nm. The result was calculated using the following formula:

$$C_1 = A/(\varepsilon.l)\ D,$$

where: C_1: concentration of total -SH group, A: adsorption at 412 nm, ε: molecular molar absorption = 13600 $M^{-1}.cm^{-1}$, l: the thickness of cuvette (nm), D: dilution factor,

The concentration of S-S bonds was calculated as followed:

$$C = (C_1 - C_o)/\ 2.$$

2.5. Determination of phobicity of *Mơ* tofu [9]

Phobicity of Mơ tofu was determined followed the hydrophobic probe binding method of Mohanan *et al*. [9]. Samples (0.02 g) of tofu was dissolved in 2.5 mL of 0.1 M phosphate buffer, pH 6.5, then were centrifuged at 9000 rpm for 15 min to remove insoluble protein. After protein determination, the supernatants were diluted with 0.1 M phosphate buffer to give solutions in the concentration range of 0.01 - 0.1 mg of protein/ml. Take 3 mL of each diluted tofu solution, added 200 μL of 0.5 mg/mL *ANS* solution **(**0.1 M phosphate buffer, pH 6.5). Ratiometric fluorescence measurements were made on a spectrofluorometer at 375 and 485 nm as the excitation and emission wavelengths, respectively. The H_O of the solubilized tofu was calculated from the slope of the relative fluorescence ***(R)*** and percent (w/v) protein concentration.

2.6. Determination of color of *Mơ* tofu [8]

Color of tofu surface was measured by a tristimulus Colorlite PH860. Results were expressed in CIE L*, a*, b* color model where L* was the lightest, a* -red (+) and green (-), b* - yellow (+) and blue (-) color. Each value was triple replications.

2.7. Determination of *Mơ* tofu texture [10]

Tofu texture was determined by TPA method on Stable Microsystems Texture Analyser TA-XT Plus using P/100 probe followed Mathare C. *et al*. method [10] with a small modification. Tofu was cut into cube pieces of 2 × 2 × 2 cm. Pre-test speed was 10 mm/s, test speed was 1.67 mm/s, strain 75 %. The hardness and elasticity of Mo tofu were recorded. Each test was repeated 10 times.

2.8. Statistical analysis

All tests were performed at least in duplicate. Analysis of variance was performed using Ducan’s multiple-range test under Microsoft Excel 2010 and SPSS software. Significance was defined at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of protein coagulating pH on moisture of tofu

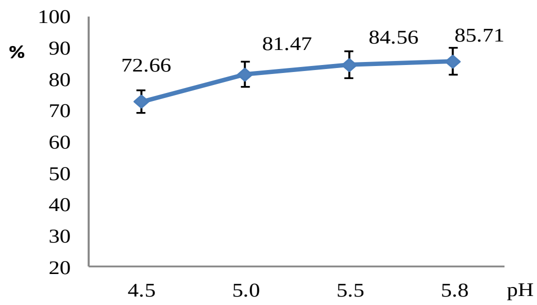

Figure 1. Effects of coagulating pH on moisture content of Mơ tofu.

The moisture plays an important role in the sensory evaluation of tofu. Suitable moisture content contributed to the softness, mouthfeel, and elasticity of tofu [2]. Effects of coagulating pH on the moisture content of tofu were presented in Fig. 1.

From Fig. 1, it was clearly seen that when coagulating pH increased from 4.5 to 5,8, and tofu moisture increased from 72.66 % to 85.71 %. Tofu moisture reached the highest value when coagulating soy milk at pH = 5.8 and the lowest value at pH = 4.5. As previously reported, soy protein contains 2 fractions: 11S and 7S protein, of which isoelectric points are 5.6 and 4.6 accordingly [11]. Thus, the number of protein molecules that participated in the coagulation process was high, the number of lypophilic and S-S linkage increased, so that the hardness of the curd was increased, the free space for water reduced, and the moisture of tofu was decreased.

3.2. Effect of pH on water holding capacity of Mơ tofu

Tofu water holding capacity was determined by centrifuging tofu then the retaining water content was measured. Effects of the coagulating pH on water holding capacity of Mơ tofu were presented in Fig. 2.

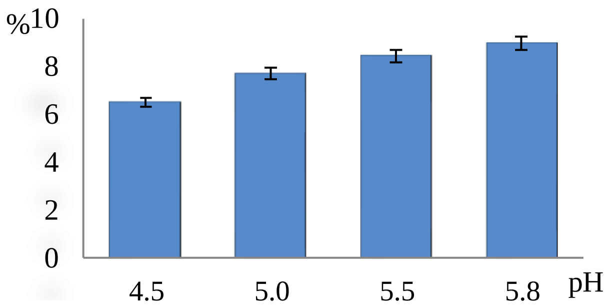

Figure 2. Effects of coagulating pH on water holding capacity of Mơ tofu.

Figure 2 showed that the lower coagulating pH of soymilk was, the lower water holding capacity of *Mơ* tofu was observed. This may due to that at pH > 5.5, the main protein fraction that could coagulate was 11S protein, which contained a high number of S-S linkages; thus the disulfide linkages in the protein gel matrix strength rose, so that gel strength was increased and hold more water molecules. In addition, the number of lypophilic linkages was decreased so that the amount of bound water was higher. When pH was reduced, the soy protein gel contained both 11S and 7S protein. However, 7S protein contained only hydrophilic and lipophobic linkages, which were easily weakened and broken by heat treatment; thus soy protein gel became weaker and held fewer water molecules. This observation was in accordance with the results of Onodera *et al.* [1].

3.3. Effect of coagulating pH on S-S linkage of Mơ tofu

A protein gel network is formed by cross-linking between polypeptide chains during processing [3]. Effects of coagulating pH on disulfide bond (S-S linkage) of Mơ tofu were indicated in Fig. 3.

Figure 3 indicated that by reducing the coagulating pH from 5.8 to 4.5, the S-S bonding number increased from 41.12 nmol/mg protein to 68.21 nmol/mg protein. It was thus reducing the coagulating pH led to the increase of S-S bonding. These results could be explained by -SH interchange of 7S (β conglycin) and 11S globulin soy protein fraction during coagulation. Heating soymilk increased the formation of free sulphydryl groups of soybean protein [2]. Then, the disulfide bond connected the unfolded polypeptides and stabilized protein network structure

[12]. At pHs around the protein's isoelectric point, the protein gel networks are heterogeneous and mostly opaque, and some syneresis. At other pH value, fine and persistent fibrillar networks or cold-set gel are formed rather than particulate stranded gel. Rod-like structures are formed side-to-side aggregation, and their continuous length varies inversely with ionic strength [13]. The 7S globulin unfolded at the lowest temperature, and the 11S globulin unfolded the highest temperature [6]. The 7S formed a gel at the pH 3.8 and 5.2, whereas 11S formed at pH 7.6 [6]. As pH raised, pH reached near pHi of 11S globulin form, 11S underwent conformation changes, less S-S bond was formed [2]. When pH was low, and near pHi of 7S globulin, more S-S bonds were created. These findings were similar to Shan *et al.* [3], Monahan *et al.* [9], and Torchinskii [14] that pH, salts, reducing agents and dissociating agents affect different molecular forces in the formation of protein gels.

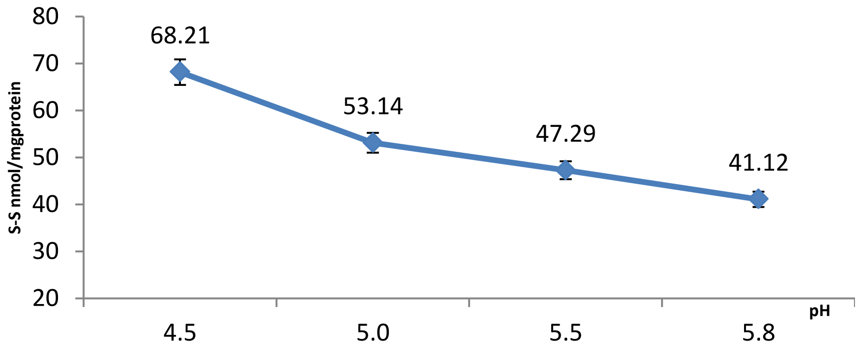

Figure 3. Effect of pH on S-S bond formation in Mơ tofu.

3.4. Effect of coagulating pH on phobicity of Mơ tofu

The effect of coagulating pH on phobicity of Mo tofu was presented in Fig. 4

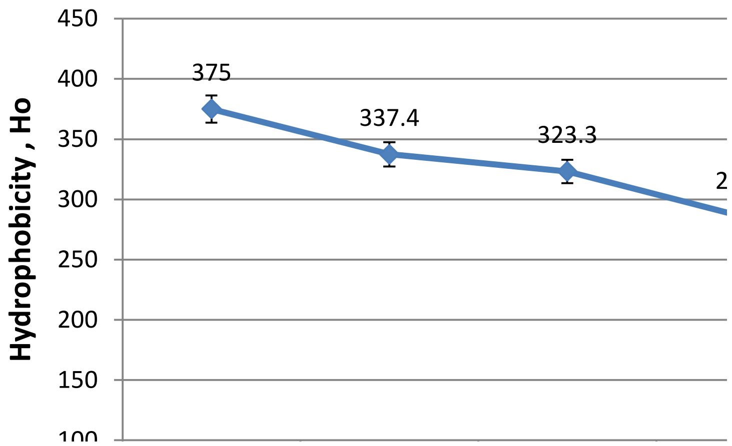

Figure 4. Effect of coagulating pH on phobicity of Mơ tofu H_o.

Accordingly, when the pH decreased, more lipophilic bonds were created. With a coagulation pH of 4.5, the lipophilic binding index was 375.0, and when the coagulation pH increased to 5.8, the Ho index was 285.1. The explanation for this phenomenon could be based on the followings:

Heating soymilk leads to the denature of soyprotein molecules where exposes the hydrophobic region and -SH groups [15]. Protein is unfolded and dispersed in water due to temporary disruption of secondary structure, exposing internal residues to the surface. There is the transmission from one region of the molecule, e.g., one binding site to another, or an active site. The pH, temperature, ionic strength or ligand binding cause protein reversible molecular changes [2, 5]. Heat the soymilk before coagulating causes the exposure of the hydrophobic area of denatured proteins. Covalent cross-linking and hydrophobic interactions among denatured protein contribute to the formation of the soy protein gel network [2]. The α-helix and β-sheet formation is influenced by hydrophobicity and hydrophobic interactions [5]. For unheated protein, the increase in pH leads to the increased exposed hydrophobicity. The combination of pH 10-11 and thermal treatments at temperatures of about 65 °C leads to higher exposure of hydrophobic groups, which would be the most suitable condition to obtain isolates with higher emulsifying capacity. Denaturing thermal treatments at this pH value induces aggregation with a resultant fall in surface hydrophobicity [2]. This phenomenon could be explained due to the destruction of the internal buried structure of a globular protein, and the ionic force in the solution. The ionic force unbalances the protein's charge by charging it. Following the polarization process, bonds are formed between the polarized protein and other molecules to form the gel system, but interactions with other external agents rarely occur, the most common is the interaction between protein macromolecules. After polarizing, the normal protein will have a negative charge and H^+ in the sour water will neutralize the protein's charge. Then protein tends to collide with each other and form a stable gel system. The lipophilic interaction is essential in this gel formation because it stabilizes the gels network by removing water from the protein-protein interaction by which the lipophilic radical is chained [5]. When the pH decreased and reached the isoelectric points of the two main proteins fraction, globulin 11S and 7S, there was an increasing number of bonds between the polypeptide chains of protein molecules, leading to the higher amount of hydrophobic bonds [8].

3.4. Effect of coagulating pH on color of Mơ tofu

Tofu color was affected by water content and fragment ratio in tofu. pH changes affect those factors, thus led to the changes in tofu color. Table 4 showed the effects of coagulating pH on the color of Mơ tofu.

Table 4. Effect of coagulating pH on tofu color.

pH	L	a*	b*
4.5	84.05 ± 0.05^{a}	0.04 ± 0.00^{a}	8.38 ± 0.58^{b}
5.0	84.49 ± 0.14^{a}	-0.11 ± 0.00^{b}	8.05 ± 0.63^{b}
5.5	86.17 ± 0.44^{b}	-0.17 ± 0.00^{b}	7.48 ± 0.45^{a}
5.8	88.32 ± 0.40^{c}	-0.48 ± 0.00^{c}	7.96 ± 0.45^{a}

From Table 4, it was indicated that when coagulating soy milk at pH from 4.5 to 5, L value of Mơ tofu did not change, and that at pH = 5.8, L value of tofu was the highest and similar to commercial Mơ tofu. b* value of tofu that formed by coagulating soy protein at pH = 4.5 was the highest, which indicated there accumulated many yellow pigments. a* value ranged from -0.48 to 0.04, showed that red or blue color were very light. When heating soymilk, a Maillard reaction may occur, so that the white color of soymilk could be changed to yellowish [16]. The pigment of tofu was because of the pigment of bean hilum [8]. Soybean with light hilum color could produce white or pale yellow curd [8]. Pigments in the seed coat diffused in soymilk during the wet milling step. When water content was lower, the lipid content was higher, so carotenoid pigments content would be higher, the color of products was darker. Darker pigments were produced in low water content [17], so at low coagulating pH, the water content of coagulated tofu was lower (Fig. 1), then darker pigment was observed in tofu. Thus, not only such coagulant (calcium sulfate, Epsom salt, *Alum*, topwater of fermented maize, etc.) affected on tofu color [18]. Obatolu *et al*. indicated that coagulating pH was also affected tofu color [19].

3.5. Effect of coagulating pH on hardness and elasticity of Mơ tofu

Tofu coagulation is the process of creating a three-dimensional gel network for the gel coagulation system. Therefore, the gel characteristics greatly determine such parameters as hardness and elasticity of the tofu. To determine these parameters, the TPA measurement method was used by TA-XT Plus equipment. Hardness is defined as the maximum force during the first compression cycle. Elasticity is defined as the height at which the food can be recovered during the period from the first compression until the start of the second compression and is calculated as a percentage [10]. The effect of pH on hardness and elasticity of tofu were presented in Figure 3 (a,b).

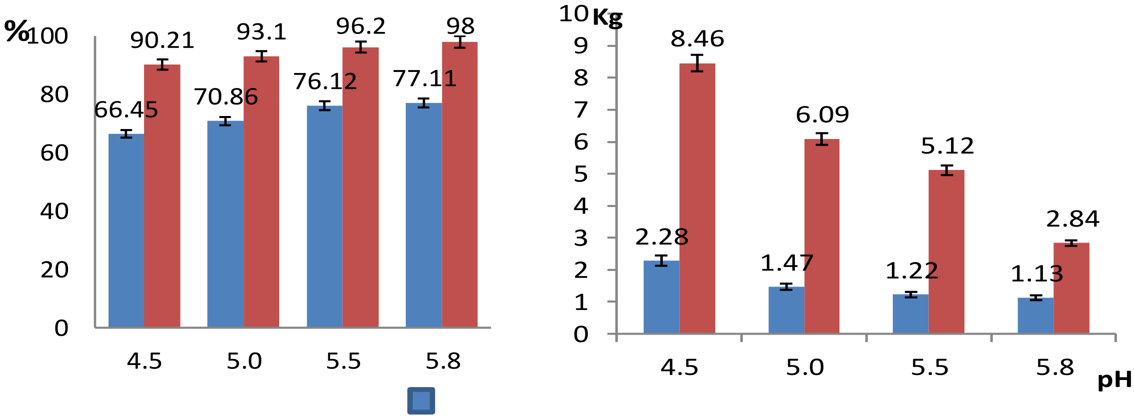

Figure 3. Effect of coagulating pH on tofu's elasticity (a) and hardness (b)

a) Fresh tofu b) ied tofu

Figure 3 showed that when pH decreased from 5.8 to 4.5, the hardness increased from 1.13 kg to 2.28 kg, and the elasticity decreased from 77.11 % to 66.45 % for fresh tofu. Whereas tofu hardness increased from 2.84 to 8.64 kg, and elasticity decreased from 98.00 % to 90.21 % for fried tofu. At pH 5.8, the minimum hardness tofu and the maximum elasticity were reached. A significant difference in hardness and elasticity between fresh and fried tofu was observed. The maximun hardness of fried tofu and fresh tofu were 8.46 and 2.28 kg, respectively. The maximun elasticity of fried tofu was also nearly 1.3 times higher than fresh tofu. The frying caused a significant lost of water, created spaces inside the product and changed the surface structure of tofu, so that the elasticity and hardness of fried *Mơ* tofu tended to increase compared to that of fresh samples [17].

From these results, it was indicated that the higher water content *Mơ* tofu contained, the softer *Mơ* tofu was. This may be explained that when coagulated soymilk to make tofu, the protein rolled back the peptide chains and kept water molecules inside. The more water molecules kept inside soy protein gel, the more spongy and softer the gel became, so that the tofu elasticity increased. Takagi and Okamoto [20] demonstrated that when linkages in tofu, especially S-S linkages, increased, hardness, as well as the cohesiveness of tofu would increase [20]. Besides, when coagulated soy protein at lower pH, *Mơ* tofu color was darker, and the tofu's moisture content was lower.

4. CONCLUSIONS

The coagulating pH affected on *Mơ* tofu qualities. At pH 5.8, the S-S bonding number was 41.12 nmol/mg protein, the Ho index (phobicity) was 285.1 *Mơ* tofu quality is best. The maximum moisture is 83.71%, the maximum water retention capacity is 8.96 g/protein, relatively high elasticity, and low hardness, high whiteness, and close to *Mơ* tofu.

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CRediT authorship contribution statement. Nguyen Quang Duc : Data collection, Data analysis, Research summary, Manuscript preparation. Nguyen Thi Que:, Data collection, Draft manuscript preparation. Nguyen Duc Doan: Data collection, Draft manuscript preparation. Nguyen Xuan Phuong: Methodology, Supervision. Luong Hong Nga: Research design, Data analysis, Methodology, Supervision, Research summary and recommendation, Manuscript revision, Corresponding for Manuscript publication.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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