Jurnal
Teknologi

Full Paper

INFLUENCE OF CHICKEN EGGSHELL POWDER AS AN ALTERNATIVE COAGULANT ON THE YIELD AND TEXTURAL CHARACTERISTICS OF TOFU

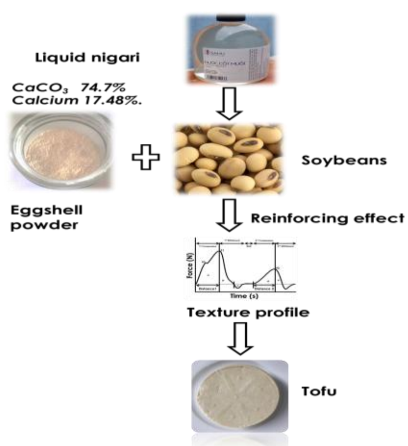
Van T. A. Nguyen*, Vuong D. Nguyen, Quoc L. P. T.

Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Vietnam

Article history
Received
30 September 2021
Received in revised form
15 October 2022
Accepted
20 October 2022
Published Online
26 December 2022

*Corresponding author
nguyenthiaivan@iuh.edu.vn

Graphical abstract



Abstract

Daily consumption of chicken eggs produces a vast number of eggshells byproducts which are a rich source of calcium salt. This paper investigated the potential of eggshell powder from eggshell byproducts as an alternative coagulant in the tofu preparation process. The eggshell powder was successfully obtained and analyzed with calcium salt ($CaCO_3$) of 74.7% followed by calcium of 17.48%. Liquid nigari as a coagulant was evaluated and showed better tofu yield (143.24 ± 1.68 g/ 100 g) at 2% of addition to the soybean milk. The tofu prepared with 2% liquid nigari and 0.25% eggshell powder showed a higher yield (154.79 ± 3.56 g/100 g) as well as the improvement of texture profile of resulting tofu with hardness of 19.1 ± 2.02 N, cohesiveness of 0.26, and springiness of 13.5 ± 0.06 mm. The studied tofu was obviously observed to have better textural characteristics than two samples prepared by traditional methods. The result suggested that eggshell powder could be a promising alternative coagulant for tofu preparation.

Keywords: Eggshell powder, liquid nigari, tofu, texture profile

1.0 INTRODUCTION

According to the report by World Wildlife Fund (WWF), the processed food from farm to fork promotes a large amount of food waste which is the most concerning risk to the planet earth [1]. Food waste is defined as a part of food loss and preferably discarded sections during food processing [2]. It was reported that one-third of food waste was considerably consumed with sufficient nutritional values (around 1.3 billion tons annually) [3]. In which, eggshell waste has been currently rated as the 15th considerable food by-product issue, leading to serious environmental problems as well as health-concerned problems due to the growth of microorganisms on these eggshells [4].

Chicken eggshells are food waste materials from domestic resources including factories, poultry farms, restaurants, and home kitchens. Eggshell contributes to 9-12% of the total egg weight which is a good source of dietary calcium [5]. Eggshells are constituted by a majority of calcium carbonate (94%), calcium phosphate (1%) and magnesium carbonate (along with some organic components and water [5,6]. The calcium carbonate could be converted to different calcium-fortified products, underlining their highly promising applicability in biomedical and food industries [7]. Therefore, the utilization of eggshells for calcium-fortified foods production could be an ideal low-cost alternative to commercial supplements to increase the consumption of calcium in the human diet, which was in agreement with many previous researchers [8–10]. Ray *et al.* (2017) supplemented eggshell powder with wheat flour to produce eggshell powder fortified chocolate cakes. The obtained results indicated that adding 6% of eggshell powder increased the calcium content of cakes (816.8 mg/100g) while remaining the physicochemical properties and sensorial properties at the acceptable level. The enrichment of eggshell powder in Polish bread spread was observed to increase calcium levels to 2.5-fold compared to the control sample [11]. Interestingly, the use of eggshell powder as a calcium supplement for rat diet was found to have higher bioavailability than the commercial calcium carbonate supplement [12].

Tofu, known as soybean curd, has been commonly used as a favorable product supplementing a rich source of protein and calcium in Asian diets for many centuries. Soybean curd is basically prepared by coagulating soymilk by using many types of coagulant [13]. The coagulant induces soy protein gel for trapping water, lipids and other chemical constituents in the matrix forming curds [14]. The quality of soybean curd is highly dependent on the coagulating agent for achieving the high yield and desired texture of tofu. Calcium sulfate is a widely used coagulating agent, but other calcium salts can considerably be an ideal calcium source such as calcium chloride, calcium lactate, calcium acetate, calcium carbonate [15,16]. Kim *et al.* (2007) also used 0.1% oyster shell powder (mostly composed of $CaCO_3$) and the yield and moisture of tofu were considerably higher than that prepared with $MgCl_2$ [17]. Therefore, the use of eggshell powder with the main component of $CaCO_3$ could be a promising alternative to tofu coagulant. Besides, liquid nigari, a by-product during salt production, is also a chloride coagulant ($MgCl_2$ and $CaCl_2$) and has been found to promote natural flavors and the original taste of soybean [18]. The combined usage of liquid nigari and eggshell powder can produce better tofu yield and textural characteristics.

Therefore, the objective of this study was to utilize the chicken eggshell powder from the eggshell byproducts as an alternative to tofu coagulant. The effect of liquid nigari and eggshell powder content on the tofu yield and texture profile was determined to better produce tofu formulation. The resulting tofu in this study was also compared with other traditional tofu products to show the discrepancy in textural characteristics. The result is expected to show the feasibility of eggshell powder as an alternative tofu coagulant as well as an effective means of easing the environmental issues due to food waste from eggshells.

2.0 METHODOLOGY

2.1 Materials

Chicken eggshells were collected as byproducts from Vinh Thanh Phat Co., ltd (Ho Chi Minh City, Vietnam). Soybean was purchased from Tam Nong VN Company limited (Ho Chi Minh City, Vietnam). Liquid nigari was supplied from Sahu company limited (Quang Ngai province, Vietnam) containing mainly MgCl2, besides Ca, Kali and Na.

2.2 Preparation of Eggshell Powder

The eggshell membrane was manually stripped off the eggshell in water and repeated washing in water. The eggshells were then blanched at 100°C for 15 min to remove impurity particles and inactivate microorganisms attached to eggshells. The eggshells were dried at 60°C for 2 h then ground by using a mortar and pestle. The fine powder was subjected to the sieving process using a 140-mesh [19]. The obtained eggshell powder was subjected to proximate analysis to determine its chemical constituents according to AOAC 968.08 method.

2.3 Preparation of Tofu

Soybean was soaked in water with the soybean: ratio of 1: 2.5 (w/v) for 4 h. The soybean was then subjected to wet grinding process with the soybean: water ratio of 1:8 (w/w). The soybean milk was filtrated to remove the soybean residual and pasteurized at 90°C for 10 min [16]. The liquid nigari varied from 1-3% (w/v) was added to the soybean milk and stirred for 90 s to investigate the effect of liquid nigari on the tofu yield and texture profile. To investigate the effect of eggshell powder on the tofu yield and texture profile, eggshell powder was added to the soybean milk at different content (0.2% to 0.35%, w/v) with fixed liquid nigari content. The mixture was poured into the tofu mold (10 cm in diameter), pressed for 10 min with pressing force of 2 N/cm^2. The tofu yield (E) was calculated as followed [20]:

$$E\ (g/\ 100\ g) = \frac{m_{tofu}}{m_{soybean}} \times 100$$

Besides, in this study, two samples of tofu in the market were also used to evaluate the structural parameters along with the best sample of the experiment (research sample). In which, sample 1 is produced manually and sold in a traditional market in Ho Chi Minh City, Vietnam; Sample 2 is produced by Vi Nguyen company, sold mostly in supermarkets, Vietnam. The purpose of this experiment is to allow readers to compare the research sample with products already available in the market and accepted by consumers.

2.4 Texture Profile Analysis

The texture profile of the resulting tofu was analyzed using texture analyzer (Brookfield CT3, AMETEK, Inc., USA) following the method of Wang *et al*. (2003). The tofu was cut into a specimen (2 x 2 x 1.5 cm) by using a sharp knife. The specimen was placed at the center of tested area to ensure the texture analyzer probe press and penetrate the center point. A TA58 probe was used with 5 mm travel distance and a speed of 1 mm/s in the cycle mode. Hardness, cohesiveness, springiness, gumminess, and chewiness were recorded from the profile based on the definitions of Saio *et al.* (1969) [21].

2.5 Statistical Analysis

Each experimental data was in three replicates. Result was expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test were carried out to compare mean values and statistical difference was expressed at the level of 5% ($p < 0.05$) by using SPSS 20.0 (SPSS, Inc., Chicago, IL).

3.0 RESULTS AND DISCUSSION

3.1 Proximate Analysis of Eggshell Powder

The chemical compositions of eggshell powder are listed in Table 1. The primary component of the eggshell powder was Calcium carbonate (74.7%) followed by calcium of 17.48% and protein of 3%. The eggshell powder was also found to contain a small number of minerals such as potassium, iron, sodium, or phosphorus. The result was inconsistent with a study done by Ray *et al.* (2017), the calcium content was found to be approximately two-fold higher than this current study [10]. Chemical analysis showed that eggshell is composed of 97% calcium carbonate [22, 23] which was considerably higher than this study. This was possibly due to the genetic factors, breeders and breeding level which affected the chemical constituents of eggshells [23].

Table 1 Compositions of obtained eggshell powder

Composition	Percentage (%)
Calcium carbonate ($CaCO_3$)	74.700
Calcium	17.480
Magnesium oxide (MgO)	0.530
Protein	3.000
Lipid	0.270
Potassium (K)	0.018
Iron (Fe)	0.002
Sodium (Na)	0.082
Phosphorus (P)	0.130

The addition of liquid nigari showed significant impacts on the yield of obtained tofu among analyzed groups ($p < 0.05$). Figure 1A showed the discrepancy in tofu yield with the addition of liquid nigari at varied concentrations. As the result, the liquid nigari at 2% was found to achieve the yield of 143.24 ± 1.68 g/ 100 g. The achieved tofu yield was comparable with previous studies. The use of 2 M $MgCl_2$ as a coagulant in the study of Zhu *et al.* (2016) revealed the tofu yield of 116.05 ± 1.06 g/100g [24]. Similarly, Shi *et al.* (2020) also prepared the tofu by adding the $MgCl_2$ with a yield of 133 g/100 g [25]. The increment in the tofu yield was attributed to the water holding capacity in the presence of liquid nigari. The mixture of salts ($MgCl_2$, $CaCl_2$) in the liquid nigari produced a uniform and continuous gel structure that was able to trap more water and soluble components in the tofu network, leading to a higher yield of tofu [26]. The increase in the tofu yield was highly associated with the increment of moisture content or water holding capacity in the tofu structure [27]. Increasing the liquid nigari content up to 3% was observed not to cause a significant influence on the tofu yield ($p \geq 0.05$).

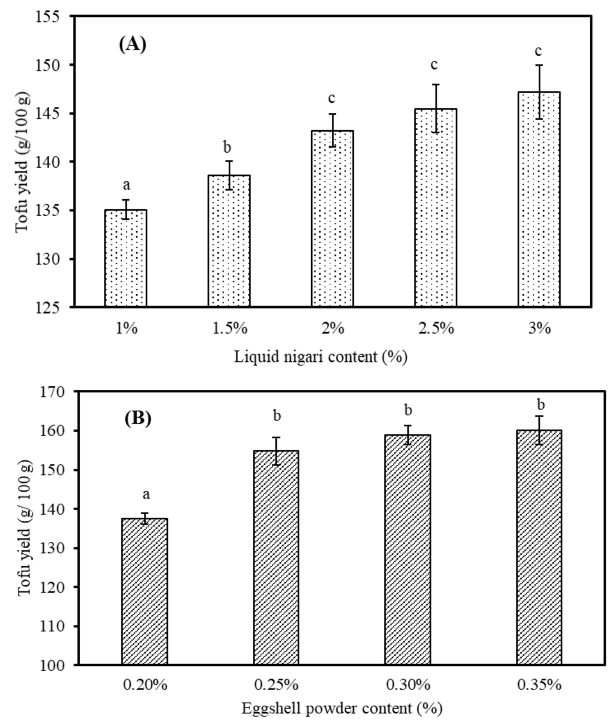


Figure 1 The yield of tofu at different content of A) liquid nigari and B) fixed liquid nigari content at 2% with eggshell powder (0.2% - 0.35%)

3.2 Effect of Liquid Nigari on the Tofu Yield and Textural Characteristics

In terms of the texture profile of tofu prepared at different contents of liquid nigari, the result also revealed a significant difference in analyzed textural characteristics of tofu ($p < 0.05$). Table 2 presents the texture profile of tofu with different contents of liquid nigari. Liquid nigari serves a role as a coagulant with the predominant compounds of $MgCl_2$ and $CaCl_2$ that contributes to the natural tofu flavor while maintaining the original taste of soybeans [28]. The hardness of tofu presents its resistance to destructive force during processing or application [29]. In this study, the highest hardness at the first compression (16.8 ± 1.55 N) of tofu was at the liquid nigari content of 1%. Under the heating process, soy protein is denatured, leading to the formation of agglomerates with negative charges on the surface [29]. The coagulation process occurs when metal ions such as Mg^{2+} or Ca^{2+} form bridges with the negatively charged protein via the electrostatic interaction between the cations and the proteins [30]. The hardness of the resulting tofu was due to the dense and compact structure of tofu by the cross-linking action of proteins and coagulants. In this study, the highest hardness of tofu at 1% of liquid nigari indicated the denser and more compact structure of tofu. Normally, the higher concentration of liquid nigari (higher content of $MgCl_2$) was found to better produce hardness of tofu by accelerating the coagulation process with the intense interaction between proteins and cations, resulting in a more compact structure of tofu [31]. The elevation of liquid nigari addition in this study, however, was found to reduce the hardness of prepared tofu. This was highly aligned to the tofu yield or the water holding capacity. The liquid nigari at 1% might contribute to a quicker coagulation process to induce a denser structure due to the loss of water inside the protein gel network [14, 32]. Meanwhile, the increase in the liquid nigari content showed the higher tofu yield which means that the higher water content is trapped in the protein gel network, leading to the loose structure of tofu or softer tofu. High holding water capacity has been highly correlated to lower hardness in tofu [14]. The difference in textural characteristics was ascribed to not only the coagulants but also various intrinsic and extrinsic factors such as protein concentration in soybean, pH, ionic strength, and temperature during the coagulation process [33].

The hardness at the second compression was observed with the same tendency that the hardness noticeably decreased from 8.63 ± 0.94 N to 4.44 ± 0.12 N when increasing the liquid nigari content from 1% to 3%. The similarly decreasing trends were observed in other textural characteristics such as gumminess and chewiness when increasing the liquid nigari content from 1 to 3%. Gumminess is the force required to break down the tofu before swallowing and chewiness is the energy required for chewing to be ready for swallowing. Cohesiveness represents the ability of tofu to resist deformation. The cohesiveness was found to be insignificant different among analyzed groups ($p \geq 0.05$). Carrageenan was noted to cause a significant reduction in the hardness but less affecting the cohesiveness of tofu [29]. Springiness values of tofu at different contents of liquid nigari were insignificantly different from each other, suggesting that tofu might nearly spring back to its originally physical shape after occurring deformation [34]. In this study, liquid nigari at 2% formulated the resulting tofu with the highest yield, and textural characteristics of cohesiveness, springiness was insignificant among analyzed groups. Therefore, the formulation of tofu was selected at 2% of liquid nigari for further analysis.

Table 2 Texture profile of the obtained tofu at different contents of liquid nigari

Liquid nigari content (%)	Hardness-first compression (N)	Hardness-second compression (N)	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (mJ)
1	16.8 ± 1.55^{d}	8.63 ± 0.94^{c}	0.25 ± 0.03^{a}	13.36 ± 0.34^{b}	4.28 ± 0.90^{c}	57 ± 10.55^{c}
1.5	11.65 ± 0.59^{c}	7.24 ± 0.44^{bc}	0.21 ± 0.03^{a}	12.66 ± 0.22^{ab}	2.55 ± 0.37^{ab}	32.33 ± 2.30^{ab}
2	11.06 ± 0.37^{c}	5.71 ± 1.16^{ab}	0.28 ± 0.06^{a}	13.06 ± 0.39^{ab}	3.06 ± 0.56^{bc}	40.09 ± 8.40^{bc}
2.5	7.50 ± 0.22^{b}	4.92 ± 0.13^{a}	0.26 ± 0.05^{a}	12.4 ± 0.22^{a}	1.96 ± 0.37^{ab}	24.36 ± 4.72^{ab}
3	6.41 ± 0.47^{a}	4.44 ± 0.12^{a}	0.28 ± 0.04^{a}	12.72 ± 0.36^{ab}	1.61 ± 0.11^{a}	20.53 ± 1.95^{a}

Data are expressed as mean ± standard deviation. Superscripts (a, b, c) indicate the statistically significant difference in mean values within the same column ($p < 0.05$).

3.3 Effect of Eggshell Powder on the Yield and Textural Characteristics of Tofu

The influence of eggshell powder addition on the tofu yield is depicted in Figure 1B. The addition of 0.2% eggshell powder was found not to cause the impact on the tofu yield but it started enhancing the tofu yield to 154.79 ± 3.56 g/100 g at 0.25% eggshell powder addition. However, the increase in eggshell powder content at 0.3% and 0.35% was not different from that at 0.25% ($p > 0.05$). Upon the results from the proximate analysis, the predominant component of eggshell powder was $CaCO_3$ which contributed to the synergistic effect with liquid nigari to better promote tofu yield. Kao *et al*. (2013) reported that 0.4% $CaSO_4$ formed a uniform protein network with more trapped water, exhibiting better tofu yield. The $CaCl_2$ was found to significantly increase the tofu yield when increasing its concentration, resulting in a great amount of tofu coagulation [13]. Some previous studies produced the tofu with a very high yield (400-600 g/100g), which was attributed to the discrepancies in types of coagulant, genetic factors of soybean, pH, cultivation, etc. [14, 29, 35, 36]. Kim *et al.* (2007) also revealed that the tofu prepared with 0.1% oyster shell powder achieved a higher yield than that with the single use of 0.2% $MgCl_2$ [17]. This confirmed the hardening effect of eggshell powder in this current study.

Table 3 Texture profile of the obtained tofu at different eggshell powder contents

Eggshell powder content (%)	Hardness-first compression (N)	Hardness-second compression (N)	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (mJ)
0.20	17.20 ± 2.99[a]	9.15 ± 2.40[a]	0.20 ± 0.01[a]	13.11 ± 0.27[a]	3.46 ± 0.68[a]	45.32 ± 9.15[a]
0.25	19.10 ± 2.02[a]	11.05 ± 1.12[ab]	0.26 ± 0.02[b]	13.50 ± 0.06[a]	4.94 ± 0.50[b]	66.68 ± 7.01[b]
0.30	20.31 ± 1.65[ab]	10.32 ± 2.24[ab]	0.24 ± 0.01[b]	13.09 ± 0.15[a]	4.88 ± 0.51[b]	63.77 ± 6.49[b]
0.35	25.59 ± 1.71[b]	14.30 ± 0.54[b]	0.19 ± 0.01[a]	13.59 ± 0.35[a]	4.81 ± 0.20[b]	65.34 ± 3.19[b]

Data are expressed as mean ± standard deviation. Superscripts (a, b, c) indicate the statistically significant difference in mean values within the same column ($p < 0.05$). The resulting tofu was prepared with the addition of 2% liquid nigari and different contents of eggshell powder (0.2% - 0.35%).

The effect of eggshell powder addition to the tofu preparation on the textural characteristics is listed in Table 3. In this study, the hardness of the resulting tofu was noticeably different when adding different content of eggshell powder ($p \leq 0.05$). The addition of 0.2% eggshell powder promoted a hardening effect on the structure of resulting tofu (hardness: 17.2 ± 2.99 N) compared to tofu prepared only with 2% liquid nigari. The more eggshell powder content was added to the soybean milk, the more hardness of resulting tofu was recorded. The highest hardness was found at 0.35% of eggshell powder addition with respect to 25.59 ± 1.71 N. A similar trend was noted at the hardness of the second compression, from 8.53 ± 0.91 N to 14.3 ± 0.54 N when increasing the eggshell powder content from 0.2% to 0.35%. The result was in agreement with previously reported studies [25, 31, 37]. The hardness of tofu was caused by the denser and more compact structure of resulting tofu via the Ca-protein bridges and hydrogen bonds also partially contribute to the hardening effect [26]. Although the eggshell powder caused a noticeable impact on the hardness of the resulting tofu ($p < 0.05$), cohesiveness and springiness were insignificantly affected by the presence of eggshell powder. A similar finding was observed in a study done by Kim *et al.* (2007) that the oyster shell powder hardened the tofu structure but was not able to increase cohesiveness [17]. Springiness in this study did not differ from each other, indicating the return to the original physical shape after applying deformation force [34]. As gumminess and chewiness were highly correlated to hardness, they appeared to have the same tendency [26]. Gumminess increased from 3.46 ± 0.68 N to 4.81 ± 0.2 N while chewiness increased from 45.32 ± 9.15 mJ to 65.34 ± 3.19 mJ when increasing the eggshell powder content from 0.2% to 0.35%. In summary, the eggshell powder at 0.25% showed an improvement in tofu yield and hardness, which could be selected as a suitable parameter for subsequent analysis. The visual appearance of soybean curd and resulting tofu prepared with 2% liquid nigari and 0.25% eggshell powder is described in Figure 2C and 2D. Tofu prepared without adding eggshell powder (Figure 2A and 2B) seemed to have more brightness while tofu with the addition of 2% liquid nigari and 0.25% eggshell powder appeared to have a more yellowish color.

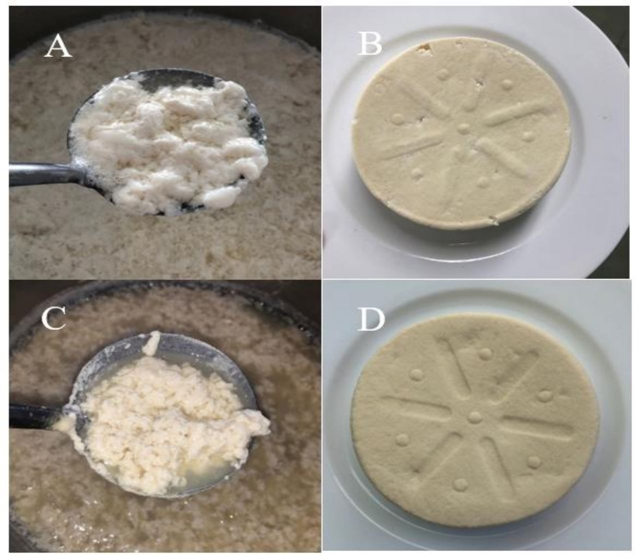


Figure 2 Visual appearance of A) bean curd and B) tofu curd in addition with 2% of liquid narigi, and C) bean curd and D) tofu curd in addition with 2% of liquid narigi and 0.25% of eggshell powder

3.4 Comparison with Traditional Tofu Products

The difference in textural characteristics between the studied tofu and two samples of traditional tofu is presented in Table 4. Two samples of traditional tofu were randomly selected for the experiment. As a result, sample 1 showed better textural characteristics than sample 2. Sample 1 showed a higher hardness (14.5 ± 1.45 N > 4.72 ± 0.23 N), cohesiveness (0.2 ± 0.02 > 0.16 ± 0.03), and springiness (12.9 ± 0.36 mm > 9.39 ± 1.51 mm) than sample 2. The textural characteristics of sample 1 were fairly similar to those of tofu prepared with only 2% liquid nigari. However, the studied tofu prepared with 2% liquid nigari and 0.25% eggshell powder showed the better textural characteristics with the highest hardness (19.1 ± 2.02 N), cohesiveness (0.26 ± 0.02), springiness (13.5 ± 0.06 mm), and many other values compared to traditional tofu products. This result confirmed the reinforcing effects of eggshell powder on the quality of tofu in terms of structural aspects.

Table 4 Texture profiles of studied tofu compared to commercial products

Tofu	Hardness-first compression (N)	Hardness-second compression (N)	Cohesiveness	Springiness (mm)	Gumminess (N)	Chewiness (mJ)
Sample 1	14.50 ± 1.45^{b}	8.53 ± 0.91^{b}	0.20 ± 0.02^{a}	12.90 ± 0.36^{b}	2.92 ± 0.15^{b}	37.66 ± 2.68^{b}
Sample 2	4.72 ± 0.23^{a}	3.56 ± 0.20^{a}	0.16 ± 0.03^{a}	9.39 ± 1.51^{a}	0.78 ± 0.17^{a}	7.49 ± 2.64^{a}
Studied sample	19.10 ± 2.02^{c}	11.05 ± 1.12^{c}	0.26 ± 0.02^{b}	13.50 ± 0.06^{c}	4.94 ± 0.5^{c}	66.68 ± 7.01^{c}

Data are expressed as mean ± standard deviation. Superscripts (a, b, c) indicate the statistically significant difference in mean values within the same column ($p < 0.05$).

4.0 CONCLUSION

In this study, the process of obtaining eggshell powder and its application on tofu preparation were successfully evaluated. The obtained eggshell powder was analyzed with the predominant component of calcium salt ($CaCO_3$). The addition of liquid nigari and eggshell powder significantly caused a significant impact on the texture profile of the resulting tofu. Addition of 2% liquid nigari was found to promote better tofu yield. Moreover, tofu prepared with 2% liquid nigari and 0.25% eggshell powder showed an improvement in textural characteristics of tofu prepared with only 2% liquid nigari. In comparison with two samples of traditional tofu, the studied tofu showed better textural characteristics. The result indicated that eggshell powder could be an alternative to coagulants used for tofu preparation.

References

[1] Waheed, M., Butt, M. S., Shehzad, A., Adzahan, N. M., Shabbir, M. A., Suleria, H. A. R. and Aadil, R. M. 2019. Eggshell Calcium: A Cheap Alternative to Expensive Supplements. *Trends in Food Science and Technology*. 91: 219-230. Doi: https://doi.org/10.1016/j.tifs.2019.07.021.

[2] Philippidis, G., Sartori, M., Ferrari, E. and M'Barek, R. 2019. Waste Not, Want Not: A Bio-economic Impact Assessment of Household Food Waste Reductions in the EU. *Resources, Conservation and Recycling*. 146: 514-522. Doi: https://doi.org/10.1016/j.resconrec.2019.04.016.

[3] FAO. 2011. Global Food Glosses and Food Waste.

[4] Ajala, E. O., Eletta, O. A. and Oyeniyi, S. K. 2018. Characterization and Evaluation of Chicken Eggshell for Use as a Bio-resource. *Arid Zone Journal of Engineering, Technology and Environment*. 14(1): 26-40.

[5] Murakami, F. S., Rodrigues, P. O., Campos, C. M. T. D and Silva, M. A. S. 2007. Physicochemical Study of $CaCO_3$ from Egg Shells. *Food Science and Technology*. 27(3): 658-662. Doi: https://doi.org/10.1590/S0101-20612007000300035.

[6] Oliveira, D. A., Benelli, P. and Amante, E. R. 2013. A Literature Review on Adding Value to Solid Residues: Egg Shells. *Journal of Cleaner Production*. 46: 42-47. Doi: https://doi.org/10.1016/j.jclepro.2012.09.045.

[7] Hou, Y., Shavandi, A., Carne, A., Bekhit, A. A., Ng, T. B., Cheung, R. C. F. and Bekhit, A. E. D. A. 2016. Marine Shells: Potential Opportunities for Extraction of Functional and Health-promoting Materials. *Critical Reviews in Environmental Science and Technology*. 46(11-12): 1047-1116. Doi: https://doi.org/10.1080/10643389.2016.1202669.

[8] Jia, H. X., Han, J. H., Li, H. Z., Liang, D., Deng, T. T. and Chang, S. Y. 2016. Mineral Intake in Urban Pregnant Women from Base Diet, Fortified Foods, and Food Supplements: Focus on Calcium, Iron, and Zinc. *Biomedical and Environmental Sciences*. 29(12): 898-901. https://doi.org/ 10.3967/ bes2016.120.

[9] Bartter, J., Diffey, H., Yeung, Y. H., O'Leary, F., Häsler, B., Maulaga, W. and Alders, R. 2018. Use of Chicken Eggshell to Improve Dietary Calcium Intake in Rural Sub-saharan Africa. *Maternal & Child Nutrition*. 14 :12649. Doi: https://doi.org/10.1111/mcn.12649.

[10] Ray, S., Barman, A. K., Roy, P. K. and Singh, B. K. 2017. Chicken Eggshell Powder as Dietary Calcium Source in Chocolate Cakes. *The Pharma Innovation*. 6(9): 1-4.

[11] Kobus-Cisowska, J., Szymanowska-Powałowska, D., Szymandera-Buszka, K., Rezler, R., Jarzębski, M., Szczepaniak, O., Marciniak, G., Jędrusek-Golińska, A. and Kobus-Moryson, M. 2020. Effect of Fortification with Calcium from Eggshells on Bioavailability, Quality, and Rheological Characteristics of Traditional Polish Bread Spread. *Journal of Dairy Science*. 103(8): 6918-6929. Doi: https://doi.org/ 10.3168/jds.2019-18027.

[12] Świątkiewicz, S., Arczewska-Włosek, A., Krawczyk, J., Puchała, M. and Józefiak, D. 2015. Effects on Performance and Eggshell Quality of Particle Size of Calcium Sources in Laying Hens' Diets with Different Ca Concentrations. *Archives Animal Breeding*. 58(2): 301-307. Doi: https://doi.org/10. 5194/aab-58-301-2015.

[13] Wang, C., Johnson, L. A. and Wilson, L. A. 2003. Calcium Coagulation Properties of Hydrothermally Processed Soymilk. *Journal of the American Oil Chemists' Society*. 80(12): 1225-1229. Doi: https://doi.org/10.1007/s 11746 -003-0846-2.

[14] Obatolu, V. A. 2008. Effect of Different Coagulants on Yield and Quality of Tofu from Soymilk. *European Food Research and* Technology. 226(3): 467-472. Doi: https://doi.org/10. 1007/s00217-006-0558-8.

[15] Lu, J. Y., Carter, E. and Chung, R. A. 1980. Use of Calcium Salts for Soybean Curd Preparation. *Journal of Food Science*. 45(1): 32-34. Doi: https://doi.org/10.1111/j.1365-2621.1980.tb03864.x.

[16] Sudarmo, S. M. and Lengkey, H. A. W. 2020. The Effect of Coagulant Type and Concentration on the Quality of Milk Tofu. *Scientific Papers Series-Management, Economic Engineering in Agriculture and Rural Development*. 20(4): 513-518.

[17] Kim, Y. S., Choi, Y. M., Noh, D. O., Cho, S. Y. and Suh, H. J. 2007. The Effect of Oyster Shell Powder on the Extension of the Shelf Life of Tofu. *Food Chemistry*. 103(1): 155-160. Doi: https://doi.org/10.1016/j.foodchem.2006.07.040.

[18] Darmajana, D. A., Afifah, N. and Indriati, A. 2020. The Effect of Extraction Method and Types of Coagulants on the Results and Physicochemical Properties of Tofu. *Food Science and Technology*. 40: 677-682. https://doi.org/ 10.1590/fst.34719

[19] Ho, W. F., Hsu, H. C., Hsu, S. K., Hung, C. W. and Wu, S. C. 2013. Calcium Phosphate Bioceramics Synthesized from

Eggshell Powders through a Solid State Reaction. *Ceramics International*. 39(6): 6467-6473. Doi: https://doi.org/10.1016/j.ceramint.2013.01.076.

[20] Cai, T. D. and Chang, K. C. 1998. Characteristics of Production-scale Tofu as Affected by Soymilk Coagulation Method: Propeller Blade Size, Mixing Time and Coagulant Concentration. *Food Research International*. 31(4): 289-295. Doi: https://doi.org/10.1016/S0963-9969(98)00091-X.

[21] Saio, K., Kamiya, M. and Watanabe, T. 1969. Food Processing Characteristics of Soybean 11s and 7s Proteins: Part I. Effect of Difference of Protein Components among Soybean Varieties on Formation of Tofu-gel. *Agricultural and Biological Chemistry*. 33(9): 1301-1308. Doi: https://doi.org/10.1080/00021369.1969.10859465.

[22] Cree, D. and Rutter, A. 2015. Sustainable Bio-inspired Limestone Eggshell Powder for Potential Industrialized Applications. *ACS Sustainable Chemistry & Engineering*. 3(5): 941-949. Doi: https://doi.org/10.1021/acssuschemeng.5b00035.

[23] Hunton, P. 2005. Research on Eggshell Structure and Quality: An Historical Overview. *Brazilian Journal of Poultry Science*. 7: 67-71. Doi: https://doi.org/10.1590/S1516-635X2005000200001.

[24] Zhu, Q., Wu, F., Saito, M., Tatsumi, E. and Yin, L. 2016. Effect of Magnesium Salt Concentration in Water-in-oil Emulsions on the Physical Properties and Microstructure of Tofu. *Food Chemistry*. 201: 197-204. Doi: https://doi.org/10.1016/j.foodchem.2016.01.065.

[25] Shi, Y. G., Yang, Y., Piekoszewski, W., Zeng, J. H., Guan, H. N., Wang, B., Liu, L. L., Zhu, X. Q., Chen, F. L. and Zhang, N. 2020. Influence of Four Different Coagulants on the Physicochemical Properties, Textural Characteristics and Flavour of Tofu. *International Journal of Food Science & Technology*. 55(3): 1218-1229. Doi: https://doi.org/10.1111/ijfs.14357.

[26] Kao, F. J., Su, N. W. and Lee, M. H. 2003. Effect of Calcium Sulfate Concentration in Soymilk on the Microstructure of Firm Tofu and the Protein Constitutions in Tofu Whey. *Journal of Agricultural and Food Chemistry*. 51(21): 6211-6216. Doi: https://doi.org/10.1021/jf0342021.

[27] Cai, T. D., Chang, K. C., Shih, M. C., Hou, H. J. and Ji, M. 1997. Comparison of Bench and Production Scale Methods for Making Soymilk and Tofu from 13 Soybean Varieties. *Food Research International*. 30(9): 659-668. Doi: https://doi.org/10.1016/S0963-9969(98)00032-5.

[28] Li, J., Cheng, Y., Tatsumi, E., Saito, M. and Yin, L. 2014. The Use of W/O/W Controlled-release Coagulants to Improve the Quality of Bittern-solidified Tofu. *Food Hydrocolloids*. 35: 627-635. Doi: https://doi.org/10.1016/j.foodhyd.2013.08.002.

[29] Shen, Y. R. and Kuo, M. I. 2017. Effects of Different Carrageenan Types on the Rheological and Water-holding Properties of Tofu. *LWT*. 78: 122-128. Doi: https://doi.org/10.1016/j.lwt.2016.12.038.

[30] Li Tay, S., Yao Tan, H. and Perera, C. 2006. The Coagulating Effects of Cations and Anions on Soy Protein. *International Journal of Food Properties*. 9(2): 317-323. Doi: https://doi.org/10.1080/10942910600596340.

[31] Prabhakaran, M. P., Perera, C. O. and Valiyaveettil, S. 2006. Effect of Different Coagulants on the Isoflavone Levels and Physical Properties of Prepared Firm Tofu. *Food Chemistry*. 99(3): 492-499. Doi: https://doi.org/10.1016/j.foodchem.2005.08.011.

[32] Noh, E. J., Park, S. Y., Pak, J. I., Hong, S. T. and Yun, S. E. 2005. Coagulation of Soymilk and Quality of Tofu as Affected by Freeze Treatment of Soybeans. *Food Chemistry*. 91(4): 715-721. Doi: https://doi.org/10.1016/j.foodchem.2004.06.050

[33] Sun, X. D. and Arntfield, S. D. 2012. Molecular Forces Involved in Heat-induced Pea Protein Gelation: Effects of Various Reagents on the Rheological Properties of Salt-extracted Pea Protein Gels. *Food Hydrocolloids*. 28(2): 325-332. Doi: https://doi.org/10.1016/j.foodhyd.2011.12.014.

[34] Joo, K. H. and Cavender, G. A. 2020. Investigation of Tofu Products Coagulated with Trimagnesium Citrate as a Novel Alternative to Nigari and Gypsum: Comparison of Physical Properties and Consumer Preference. *LWT*. 118: 108819. Doi: https://doi.org/10.1016/j.lwt.2019.108819.

[35] Zuo, F., Chen, Z., Shi, X., Wang, R. and Guo, S. 2016. Yield and Textural Properties of Tofu as Affected by Soymilk Coagulation Prepared by a High-temperature Pressure Cooking Process. *Food Chemistry*. 213: 561-566. Doi: https://doi.org/10.1016/j.foodchem.2016.07.008.

[36] Hou, H. J. and Chang, K. C. 2003. Yield and Textural Properties of Tofu as Affected by the Changes of Phytate Content during Soybean Storage. *Journal of Food Science*. 68(4): 1185-1191. Doi: https://doi.org/10.1111/j.1365-2621.2003.tb09622.x.

[37] Zhao, Y. Y., Cao, F. H., Li, X. J., Mu, D. D., Zhong, X. Y., Jiang, S. T., Zheng, Z. and Luo, S. Z. 2020. Effects of Different Salts on the Gelation Behaviour and Mechanical Properties of Citric Acid-induced Tofu. *International Journal of Food Science & Technology*. 55(2): 785-794. Doi: https://doi.org/10.1111/ijfs.14348.