Optimization of Pre-treatment Parameters on the Protein Content of the Liquid Extract of Sweet Potato (*var*. VitAto) for Developing a Plant-based Beverage Intended as a Milk Alternative

ABSTRACT

Aims: This studyaimed to identify the most effective method and optimal parameters for enhancing the protein content during the pre-treatment phase of extracting the liquid component from a local sweet potato (*var*. VitAto) in developing a plant-based beverage as a milk alternative (PBMA).
Study design: Response surface methodology (RSM) which involved investigating three key factors—percentage of sodium carbonate, ratio of VitAto to alkaline solution, and soaking time—each at three different levels was implemented in this study.
Place and Duration of Study: The study was conducted at the Food Science & Technology Research Center, Malaysian Agricultural Research & Development Institute (MARDI), utilizing VitAto sourced from a local farm in Bachok, Kelantan, between August 2021 to July 2022.
Methodology: Various treatment approaches were explored, including soaking with water, acidic or alkaline solutions, blanching, boiling, and steaming. Following treatment, the liquid portion was extracted using a heavy-duty juicer and subsequently filtered through cheesecloth. The protein content of the extracts was then quantified using the AOAC method. The pre-treatment method resulting in the highest protein content was chosen for further optimization using RSM.
Results: Soaking the sweet potato in an alkaline solution (sodium carbonate), proved to be the most effective pre-treatment method, resulting in a fivefold increase in protein content compared to other methods. The optimal extraction parameters were determined to be a 3.0% concentration of sodium carbonate, a 1:1 ratio of VitAto to alkaline solution, and a soaking time of 20 hours. The predicted protein content under these conditions (2.8 g/100g) exceeded the proposed minimum protein content for PBMA (2.2 g/100g).
Conclusion: The optimum parameters for the protein content of pre-treatment in extracting the liquid part of sweet potato (VitAto) for developing PBMA were determined to be 3% sodium carbonate, a 1:1 ratio of VitAto to alkaline solution, and a soaking time of 20 hours.

Keywords: Plant-based beverage, milk alternative, sweet potato, optimization, pre-treatment, response surface methodology

1. INTRODUCTION

The rising demand for plant-based beverage as milk alternatives (PBMA) reflects a significant shift in consumer preferences towards sustainable, ethical, and health-conscious dietary choices. One of the primary motivations for replacing traditional cow's milk with plant-derived alternatives lies in addressing various dietary restrictions and health concerns, including lactose intolerance, milk protein allergies, ethical considerations associated with veganism, and also occurrence of hypercholesterolemia [1]. Lactose intolerance, a prevalent condition affecting a substantial portion of the population worldwide, necessitates the exploration of dairy-free alternatives to meet the nutritional needs of individuals unable to digest lactose, the sugar present in dairy milk. Plant-based milk alternatives offer a lactose-free solution, without causing gastrointestinal discomfort [2]. However, it is essential to note that some individuals may experience allergic reactions to certain plant-based ingredients used in PBMA formulations, highlighting the importance of allergenicity considerations in product development.

Common examples of plant-based milk alternatives include soy milk, almond milk, oat milk, and coconut milk; each offering distinct sensory profiles and nutritional compositions. Soy milk, derived from soybeans, is a popular choice among consumers due to its high protein content and versatility in culinary applications. However, soy allergies affect a subset of the population, necessitating alternative options for individuals with soy protein sensitivities. Almond milk, made from ground almonds and water, has gained popularity for its delicate nutty flavor and low calorie content. Despite its nutritional benefits, almond milk may pose allergenic risks for individuals with tree nut allergies, underscoring the importance of allergen labeling and product transparency in the food industry. Oat milk, produced from oats and water, has emerged as a sustainable and environmentally friendly alternative to dairy milk, offering a creamy texture and neutral taste suitable for various beverages and culinary recipes. Oats are naturally gluten-free; however, cross-contamination during processing may occur, posing a risk for individuals with gluten sensitivities or celiac disease. Coconut milk, derived from the grated flesh of mature coconuts, boasts a rich, creamy texture and distinctive tropical flavor profile. While coconut allergies are relatively rare, individuals with tree nut allergies should exercise caution when consuming coconut-based products due to potential cross-reactivity [3].

In addition to addressing dietary restrictions and allergenic concerns, the adoption of plant-based milk alternatives aligns with the principles of veganism, a lifestyle choice characterized by the avoidance of animal products for ethical, environmental, and health reasons. Vegan-friendly plant-based beverages offer a compassionate and sustainable alternative to conventional dairy milk, supporting the growing demand for cruelty-free and environmentally conscious food options. Due to these reasons, the present study introduce sweet potato as the main ingredients for developing a plant-based milk alternative.

Sweet potatoes, notably the orange-flesh 'VitAto' variety, are a versatile and nutrient-rich crop that has found its way into various culinary applications. However, their potential as a source of plant-based milk alternative remains relatively unexplored. The liquid part of sweet potatoes, often an overlooked by-product, has the potential to be a valuable source of protein and other essential nutrients [4]. This present study aims to optimize the pre-treatment parameters of extracting the liquid fraction of sweet potato (var. VitAto), focusing on high protein content as the essential macronutrient in milk alternatives. Understanding the pre-treatment methods and their effects on the final product can help manufacturers optimize their production processes and produce high-quality plant-based milk alternatives.

2. MATERIAL AND METHODS

2.1 Materials

Local sweet potato *var*. VitAto was purchased from farmers in Bachok, Kelantan, Peninsular Malaysia. The tuber was sorted; only the good ones were washed to remove the soil and dirt and then ready for extraction or pre-treatments. All the chemicals (sodium carbonate and acetic acid) were food-grade and purchased from local manufacturers.

2.2 Pre-treatment and extraction of the liquid part

Few pre-treatment techniques were applied to the sweet potato, as in Table 3. The solution was drained if soaked and directly put into an industrial stainless steel juice extractor (model WF-B3000, China) for liquid extraction. Then, it was stored at a chilled temperature for two hours to precipitate the starch. After that, the liquid was filtered using cheesecloth and centrifuged for 30 minutes at 4800 rpm. Next, the supernatant was packaged and kept frozen for protein analysis.

2.3 Protein analysis

Protein was determined using the AOAC 988.05 [5] Kjeldahl method, where the total nitrogen was converted to protein using factor 6.25.

2.4 Experimental design

Parameters for the selected pre-treatment were optimized using Response surface methodology (RSM). The independent variables were the percentage of sodium carbonate (x_1), the ratio of the sweet potato and alkaline solution (x_2), and soaking time (x_3). Table 1 shows the variables and their levels. A face-centered central composite rotatable design was applied in designing the experiments using MINITAB 14 statistical package (Minitab Inc., State College, USA). Seventeen combinations of the variables were generated (Table 2) for the liquid extraction from VitAto.

Response surface regression analyses were used to model the effect of the independent variables on the protein content of extracted liquid from VitAto using Minitab 14 statistical package. Experimental data were fitted to a quadratic polynomial model; its adequacy was checked based on R_2 and adjusted-R_2 and the lack of fit error. The model proposed for the response (Y) was as follows:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_{21} + b_{22}x_{22} + b_{33}x_{23} + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$$

Table 1. Independent process variables and their corresponding levels

Variables	Levels			
	Symbol	**-1**	**0**	**1**
Sodium carbonate (%)	x_1	3.0	7.5	12.0
VitAto: Alkaline solution (w/v)	x_2	1:1	1:2	1:3
Soaking time (hour)	x_3	15.0	19.5	24.0

Table 2. Seventeen combinations of independent variables for the extraction of the liquid part of VitAto*

Run Order	x_1	x_2	x_3	Protein content

				(g/100g)
1	-1 (3.0)	0 (1:2)	0 (19.5)	1.4
2	0 (7.5)	1 (1:3)	0 (19.5)	1.6
3	0 (7.5)	0 (1:2)	0 (19.5)	2.3
4	0 (7.5)	0 (1:2)	0 (19.5)	2.4
5	0 (7.5)	0 (1:2)	0 (19.5)	2.2
6	1 (12.0)	1 (1:3)	1 (24.0)	2.1
7	0 (7.5)	0 (1:2)	-1 (15.0)	1.4
8	-1 (3.0)	1 (1:3)	-1 (15.0)	1.6
9	0 (7.5)	-1 (1:1)	0 (19.5)	2.3
10	1 (12.0)	1 (1:3)	-1 (15.0)	1.3
11	-1 (3.0)	1 (1:3)	1 (24.0)	1.1
12	1 (12.0)	-1 (1:1)	1 (24.0)	1.1
13	1 (12.0)	-1 (1:1)	-1 (15.0)	1.4
14	-1 (3.0)	-1 (1:1)	-1 (15.0)	1.6
15	1 (12.0)	0 (1:2)	0 (19.5)	1.5
16	-1 (3.0)	-1 (1:1)	1 (24.0)	2.6
17	0 (7.5)	0 (1:2)	1 (24.0)	1.8

*****The table represents the coded value (and absolute value)

2.5Extraction procedure of the liquid part from VitAto

VitAto was washed to remove the dirt and soil. Then, they were soaked in soda ash (sodium carbonate) solution according to the combinations of the variables in Table 2. After draining, the VitAto was washed two times with potable water and peeled off using stainless steel industrial tuber crops peeling machine (Model P-10, Osaka, Japan).Next, three-minute steaming of the VitAto was employed, and the liquid part was extracted using an industrial juicer (Model WF-B3000, China). The extract was then placed in a cold room for two hours for starch sedimentation and filtered through double cheesecloth. The liquid was centrifuged at 4800 rpm for 30 minutes before being packed and sent for protein analysis.

2.5 Statistical analyses

Each analysis was conducted in triplicate, and the results were averaged and expressed as mean ± standard deviation. Analysis of variance was carried out to analyze the differences between samples, and the significant differences were determined using the Tukey test at *P* = .05 using MINITAB 14 statistical software (Minitab Inc., State College, USA).

3. RESULTS AND DISCUSSION

3.1 Selection of pre-treatment technique

Pre-treatment of raw materials before extracting the soluble part is necessary for producing PBMA. This step is essential to improve the final product's solubility, yield, and sensory properties [3]. Soaking is one of the most common pre-treatment methods in plant-based milk production. Immersing the raw material in water or other solutions for a specific period can soften and hydrate the plant material, making the extraction of the soluble part easier and increasing the extraction yield [6]. Besides, soaking also helps deactivate nutrient inhibitors, release toxins, and improve its physicochemical properties [7] [8] [9]. As shown in Table 3, soaking increased the extract's protein content, although it was not significantly different from the control (sample A). This result is consistent with the studies by Thakur et al. [10] and Adekanmi et al. [11], which also found that soaking increased the protein in buckwheat, quinoa, amaranth, and tiger nut. Meanwhile, adding acid and alkaline substances during soaking significantly increased the protein content (Samples C and D). This is likely due to the modification of pH affecting protein solubility [12]. Blanching and steaming (Samples E and F) also increased the protein content, but they were not significantly different from the control. In a study conducted by Tunde-Akintunde and Souley [13], both soaking and blanching led to increased protein content in soymilk. The increase in protein content was attributed to the finer slurry obtained through blanching, which generated more filtrate. Consequently, a higher yield of soymilk was obtained, raising its protein content. Based on the results obtained, soaking with an alkaline solution (sodium carbonate) was selected for further optimization of its parameters as the pre-treatment method, due to the highest protein content in the extract.

Table 3. Protein content in samples of different pre-treatments of the liquid part of VitAto

Sample	**Pre-treatment**	**Protein content (g/100g)***
A	None	1.15 ± 0.14^{a}
B	Soak overnight (VitAto: water; 1:3)	1.30 ± 0.07^{a}
C	Soak with 5% citric acid (VitAto: acid solution; 1:3)	1.80 ± 0.14^{bc}
D	Soak with 5% soda ash (VitAto: alkaline solution; 1:3)	2.20 ± 0.14^{b}
E	Blanch for 3 minutes	1.55 ± 0.07^{ac}

F	Steam for 5 minutes	1.70 ± 0.14^{abc}

***Values are the mean ± standard deviations (n=3). Means with different superscript alphabets indicate significantly different (P = .05)

3.2 Protein content in VitAto extracts treated with alkaline soaking with different combinations of independent variables

According to the data presented in Table 2, VitAto extracts subjected to various combinations of independent variables recorded protein contents ranging from 1.1 g/100g to 2.6 g/100g. These observed values of protein content in the extracts surpassed those reported in fresh VitAto by Zulkifli et al. [4]. This discrepancy may be attributed to alkaline soaking, which disrupts plant cell structures, thereby facilitating the release of proteins and inducing conformational changes that impact extraction efficacy [14]. Subsequently, the obtained protein content data were fitted to regression models, and Table 4 displays the fit statistics for the selected quadratic predictive model for protein content.

Examining variance within the comprehensive regression models (Table 4) indicates consistent results across all instances, revealing significant model and not significant lack of fit. These models collectively exhibited an adjusted R^2 value of 89.3%, signifying their robustness in predicting the effects of independent variables: the percentage of sodium carbonate (Na_2CO_3) represented by x_1, the ratio of VitAto to the alkaline solution denoted as x_2, and the soaking time that is marked as x_3. Hence, these models prove sufficiently adequate for forecasting the impact of these specified independent variables on protein content.

Table 4. Coefficients of variables in regression models for expected protein content from VitAto extract

Source	d.f.	Coefficient	***P*-value**
Model	9	-9.36222	0.005*
Linear	3		
x_1	1	1.14630	0.091
x_2	1	-6.42833	0.002*
x_3	1	1.36037	0.002*
Square	3		
x_{11}	1	-0.04198	0.002*
x_{22}	1	-0.35000	0.056

x_{33}	1	-0.03457	0.004*
Interaction	3		
x_{12}	1	0.33333	0.003*
x_{13}	1	-0.05926	0.007*
x_{23}	1	0.25556	0.008*
Residual Error	5		
Lack of fit	3		0.240
Pure Error	2		
Total	14		
R^2		96.20%	
Adj-R^2		89.3%	

* Values are significant (P = .05)

3.3Effect of independent variables on the protein content of VitAto extracts

Analysis of the regression models presented in Table 4 reveals that soaking time positively influences protein content, while the ratio of VitAto to alkaline solution has a negative impact. These parameters significantly affect protein content in the extracts, except for the percentage of sodium carbonate. However, the interaction between the three factors has a significant effect on protein content in the extracts. These findings suggest that soaking time has the most significant effect on protein content in VitAto extract. Previous studies have demonstrated that an alkaline solution enhances protein content in plant protein extraction [14] [15], with significant influence observed as the concentration increases [16]. A recent study by Kebede and Teferra [17] similarly showed that soaking lupine in sodium carbonate significantly enhances protein extraction efficiency. Additionally, the ratio of solvent to material during protein extraction is another factor affecting protein content. Zhang et al. [15] identified this factor as a key parameter for protein yield, while Sari et al. [14] demonstrated its influence on protein extraction, albeit in a different trend. Their results indicate a negative correlation between protein concentration and this factor, suggesting that an excess amount of solvent may lead to low protein solubility in the extracts, consistent with the findings of the current study.

However, limited studies have been conducted to investigate the influence of soaking time in an alkaline solution on the protein content of extracts. One such study by Abdul Fattah et al. [18] bears similarities to the current research. They employed sodium bicarbonate ($NaHCO_3$) to explore the effect of soaking conditions on the protein content of extracted mung bean. Their findings suggested that longer soaking times led to higher protein content, supported by a positively significant effect indicated by analysis of variance. Similarly, Shasego [19] and Munu et al. [20] observed that soaking time significantly affected the protein content of soy milk, albeit using tap water as the solvent. These results align with the current study, suggesting that increased cell-wall rupture during soaking may enhance protein recovery.

The surface plot shown in Figure 1(a) illustrates that protein content gradually decreases as the concentration of soda ash (sodium carbonate) increases, while Figure 1(b) depicts the opposite trend under different constant factors: soaking time for the former and material-to-solvent ratio for the latter. These quadratic trends are consistent with the results presented in Table 4, which demonstrate a significant effect on protein content when the independent variables are combined or interacted. Conversely, Figure 1(c) clearly demonstrates the negative linear effect of the VitAto to solvent ratio and the positive linear effect of soaking time on the protein content of the extracted liquid when the soda ash concentration is held constant. The decrease in protein content with an increase in the amount of alkaline solution is likely due to the elevated water polarity, which can induce the deterioration of electrostatic interactions and hydrogen bonding within the hydrophilic side chains of protein molecules [21]. Meanwhile, longer soaking times result in greater diffusion of water into the VitAtocotyledonous cells, leading to cellular swelling attributed to the accumulation of turgor pressure within the cell walls. Consequently, more ruptured cells are formed, releasing more reserve proteins [20]. However, soaking time has its limit in reaching the highest protein content and will gradually decrease beyond that period (Figure 1(b)). Prolonged exposure to alkaline conditions can promote the solubilization of proteins, causing them to leach into the soaking solution and reduce the protein content in the liquid extract.

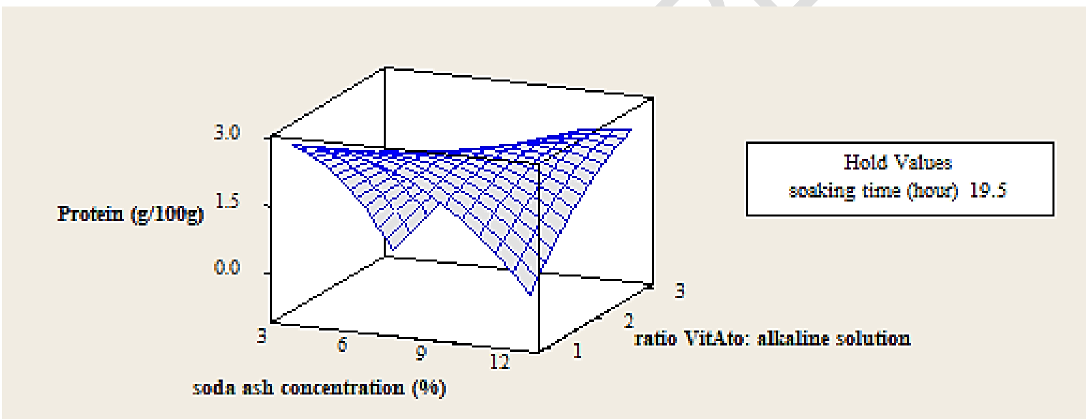

Fig. 1(a). Surface plot for protein content (*Y*) as a function of concentration of soda ash (x_1) and ratio of VitAto to alkaline solution (x_2)

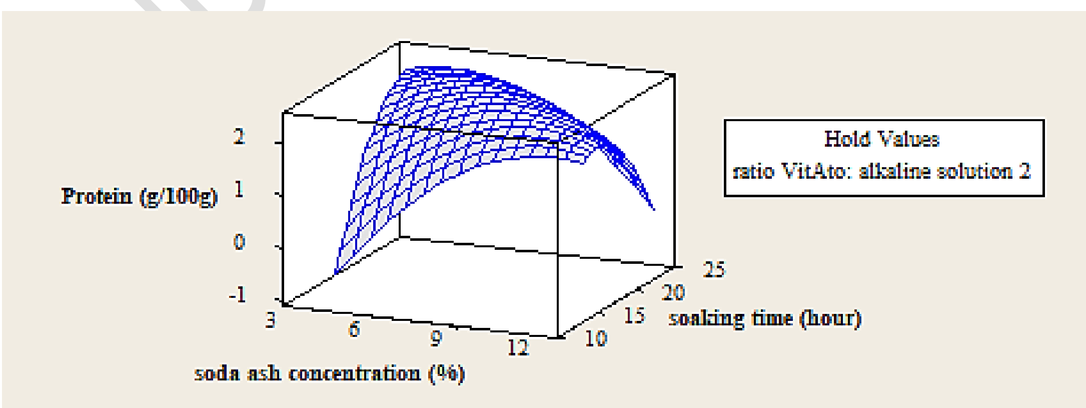

Fig. 1(b). Surface plot for protein content (*Y*) as a function of concentration of soda ash (x_1) and soaking time (x_3)

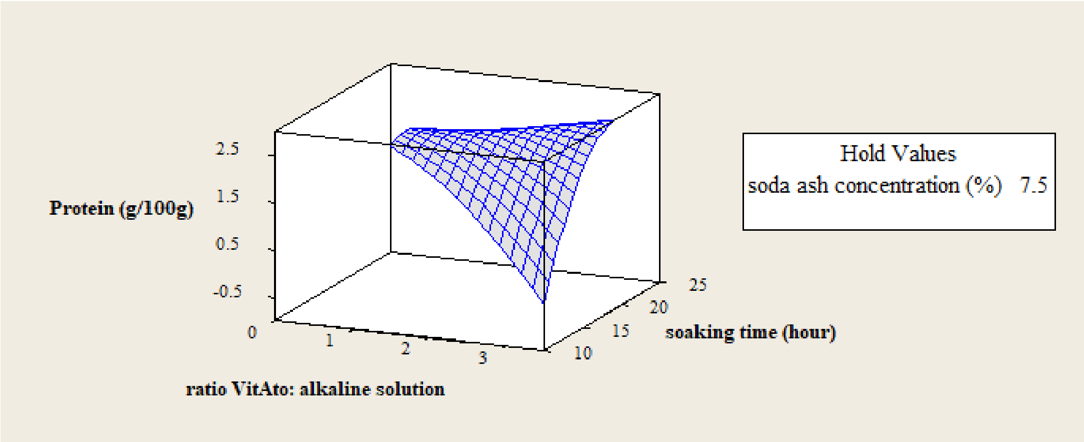

Fig. 1(c). Surface plot for protein content (*Y*) as a function of ratio of VitAto to alkaline solution (x_2) and soaking time (x_3)

3.4Optimization of the independent variables

After analysing the three key influencing factors and conducting response surface test, a set of optimal parameters were determined: percentage of soda ash 3%, ratio of VitAto to alkaline solution 1:1, and soaking time 20 hours. The optimum parameter provides an expected protein value of 2.8 g/100g with a high desirability of 1.0. Three parallel experiments using the optimal pre-treatment parameters were conducted to extract the soluble liquid from VitAto. The actual protein content of the extract was 2.6 g/100g, closely aligning with the predicted value and also the proposed protein content for PBMA (2.8 g/100g) [22]. This result indicates the effectiveness of the optimized process parameters derived from the successful response surface methodology and thus verifies the model. The results are also comparable and hold practical application value.

4. CONCLUSION

This study demonstrates that the best pre-treatment method for extracting the liquid portion of sweet potato (var. VitAto) with the highest protein content, for the development of plant-based milk alternatives, is soaking the VitAto in an alkaline solution (soda ash/sodium carbonate). The optimal conditions for the pre-treatment, analyzed using response surface methodology, were determined to be 3% soda ash, a 1:1 ratio of VitAto to alkaline solution, and 20 hours of soaking time. Verification of the results indicates that the mathematical model was acceptable, with a high desirability value of 1.0, and the difference between the predicted and actual values of the protein content of the extracts was minimal. These findings suggest that the results can be effectively applied for further development.

REFERENCES

1. SethiS, Tyagi SK, Anurag RK. Plant-based milk alternatives an emerging segment of functional beverages: a review. J Food Sci Technol. 2016; 53(9): 3408–3423.

2. Silva ARA, Silva MMN, Ribeiro BD. Health issues and technological aspects of plant-based alternative milk. Food Res. Int. 2020; 131: 108972. https://doi.org/10.1016/j.foodres.2019.108972

3. Reyes-Jurado F, Soto-Reyes N, Dávila-Rodríguez M, Lorenzo-Leal AC, Jiménez-Munguía MT, Mani-López E, López-Malo, A. Plant-based milk alternatives: Types, Processes, Benefits, and Characteristics. Food Rev. Int. 2021; DOI: 10.1080/87559129.2021.1952421

4. Zulkifli NA, Nor MZM, Omar FN, Sulaiman A, Mokhtar MN. Proximate composition of Malaysian local sweet potatoes. Food Res. 2021; 5(Suppl. 1): 73-79.

5. AOAC – Association of Official Analytical Chemists. Official methods of analysis of AOAC international (20th ed.). Arlington;2016.

6. Asante FA, Ellis WO, Oduro I, Saalia FK. Effects of soaking and cooking methods on extraction of solids and acceptability of tiger nut (*Cyperusenculentus*) milk. J. Agric. Stud. 2014; 2(2): 76-86.

7. Ahmadian-Kouchaksaraei Z, Varidi M, Varidi, MJ, Pourazarang H. Influence of processing conditions on the physicochemical and sensory properties of sesame milk: A novel nutritional beverage. LWT - Food Sci. Technol. 2014; 57: 299-305.

8. Nowshin H, Devnath K, Begum AA, Mazumder MAR. Effects of soaking and grinding conditions on anti-nutrient and nutrient contents of soy milk.J. Bangladesh Agric. Univ. 2018; 16(1): 158-163.

9. Padma M, Jagannadarao PVK, Edukondalu L, Ravibabu G, Aparna K. Physico-chemical analysis of milk prepared from broken rice.Int. J. Curr. Microbiol. Appl. Sci. 2018; 7(2): 426-428.

10. Thakur P, Kumar K, Ahmed N, Chauhan D, Rizvi QUEH, Jan S, Singh TP, Dhaliwal HS. Effect of soaking and germination treatments on nutritional, anti-nutritional, and bioactive properties of amaranth (*Amaranthushypochondriacus* L.), quinoa (*Chenopodium quinoa* L.), and buckwheat (*Fagopyrum esculentum* L.). Curr. Res. Food Sci.2021; 4: 917-925.

11. Adekanmi OK, Oluwatooyin OF, Yemisi AA. Influence of processing techniques on the nutrients and antinutrients of tigernut (*Cyperusesculentus* L.).World J. Dairy Food Sci. 2009; 4(2): 88-93.

12. Ma KK, Greis M, Lu J, Nolden AA, McClements DJ, Kinchla AJ. Functional performance of plant proteins. Foods. 2022; 11(4): 594.

13. Tunde-Akintunde YT, Souley A. Effect of processing methods on quality of soymilk.Pak. J. Nutr.2009; 8(8): 1156-1158.

14. Sari YW, Mulder WJ, Sanders JPM, Bruins ME. Towards plant protein refinery: Review on protein extraction using alkali and potential enzymatic assistance.Biotechnol. J. 2015; 10: 1138-1157.

15. Zhang C, Sanders JPM, Bruins ME. Critical parameters in cost-effective alkaline extraction for high protein yield from leaves.Biomass Bioenerg. 2014; 67: 466-472.

16. Baby C, Dileep A, Gopika B. Optimization of protein extraction from velvet beans.Pharma Innov. J. 2022; SP-11(7): 3479-3488.

17. Kebede YS, Teferra TF. Isoelectric point isolation and characterization of proteins from lupine cultivars as influenced by chemical and thermal treatments. Heliyon. 2023; 9: e14027.

18. Abdul Fattah AR, Ting UA, MohdSyafiq A, Norhasmillah AH. The effect of soaking condition on mung bean *Vigna radiata* towards water absorption and mung bean extracted crude protein content.Borneo J. Sci. Technol. 2019; 1(2): 9-15.

19. Shashego Z. Effects of soaking time and temperature on the nutritional content and sensory quality of soybean flour and milk.Glob. J. Sci. Front. Res. D Agric. Vet. 2019; 19(3): 25-38.

20. Munu N, Kigozi J, Azziwa A, Kambugu R, Wassawa J, Tumutegyereize P. Effect of ambient-soaking time on soybean characteristics for traditional soymilk extraction. J. Adv. Food Sci. Technol.2016; 3(3): 119-128.

21. Feyzi S, Varidi M, Zare F, Varidi MJ. Fenugreek (*Trigonellafoenum-graecum*) seed protein isolate: extraction optimization, amino acid composition, thermo and functional properties.J. Sci. Food Agric. 2015; 95(15): 3165-3176.

22. Drewnowski A, Henry CJ, Dwyer JT. Proposed nutrient standards for plant-based beverages intended as milk alternatives. Front. Nutr. 2021; 8:761442. doi: 10.3389/fnut.2021.761442