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.

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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

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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*

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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)

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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 the 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 hydrates the plant material, making extracting the soluble part easier and increasing the extraction yield [6]. Besides, soaking also helps deactivates nutrient inhibitors, release toxins, and improve its physicochemical properties [7][8][9]. As shown in Table 3, soaking increased the extract's protein content even though it was not significantly different from the control (sample A). This result is in line with the study fromThakur *et al*. [10] andAdekanmi*et al*. [11], which also found that the protein in buckwheat, quinoa, amaranth, and tiger nut was increased with soaking. Meanwhile,adding acid and alkaline substances during soaking significantly increased the protein content (Sample C and D). This is probably due to the modification of pH that affects the protein solubility [12]. Blanching and steaming (Sample 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], it was found that 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. As a result, a higher yield of soymilk was obtained, raising its protein content. With 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.

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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

From the data in Table 2, VitAto extracts with different combinations of independent variables recorded 1.1 g/100g to 2.6 g/100g protein. The observed values of protein content in the extracts were higher than those reported in fresh VitAto by Zulkifli et al. [4]. This situation may be due to alkaline soaking that disrupted plant cell structures, facilitating the release of proteins and conformational changes, impacting extraction efficacy [14]. The data obtained for the protein content were fitted to regression models. Table 4 shows the expected protein content fit statistics for the selected quadratic predictive model.

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		

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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

From the regression models in Table 4, the protein content was positively influenced by soaking time but negatively by the ratio of VitAto to alkaline solution. The parameters significantly affected the protein content in the extracts, except for the percentage of sodium carbonate. However, the interaction between the three factors significantly affected the protein content in the extracts. These results revealed that soaking time has the most significant effect on the protein content in VitAto extract. Previous studies have proved that alkaline solution enhanced protein content in plant protein extraction [14][15] and significantly influenced protein extraction from velvet beans as the concentration increased [16]. A recent study by Kebede and Teferra [17] also showed that soaking lupine in sodium carbonate significantly increased the protein extraction efficiency. Besides, the ratio of solvent and material during protein extraction is another factor affecting protein content. Zhang et al. [15] regarded this factor as a key parameter for protein yield in their study of the critical parameters for protein extraction in leaves. Sari et al. [14] also demonstrated the influence of material-to-solvent ratio on protein extraction but in a different trend. The results explained that the protein concentration is negatively correlated with the factor as an excess amount of solvent will force the protein in the material to go into the solution and result in low protein solubility in the extracts, which is in line with the findings in this current study.

However, limited studies have been done on investigating the influence of soaking time of alkaline solution on the protein content of the extracts. There was a study by Abdul Fattah et al. [18] which is almost similar to the current study. They used sodium bicarbonate ($NaHCO_3$) to investigate the effect of soaking conditions on the protein content of the

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extracted mung bean. They recommended a longer soaking time to achieve high protein content, and the analysis of variance also showed that the soaking time is positively significant. Shasego[19] and Munu et al. [20] also reported that soaking time significantly affected the protein content of soy milk but only used tap water as the solvent. These results were in agreement with the current findings. It is probably due to increased cell-wall rupture during the soaking time that improved the protein recovery.

As illustrated by the surface plot shown in Figure 1(a), protein content gradually decreased as the concentration of soda ash increased but acted conversely in Figure 1(b) at different constant factors: soaking time for the former and material-to-solvent ratio for the latter. These quadratic trends aligned with the results in Table 4 that exhibited a significant effect on the protein content when the independent variables combined or interacted. On the contrary, Figure 1(c) clearly shows 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 was on hold. The decrease in protein content when the amount of alkaline solution increases is probably due to the elevated water polarity potential to induce the deterioration of electrostatic interactions and hydrogen bonding within the hydrophilic side chains of protein molecules [21]. Meanwhile, the longer the soaking time, the more water diffused into the VitAtocotyledonous cell. This phenomenon resulted in cellular swelling attributed to the accumulation of turgor pressure within the cell walls. Consequently, more ruptured cells will form and release more reserve proteins [20]. However, the soaking time has its limit to reach the highest protein content and will gradually decrease beyond that period (Figure 1(b)). The prolonged exposure to alkaline conditions can promote the solubilization of proteins, causing them to leach into the soaking solution and lessen the protein in the liquid extract.

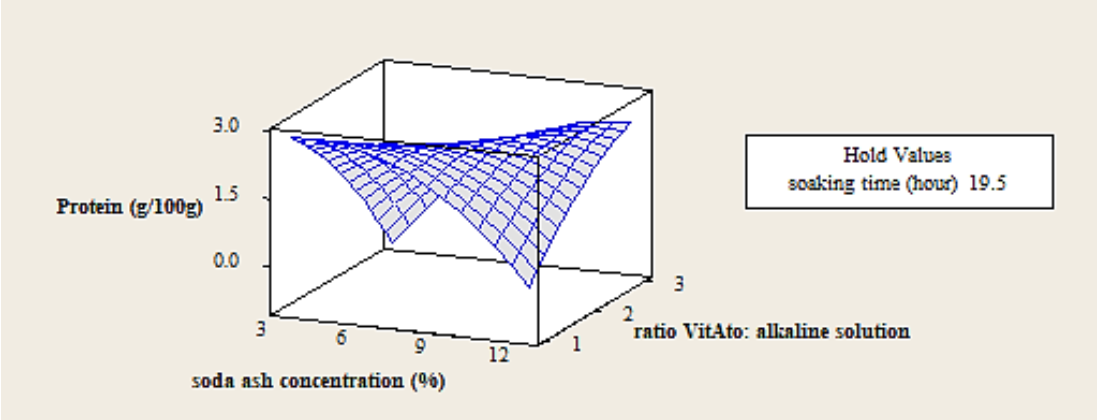

Fig. 1(a). Surface plot for protein content (*Y*) as a function of concentration of soda ash (x_1) and ratio of VitAto to alkaline solution (x_2)

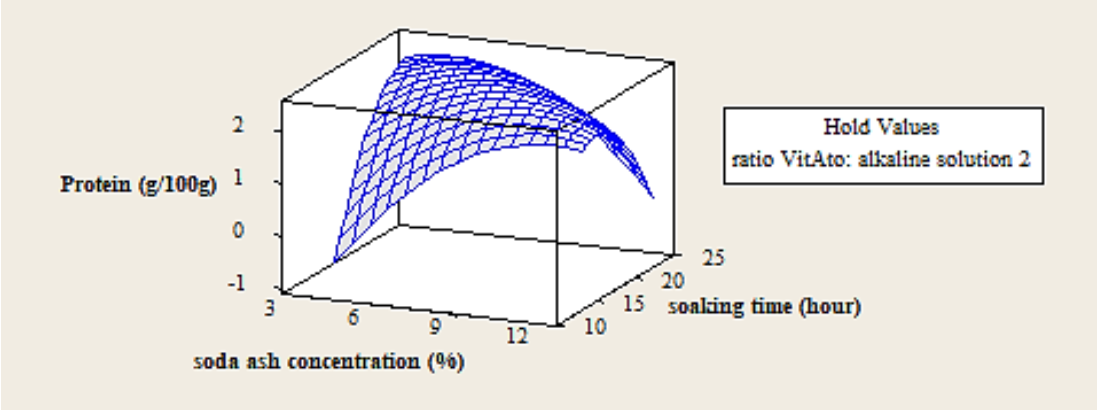

Fig. 1(b). Surface plot for protein content (*Y*) as a function of concentration of soda ash (x_1) and soaking time (x_3)

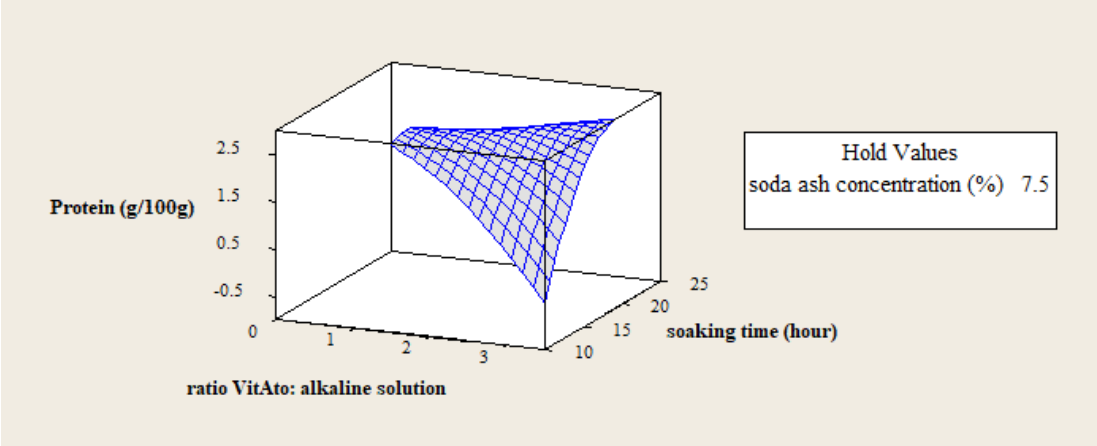

Fig. 1(c). Surface plot for protein content (*Y*) as a function of ratio of VitAto to alkaline solution (x_2) and soaking time (x_3)

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, in developing 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.

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