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Optimization and proximate analysis of prosomillet milk using response surface methodology

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Abstract The milk extracted from plant-based sources is an upsurging fragment in augmenting new food products. The Minor Millet Proso(*Panicummiliaceum* L.) is a drought-resistant crop and well-provided nutrients such as dietary fiber, trace elements, and vitamins. The results depicted that soaking time, water for soaking, and extraction time had significantly affected on responses ($p<0.05$). The R^2 for milk extracted, pH, and overall acceptability of the millet milk were 0.9475, 0.9017, and 0.9211 respectively. The results were considered as optimized values to obtain for soaking hours, water for soaking time, and extraction time with uppermost desirability index of 0.93was 12hours, 301ml, and 30minutes, respectively, while the optimal values for responses were milk extracted as 449.99ml, and pH6.43, Overall acceptability showed7.90.The experimental results recommended that the obtained model is accepted for the maximum yield of milk and enhanced density of the quality.

Keywords: Prosomillet-Millet milk, Optimization, Central composite design, RSM

Introduction

Millets exist with broad adaptability under distinct environmental conditions are considered as one of the appropriate crops for viable agriculture and food security (Das *et al.*, 2019; Habiyaremye *et al.*, 2017). Small millets are an adequate source of energy, vitamins, minerals, dietary fiber. They are also rich in antioxidants and phytochemicals (Bhat *et al.*, 2018; Bouis, 2000) and are generally called "small-seeded crops" which give more therapeutic advantages (Nithiyanantham *et al.*, 2019). Prosomillets are grown in dry weather conditions with less rain in tropical areas of Asia and Africa (Bhat *et al.*, 2018; Sankar *et al.*, 2008). Prosomillets are the provenance of nutrients like iron, calcium Iron, manganese, potassium and zinc and also rich in all essential amino acids (Das *et al*., 2019; Saleh *et al*., 2013). Products made from

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prosomillet have a lower glycemic index[GI] than corn. (McSweeney *et al*., 2017; Shen *et al*., 2018).

Non-dairy beverages can be extracted from plant-based foods like millets, rice, soy, almonds, peanuts etc. and they are similar to cow's milk in appearance but their nutritional value depends upon the type of plant source, processing methods (Constantine *et al*., 2017; Mäkinen *et al*., 2016) Mostly non-dairy milk is generally recommended for food allergies during childhood which is represented as cow's milk protein allergy [CMPA] but it is generally muddled with lactose intolerance (Constantine *et al*., 2017; Lifschitz and Szajewska, 2015). Non-dairy alternatives have popularized among people due to allergy, vegan diet, and plant-based foods, also so much bothered towards saturated fat, hormonal levels and antibiotics generally added in dairy cattle. Many people are allergic to dairy milk and some are changing their dietary practices to vegan style which changes their mind to choose dairy-free alternatives and also very low in cost. Plant-based milk serves the purpose and it can be a better alternative for cow milk allergy and lactose intolerance (Sethi *et al.*, 2016).

Materials and methods

Prosomillet(*Panicummiliaceum*) was procured from Tamil Nadu Agricultural University, Coimbatore, Tamil nadu, India.

Experimental design and data analysis

A well-defined tool is stated as Response Surface Methodology (RSM) used for the optimization process. RSM describes an appropriate experimental design that associated with all the independent variables and accomplished data input from the experiment and finally, the well-designed outputs are acquired with regression analysis and the dependent variable can also be predicted(Drake, 2007; Said *et al*., 2015) Central composite design is a non-linear model that used to regulate the variables and regression equations from the process of experiments (Chattoraj *et al*., 2013; Sadhukhan *et al*., 2016). The recorded data investigated was proceeded with the research design using RSM by applying central composite design and evaluated by the response surface eregression using the second-order regression. Regression analysis for every model represented that the fitted quadratic models applicable for more than 80% of the variation in the recorded data (Jha *et al*., 2013)**.** The optimized dairy pearl millet dessert was performed with a 3-factor central composite design to find the best-predicted values for the development of pearl millet kheer mix. The sensory parameters were optimized by (Chakraborty *et al*., 2011) for texture and overall acceptability of millet enriched biscuit using RSM and Pearl millet kheer mix to improve the shelf life was also optimized by Bunkar *et al*. (2014). The independent variables for milk extraction were soaking time (X1) and water for soaking(X2) and extraction time(X3). The variables and their levels are given in Table 1. A central composite rotatable design was enforced using design-expert software. Twenty experimental runs are accomplished in Table 2 for the Milk extraction.

Extraction of prosomillet milk

One hundred grams(100gm) of millet was cleaned and washed well to remove the dirt particles. The process parameters soaking time, and water needed for soaking, and extraction minutes for the preparation of the prosomilk were followed according to experimental design as shown in Table 2. It was ground well in a mixer-grinder with a high speed of 12minutes to a fine slurry and it was filtered in a muslin cloth with a pore size of 0.7mm to 1.0mm to obtain prosomilk. The milk was boiled for 74.6 ^{o} Celsius for 8minutes and allowed to cool at room temperature for further analysis.

Determination of product responses

Experimental responses were examined milk extraction, pH, proximate and chemical composition, microbial analysis, and overall acceptability. The amount of milk extracted was measured in a measuring cylinder after the extraction process. The method of analyzing pH was done by measuring 100ml of the extracted milk in a beaker using a digital pH meter(AM-P-Aquasol).

Microbial analysis of the developed prosomillet milk: The millet milk was extracted was observed for total plate count by IS 5402:2012 (RA.2018) and yeast and mold count by IS 5403:1999 (RA.2013).

Sensory evaluation of the millet milk

Sensory evaluation was determined by the scale which consisted of 9 parameters generally followed in foodscience (Lim, 2011). The sensory scale comprised nine scoring categories which include disliking extremely to like extremely and considered in the various organoleptic evaluation. The prepared millet milk was observed for consumer acceptability comprising of 20untrained panels and it was acceptable with an overall acceptability score of 8.5. Proximate and chemical composition of the prosomilk, the chemical and nutrient compositions of the best optimized prosomillet milk were analyzed by the AOAC(2005)method by triplicates and the results were obtained.

Results

Response surface methodology was used to extrapolate the conditions for splendid millet milk formulation with a central composite rotatable design. The experimental data represent the effect of three independent variables and their effect on the quantity and quality of the prosomilk extracted as responses that commanded twenty runs. The conditions like soaking time(hours,X1), water for soaking(ml,X2), and extraction time(minutes,X3) were adopted as factors and the responses such as Milk Extracted(%,Y1), pH(Y2), and overall acceptability(Y3)were used as a quality of the extracted prosomilk. The derived value of the experimental design to various responses of the millet milk with different concentrations is given in Table 1. It was depicted that the responses range from 155 to 450ml of milk yield, pH- 4.9 to 6.6, and overall acceptability-5.1 to 8.5 respectively. The maximum quantity of the extracted milk could be remarked during 12hours of soaking time, 350ml of water added for soaking the pros millet with 30minutes extraction time but the exquisite overall acceptability 8.5 scores of the millet extracted milk could be noticed during 8 hours of soaking time, 275ml of water added for soaking and 20minutes of extraction time and the pH remained around 6.4 to 6.6 for both the hours of soaking time. The least most quantity of 155ml of the extracted pros milk attained in 4hours of soaking time with 200ml of water and 10 minutes of extraction time and the overall acceptability score was 6.1 with 5.1 as pH. This predicted that the soaking time and water for soaking with more extraction time that revealed a greater impact on the quality attributes of the prosomilk.

Table 1. Experimental values of responses for prosomilk extraction

	INDEPENDENT VARIABLES			DEPENDENT VARIABLES		
Run	soaking time[hrs]	Water for soaking[ml]	Extraction time[min]	Milk Extracted[ml]	pH	Overall acceptability
	Hours	Ml	Minutes	Ml	-	
1	1.272829	275	20	276	4.9	5.1
2	8	275	20	386	6.6	8.5
3	8	275	20	386	6.6	8.5
4	4	350	10	330	5.5	6.1
5	4	200	30	280	5.1	6.1
6	12	350	30	450	6.4	7.7
7	12	200	30	300	6.1	7.5
8	8	275	3.182072	285	6.1	8.5
9	8	275	20	386	6.1	8.5
10	8	275	20	386	6.1	8.5
11	12	350	10	340	6.2	7.7
12	8	148.8655	20	155	6.1	6.1
13	8	401.1345	20	410	6.3	6.1
14	8	275	36.81793	380	6.1	8.5
15	14.72717	275	20	450	6.2	5.5
16	4	200	10	195	5.1	6.1
17	8	275	20	386	6.6	7.6
18	12	200	10	200	6.1	7.2
19	8	275	20	386	6.6	8.5
20	4	350	30	390	5.2	6.1

Table 2. Effect of the independent variable on the yield of prosomilk

Source	Coefficient	Sum square	Df	F-value	P-value
Model		126091	9	20.0614	< 0.0001***
A	28.38369	11002.4	1	15.7546	0.0026**
B	70.57681	68026	1	97.4079	< 0.0001***
C	37.69318	19403.3	1	27.7841	0.00036***
AB	5.625	253.125	1	0.36246	0.56055
AC	8.125	528.125	1	0.75623	0.4049
BC	-1.875	28.125	1	0.04027	0.84497
A^2	-10.6082	1621.75	1	2.32222	0.15852
B^2	-39.0692	21997.5	1	31.4987	0.00022***
C^2	-21.3916	6594.59	1	9.44294	0.01178*
Lack of fit	-	6983.62	5	-	-
R^2	0.947521				
Adj R^2	0.90029				
Pred R^2	0.59968				
Adeqprec	16.41087				

A-Soaking time,B-water for soaking, C-extraction time, df-degrees of freedom, *significant at P>0.05,**significant P>0.01,***Significant at P>0.0001 NS-Not significant, R^2-coefficient of determination, AdjR^2-Adjusted R^2, Pred R^2-predicted R^2, Adeq pre-Adequate precision.

The regression coefficient, the sum of a square, F value, and the P-value of a coded form of process for the yield of milk are shown in Table 2.The quadratic model for millet milk in the form of uncoded[actual] process variables is as follows:

Milk extracted was =-597.82+8.48535*A+4.66113*B+11.3884*C+0.01875*A*B+0.20312*A*C-0.0025*B*C-0.663*A^2-0.0069*B^2-0.2139*C^2.

The coded form of process variables, the equation was shown as follows:

MilkExtracted=+386.3789+28.38369*A+70.57681*B+37.69318*C+5.625*A+B8.125*AC-1.875*BC-10.6082 *A^2-39.0692* B^2-21.3916*C^2

The obtained quantity of the prosomilk ranged from 155ml to 450ml and the coefficient of determination(R^2) was 0.947 and indicated a good model(Table 1 and 2).The quantity yield of the milk was achieved the maximum at 450ml when the soaking hours, water for soaking, and extraction minutes increased from 12 to 14hours, 275 to 350ml and20 to 30 minutes respectively. The independent variables gave a greater impact on the yield of the milk quantity and the maximum yield of the prosomilk(450ml) was obtained during 12hours of soaking time with 301ml of water (Figure 1).

The response surface plot showed the effect of soaking time and water added for soaking on the Extracted milk (Figure 1). Increasing the soaking time had a positive effect on the yield of the milk. The quantity of the milk yield gradually increased when the maximum extraction time(20 to 30 minutes) of the prosomilk (Figure 2). It showed that the noticeable increase in yield of the prosomillet milk and significantly decreased in yield of the prosomilk when the water for soaking decreased to 200ml(Figure3).

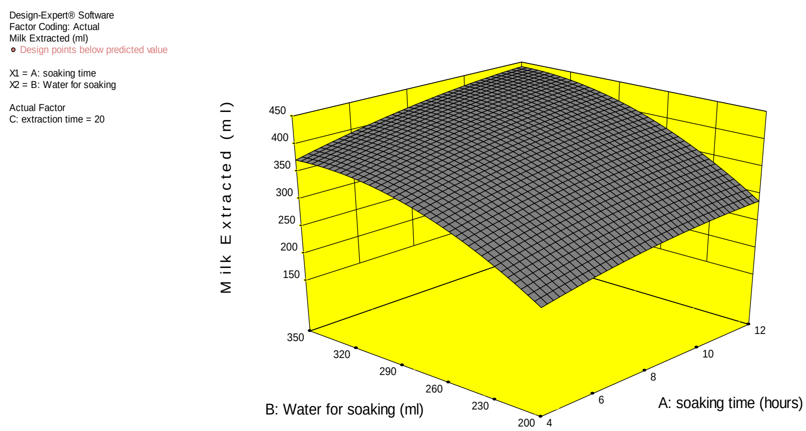

Figure1. Response surface plot showing the effect of water soaking and Soaking time on Milk yield

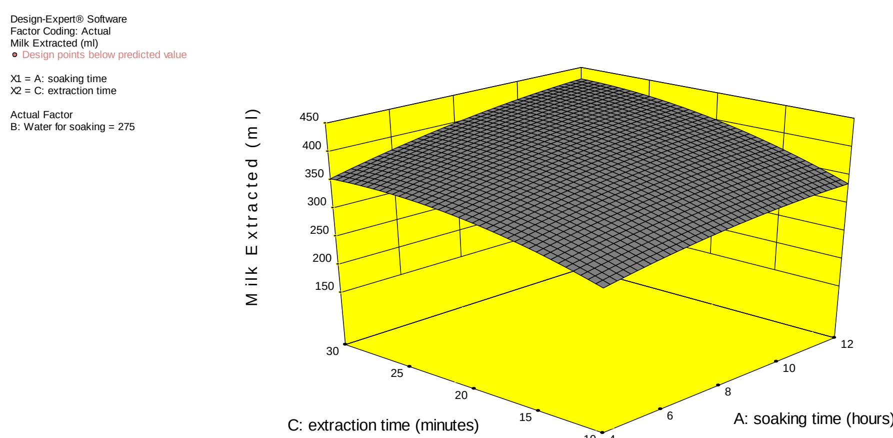

Figure 2. Response surface plot showing the effect of extraction time and soaking time on milk yield

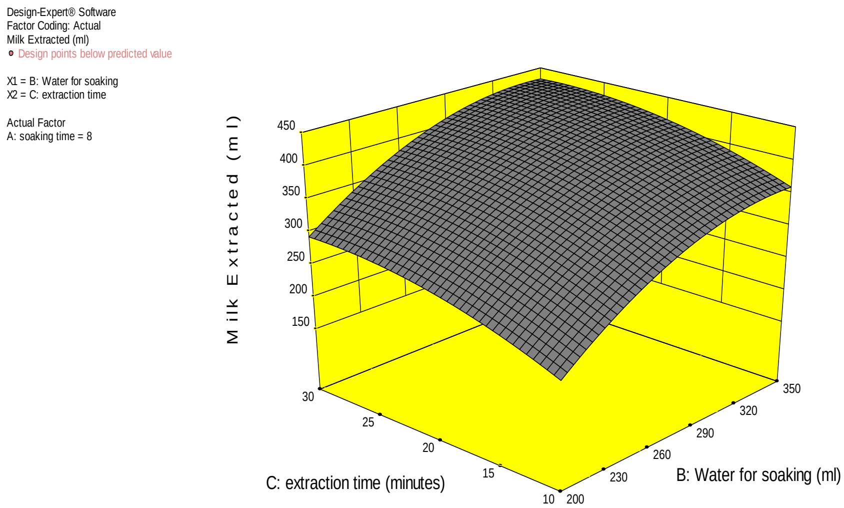

Figure 3. Response surface plot showing the effect of extraction time and Water for soaking on milk yield

Table 3. Effect of the independent variable on the pH of the milk

Source	Coefficient	Sum square	Df	F-value	P-value
Model		5.013354	9	10.19014	0.0006***
A	0.44566	2.712442	1	49.61975	< 0.0001***
B	0.09053	0.111928	1	2.04754	0.182951
C	-0.0073	0.000732	1	0.013395	0.910152
AB	-0.0125	0.00125	1	0.022867	0.882811
AC	0.0625	0.03125	1	0.571668	0.467034
BC	-0.0125	0.00125	1	0.022867	0.882811
A^2	-0.3562	1.828859	1	33.45602	0.000177***
B^2	-0.1264	0.230347	1	4.213825	0.067197*
C^2	-0.1618	0.377194	1	6.900159	0.0253*
Lack of fit		0.213312	5	0.639	0.6819^{NS}
R^2	0.901682				
Adj R^2	0.813197				
Pred R^2	0.614687				
Adeqprec	10.62817				

A-Soaking time, B-water for soaking, C-extraction time, df-degrees of freedom, *significant at P>0.05,**significant P>0.01,*** significant at P>0.0001 NS-Not significant, R^2-Coefficient of determination, AdjR^2-Adjusted R^2, pred R^2-Predicted R^2, Adeq pre-Adequate precision.

The regression coefficient, a sum of the square, F value, and the P-value of a coded form of process for pH of the milk are shown in Table 3. The quadratic model for millet milk in the form of uncoded (actual) process variables found to be$pH=1.526283+0.44786*A+0.014235*B+0.056064*C-4.2E-05*A*B+0.001563*A*C-1.7E-05*B*C-0.02226*A^2-2.2E-05*B^2-0.00162*C^2$.

The coded form of process variables, the equation was as follows: $pH= 6.440055+0.445661*A0.09053*B+0.00732*C+0.0125*AB+0.0625*AC+0.0125*C+0.35624* A^2-0.12643* B^2-0.16178* C^2$.

The pH value was in a range from 4.9 to 6.6as seen in Table 1. The pH was acidic and slightly alkaline. The soaking time had a significant effect on the pH of the millet milk at $P<0.05$(Table 2). It revealed that when the soaking time increased from 8hrs to 12hrs, there was no considerable increase in the pH but another variable water needed for soaking could not give much effect on the pH of the milk (Figure 4).

The response surface plot graph showed that the effect of soaking time and water added for soaking on the pH of the milk (Figure 4). Increasing the soaking time showed a negative effect on the pH of the milk. There was not much impact of extraction time on the pH of the prosomilk (Figure 5). A similar range of pH from 5.1 to 6.1 was obtained with the extraction time of 10 to 30minutes. There was a constant value of the maintained pH when the water needed for soaking and extraction time either increased or decreased (Figure 6).

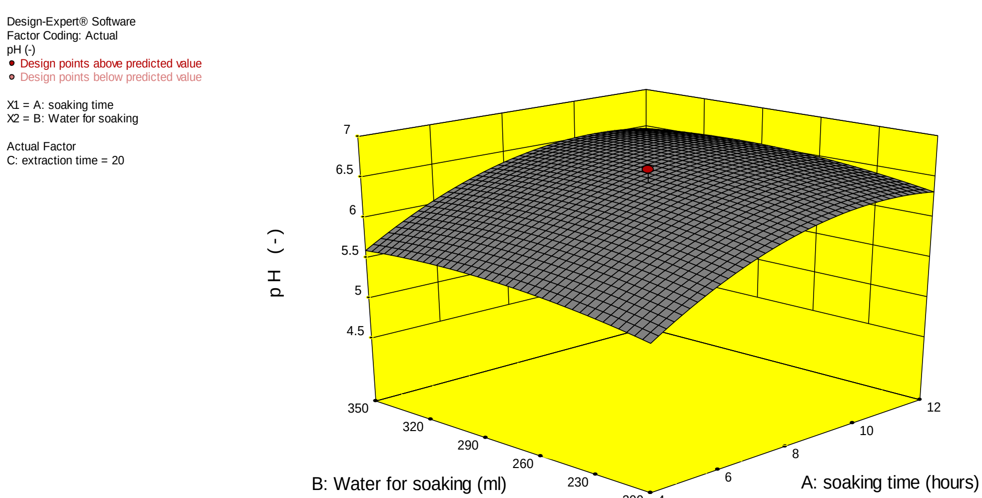

Figure 4. Response surface plot showing the effect of Soaking time and Water for soaking on pH of the milk

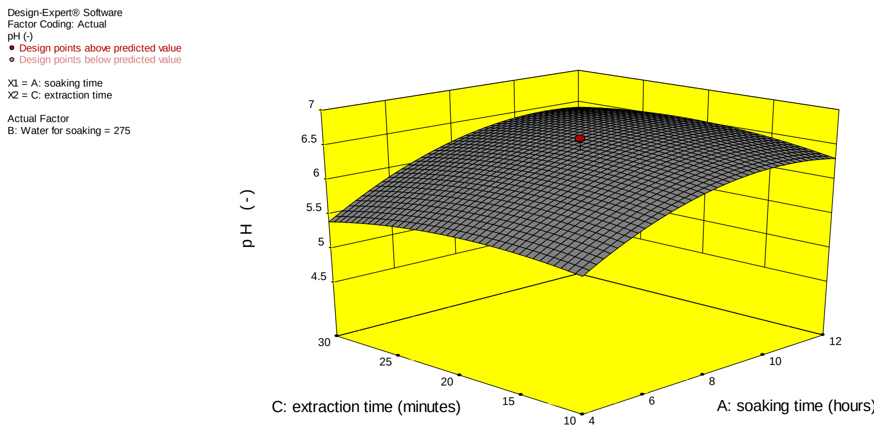

Figure 5. Response surface plot showing the effect of extraction time and soaking time on pH of the milk

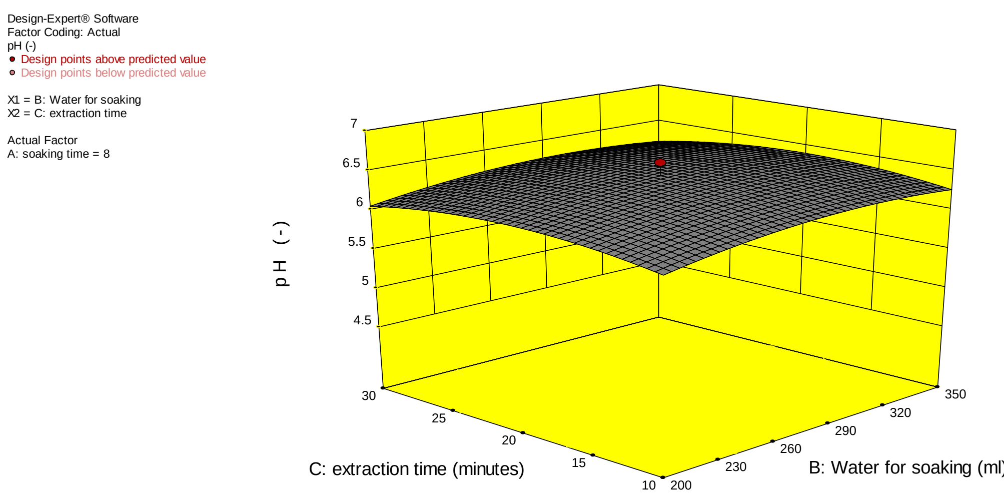

Figure 6. Response surface plot showing the effect of extraction time and Water for soaking on pH of the milk

The quadratic model for millet milk in the form of uncoded (actual) process variables found to be

Overall acceptability (OA)=-5.82181+1.036254*A+0.071286*B-0.03668*C+0.000292*A*B+0.000937*A*C-5E-05*B*C-0.06366*A^2-0.00013*B^2+0.001128*C^2.

The coded form of process variables, the equation was overall acceptability=8.340852605+0.466631408*A+0.051256313*B+0.021966991*C

+0.0875*AB+0.0375*AC0.0375*BC1.018554733*$A^2$0.735712021*B^2+0.112816117*C^2.

Table 4. Effect of independent variables on overall acceptability of the milk

Source	Coefficient	Sum square	Df	F-value	P-value
		24.99185	9	12.97516	0.000206***
Model					
A	0.466631	2.97371	1	13.89489	0.003926*
B	0.051256	0.035879	1	0.167649	0.690842
C	0.021967	0.00659	1	0.030793	0.864206
AB	0.0875	0.06125	1	0.286195	0.60436
AC	0.0375	0.01125	1	0.052567	0.823277
BC	-0.0375	0.01125	1	0.052567	0.823277
A^2	-1.01855	14.95105	1	69.85995	8.01E-06
B^2	-0.73571	7.800431	1	36.44813	0.000126***
C^2	0.112816	0.183419	1	0.857041	0.376369
Lack of fit		1.465146	5	2.170586	0.207527 ^{NS}
R^2	0.921121				
Adj R^2	0.85013				
Pred R^2	0.553789				
Adeqprec	12.29434				

A-Soaking time, B-water for soaking, C-extraction time, df-degrees of freedom, *significant at P>0.05,**significant P>0.01,***Significant at P>0.0001 NS-Not significant, R^2-Coefficient of determination, AdjR^2-Adjusted R^2, Pred R^2-Predicted R^2, Adeq pre-Adequate precision.

The results showed that the quadratic model A, B^2had significant effects (p<0.05) on the overall acceptability (OA) of the prosomilk (Table 4).The score of OA ranged from 5.1 to 8.5(Table 1). It was observed that when the soaking hours increased the overall acceptability score also greatly increased when compared to decreased soaking hours of 4 and 8.The response surface graph showed the effect of soaking time and water added for soaking on the overall acceptability of the extracted milk (Figure 7). Increasing the soaking time of 8 to 12hours had a positive effect on the OA score. There was a significant decrease in the overall acceptability of the prosomilk, when the soaking time decreased and the water for soaking had not showed the impact on overall acceptability. When 275to 350ml of water was added for soaking prosomillet showed the same range of OA score of 5.1 to 8.5 along with the soaking time (Figure 7). When the maximum soaking time of 12and 20 minutes of extraction increased the overall acceptability of the prosomilk, but the decreased soaking time of 4hours with similar 20minutes of extraction showing the least most OA score of 5.1(Figure 8). When extraction time and water for soaking increased the OA score remained constant. Because the increased water for soaking and extraction time or the decreased variables did not showed much impact on the overall acceptability of the milk yield (Figure 9).

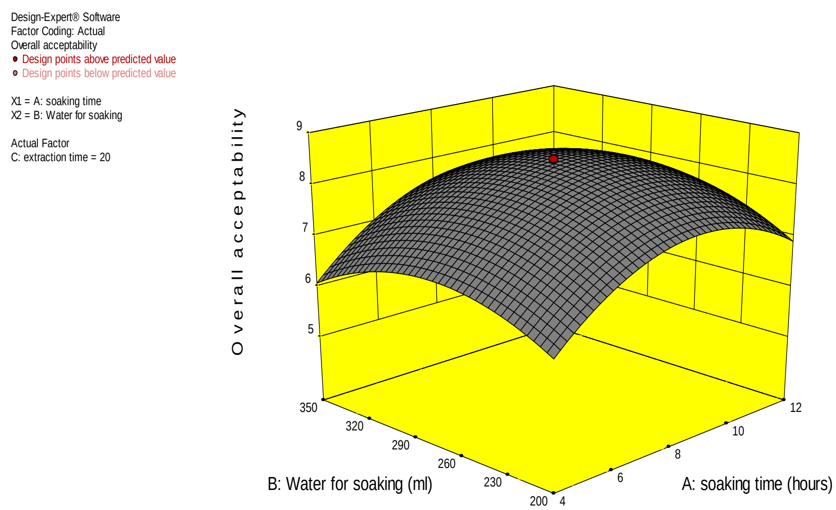

Figure 7. Response surface plot showing the effect ofwater for soaking and soaking time on Overall acceptability of the milk

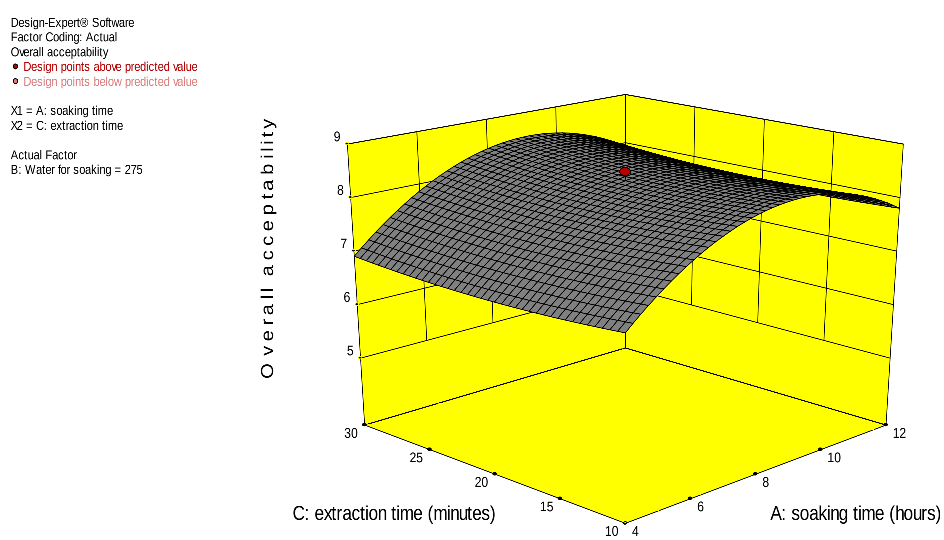

Figure 8. Response surface plot showing the effect of extraction time and soaking time on overall acceptability of the milk

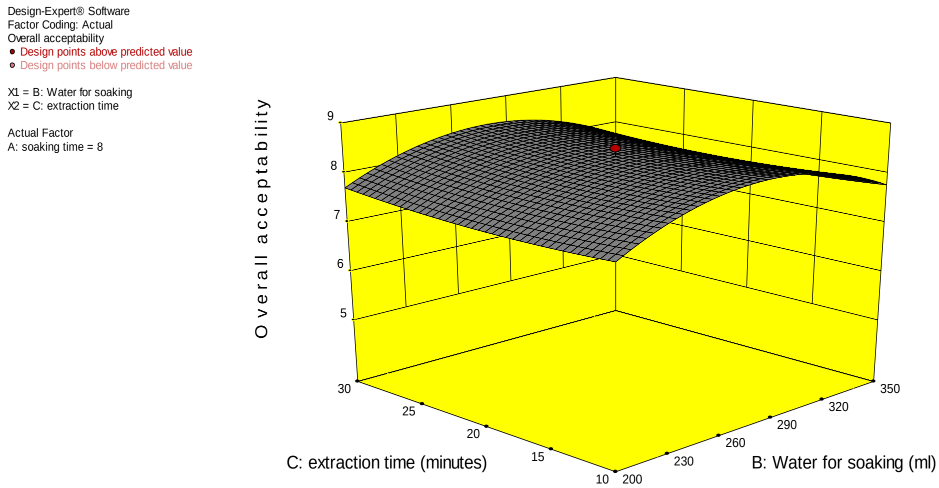

Figure 9. Response surface plot showing the effect of extraction time and water for soaking on the overall acceptability of the milk

Table 5. Levels of responses nailed for optimization of prosomillet milk

Constraints	Goal	Lower limit	Upper limit	Importance	Desirability index
A:Soaking time	maximize	4	12	3	
B:Water for soaking	is in range	200	350	3	
C:Extraction time	is in range	10	30	3	0.93
Milk Extracted	Maximize	155	450	3	
pH	is in range	4.9	6.6	3	
Overall acceptability	Maximize	5.1	8.5	3	

The desired goals for each factor and response values are shown in Table 5. Responses obtained after the 20 trials were analyzed to envisage the interactive effect of various parameter processes on the properties of prosomilk. The factors and responses of the process variables of the prosomilk were given similar importance of 3 to achieve the best-optimized milk with three parameters and three responses. The measure of optimization was selected on quantity and sensory acceptability of prosomillet milk like maximum level of soaking time, the quantity of milk extracted and overallacceptability whereas the water needed for soaking millets, extraction minutes, and pH were fixed within the range value. Optimization was completed with the support of a multiple response approach called desirability. Out of 20 suggested formulations, the optimum conditions of prosomilk was derived with RSM which had superior desirability index of 0.93 was 12hrs of soaking time, 301ml of water and 30minutes of Extraction time which had the predicted values of responses are Milk Extracted-449.99ml, pH-6.43 and Overall acceptability-7.90.

Table 6. Proximate and chemical composition of the proso millet milk

S.no	Nutrients	Gm/100gm
1.	Energy[gm]	20.66±0.57
2.	Carbohydrate[gm]	2.52±0.13
3.	Total Fat[gm]	0.42±0.02
4.	Protein[gm]	1.65±0.05
5.	Dietary fiber[gm]	0.86±0.05
6.	Calcium[mg]	5.75±0.28
7.	Iron[mg]	0.61±0.02
8.	Moisture[gm]	95.06±0.25
9.	Ash[gm]	0.10±0.02

The energy, carbohydrate, total fat, protein, dietary fiber, calcium, iron, and ash were tested in triplicates and presented in mean and standard deviation. The prosomilk had a higher moisture content(95.06±0.25) and low dietary fiber (0.86±0.05), fat(0.42±0.02). The milk had a sufficient amount of energy(20.66±0.57), protein(1.65±0.05), carbohydrate(2.52±0.13), and ash (0.10±0.02)content. The calcium(5.75±0.28) and Iron(0.61±0.02) were lower than in the range. The proximate composition of the prosomilk was the congenial limit, it can be utilized for formulating a product. The extracted prosomilk was tested for the total plate count, yeast, and mold counted for the microbial analysis of the product. Accumulation or increased level of spoilage organisms may cause a disagreeable effect on the quality of the food products and leading to foodborne illness after consumption. The total plate count showed $3.0x10^{7}$CFU/ml, which was the acceptable limit whereas yeast and mold count was not detected in the extracted sample. The observed total plate count was less than 10cfu/gm and the yeast, and mold count of the millet milk was not detected and the developed millet milk was prepared in a well hygienic environment.

Discussion

The prosomillet milk was effectively optimized using response surface methodology. Experimental results delineated that soaking time, water for soaking, and extraction time considerably affected the Amount of milk yield, pH, and overall acceptability. Our of 20 suggested formulations, which had a superior desirability index of 0.93 in 12hrs of soaking time, 301ml of water, and 30minutes of extraction time which had predicted values of responses in the extracted milk of 449.99ml, pH6.43, and overall acceptability was7.90 which also reported by Jusoh *et al.*(2013) who stated that R^2 between 0.8 and 1 indicated a good model. Thus, R^2 of 0.947, 0.901, and 0.9211 for milk extracted, pH, and overall acceptability indicated a good model. And also 94% of variations were described by the Quadratic model. The predicted $R^2$and adjusted R^2 differed less than 0.2 which implies a magnificent correlation between independent variables in predicting the responses. The observed results stated that the independent variables had significant effects($p<0.05$) on dependent variables of the yield of the prosomilk when compared to the other two responses, pH and overall acceptability where the soaking time had significantly affected. A study of soybean reported that an increase in soaking time increased the water absorption of soybeans which was reported by Luo *et al*. (2009) and Wang*et al*.(1979). Punniyamoorthy (2018)stated that there was a higher percentage of extracted milk when the millets like foxtail millet, little millet, Kodomillet, prosomillet, and barnyard millet were soaked for 8 hours. Hence, the yield of the milk would be automatically increased with maximum soaking hours. The human body required a pH level near 7.4 that was slightly alkaline that the enzymes present inside the body will function. Generally, non-dairy sources of milk from soy milk, almond milk, peanut milk would be reached a pH level of 6. The nutritional components and pH were analyzed for soya milk when compared to cow milk. Soya milk contained pH-6.74 and acidity was 0.24% which reported Aidoo *et al.* (2010) who described that the plant-based milk would be slightly acidic with a pH range of 6.33 to 6.97. Hence, the pH of the prosomillet milk varied from 4.1 to 6.25 and it was slightly alkaline and acceptable for the human body as stated by Shunmugapriya *et al.*, (2020) which formulated finger millet milk by enzymatic extraction method and analyzed the protein content of the finger millet milk as 1.38 ± 0.03 to 1.12 ± 0.02 gm and the prosomillet milk had also met the required values which can be utilized for homemade purposes and industrial level value-added products. The extracted milk was good in nutritional, chemical characteristics, and acceptable microbial range. The final result of the study suggested the obtained model is suitable and acceptable for maximum yield of the millet milk and standardized non-dairy milk which can be used for further development of the new product.

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