Optimization of vegetable milk extraction from whole and dehulled *Mucuna pruriens* (Var *Cochinchinensis*) flours using central composite design

Dimitry Y. Mang · Armand B. Abdou · Nicolas Y. Njintang ·
Edith J. M. Djiogue · Emmanuel A. Panyo · Clemence Bernard ·
Robert Ndjouenkeu · Benoît B. Loura · Carl M. F. Mbofung

Revised: 31 January 2015 / Accepted: 5 February 2015 / Published online: 12 September 2015
© Association of Food Scientists & Technologists (India) 2015

Abstract Extraction conditions for maximum values of protein yield, protein content, sugar content and dry matter of vegetable milk extracts from dehulled *Mucuna cochinchinensis* bean flour and whole *Mucuna cochinchinensis* bean flour were investigated using response surface methodology. A Central Composite Design (CCFD) with three factors: temperature (25 to 95 °C); extraction time (6 to 74 min.) and water to flour ratio (6 to 24 mL/g) were used. Data analysis showed that all the factors significantly ($p < 0.05$) affected the responses variables. The optimal

conditions determined for extraction were temperature 63–66 °C, water to flour ratio 12–13 mL/g and extraction time of 57–67 min. At these optimum points the protein and sugar contents, extraction yield of protein and dry matter were respectively 14.0 g/100 mL, 4.8 g/100 mL, 53.8 g/100 g, 12.1 g/100 g for vegetable milk produced from dehulled *M. cochinchinensis* bean flour and 6.4 g/100 mL, 3.5 g/100 mL, 50.0 g/100 g and 8.0 g/100 g for vegetable milk extracted from whole *M. cochinchinensis* bean flour milk. The optimal condition was

- Vegetable milks from whole and dehulled mucuna seeds flours were successfully produced by optimization using a central composite design;
- Second polynomial models were established to significantly describe the variation in protein content and yield as a function of extraction time, temperature and water to flour ratio;
- The protein content, protein yield and dry matter of the milk increased as the extraction time increase, while increase followed by decrease was observed when temperature and ratio increased;
- Optimal conditions determined by superposition of response contours plot and by using desirability function did not varied significantly with dehulling; treatment;
- The protein content of milk produced at optimal condition was 14 % for dehulled mucuna flour and 6.4 % for whole mucuna flour, while no consistent differences were observed on protein yield, dry matter and carbohydrates content.

D. Y. Mang (✉) · A. B. Abdou
Department of Agriculture, Livestock and By products, Higher
Institute of Sahel, University of Maroua, PO Box 46,
Maroua, Cameroon
e-mail: mangyannick@yahoo.fr

N. Y. Njintang
Department of Biological Sciences, Faculty of Science, University of
Ngaoundere, PO Box 454, Ngaoundere, Cameroon

E. J. M. Djiogue · R. Ndjouenkeu · C. M. F. Mbofung
Department of Food Science and Nutrition, ENSAI, University of
Ngaoundere, PO Box 455, Ngaoundere, Cameroon

C. Bernard
Ecole de Biologie Industrielle, Laboratoire EBInnov, 32 boulevard
du port, 95094 Cergy Pontoise, Cedex, France

E. A. Panyo
Food and Nutrition Research Center, IMPM, Yaounde PO Box 6163,
Cameroon

B. B. Loura
Department of Textile Engineering and Leather, Higher institute of
Sahel, University of Maroua, PO Box 46, Maroua, Cameroon

verified at the optimum points for model validation and the response values were not significantly different from the predicted values.

Keywords *Mucuna* bean flours · Vegetable milk · Optimization · Response surface methodology

Introduction

Mucuna pruriens var. *cochinchinensis* is an underutilized tropical legume which has a nutritional quality comparable to soya beans and other conventional legumes as it contains similar proportions of proteins, lipids, minerals, and other nutrients. They are traditionally used as a soup thickener by rural population in Far-North region of Cameroon. Outside Cameroon, the seeds are also eaten by Ibos in south-eastern Nigeria, Indian tribal sects, Mundari and Dravidian groups (Adebawale et al. 2005). Although *Mucuna* seeds contain high levels of protein and carbohydrate, their utilization is limited due to the presence of a number of anti-nutritional/anti-physiological compounds such as phenolics, tannins, L-Dopa, lectin and protease inhibitors which may reduce the nutrients utilization (Balogun and Olatidoye 2010).

In order to improve *Mucuna* utilization, several investigations have attempted to eliminate anti-nutritional factors by simple processing techniques (Diallo and Berhe 2003; Egounlety 2003; Gurumoorthi et al. 2013; Mugendi et al. 2010). In our previous work, we evaluated the effects of pre-boiling and dehulling on the physico-chemical, functional and pasting properties of two varieties of *Mucuna pruriens* bean flour (Mang et al. 2014). The results showed that pretreated *M. pruriens* var. *cochinchinensis* beans flours exhibited high water solubility index (50 to 70 %) which was correlated with their high protein content. This property makes them a good candidate for the production of vegetable milk, an alternative to cow milk for the management of protein malnutrition in developing countries and reduction of metabolic disorders (Ngatchic et al. 2013). In addition our recent study revealed that the pre-soaking/boiling process significantly reduced the antinutrients in the flour (Mang et al. 2014).

Vegetable milk is a water extract of leguminous seeds/flours that is a source of proteins and calories for human consumption (Touba et al. 2013). It may be produced by soaking and grinding full-fat raw beans with water to produce a slurry, subject to filtration (Chan and Beuchat 1992). Alternatively, it may also be produced by grinding unsoaked roasted beans, raw full-fat, or partially defatted beans to form flour to which water may later be added to make an emulsion (Isanga and Zhang 2009). Heating is commonly applied during the vegetable milk process, mostly to ensure food safety and extend the

shelf life of the product. It has been advocated that cow milk production should be substituted with vegetable milk production, especially where the former is difficult and expensive. It often has lower fat content than cow milk and contains no cholesterol (Rehman et al. 2007). This is regarded as one of its positive health benefits. The absence of lactose in vegetable milk also positions it as a solution to lactose intolerance for some consumers of dairy milk, especially infants with such biochemical challenge (Ikya et al. 2013). Beside mucuna protein isolate has been shown to exhibit antioxidant and hypolipidemic activity (Ngatchic et al. 2013).

Several studies are reported on production of soya milk, sesame milk, and peanut milk (Isanga and Zhang 2009; Malaki et al. 2008; Mullin et al. 2001; Rinaldoni et al. 2012). In all cases, the bean flour to water ratio, temperature and time of extraction vary greatly from one legume seed variety to another and between producers. In overall and in the limit of our knowledge, very few if not such studies have been conducted on production of *Mucuna* milk. It is important therefore to find out the optimum conditions for extraction of *Mucuna* milk in order to obtain the highest extraction yield of proteins, proteins content, sugar content and dry matter. In fact, in the extraction processes, there are multiple independent variables affecting the responding factors. In addition, the possibility of interactions between the independent variables should be considered in order to determine the optimal experimental conditions (Cui et al. 1994). Response surface methodology (RSM) has been reported to be an effective tool and successfully used for optimization of a process when the independent variables have a combined effect on the desired response (Koocheki et al. 2008; Wu et al. 2007). The technique provides mathematical and statistical procedures to study relationships between one or more responses (dependent variables) and a number of factors (independent variables) (Diniz and Martin 1996).

Therefore, the present work aims at optimizing the water to flour ratio, time and temperature of extraction for *Mucuna cochinchinensis* milk production using the response surface methodology.

Materials and methods

Sampling and production of *Mucuna* bean flours

Mucuna bean flour was produced using the modified method of Ngatchic et al. (2013). *Mucuna pruriens* seeds (var. *cochinchinensis*) were obtained from the International Institute of Tropical Agriculture (IITA) of Yaounde, Cameroon. Dried beans were carefully cleaned, sorted to remove defective ones and graded according to size. Bean samples were soaked in distilled water (1:10, w/v) in a controlled temperature water bath at 35 °C for 48 h. Steep water was

replaced every 12 h. At the end of soaking, seeds were divided into two sets: one part of seeds was dehulled manually and the other one was left whole. Each of the two portions of beans (whole and dehulled) was poured into boiling water (100 °C) and cooked for 1 h. The cooked beans were then dried in a ventilated electric turning dryer (brand Riviera & Bar) at 40 ± 2 °C for 48 h. After drying, processed bean samples were separately ground into fine flour using an electric grinder (Culatti, Polymix, France) equipped with a sieve of diameter 500 µm mesh. The obtained whole and dehulled *cochinchinensis* bean flours were sealed in polyethylene bags and stored at 4 °C until analysis. Table 1 (Mang et al. 2014) shows the proximate composition of the whole and dehulled mucuna flours.

Production of mucuna milk

The production of vegetable milk from *Mucuna cochinchinensis* bean flour was performed using a method described by Amir et al. (2011) with some modifications. The flour was blended with distilled water (water to flour ratio variations 6 to 24 mL/g). The slurry was stirred at 3500 rpm using an electric stirrer (TECHNICON stirrer motor, England) under different extraction time (6 – 74 min.) and temperature (25 – 95 °C) conditions. After incubation, the sample was centrifuged at 1500 g for 15 min. at 20 °C using refrigerated ultracentrifuge. The supernatant was collected and the residues were re-extracted in the same conditions. The collected supernatants were combined and packaged in 100 mL volumetric glass vessels and stored at 4 °C in a refrigerator for analysis within a maximum of 4 h. The experimental design for conditions of mucuna milk production is shown in Table 2.

Determination of the response variable

Four response variables were used in this work: the protein and total sugar contents, protein yield, and dry matter of vegetable milks. Total protein content was determined using the

method of Lowry et al. (1951). The yield extraction of protein, expressed in percentage, was calculated as the ratio of total proteins extracted to the initial amount in flour. Total sugar content of milk was determined after digestion in concentrated sulfuric acid followed by analysis with aqueous phenol as described by Dubois M and Hamilton JK (1956). Dry matter of vegetable milk was determined after drying at 105 °C following the AOAC (2003) gravimetric method.

Experimental design and statistical analysis

Response surface methodology (RSM) was used to estimate the effect of independent variables (extraction time, X_1 ; extraction temperature, X_2 ; and water to flour ratio, X_3) on the protein and carbohydrate contents (g/mL), yield extraction of protein (%), g/100 g of flour) and dry matter (%), g/100 g of milk) of *Mucuna cochinchinensis* milk. A Central Composite Rotatable Design was employed for designing the experimental data. The design variables used in this study with actual and coded levels are shown in Table 2. All experiments were conducted at three replicates.

The RSM was applied to the experimental data using commercial statistical package Minitab.16 software (Montgomery 2001). Experiments were randomized in order to minimize the effect of unexplained variability in the observed responses due to extraneous factors. The experimental design was composed of 17 experiments including 2^3 full factorial design points, six star points and three center points to calculate the repeatability of the method. Correspondences between coded and actual values investigated are presented in Table 2. The response variables were related to the coded factors (X_i , $i = 1, 2$ and 3) by a second order polynomial using the equation below:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

The coefficients of the polynomial model were determined using the numerical optimization analysis design option of Minitab statistical package. The significant ($p < 0.05$) terms in the model were found by analysis of variance (ANOVA) for each response. The model adequacies were checked by lack-of-fit test, R^2 , adjusted- R^2 (adj- R^2), and p-values as outlined by previous studies (Hamed et al. 2008; Karazhiyan et al. 2011). The coefficient of determination, R^2 , is defined as the proportion of the variation in the response variable attributed to the model. It was suggested that for a good fitting model, R^2 should not be less than 80 % (Joglekar and May 1987).

Optimization procedure

After generating the polynomial regression equations relating the responses to the independent variables studied, the

Table 1 Proximate composition of *M. cochinchinensis* bean flours (Mang et al. 2014)

Parameters (%)	Treatments	
	Dehulled bean flour	Whole bean flour
Moisture	9.59 ± 0.13^a	10.55 ± 0.15^b
Proteins	32.94 ± 0.65^c	26.63 ± 0.33^b
Carbohydrates	55.35 ± 1.01^c	46.49 ± 1.29^a
Lipids	6.16 ± 0.28^{ab}	5.98 ± 0.48^{ab}
Ash	2.21 ± 0.14^a	2.92 ± 0.13^b

Means $\pm$ SD values with different letters within the same row differed significantly ($P < 0.05$) as determined by Duncan's multiple range test ($n = 03$)

Table 2 Matrice of central composite design of independent variables (actual and coded levels) for extraction of *M. cochinchinensis* milks.

Run	Independent variables			Dependent variables			
	Coded and Actual level			Whole <i>M. cochinchinensis</i> bean flour			
	Time (min.)	Temperature (°C)	Ratio (W/F)	Protein (g/100 mL of milk)	Extraction yield (g/100 g of flour)	Carbohydrates (g/100 mL of milk)	Dry matter (g/100 g of milk)
Dehulled <i>M. cochinchinensis</i> flour							
1	0 (40)	0 (60)	0 (15/1)	4.16	53.41	2.95	10.12
2	1 (60)	1 (80)	−1 (20/1)	1.15	38.07	1.21	3.41
3	−1 (20)	1 (80)	−1 (20/1)	0.19	16.67	1.16	2.11
4	1 (60)	−1 (40)	−1 (20/1)	2.19	47.04	1.96	6.17
5	1 (60)	1 (80)	1 (10/1)	4.25	28.23	2.61	6.03
6	−1 (20)	−1 (40)	−1 (20/1)	0.64	25.10	0.51	2.21
7	−1 (20)	−1 (40)	1 (10/1)	0.99	11.37	1.66	3.27
8	0 (40)	0 (60)	1.68 (6/1)	5.44	17.93	4.32	8.12
9	0 (40)	0 (60)	0 (15/1)	3.95	55.90	2.82	9.78
10	1.68 (74)	0 (60)	0 0	4.87	65.55	2.25	7.63
11	0 (40)	0 (60)	−1.68 (24/1)	1.21	38.72	2.23	3.45
12	0 (40)	−1.68 (25)	0 (15/1)	0.61	23.17	1.25	4.10
13	0 (40)	0 (60)	0 (15/1)	4.41	51.14	3.02	10.17
14	0 (40)	1.68 (95)	0 (15/1)	0.28	19.27	1.21	1.95
15	−1 (20)	1 (80)	1 (10/1)	0.72	11.68	2.29	2.30
16	−1.68(6)	0 (60)	0 (15/1)	0.38	16.07	0.76	1.46
17	1 (60)	−1 (40)	1 (10/1)	4.46	21.63	3.76	8.76

optimization procedure was performed to obtain the optimal levels of the three factors (X_1 , X_2 ; and X_3). Numerical optimization was also carried out to determine the exact optimum level of independent variables leading to the desirable vegetable milk in terms of the response variables. The desired goals for each response variable were chosen as maximized while all the factors were kept within range. The optimal condition that depends on the factors was obtained using the predicted equations determined by RSM. These equations were computed graphically for deduction of workable optimum conditions. Therefore, the fitted regression models were expressed as contour plots in order to visualize the relationship between the response variables and experimental levels of each factor and to deduce the optimum conditions. For graphical optimization, contours plots were plotted by keeping one variable constant at the center point and varying the other two variables within the experimental range (Razavi et al. 2009; Mason et al. 2003). In addition the desirability function was used for achieving optimization with multiple responses. In fact multiple responses can be combined into one response called “desirability function” by choice of value from 0 (one or more product characteristics are unacceptable) to 1 (all product characteristics are on target). The general idea of this method is to compute a desirability value, d_i , for each response that is a measure of how close the fitted value with the optimal settings of the factors is to the desired value, and use these values to form the composite desirability for k response variables given by $D = (d_1 \times d_2 \times \dots \times d_k)^{1/k}$ (Derringer and Suich 1980). Response surface and contours plots were generated with Sigma plot 12.0 software.

Validation of models

The adequacy of response surface models for predicting the optimum response values was verified by conducting experiments in triplicate under the recommended optimum conditions. The experimental and predicted values of the responses were compared using a t-test analysis for comparison of a mean to a theoretical value in order to check the validity of models.

Results and discussion

Model fitting

The analysis of variance of the second-order polynomial response surface model and significance of the terms of the models are presented in Tables 3 and 4 for whole and dehulled mucuna, respectively. The analysis revealed that adding terms up to quadratic level significantly improved the model and, therefore, could be the most appropriate model for the four response variables. The estimated regression coefficients of

the polynomial response surface models along with the corresponding R^2 values and lack of fit tests are also given in Tables 3 and 4. All the terms in the models were significant as revealed by F-ratio and p-value. The most significant variables affecting the variation of the four response variables studied were the linear terms. In all cases the regression models were significant ($p < 0.05$) as shown by the analysis of variances, the lack of fit analysis and the R^2 . The R^2 values for these response variables ranged 0.962–0.999 higher than 0.80, thus ensuring a satisfactory fitness of the regression models to the experimental data.

Effect of factors on the protein content and protein yield of whole and dehulled mucuna milk

The protein content in dehulled mucuna milk was significantly ($p < 0.05$) higher than that produced with whole bean flour in all the experimental points. This difference has been ascribed to the complexation of proteins by tannins and polyphenols present in the bean hull. From Tables 3 and 4, it may be observed that among the model coefficients, three were not significant for whole mucuna flour: temperature and its interaction with time or flour to water ratio, while all coefficients were significant for dehulled mucuna. In all cases, temperature and water to flour ratio together with their interaction had positive effect on the protein content while their quadratic effects were negative. The contour plots showing the combined effect of temperature and flour to water ratio on the protein content in mucuna milk are presented in Fig. 1. Similar behaviors were observed for both whole and dehulled mucuna milks but the protein content was lower and did not varied significantly with temperature for whole mucuna milk. Figure 1 also shows a strong interaction effect of all the factors on the protein content of the vegetable milk. In fact at low water to flour ratio, increase in time led to systematic significant increase in proteins while at higher ratio ($>15/1$), increase in time led to an increase in protein content up to a maximum around 30–40 min. over which a decrease was observed. Reversely at low extraction time, increase in ratio led to significant increase in protein content up to a maximum around the ratio 15/1 over which the protein content decreases; while at higher extraction time increase in ratio tends to a systematic decrease in protein content. This result reflects the solubility dynamism involved during the extraction process. In fact at low water level, proteins diffused from flour into water and are solubilized by establishment of hydrogen bonds with water. Theoretically diffusion of proteins will continue until a saturation of the solution is achieved. Higher will be the quantity of flour in solution, faster the saturation will be achieved. At saturation, proteins tend to aggregate and precipitate. Increase in temperature seemed to accelerate the achievement of this saturation observed around temperature 55 °C over which a decrease in protein content was observed (Fig. 1). Moreover, increasing temperature could promote mass transfer and solubility, reduce viscosity

Table 3 ANOVA and regression coefficients of the second-order polynomial model for the response variables (whole *M. cochinchinensis* bean flour)

Source	Proteins (%)				Extraction Yield of proteins (%)				Carbohydrates (%)				Dry matter (%)				
	DF	Coefficients	Sum of squares	F-ratio	P-value.	Coefficients	Sum of squares	F-ratio	P-value.	Coefficients	Sum of squares	F-ratio	P-value.	Coefficients	Sum of squares	F-ratio	P-value.
Linear																	
b_1	1	1.249	21.34	129.05	0.000	11.230	1722.41	304.19	0.003	0.442	2.67	259.85	0.003	1.821	45.297	987.28	0.001
b_2	1	-0.185	0.47	2.84	0.140	-1.248	21.29	3.76	0.19	-0.013	0.003	0.31	0.640	-0.745	7.589	165.42	0.006
b_3	1	0.979	13.11	79.28	0.000	-6.510	578.93	102.24	0.009	0.622	5.28	513.02	0.001	1.048	15.014	327.25	0.003
Quadratic																	
b_{11}	1	-0.587	3.90	23.57	0.002	-5.307	317.59	56.09	0.017	-0.504	2.868	2.78	0.003	-1.9	40.731	887.77	0.0011
b_{22}	1	-1.358	20.81	125.88	0.000	-12.234	1687.46	298.02	0.003	-0.602	4.105	298.56	0.002	-2.438	67.038	1461.14	0.001
b_{33}	1	-0.340	1.31	7.91	0.026	-9.722	1065.61	188.19	0.005	0.120	0.165	16.12	0.053	-1.46	24.053	524.25	0.002
Interaction																	
b_{12}	1	-0.064	0.03	0.20	0.666	0.718	4.13	0.73	0.483	-0.46	1.692	164.35	0.006	-0.554	2.457	53.56	0.018
b_{13}	1	0.560	2.51	15.19	0.006	-2.066	34.16	6.03	0.133	0.177	0.252	24.47	0.385	0.495	1.965	42.85	0.023
b_{23}	1	0.127	0.13	0.79	0.405	3.039	73.92	13.05	0.069	-0.101	0.08	7.77	0.100	-0.105	0.089	1.96	0.297
b_0		4.184				53.724				2.930							
Lack of fit	5		1.054	4.08	0.209		168.152	5.94	0.1504		0.003	0.07	1.08		1.418	6.18	0.145
Pure error	2		1.157				11.324				0.020				0.091		
Total	16		60.127				4763.77				17.25				161.064		
R ²		98.08				96.23				99.87				99.06			
Adj-R ²		95.60				91.39				99.72				97.86			

 b_1 time, b_2 temperature, b_3 ratio, b_{11} time $\times$ time, b_{22} temperature $\times$ temperature, b_{33} ratio $\times$ ratio, b_{12} time $\times$ temperature, b_{13} time $\times$ ratio, b_{23} temperature $\times$ ratio, (b_0): constant

Table 4 ANOVA and regression coefficients of the second-order polynomial model for the response variables (Dehulled *M. cochinchinensis* bean flour)

Source	Proteins (%)			Extraction Yield of Proteins (%)			Carbohydrates (%)			Dry matter (%)			
	DF	Coefficients	Sum of squares	F-ratio	P-value.	Coefficients	Sum of squares	F-ratio	P-value.	Coefficients	Sum of squares	F-ratio	P-value.
Linear													
b_1	1	2.310	72.91	1665.27	0.001	14.398	2831.34	235.34	0.004	0.682	6.371	343.52	0.002
b_2	1	-0.883	10.67	243.73	0.004	-8.215	921.69	76.61	0.013	-0.038	0.021	1.10	0.043
b_3	1	1.256	21.55	492.21	0.002	-5.380	395.36	32.86	0.029	0.377	1.944	104.94	0.009
Quadratic													
b_{11}	1	-1.326	19.83	452.96	0.002	-5.753	373.16	31.02	0.031	-0.375	1.588	85.72	0.011
b_{22}	1	-2.470	68.82	1571.80	0.001	-14.707	2438.40	202.68	0.005	0.281	0.894	48.27	0.020
b_{33}	1	-0.996	11.19	255.66	0.004	-9.665	1053.08	87.53	0.011	-0.089	0.089	4.82	0.159
Interaction													
b_{12}	1	-1.113	9.91	226.45	0.004	-6.964	387.98	32.25	0.029	-0.268	0.577	31.18	0.030
b_{13}	1	0.923	6.82	155.81	0.006	-0.164	0.22	0.02	0.906	0.066	0.351	1.89	0.302
b_{23}	1	-0.540	2.34	53.39	0.018	1.482	17.58	1.46	0.350	-0.258	0.535	28.90	0.032
b_0		9.467				57.077					3.032		12.255
Lack of fit	5		2.20	10.07	0.093		94.27	1.57	0.434		0.38	4.10	0.207
Pure error	2		0.09				24.06				0.037		
Total	16		199.93				7461.03				11.894		
R ²		98.85				98.41				96.49			
Adj-R ²		97.38				96.37				99.97			
										99.93			

 b_1 time, b_2 temperature, b_3 ratio, b_{11} time $\times$ time, b_{22} temperature $\times$ temperature, b_{33} ratio $\times$ ratio, b_{12} time $\times$ temperature, b_{13} time $\times$ ratio, b_{23} temperature $\times$ ratio, (b_0): constant

Fig. 1 Typical contour plots for the effect of time extraction and temperature (a) and water to flour ratio (b) on protein content of *Mucuna cochinchinensis* milk (dehulled bean flour sample)

of solution and thus increases extraction rate (Zhang et al. 2009). The decrease in protein content with increasing temperature over 60 °C might also be due to the thermal denaturation of the proteins, hence the lower proteins solubilization (Kumoro et al. 2010).

The extraction yield is a parameter that gives an idea of the relative quantity of proteins extracted from the flour, as

compared to the protein content which is an indicator of the absolute quantity of proteins in the extract. For an extraction of proteins to be optimized, it should maximize not only the content of protein in the extract, but also the quantity of proteins removed from the flour. Fortunately some similarities were observed between the behaviors of both (protein content and protein yield) response variables with function of extraction time, extraction temperature and water to flour ratio as shown by the correlation between both parameters. In fact significant linear correlation coefficients of 0.54 ($p < 0.02$) for whole mucuna and 0.77 ($p < 0.001$) for dehulled mucuna, were observed between protein content and protein yield. Compared to protein content, the coefficients of the polynomial models related to the change in protein yield revealed similar linear effect of time and quadratic effect of all factors (Tables 3 and 4). The typical contour plots showing the combined effect of extraction time, temperature and water to flour ratio on protein yield are presented in Fig. 2. The curves showed less interaction effects between the factors as confirmed by the coefficient in Tables 3 and 4 which were not significant excepted time vs. temperature interaction which was significant ($p < 0.03$) for dehulled mucuna flour. In addition and in conformity with the variation in protein content, the curves of change in protein yield were similar for whole and dehulled mucuna. In this respect, protein extraction yield increased significantly ($p < 0.05$) with increase in temperature to a maximum around 60 °C from which further increase in temperature resulted in decrease. In addition increase in time led to linear significant increase in protein yield. This change in yield with time was observed irrespective of the temperature for whole mucuna flour. However for dehulled mucuna flour, no significant effect of time was observed at high extraction temperature. Similar quadratic change in protein yield with extraction temperature has been reported for boat-fruited sterulia seeds (Wu et al. 2007). The water to flour ratio followed an effect similar to that of temperature. In fact, an increase in yield was observed as the ratio increased up to an optimum around a ratio of 16 mL/g from which a decrease in yield was observed.

Total sugar content

Vegetable milk is a combination of proteins and sugars which formed the bulk and nutritive part of the product. This study was performed in order to maximize both nutrients which behaviors were shown to be quite different. In fact no significant correlation was observed between the total sugar content and neither the protein content ($r = 0.41$, $p = 0.10$ for whole and $r = 0.26$, $p = 0.10$ for dehulled mucuna flour), nor the protein yield ($r = 0.03$, $p = 0.89$ for whole and $r = 0.29$, $p = 0.27$ for dehulled mucuna flour). In addition all the linear, interaction and quadratic effects were significant ($p < 0.05$) in the exception of the quadratic effect of time for dehulled

Fig. 2 Typical contour plots for the effect of extraction time and temperature (a) and water to flour ratio (b) on the proteins yield of *Mucuna cochinchinensis* milk (dehulled bean flour sample)

mucuna flour (Tables 3 and 4). All the linear effects were significant in the case of either whole mucuna or dehulled mucuna suggesting that increase in either extraction time, temperature, and water to flour ratio tends to increase the total sugar content in the milk. The largest effects were the linear terms followed by the interactions terms. The corresponding graph showing these effects are presented in Fig. 3. From the

Fig. 3 Typical contour plots for effect of extraction time and temperature (a) and water to flour ratio (b) on the total sugar content of *Mucuna cochinchinensis* milk (dehulled bean flour sample)

plot it can be seen that when extraction temperature was increased from 25 to 95 °C, the sugar content of mucuna milk increased. In addition increase in time led to significant increase in total sugars. The effect of time was much important at high temperature and vice versa, suggesting synergism effects of both factors on the sugar content in the milk as confirmed by the significant and positive interaction observed in

the Anova (Tables 3 and 4). As for protein content and yield, similar curves were observed for whole and dehulled mucuna flour with dehulled mucuna exhibiting high contents of sugar. The high content of sugar in dehulled sample might be due to the increase hydrolysis of polysaccharides at higher extraction temperatures and long exposure time as demonstrated recently on *Lepidium sativum* seed (Karazhiyan et al. 2011). Reverse effect of water to flour ratio was observed on sugar content. In fact increase in water to flour ratio tends to decrease the total sugar level and this effect seems to be more important at high extraction time and temperature. The effect of water to flour ratio seemed to follow the normal order, in the way that increase in water normally lead to the dilution of the constituent, and then a decrease in the content. The difference between the change in protein and sugar content in the vegetable milk is probably a consequence of the low solubility of sugars mainly composed of whole starch, amylose and amylopectine in our samples, although mucuna seeds were boiled before extraction.

Dry matter

The dry matter represents all the components other than water in the extract such as proteins and sugars. In the present study the change in dry matter was mostly influenced by the change in protein content as referred to the highly significant correlation between dry matter and protein content ($r = 0.91$, $p < 0.001$ for whole; $r = 0.95$, $p < 0.001$ for dehulled mucuna flour) while the correlation coefficients with sugar content were not significant ($r < 0.24$). Similarities between protein content and dry matter variations can also be observed on the linear, interaction and quadratic effects in the models shown in Tables 3 and 4. These data showed that irrespective of the pretreatment, linear and interaction effects of time and water to flour ratio were significantly ($p < 0.01$) positive, while the quadratic effects were negative. Linear and interaction effects of temperature were negative and less important compared to time and water to flour ratio. In addition the linear and interaction effects of time and water to flour ratio were significantly higher for dehulled mucuna than for whole mucuna flour, suggesting high extraction of dry matter in dehulled sample. However the behavior of the variations in dry matter of dehulled or whole mucuna samples with the factors were quite similar. The typical contour plot of changes in dry matter vs. time, temperature and water to flour ratio is presented in Fig. 4. This plot revealed that dry matter content of vegetable milk increased with increase in temperature extraction up to 60 °C from which further increase in temperature resulted in decrease of dry matter. In addition increasing extraction time was accompanied by an increase in the dry matter content of vegetable milk. Many studies on other food stuffs revealed

Fig. 4 Typical contour plots for effect of temperature, time extraction (a) and water to flour ratio (b) on dry matter of *Mucuna cochinchinensis* milk (dehulled bean flour sample)

similar effects of extraction time and temperature (Dua et al. 2009; Fikselová et al. 2008). These results suggested that prolonged extraction time might have resulted in leaching out of high amount of compounds into water, and in particular proteins. The quadratic effect of water to flour ratio can also be visualized in Fig. 4 which shows an optimum dry matter, around the ratio of 16 ml/g.

Optimization procedure

Optimum was achieved graphically by identifying zones of maximum protein content and yield, total sugar and dry matter. Intersection of optimal extraction zones identified in all the contours plots shown above for milk extracted from whole *cochinchinensis* bean flour corresponded to the range temperature of 40–70 °C, water to flour ratio 6–17 mL/g and time of extraction of 30–74 min. while the corresponding optimal zone for dehulled *M. cochinchinensis* bean flour was 40–65 °C for temperature, water to flour ratio 6–17 mL/g and time of extraction 35–74 min. As expected the computed optimal condition of milk extraction given in Table 5 was within the optimum zone determined graphically. No remarkable difference existed between the optimal condition of extraction from dehulled mucuna and whole mucuna flour. However, as expected the predicted response variables at the optimal condition were significantly lower for whole mucuna milk than for dehulled mucuna milk. Protein extraction yield, total sugar and dry matter were quite in the same range values, but the protein content in dehulled was twice the value in the whole mucuna milk.

In order to achieve the optimization with all the responses, the desirability function was used. It describes the variation in probability to achieve the maximization of the response variables. Figure 5 presents the typical surface plot of the variation in desirability function as influenced by the factors variables. The plot revealed that desirability increased with increase in extraction time in a linear manner while peak desirability was observed at temperature around 60 °C and water to flour ratio around 16 mL/g. In addition significant interaction can be visualized between water to flour ratio and extraction time. The optimization procedure indicated that the overall optimum regions had a desirability of 0.94 for dehulled *M. cochinchinensis* bean flour milk and 0.92 for whole *M. cochinchinensis* bean flour milk.

Validation of the models

The observed experimental values and fitted values predicted by the response surface models are presented in Table 6.

Table 5 Predicted optimum conditions of extraction of *M. cochinchinensis* milks

Factors	Low	High	Optimum
Dehulled bean flour			
Time (min.)	6	75	57
Temperature (°C)	25	95	66
Ratio (mL/g)	6/1	24/1	13/1
Whole bean flour			
Time (min.)	6	75	67
Temperature (°C)	25	95	63
Ratio (mL/g)	6/1	24/1	12/1

Fig. 5 Typical contours plot of the desirability for the optimization of *M. cochinchinensis* milk production as affected by the extraction time and temperature (a) and water to flour ratio (b)

Protein and sugar contents of *M. cochinchinensis* milk produced at optimum condition were higher than corresponding values of 3.71 and 0.84 % reported for peanut milk (Isanga and Zhang 2009). Equally corresponding values of 2.84 and 5.49 % reported for cow milk were significantly lower than ours (Isanga and Zhang 2009). Much high value of protein content (7.64 %) was recently reported for soya milk (Manhal and Kamal 2010). The high protein and sugar contents of

Table 6 Predicted and experimental values of the responses obtained at optimum conditions

Responses	Predicted value	Experimental value	p-value (t-test)
Dehulled <i>M. cochinchinensis</i> flour			
Proteins (% w/v)	14.58	14.03 ± 0.67	0.232
Extraction Yield of Proteins (% w/w)	55.25	53.75 ± 1.02	0.971
Carbohydrates (% w/v)	4.73	4.75 ± 0.42	0.955
Dry matter (% w/w)	12.19	12.14 ± 0.06	0.222
Whole <i>M. cochinchinensis</i> flour			
Proteins (% w/v)	6.55	6.40 ± 0.2	0.263
Extraction Yield of Proteins (% w/w)	52.40	49.95 ± 2.11	0.954
Carbohydrates (% w/v)	3.51	3.52 ± 1.31	0.891
Dry matter (% w/w)	8.03	8.39 ± 0.95	0.550

Means ± SD; $n = 3$

mucuna milk make it a good potential candidate for the management of protein calorie malnutrition.

The adequacy of the model equations for predicting the response values was tested by comparing the experimental and predicted values at optimum condition. In this respect no significant difference was observed between the experimental and the predicted values. Closeness between the experimental and predicted values under the optimum region indicated the suitability of the corresponding models. In addition very high correlation coefficients (0.95 to 0.99) between experimental responses and predicted values in the experimental domain also confirmed the adequacy of the model.

Conclusion

The protein content, protein yield, sugar content and dry matter of mucuna milk vary significantly with extraction time and temperature, and water to flour ratio. All the response variables increase continuously with increase in extraction time, while for ratio and temperature the response variables firstly increase followed by a decrease in a quadratic manner. Similar effects of the factors were observed for whole and dehulled mucuna flour but the response variables were much high for dehulled mucuna flour. The optimization procedure indicated that the overall optimum region with high overall desirability ($D = 0.937$ for dehulled bean flour milk and $D = 0.921$ for whole bean flour milk) was obtained by setting the experiment at temperature 63 °C, extraction time 67 min., and water to flour ratio 12:1 mL/g for whole *M. cochinchinensis* bean flour; 66 °C, 57 min. and 13:1 mL/g for dehulled *M. cochinchinensis* bean flour. In these conditions, the dehulled mucuna milk contains up to 14.6 g/100 mL protein content and 4.7 g/100 mL total sugar content, values which provide to mucuna milk a potential role in the management of protein malnutrition in developing countries. The respective values for the whole mucuna milk are 6.6 g/100 mL and 3.5 g/100 mL. Mucuna milk also represents a food value for food

industries which can add a new source of proteins in their list of ingredients. But the nutritional properties of the mucuna milk needs to be investigated. These results provide innovativeness approach for recovery proteins from underutilize *M. cochinchinensis* beans, a low cost source of proteins.

Acknowledgments Part of this study was carried out within the team TQ2A (Technologie, qualité, innovations agro-alimentaires). In this respect we are grateful to the financial support of AIRD. The authors also declared no conflict of interest.

References

- Adebawale AY, Adeyemi AI, Oshodi AA (2005) Functional and physicochemical properties of flours of six *Mucuna* species. *Afr J Biotechnol* 4(12):1461–1468
- Amir MN, Marcela A, Vaino P, Loma W, Milena C (2011) Effect of Soy Protein Subunit Composition on the Rheological Properties of Soymilk during Acidification. *Food Biophys* 6:26–36
- AOAC (2003) Official methods of analysis of the association of official's analytical chemists, 17th edn. Association of official analytical chemists. Arlington, Virginia
- Balogun IO, Olatidoye OP (2010) Functional properties of dehulled and undeveloped velvet beans flour (*Mucuna* sp.). *J Biol Sci Bioconserv* 2:1–10
- Chan L, Beuchat LR (1992) Chemical, physical and sensory characteristics of peanut milk as affected by processing conditions. *J Food Sci* 57:401–405
- Cui W, Mazza G, Oomah BD, Biliaderis CG (1994) Optimization of an aqueous extraction process for flax seed gum by response surface methodology. *LWT-Food Sci Technol* 27:363–369
- Derringer G, Suich R (1980) Simultaneous optimization of several response variables. *J Qual Technol* 25:199–204
- Diallo OK, Berhe T (2003) Processing of *Mucuna* for human food in the Republic of Guinea. *Trop sub trop agroecosyst* 1:193–196
- Diniz FM, Martin AM (1996) Use of response surface methodology to describe the combined effects of pH, temperature and E/S ratio on the hydrolysis of dogfish (*Squalus acanthias*) muscle. *J Food Sci Tech* 31:419–426
- Dua S, Odean ML, Gavin H, Kathy A (2009) Effect of Extraction Methods and Wheat Cultivars on Gluten Functionality. *Open Food Sci J* 3:84–92

- Dubois M, Gilles KA, Hamilton JK, Roberts PA, Smith F (1956) Colorimetric method for determination of sugar and related substances. *Anal Chem* 28: 350–356.
- Egounlety M (2003) Processing of velvet bean (*Mucunapruriensvarutilis*) by fermentation. *Trop subtropagroecosyst* 1:173–181
- Fikselová M, Šilhár S, Mareček J, Frančáková H (2008) Extraction of Carrot (*Daucuscarota* L.). Carotenes under Different Conditions. *Czech J Food Sci* 26(4):268–274
- Gurumoorathi P, Janardhanan K, Kalavathy G (2013) Improving nutritional value of velvet bean, *Mucunapruriens* (L.) DC. Var. utilis (Wall.ex.wight) L. H. Bailey, an under-utilized pulse, using microwave technology. *Indian J Tradit Know* 12(4):677–681
- Hamed M, Chin-Ping T, Nazimah SA, Hamid SY (2008) Optimization of the contents of Arabic gum, xanthan gum and orange oil affecting turbidity, average particle size, polydispersity index and density in orange beverage emulsion. *Food Hydrocoll* 22:1212–1223
- Ikya JK, Gernah DI, Ojobo HE, Oni OK (2013) Effect of Cooking Temperature on Some Quality Characteristics of Soy Milk. *Adv J Food Sci Technol* 5(5):543–546
- Isanga J, Zhang G (2009) Production and evaluation of some physico-chemical parameters of peanut milk yoghurt. *LWT-Food. Sci Technol* 42:1132–1138
- Joglekar AM, May AT (1987) Product excellence through design of experiments. *Cereal Foods World* 32:857–868
- Karazhiyan H, Seyed MA, Razavi G, Phillips O (2011) Extraction optimization of a hydrocolloid extract from cress seed (*Lepidiumsativum*) using response surface methodology. *Food Hydrocoll* 25:915–920
- Koocheki A, Mortazavi SA, Shahidi F, Razavi SMA, Kadkhodae R, Milani J (2008) Optimization of mucilage extraction from QodumeShirazi seed (*Alyssum homolocarpum*) using response surface methodology. *J Food Process Eng* 33(5):861–882
- Kumoro AC, Sofiah NA, Diah SR, Catarina SB (2010) Effect of temperature and particle size on the alkaline extraction of protein from chicken bone waste. *Reaktor* 13(2):124–130
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
- Malaki NA, Susan T, Vaino P, Lorna W, Milena C (2008) Physicochemical characterization of soymilk after step-wise centrifugation. *Food Res Int* 41:286–294
- Mang YD, Abdou BA, Njintang YN, Djiogue MJE, Clémence B, Mbofung CM (2014). Effect of dehulling and boiling on the physico-chemical, functional and pasting properties of two varieties of *Mucuna* bean (*Mucunapruriens* L.) flours. submitted for publication in *Food Bioproc Tech*.
- Manhal MDO, Kamal AAR (2010) Quality Attributes of Soy-yoghurt During Storage Period. *Pak J Nutr* 9(11):1088–1093
- Mason RL, Gunst RF, Hess JJ (2003) Statistical design and analysis of experiments with applications to engineering and science. Hoboken, NJ-Wiley, an International Thomson Publishing, Europe, London, 1V7AA.
- Montgomery DC (2001) Design and analysis of experiments, 5th edn. Wiley, New York, pp. 455–492
- Mugendi JBW, Njagi ENM, Kuria EN, Mwasaru MA, Mureithi JG, Apostolides Z (2010) Nutritional quality and physicochemical properties of *Mucuna* bean (*Mucunapruriens* L.) protein isolates. *Food Res Int* 17:357–366
- Mullin WJ, Fregeau-Reid JA, Butler M, Poysa V, Woodrow L, Jessop DB (2001) An inter laboratory test of a procedure to assess soybean quality for soymilk and tofu production. *J Food Res* 34:669–677
- Ngatchic MTJ, Dongmo SS, Njintang YN, Maoundombaye T, Oben J, Mbofung FCM (2013) Evaluation of some selected blood parameters and histopathological of liver and kidney of rats fed protein-substituted *Mucuna* flour and derived protein rich product. *Food Chem Toxicol* 57:46–53
- Razavi MAS, Seyed AM, Lara MM, Seyed HHP, Ali M, Elham K (2009) Optimisation study of gum extraction from Basil seeds (*Ocimumbasilicum* L.). *Int J Food Sci Technol* 44:1755–1762
- Rehman SH, Nawaz MM, Ahmad S, Hussain A, Murtaza SSH (2007) Physico-chemical and sensory evaluation of ready to drink soy-cow milk blend. *Pak J Nutr* 6(3):283–285
- Rinaldoni AN, Mercedes EC, Antonio PP (2012) Physico-chemical and sensory properties of yogurt from ultrafiltered soy milk concentrate added with inulin. *LWT-Food Sci Technol* 45:142–147
- Touba I, Zahra I, Mostafa MT, Mandana AP, Mohammad ZM, Mohammad AS (2013) Investigation of Optimized Methods for Improvement of Organoleptical and Physical Properties of Soy milk. *Intl J Farm & Alli Sci* 2(10):245–250
- Wu Y, Cui SW, Tang J, Gu X (2007) Optimization of extraction process of crude polysaccharides from boat-fruited *sterculia* seeds by response surface methodology. *Food Chem* 105:1599–1605
- Zhang B, Cui Y, Yin G, Li X, Zhou X (2009) Alkaline Extraction Method of Cotton seed Protein Isolate. *Mod Appl Sci* 3(3): 77–82.