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Production of *Mucuna pruriens* (var. utilis) Proteins Isolates Using Central Composite Design and Effect of Drying Techniques on Some Properties

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Authors' contributions

This work was carried out in collaboration between all authors. Author KB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BC and NYN managed the analyses of the study. Author KR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Response surface methodology was used to model the effect of temperature, NaCl concentration and pH on the production of protein from *Mucuna pruriens* seeds. The analysis of response surface graphs showed that the optimal parameters correspond to a temperature of 27°C, a concentration of NaCl of 0 mol/L and a pH of 6. The water absorption capacity of the *Mucuna* protein isolates was 171°g and 124 g of water per 100 g of dry matter respectively for freeze-dried and spray-dried isolates. The granulometric distribution of emulsions was monomodale. The emulsion made with bottom proteins of *Mucuna* has presented a viscoelastic character.

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1. INTRODUCTION

The research of new proteins sources has become today a priority not only because of the growing number of consumers, but also because of low availability of animal proteins. Researchers have therefore found themselves looking for available and accessible vegetal proteins in developing countries. Among eadible protein content plants leguminous occupy an important place because of their low prices. This group of plants has many varieties that are widely used in Africa especially in Cameroon [1]. Among the different leguminous plant species, *Mucuna pruriens (var. Utilis)* presents functional characteristics comparable to others with protein contents up to 29% [2,3]. In fact, *Mucuna pruriens* constitutes a source of food for some tribe and ethnic group in Asia and Africa continents [4,5]. Unfortunately, *M. pruriens* is not well exploited because of its anti-nutritional factors such as phytate, tannins, phenols, trypsin inhibitor and L-dopa, which reduce the bioavailability of nutrients. Some tribes used to cook, ferment or germinate *Mucuna pruriens* seeds before consumption; these processes lead to the elimination of the anti-nutritional factors from their seeds. However, several works were done on *Mucuna* species to eliminate or reduce antinutritional factors while using simple processes like heating or dehulling [6–8,9] showed that fermentation and germination permit to obtain *Mucuna* proteic isolates with protein contents of up to 89 and 88% respectively and that their functional properties (water and oil absorption capacity, emulsifying capacity) are similar to those of soy. In addition, [10] showed that emulsifying capacity varied between 78% and 90% on flours and between 56% and 68% on delipidated flours of some *Mucuna* species. Likewise, [11]showed that dark color (L = 36.39) limited the use de *Mucuna pruriens* proteic isolates as a food ingredient. According to its proteic potential, it is important to find apart from food usage, others domains of valorisation of *Mucuna pruriens* proteic isolates.

Endeed, industries are looking for new ingredients to formulate or elaborate various products. Thus, the search for new ingredients with specific properties is becoming an emergency requirement. That is why it is important and urgent to dispose these products in high quantity and in most pure possible state. Optimization of proteinic isolates extraction of some leguminous have been reported in literature [12–15]. It comes from these studies that, proteins of most leguminous plants present a best solubility at high pH (pH between 11 and 12), but optimization of extraction of soluble proteins of *M. pruriens* have not been realized. That is why we found important to optimize the extraction of soluble proteins from *Mucuna pruriens (var. utilis*) while taking into account some independent variables which could affect the quantity of soluble proteins and their interactions.

Numerous products, liquid or solid are dehydrated or transformed in powder to obtain long term stability in addition to a great functionality and an ease of use. Nowadays, the spray-drying and freeze-drying technology is considered as one of the principal methods of powder production in various domains given its several advantages. In addition to optimize the extraction of soluble proteins of *Mucuna pruriens*, other objective of this work was to determine some properties according to the technique of drying.

2. MATERIALS AND METHODS

2.1 Raw Material and Preparation of *Mucuna pruriens* Flours

The raw material used in this study was made of leguminous flours from *M. pruriens* grains obtained from local markets of Ngaoundere (Cameroon). Grains were previously sorted manually to remove impurities and infested grains.*M. pruriens* flours chosen were produced according to the method of [16], which consists of steeping-drying-dehusking and drying. *M. pruriens* grains were therefore steeped in distilled water in a ratio of 1/5 (w/v) for 12 hours at 25°C, then wrung and dried for 48 hours in a ventilated electric dryer at 40°C. Dried grains were then dehusked with a manual abrasive, winnowed, ground and sieved with a of 500 µm mesh sieve. Powders obtained were sealed in polyethylene bags and kept at 4°C for further analyses.

2.2 Protein Solubility

The effect of pH on *M. pruriens* protein solubility was determined according to the method described by [17] with some modifications. A

solution of *M. pruriens* flours was prepared with distilled water with a ratio of 1/5 (w/v) and the pH adjusted with HCl 1M or NaOH 1M to values ranging from 2 to 9. The obtained solution was agitated at 600 rpm for 2 hours and centrifuged at 4000 rpm for 1 hour. The proteins content of the collected supernatant was measured by BCA (Biccinchonic Acid) method. The quantity of soluble proteins was expressed in percentage brought back to dry mater.

2.3 Experimental Design and Statistical Analysis

Surface response methodology was used to estimate the effect of three independent variables (temperature of extraction, X_1; concentration of NaCl, X_2; and pH, X_3) and the optimum conditions on the soluble proteins content [12]. A central composite design was used to reach the fixed levels. Relationships between coded and real variables are given in Table 1 according to the following relations:

$$\text{Extraction temperature} = 37.5 + 7.5X_1$$
$$\text{Concentration in NaCl} = 0.3 + 0.2X_2$$
$$\text{pH} = 4 + 2X_3.$$

Table 2 presents the experimental design with the obtained response. Each experience was done in triplicate. The data were then subjected to a multiple regression analysis to obtain an empirical model that could relate the response measured to the independent variables. It's a second order polynomial equation with interactions between the different parameters.

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X^2_1 + a_{22}X^2_2 + a_{33}X^2_3 + a_{12}\,X_1X_2 + a_{13}\,X_1X_3 + a_{23}\,X_2X_3 + a_{123}\,X_1X_2\,X_3$$

The use of Minitab 15 software allowed us to determine the coefficients of each parameter, and the analysis of variance (ANOVA) was used to the significant ($p<0.05$) terms in the mode. Soluble protein extraction yield was taken as a response (Y).

2.4 Proteins Extraction

Soluble proteins were extracted from *M. pruriens* seeds flours according to the method described by [18] with some modifications. *M. pruriens* seeds flour was solubilized in NaCl. The pH was adjusted with a solution of NaOH 1M or HCl 1M. The mixture obtained was heated to desired temperature with an electrical agitator (Lab-Mix 20) for 2 hours at 600 rpm, and centrifuged at 4000 rpm for 1 hour. The supernatant was then collected and the soluble proteins level was measured by BCA (Biccinchonic Acid) method. Each test was carried out in duplicate. The collected supernatant was precipitated at the isoelectric point and then centrifuged 4000 rpm for 1 hour. Proteins obtained as paste were collected, freeze-dried (Freeze-dryer Serail RPEV). In the same way, when the supernatant was precipitated at the isoelectric point, precipitates of protein extracts were spray dryied using a pilot plant's Pignat atomiser at 170°C.

2.5 Properties of Proteins Isolates

2.5.1 Physicochemical properties analysis

A proximate analysis was carried out on *M. pruriens* seeds flours and proteins isolates. The dry matter content, ash content and total protein content was determined following the standard methods described by [19].

2.5.2 Water and oil absorption capacity

Water and oil absorption capacity was determined following the modified method of [10]. 0.5 g of sample was mixed with 10 mL of distilled wateror 5 mL of sunflower oil (d= 0,865 ± 0,008) respectively for water absorption capacity and oil absorption capacity. The mixture was then vigorously stirred at 250 rpm for 30 min with a Lab-Mix- 20 magnetic stirrer and then centrifuged at 1789 g for 30 min. The sediment was collected and the water and oil absorption capacity was calculated.

Table 1. Independent variable values of the process and their corresponding levels

Variable parameters	Levels of variation					
	Symbol	-1,682	-1	0	+1	+ 1,682
Extraction temperature (°C)	X_1	25	30	37.5	45	50
NaCl concentration (mol/L)	X_2	0	0.1	0.3	0.5	0.6
pH	X_3	1	2	4	6	7

2.5.3 Least gelling concentration

The method of [20] was used. 5 mL of proteins isolates suspensions of 2 to 20% (w/v) were prepared with distilled water in test-tubes, heated in a boiling water bath (95°C) for 1 hour, cooled at 20°C and kept at 4°C for 2 hours. The least gelling concentration was estimated visually as those where the test tube didn't left the paste sunken when the test-tube was returned.

2.5.4 Foaming properties

The foaming power in water was determined by the modified method used by [10]. Thus, a proteic solution at 2% (w/v) was prepared, introduced in a test-tube and intensely agitated mechanically with a rotor-stator system (Ultra-turax) for 5 min at 13500 rpm. The foaming power (FP) was determined starting from the augmentation of volume due to incorporation of gas. The foaming stability (FS) expressed in minutes is obtained after foaming power determination. It corresponds to time (t) necessary for the foam volume to decrease after a time t.

2.5.5 Emulsifying properties

The method of [21] was used to prepare the emulsion. Proteic solutions were prepared at the concentration of 5% (w/v) in distilled water at 20°C. These solutions were agitated at 500 rpm for 2 hours in order to solubilize the powder. Sunflower oil (d=0.865±0.008) was used to prepare oil in water emulsions at 20% (w/w) by mixing at 13500 rpm an appropriate mass of oil and proteic solution during 10 min with an Ultra turrax (Janke and Kunkel, Staufen, Germany) equipped with a rotor-stator system. The physico-chemical characterization of the different emulsions was done the same day of their preparation. The emulsion stability at 25°C was studied using a Turbiscan LAB instrument (Formulaction, L'Union, France). Measures were carried out all hours during 24 hours. The sizes of emulsions droplet were measured with laser diffraction by using Mastersizer Malveen in wet way (Hydro 2000S, Malvern Instruments SA, Orsay, France) at 25°C. The distribution curves obtained were represented in volumic fraction (%) and corresponded to the mean of three independent measures.

3. RESULTS AND DISCUSSION

3.1 Protein Solubility

Study of *M. pruriens* protein solubility with pH is useful to determine the isoelectric point of the protein (Fig. 1). This figure shows that the quantity of soluble protein decrease quickly when the pH increase to reach a minimum at pH 4 and increase with pH until pH value of 8. This solubility plot of *M. pruriens* protein with pH is similar to those obtained by [10] and [18] on the protein isolates of flower of some species of *Mucuna.* They found that the protein solubility is influenced by the pH of the medium into which the protein is found. Indeed, the behavior of protein in solution at a giving pH is influenced by the electrical charges of the protein [17]. When the pH is equal to pI (isoelectric point), the protein is in the zwitterion form. Thus when the protein is charged positively or negatively, electrostatic forces will repulse ions, it will favor their solubilization in water whereas the zwitterion form will facilitate protein-protein interaction because the positive charges of the zwitterion will be fixed to the negative charges of the other zwitterion [1]. From pH = 8, it wasn't easy to determine the percentage of soluble protein of *M. pruriens* because of the browning of the medium due to the presence of L-dopa.

3.2 Model Fitting and Effect of Factors on the Protein Solubility Yield

The application of Response Surface Methodology was selected to gives the models conditions for maximizing the yield of soluble protein extraction from *Mucuna pruriens* [22]. The analysis of variance of the second order polynomial response surface model with interactions between parameters and significance of the terms of the models are presented in Table 3. Many significant effects can be observed. Temperature effect presented a positive significant effect (1.124) and it quadratic effect presented a negative significant effect (-0.258) at $p<0.05$ on the yield of protein extraction. These results could be explained by the fact that, when temperature is high, it could induce denaturising of soluble proteins. In the same way, temperature and NaCl concentration negatively influenced (-0.301) the protein extraction yield. NaCl concentration influenced negatively (-0.925) the protein yield. It could be explained by the fact that the presence of salts in aqueous solution induces antagonistic effect due to electrostatic and hydrophobic interaction on the protein solubility. pH have a positive effect (0.878 at $p<0.05$) on the protein yield while it quadratic effect negatively (-0.159 at $p<0.05$) influences the response. This could suggest that the protein solubility is highly linked to the pH of the medium where the protein is found. In

addition, the global charge of protein (positive or negative) depends on the pH of the medium.

The mesh plot was used to present the influence of factors on the yield of soluble protein and to illustrate the main and interactive effects of the variables. When the NaCl concentration was fixed at its optimal value, the best responses were obtained from means values of pH and higher value of temperature in the extraction medium (Fig. 2). Weakest responses were obtained for weaker values of pH and temperature. The increase in the solubility of the protein is then linked to the increase of pH.The influence of temperature and NaCl concentration of *M. pruriens*soluble protein is presented in Fig. 3. It can be observed that the solubility increase with temperature and NaCl concentration, but it highly decreases with reduction in NaCl concentration and temperature. These results show the high influence of NaCl concentration on the extraction process. Nevertheless, if the two parameters are weak, the obtained response will be weak. However, when they are very high, they could induce extraction of others undesirable compounds in *M. pruriens*flour like anti nutrients (Fig. 3). Also, the Fig. 4 shows that the best response is obtained for weaker value of NaCl concentration. The solubility of protein decreases for higher value of pH and NaCl concentration. The influence of NaCl concentration on the solubility of *M. pruriens*protein is globally weak. However, the best responses can be obtained with means values of NaCl and pH (Fig. 4).

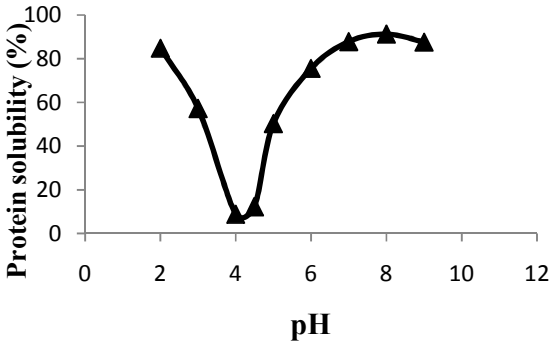

Fig. 1. Effect of pH value on protein solubility of *M. pruriens* flour

Table 2. Central composite design for independent variables and the result

N° of experience	Code variables			Real variables			Response
	x_1	x_2	x_3	X_1	X_2	X_3	Y
1	-1	-1	-1	30	0,1	2	46.90
2	+1	-1	-1	45	0,1	2	39.06
3	-1	+1	-1	30	0,5	2	45.54
4	+1	+1	-1	45	0,5	2	20.90
5	-1	-1	+1	30	0,1	6	44.37
6	+1	-1	+1	45	0,1	6	37.13
7	-1	+1	+1	30	0,5	6	50.15
8	+1	+1	+1	45	0,5	6	29.00
9	-1,682	0	0	25	0,3	4	39.67
10	+1,682	0	0	50	0,3	4	28.10
11	0	-1,682	0	37,5	0	4	40.87
12	0	+1,682	0	37,5	0,6	4	51.93
13	0	0	-1,682	37,5	0,3	1	47.57
14	0	0	+1,682	37,5	0,3	7	42.95
15	0	0	0	37,5	0,3	4	45.31
16	0	0	0	37,5	0,3	4	41.90

Table 3. ANOVA and regression coefficients of the second-order polynomial model for the response variables

Source		Soluble proteins (%)			
Linear	**Df**	**Coef**	**Sun of squares**	**F-ratio**	**P-value**
a_1	1	1.124	20.11	101.33	0.000
a_2	1	-0.925	0.78	4.59	0.280
a_3	1	0.878	18.78	90.97	0.000
Quadratic					
a_{11}	1	-0.258	7.88	28.75	0.007
a_{22}	1	-0.721	17.11	159.23	0.000
a_{33}	1	-0.159	3.15	10.71	0.048
Interaction					
a_{12}	1	-0.301	0.11	1.73	0.787
a_{13}	1	0.267	1.27	19.71	0.008
a_{23}	1	0.670	0.97	2,23	0.783
a_{123}		0.781			
a_0		40.490			
Lack of fit	5		0.211	8.197	0.489
Pure error	2		0.107		
Total	16		48.131		
R^2		90.70			
Adj-R		89.16			

$a_1$temperature, a_2 concentration of NaCl, a_3 pH, a_{11} temperature x temperature, a_{22} concentration of NaCl x concentration of NaCl, a_{33} pH x pH, a_{12} temperature x concentration of NaCl, a_{13} temperature x pH, a_{23} concentration of NaCl x pH, a_{123} a_1 temperature x concentration of NaCl x pH, a_0 constant

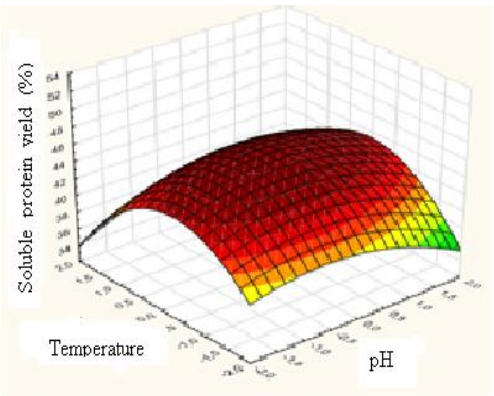

Fig. 2. 3D Surface plots for soluble protein yield of *M. pruriens* versus pH and temperature

It comes from analysis of response surface graph that the optimal point for the combination of factors correspond temperature of -1.4, pH of 1 and NaCl concentration of -1.5 in coded values. In real value, it corresponds to temperature of 27°C, pH of 6 and NaCl concentration of 0 mol/L according to the relationship between coded and real variables.

3.3 Confirmative Tests

The suitability of the equation model for predicting the optimum response values was tested using the obtained optimum conditions. When the optimum values of independent variables (temperature, NaCl concentration and pH) were incorporated into the polynomial equation, 38.36 g/100 g of soluble protein yield was obtained whereas experiments at optimum conditions gave a soluble protein yield of 40.07±0.23 (Table 4). We observe that there is no significant gap between predicted (by the model) and experimental responses (*Mucuna pruriens* soluble proteins).

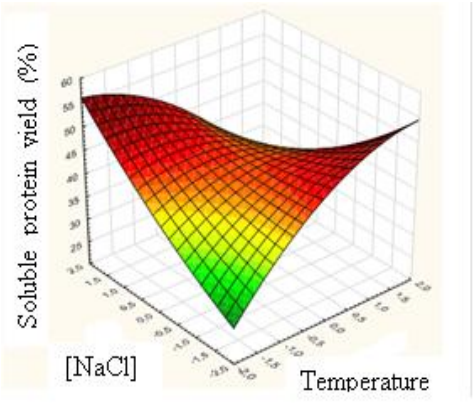

Fig. 3. 3D Surface plots for soluble protein yield of *M. pruriens* versus temperature and NaCl concentration

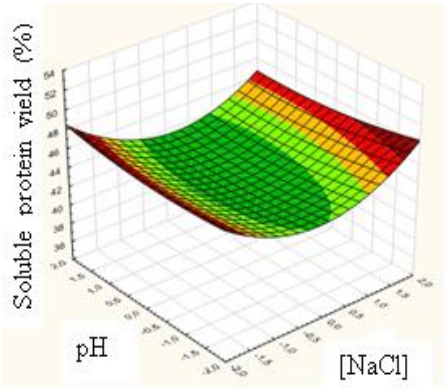

Fig. 4. 3D Surface plots for soluble protein yield of *M. pruriens* versus pH and NaCl

3.4 Physicochemical Properties of *M. pruriens* Flour and Its Proteins Isolates

Table 5 presents the protein, water and ash contents of *Mucuna pruriens* flour including the ones of its proteic isolates freeze-dried and spray-dried.The dry matter content of the *Mucuna* bean flour is higher than their isolates. This shows that the freeze-dried and spray dried proteins isolates of *M. pruriens* have a better storability than flours. The flour protein content is slightly lower than that of [23] (30.1 g / 100 g dry matter), but close to 27.50 g / 100 g of dry material obtained by [24]. In addition, the protein content of 26.12 g / 100 g of dry matter in flour is deemed sufficient compared to other unconventional protein sources such as Red pepper seed (*Capsicum frutescens*) (26.02 g / 100 g of dry weight) [14], germinant *Pumpkin* seed (24.5 to 36 g / 100 g) [13], *pigeon pea* (15.5-28.8 g / 100 g) [25]. Concerning the proteic isolates, 86.84 and 85.94% were obtained respectively for the freeze-dried and spray-dried ones. These values are similar to those obtained by [26] on proteic isolates of dried *Mucuna pruriens* in an electric dryer which is of 85.28%. In addition, there is no a significant difference ($p<0.05$) among protein contents of proteic isolates no matter the drying process.

3.5 Functional Properties of *M. pruriens* Protein Isolates

Fig. 5 shows the water and oil absorption capacities of proteic isolates from freeze-dried and spray-dried *Mucuna pruriens*. It comes from this figure that the water absorption capacity of the freeze-dried and spray-dried *Mucuna* protein isolates are 171 g and 124 g respectively of water per 100 g of dry matter. The water absorption capacity is an essential function of the proteins of various food and non-food products. The water absorption capacity by the *Mucuna* proteins is reported by many authors (10.9). The water absorption by the protein is largely influenced by their structures. The water absorption capacity of the protein isolates showed that the isolate has a high capacity to bind water. This suggests that the proteins have a granular structure which is not very compact. This implies that *Mucuna* proteins could be incorporated into food and non-food formulations. The oil absorption capacity of 142.7 and 133.4 g / g respectively from freeze-dried and spray-dried proteic isolates of *Mucuna pruriens* (Fig. 6) show the binding capacity of the protein isolate of *Mucuna* with oil. The least gelling concentration which represents the least concentration of protein necessary for the gel to

Table 4. Optimum of condition based on graphical optimisation predicted and experimental value of response at that condition

Optimum condition	Coded levels	Real levels
Temperature	-1.4	27°C
NaCl concentration	-1.5	0mol/L
pH	1	6
Response	Predicted value	Experimental value
Soluble protein yield	38.36%	40.07±0.23%

Table 5. Protein, water and ash composition of *M. pruriens* flour and its proteins isolates

	Protein	Water	Ash
*Mucuna*beanflour	26,12±0,12^{a}	6,70 ±0,28^{c}	3,55±0,11^{e}
Freeze-dried proteins isolates	86,84±1,12^{b}	4,82±0,28^{d}	3,98±0,11^{e}
Spray-dried proteins isolates	85,94±1,29^{b}	4,78±0,29^{d}	4,14±0,29^{e}

Values having the same letter in the same column are not statistically different (P<0.05)

be formed is 12% and 10 % respectively for freeze-dried and spray-dried proteic isolates. These values are lower than those reported by [10] on powder of some varieties of *Mucuna* which are between 14 and 20%. This can be explained by the fact that the powder contains in addition to proteins, other compounds such as starch in large quantities. The gelling properties were associated with the ratio of the different components such as proteins, lipids and carbohydrates in various legumes [17].

The foaming capacities of proteic isolates of *Mucuna* are respectively 140 and 92 for the freeze-dried and spray-dried ones. It is greater than that of the protein isolates of fermented *Mucuna cochinchinensis*, but substantially equal to those germinated after 48 and 72 hours [9]. This foaming ability is lower than that obtained on soy isolate [27]. The foaming stabilities of the proteic isolates from *Mucuna pruriens* are given in Fig. 6. This figure shows that foams of the proteic isolates are unstable after 30 minutes, but stable after 90 minutes. The heat treatment applied during the spray drying process led to a partial denaturation of proteins, which had reduced the foaming power of proteic isolates obtained after spray drying. This result is in agreement with the one of [28] who has shown that foaming properties are more often reduced by heat treatments. The importance of foam stability is in its capacity to maintain the foam as long as possible in order to use it as a foaming agent in food and non-food formulations where foams are necessary.

3.6 Emulsions Analysis

Fig. 7 shows the retrodiffusion profile of emulsions obtained with a turbiscan. Emulsion contained in a tube of about 45 mm of height was scanned one time each hour during 24 hours. We observe that the retrodiffusionprofiles of emulsions obtained are similar and that the stability of emulsions has grown up within those 24 hours. In fact, no matter the drying process of protein extracts, there is a clarification at the bottom of the tube and the formation of cream on top. However, the clarified part is more important in the emulsions where the samples were spray-

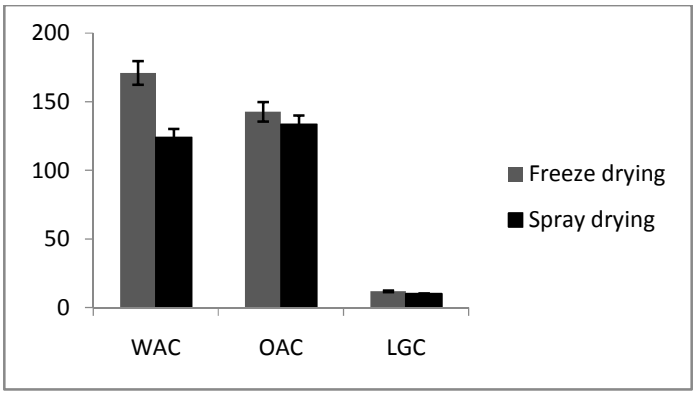

Fig. 5. Water absorption capacity (WAC), Oil absorption capacity (OAC) and least gelling concentration (LGC) of proteic isolates from freeze-dried and spray-dried *Mucuna pruriens*

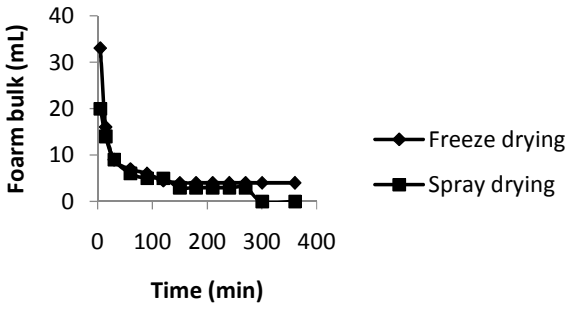

Fig. 6. Foaming stability

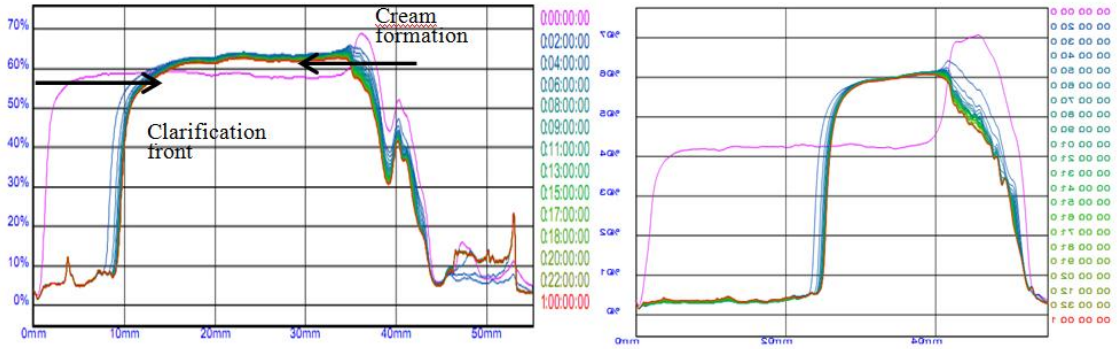

Fig. 7. Emulsion stability. (a): Emulsion from freeze-dried protein extracts; (b): Emulsion from spray-dried protein extracts

Table 6. Average diameter and span of emulsions droplets

Emulsion	D0.5 (µm)	span
Freeze-driedextract	22.830±0,191	1.710±0.027
Spray-driedextract	23.330±0.082	1.880±0.024

dried than those freeze-dried. This can be explained by the fact that during the spray-drying process, a part of proteins could have been denatured due to the temperature [28]; which could have led to a dispersing of proteins in the medium allowing to their droplets to fuse among themselves. In the range of concentration of the studied solution, a slightly decrease of retro diffusion flow signal at the bottom of the sample was noticed. This was due to the decrease of the concentration of particle of the emulsion at the bottom of the sample (clarification) (Fig. 7). This indicates that the phase formed in at the first time is the heaviest (concentrate phase). By the way, above the sample, the retro diffusion flow increases slightly due to a weak increase of the dispersed phase concentration (creaming). This phenomenon of emulsion destabilization is in accordance with the D (0.5) of the granulometric profile of emulsion which seems to be higher.

The size of the particles of an emulsion is an important parameter in the sense that it influences its texture and stability [29]. Fig. 8 thus shows the granulometric profile of emulsions made with freeze-dried protein extracts and spray-dried ones from *Mucuna pruriens* and Table 6 gives the median diameter (D 0.5) and the span. It is observed on this figure that the granulometric distribution of emulsions is monomodal with a principal pic between 15 and 25 µm. Emulsions made with freeze-dried extracts have a higher volumic percentage (about 10%) than those made with spray-dried ones. In addition, Table 6 gives the average diameter ($D_{0.5}$) of 22.830 for the emulsions made with freeze-dried protein extracts with a span of 1.710 while for spray-dried extracts; it is 23.330 and 1.880 for the span. This difference can be explained by the capacity of proteins to stabilise the droplets of the emulsion.

This result is in accordance with the one obtained using the turbiscan ; this may suggest a rapid destabilization of the emulsion because it seemed high and shows that the drying process influences the stability of emulsions due to a partial denaturation of spray-dried proteins [30].

Fig. 9 shows the textural profile of emulsions. This profile, as a graph, represents the practised force of the sample on the borer with the distance covered by the hand of the mobile. It results from this curve that no matter the drying process of protein extracts, the force increase with the lengthening up to a maximum, then decreases in spite of the increase of the lengthening. This figure also allows us to see the primary mechanic characteristics of emulsions which are the hardness, the viscosity and elasticity [31]. The maximum point of force's compression corresponds to the hardness and represents the necessary force to cause a deformation of emulsions droplets. It is respectively of 0.8 N and 0.6 N for emulsions made with freeze-dried and spray-dried extracts. After this maximum point, we can see a light plateau assimilable to the viscosity, a decrease of the force with a slope which expresses the elasticity of emulsions. This phenomenon

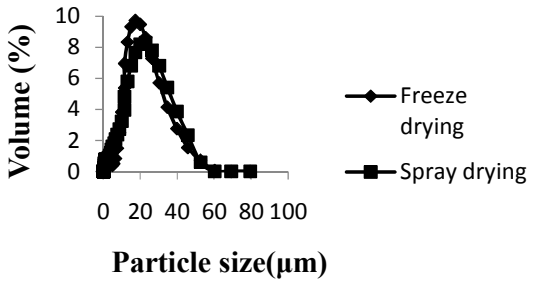

Fig. 8. Granulometric distributions of emulsions

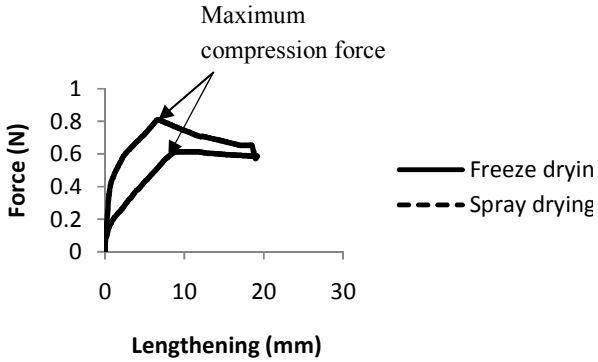

Fig. 9. Textural profiles of emulsions

observed on the textural curve expresses the viscoelastic character of emulsions [32].These low values of necessary forces to reach a deformation of emulsions droplets show that the obtained result is in accordance with the ones obtained using the Turbiscan, which show a rapid destabilisation of emulsions droplets with time, with a large distribution of sizes of emulsions particles. Therefore, the development of new textures can be considered as an important source of innovation in the food and non-food domains, for textures informations vary a lot.

4. CONCLUSION

The protein extraction process of *M. pruriens* seeds was optimized using Central composite design. The three independent variables involved in the optimization were temperature of extraction (X_1), concentration of NaCl (X_2), and pH (X_3). Optimum extraction of *M. pruriens* seeds proteins corresponded to temperature of 27°C, pH of 6.These optimum conditions could be used to produce protein concentrate from *M. pruriens* seed flours. The functional properties of *M. pruriens* proteins isolates showed that the species could be an economic and alternative protein source with a great potential to alleviate protein malnutrition in developing countries and can also be used to improve the cosmetic proprieties of the formulations in developed countries. The influence of heat on functional properties of proteic isolates greatly depended on the time where there were applied during the process. Thus, a too high temperature led to a thermal denaturation of soluble proteins and an aggregation almost irreversible of a part of proteins which would then influence obtained emulsions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abdel Rahman SM Eltayeb, Ali O Ali, Azza A, Abou-Arab, Ferial M. Chemical composition and functional properties of flour and protein isolate extracted from

Bambara groundnut (*Vigna subterranean*). African J. Food Sci. 2011;5(2):82–90.
2. Ezeagu GIE, Maziya-Dixon B, Tarawali. Seed characteristics and nutrient and antinutrient composition of 12 *Mucuna* accessions from Nigeria. Trop. Subtrop. Agroecosystems. 2003;1:129–140.
3. Vadivel V, Janardhanan K. Nutritional and antinutritional characteristics of seven south Indian Wild Legumes. Plant Foods Hum. Nutr. 2005;60:69–75.
4. Iyayi EA, Egharevba JI. Biochemical evaluation of seeds of an underutilized legume (*Mucuna utilis*). Niger. J. Anim. Prod. 1998;25:40–45.
5. Ukachukwu SN, Obioha, F. Chemical evaluation of *Mucuna cochinchinensis* as alternative protein feedstuff. J. Appl. Chem. Agric. Res. 1997;4:33–38.
6. Fathima KR, Soris PT, Mohan VR. Nutritional and antinutritional assessment of *Mucuna pruriens* L DC var. Pruriens an Underutilized Tribal Pulse. 2010;1:79–89.
7. Mugendi JB, Njagi ENM, Kuria EN, Mwasaru MA, Mureithi JG. Effects of processing technique on the nutritional composition and anti-nutrient content of mucuna bean (*Mucuna pruriens* L). 2010;4:156–166.
8. M Mang YD, Abdou B, Njintang YN, Djiogue M, Clémence B, Mbofung C. Effect of dehulling and boiling on the physico-chemical, functional and pasting properties of two varieties of Mucuna bean (*Mucuna pruriens* L) flours. Food Bioproc Tech; 2014.
9. Udensi EA, Okoronkwwo KA. Effects of fermentation and germination on the physicochemical properties of *Mucuna cochinchinensis* protein isolate. African J. Biotechnol. 2006;5(10):896–900.
10. Adebowale YA, Adeyemi IA, Oshodi AA. Functional and physicochemical properties of flours of six *Mucuna* species. 2005; 4:1461–1468.
11. Mugendi JBW, Njagi ENM, Kuria EN, Mwasaru MA, Mureithi JG, Apostolides Z. Nutritional quality and physicochemical properties of Mucuna bean (*Mucuna pruriens* L.) protein isolates. Food Res Int. 2010;17:357–366.
12. M Wang, Jiang L , Li Y, Liu Q, Wang S, Sui X. Optimization of extraction process of protein isolate from mung bean. Procedia Eng. 2011;15:5250–5258.
13. Quanhong L, Caili F. Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein. Food Chem. 2005; 92(4):701–706.
14. Firatligil-Durmus E, Evranuz O. Response surface methodology for protein extraction optimization of red pepper seed (*Capsicum frutescens*).LWT-Food Sci. Technol. 2010; 43(2):226–231.
15. Horax R, Hettiarachchy N, Kannan A, Chen P. Protein extraction optimisation, characterisation, and functionalities of protein isolate from bitter melon (*Momordica charantia*) seed. Food Chem. 2011;124(2):545–550.
16. G Kaptso K. Potentiel technologique des farines de niébé *(Vigna unguiculata)* et de voandzou *(Vigna subterranea)* pour la préparation du koki *(gâteau de pate cuite à la vapeur)*. Ngaoundéré: Thèse de doctorat/Ph.D, Département des Sciences Alimentaires et Nutrition, ENSAI, Université de N'Gaoundéré; 2008.
17. Sathe SK, Deshpande SS, Salunkhe DK. Functional properties of winged bean *(Psophocarpus tetragonolobus* L) proteins. J. Food Sci. 1982;47:503–506.
18. Yemisi O, Adebowale A, Kayode. Emulsifying properties of Mucuna flour and protein isolates. J. Food Technol. 2008; 6(2):66–79.
19. AOAC, Official methods of analysis, 16th Ed. Th. Gaithersburg MD: Official Methods of Analysis; 1997.
20. Sathe SK, Salunkhe DK. Functional properties of great northern bean (*Phaseolus vulgaris*) proteins: Emulsion, foaming, viscosity and gelation properties. J. Food Sci. 2005;46:71–75.
21. Bernard C, Regnault S, Gendreau S, Charbonneau S, Relkin P. Enhancement of emulsifying properties of whey proteins by controlling spray-drying parameters. Food Hydrocoll. 2011;25(4):758–763.
22. Wani AA, Sogi DS, Grover L, Saxena D. Effect of temperature, alkali concentration, mixing time and meal/solvent ratio on the extraction of water-melon seed proteins-a response surface approach. Biosyst. Eng. 2006;94(1):67–73.
23. Siddhuraju PK, Becker. Nutritional and antinutritional composition, in Smith PK. (1985). Measurement of protein using bicinchoninic acid. Anal Biochem. 2005;150:76–85.
24. Agbede JO, Aletor VA. Studies of the chemical composition and protein quality evaluation of differently processed

Canavalia ensiformis and *Mucuna pruriens* seed flours. J. Food Compos. Anal. 2005; 18:89–102.

25. Mizubuti IY, Biondo Júnior O, De Oliveira Souza LW, Dos Santos Ferreira Da Silva RS, Ida EI. Response surface methodology for extraction optimization of pigeon pea protein. Food Chem. 2000; 70(2):259–265.
26. Ngatchic TJ, Yanou NN, Oben JE, Mbofung CMF. Protein quality and antigrowth effect of protein isolate of Mucuna (*Mucuna pruriens*) and Canavalia (*Canavalia ensiformis*) seeds. Sch. Acad. J. Biosci. 2013;1(5): 183–191.
27. Okerie BO, Bello AB. Physicochemical and functional properties of winged bean flour and isolate compared with soy isolate. J. Food Sci. 1988;53:450–454.
28. Bruhn CM, Bruhn JC. Obserrvations on the whipping characteristics of cream. J. Dairy Sci. 1988;71(3):857–862.
29. Huang X, Kakuda Y, Cui W. Hydrocolloids in emulsions : Particle size distribution and intefacial activity. Food Hydrocoll. 2001;15: 533–542.
30. Hunt JA, Dalgleish DG. Heat stability of oil-in-water emulsions containing milk proteins: Effect of ionic strength and pH. J. Food Sci. 1995;69:1120–1123.
31. Aghazarian LV, Piccerelle P, Reynier JP, Joachim J, Phan-Tan-Luu R. Texture optimization of water-in-oil emulsions. 2004;9(2):125–134.
32. Virangkumar NL, Zagabathuni VPM. Enhancing the stability of oil-in-water emulsions emulsified by coconut milk protein with the application of acoustic cavitation. Ind. Eng. Chem. Res. 2012;51(11):4222–4229.

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